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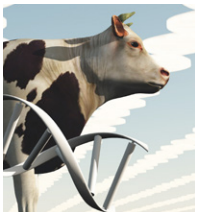
Genome-edited livestock: Ethics and social acceptance



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Implications

- With the advent of robust genome editing tools, strains of cattle, pigs, sheep, goats, and fowls with no transgenes have been bred.
- Food products derived from genome-edited livestock are expected to enter the market soon after the safety is confirmed in a country. However, previous controversy over genetically modified (**GM**) animals and cloned animals suggests that many people will be unlikely to accept the products from genome-edited animals.
- The social acceptance of such farm animal products would depend on the major premise that animal breeding by genome editing is performed after due considerations with regard to people's sense of ethics as well as animal welfare.

Key words: animal welfare, ethics, genome editing, livestock breeding, social acceptance

Introduction

The agricultural application of genetic engineering has advanced in the field of crop breeding. In 1994, the US Food and Drug Administration (**FDA**) approved a genetically modified (**GM**) tomato variety, the world's first GM crop for food consumption (Bruening and Lyons, 2000). In this GM tomato (the *Flavr Savr*), ripening was delayed by the insertion of an antisense gene that interferes with polygalacturonase production. Although the regulatory approval of GM crops largely demands strict assessments of the environmental risks and food safety, the commercial cultivation of GM crops with an exogenous gene (termed transgene) has spread to at least 28 countries, including the USA, Brazil, Argentina, India, Canada, China, and some European countries (Ishii and Araki, 2016). Conversely, there have been few regulatory approvals regarding GM livestock, with the exception of GM goats for "pharming" in which biopharmaceuticals are manufactured using transgenesis (FDA, 2009).

Currently, older genetic engineering practices, such as transgenesis, are giving way to genome editing. Genome editing tools, such as zinc-finger nucleases (**ZFNs**; Klug, 2010), transcription activator-like effector nucleases (**TALENs**; Joung and Sander, 2013), and the clustered regularly interspaced short palindromic repeat (**CRISPR**)/Cas 9 (Barrangou and Doudna, 2016), can break DNA double strands at target sites and then achieve various types of genetic modification via non-homologous end-joining (**NHEJ**) or homology-directed repair (**HDR**), thus potentially adding new value to agriculture (Figure 1). Recent reviews suggest that NHEJ is preferred in crop genome editing because the resultant plants are considered to contain no transgenes, which is one of the major concerns over GM crops from regulatory and social aspects (Hartung and Schiemann, 2014; Voytas and Gao, 2014; Araki and Ishii, 2015). Genome editing has also been applied in livestock breeding (Carlson et al., 2012; Hai et al., 2014; Crispo et al., 2015; Cui et al., 2015; Proudfoot et al., 2015; Wang et al., 2015a; Wang et al., 2015b; Wang et al., 2015c; Carlson et al., 2016; Fischer et al., 2016; Oishi et al., 2016; Petersen et al., 2016; Tanihara et al., 2016; Wang et al., 2016; Whitworth et al., 2016). Animals modified via NHEJ are unlikely to impose substantial risks on the environment because they can be managed within a farm, unlike GM crops, which are intentionally released into the environment (field cultivation). Thus, one can presume that the products derived from genome-edited livestock will soon be accepted in society if the food safety can be confirmed.

However, it would be inappropriate to presume that such a favorable course of events is the only possibility. In November 2015, the FDA approved a GM salmon for food consumption (FDA, 2015). Nonetheless, citizen groups and environmentalists still loudly oppose the FDA's decision about its safety. In addition, they questioned the environmental risk that it posed to wild salmon populations; despite that the sterile GM fish is only raised in landlocked tanks (Pollack, 2015). Such public movements may have prolonged the FDA review of the GM salmon. It took nearly a quarter of a century and cost more than $77 million (Van Eenennaam and Muir, 2011). Psychological investigations have suggested that GM animals are viewed as less acceptable than GM plants and that people's sense of ethics has a more significant effect on the acceptance than other factors such as the perceived risks, the recognized benefits, or the trust in regulators and researchers (Zechendorf, 1994; Siegrist, 2000). Likewise, complex situations are likely to emerge in the case of livestock genome editing because animals modified via NHEJ are also genetically modified. In the present article, we consider the practical and ethical bottlenecks in obtaining the social acceptance of animal breeding by genome editing, focusing on the development of livestock strains.

Genome Editing in Livestock

Zinc-finger nucleases and TALENs are artificial DNA cutting enzymes (nucleases) with a DNA–protein binding domain that directs the nucleases to a target sequence in the genome. CRISPR/Cas9 adopts a separate type of DNA-RNA binding system that can be readily prepared in most laboratories. Thus, the use of CRISPR/Cas9 has been particularly spreading worldwide.

The microinjection of the site-directed nucleases (in the form of plasmids, mRNAs, or proteins) into one-cell-stage animal embryos (zygotes) can effectively generate genome-edited offspring (Ishii, 2015). This approach is much

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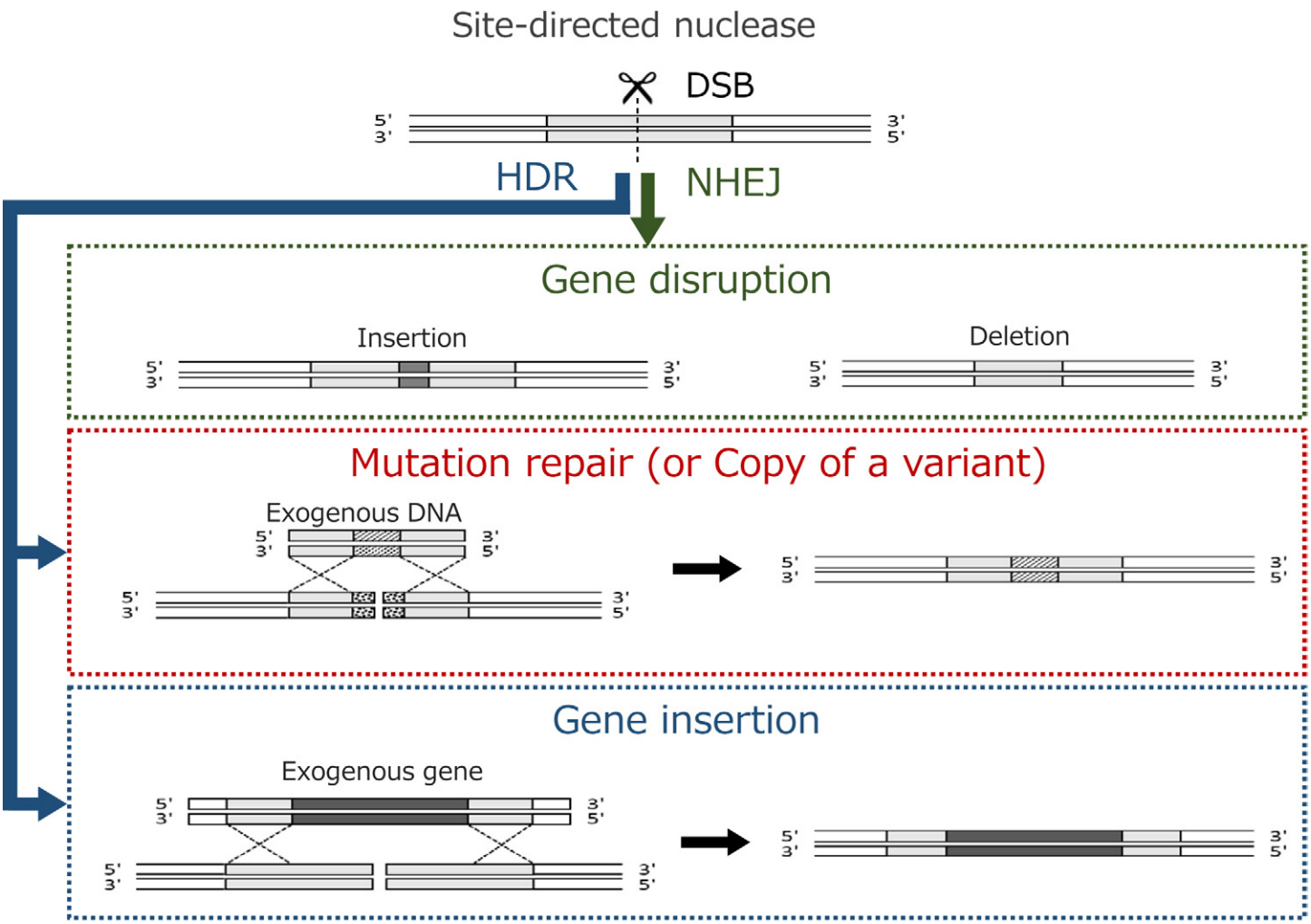

Figure 1. Two major pathways of genome editing. Double-stranded break (DSB) is induced at a targeted sequence by introducing site-directed nuclease. Non-homologous end-joining (NHEJ) is a DSB repair pathway that ligates or joins two broken ends together, resulting in the introduction of small insertions or deletions (indels) at the site of the DSB (gene disruption). Homology-directed repair (HDR) is a DNA template-dependent pathway for DSB repair, using a homology-containing donor template along with a site-specific nuclease, enabling the insertion of single or multiple transgenes (gene insertion) in addition to some nucleotide changes in which amino acid substitutions of a protein occur (copy of a variant), or a mutation is completely repaired in the resultant organism genome (mutation repair).

simpler than GM animal production involving the transfer of embryonic stem (**ES**) cells into animal embryos. In addition, the one-step-generation approach is applicable even in animal species for which no ES cell line is available. This methodology has been employed for NHEJ, primarily using the cytoplasmic injection of CRISPR/Cas9 mRNA and single-guide (**sg**) RNA into bovine, swine, ovine, and caprine zygotes (Table 1). The efficiency of genetic modification in neonates is largely high, as illustrated in the bovine [19% (Proudfoot et al., 2015)], swine [50%: biallelic modification (Petersen et al., 2016)], ovine [23%: homozygous KO (Crispo et al., 2015)], and caprine [13%: double KO (Wang et al., 2015a)] cases. Other approaches adopted NHEJ in primordial germ cells to generate knockout fowls (Oishi et al., 2016), NHEJ in somatic cells to generate double-knockout pigs via somatic cell nuclear transfer (**SCNT**; Fischer et al., 2016), and HDR in somatic cells to develop cattle and goats in which a variant was copied or a transgene was introduced via SCNT (Wang et al., 2015a; Carlson et al., 2016).

Overall, CRISPR/Cas9 is predominantly used in livestock genome editing. Moreover, one-step-generation via NHEJ is frequently used for gene disruption. Meanwhile, SCNT following HDR in somatic cells is applied for efficiently attaining transgenesis or copying a variant in the animal genome. Such reports have rendered precision livestock breeding technically feasible in the era of genome editing.

Practical Aspects

Consider the GM salmon again. The GM fish can grow twice as fast as conventional Atlantic salmon, through the introduction of two different transgenes: a growth hormone gene from a Chinook salmon and a promoter sequence of an anti-freezing protein gene from the eel-like ocean pout. As mentioned above, the FDA concluded that this food product is safe in 2015. Subsequently, two Canadian regulators also declared that the same GM fish is safe for use as a food and as livestock feed in 2016 (Health Canada, 2016). In the case of genetic modification via NHEJ, the resultant livestock have no transgenes, and thus potentially bypass current GM organism (GMO) regulations. However, will the deregulation based on the lack of transgenes lead to the social acceptance of products derived from genome-edited animals?

Table 1. Examples of genome editing-mediated genetic modification in livestock

Subject	Target Gene	Efficiency in zygotes*	Efficiency in Live Born	Off-target Mutation	Gene Editing	Delivery	Remarks	Reference
NHEJ (non-homologous end-joining)								
Bovine zygotes	MSTN	-	19%**	N.D.	TALEN	mRNA	Cytoplasmic injection	Proudfoot et al., 2015
Bovine zygotes	LDLR	3.8%	–	N.D.	TALEN	mRNA	Cytoplasmic injection	Carlson et al., 2012
Porcine zygotes	MSTN	–	20% (biallelic)	No	Cas9	Cas9 protein, sgRNA	Mosaicism, electroporation	Tanihara et al., 2016
Porcine zygotes	GGTA1	–	50% (biallelic)	No	Cas9	Plasmid	Mosaicism, cytoplasmic injection	Petersen et al., 2016
Porcine zygotes	CD163	–	9%**	N.D.	Cas9	mRNA/sgRNA	Cytoplasmic injection	Whitworth et al., 2016
Porcine zygotes	MITF	–	5% (biallelic)**	No	Cas9	mRNA/sgRNA	Cytoplasmic injection	Wang et al., 2015b
Porcine zygotes	Npc111	–	11%**	No	Cas9	mRNA/sgRNA	Mosaicism. cytoplasmic injection	Wang et al., 2015c
Porcine zygotes	vWF	–	15%**	No	Cas9	mRNA/sgRNA	Cytoplasmic injection	Hai et al., 2014
Porcine zygotes	RELA	0.5%	–	N.D.	TALEN	mRNA	Cytoplasmic injection	Carlson et al., 2012
Ovine zygotes	MSTN, ASIP, BCO2	–	6% (triple KO)	No	Cas9	mRNA/sgRNA	Cytoplasmic injection	Wang et al., 2016
Ovine zygotes	MSTN	4.6%	23% (homo-zygous KO)	Yes (20% in mutants)	Cas9	mRNA/sgRNA	Mosaicism, cytoplasmic injection	Crispo et al., 2015
Ovine zygotes	MSTN	–	4%**	N.D.	TALEN	mRNA	Cytoplasmic injection	Proudfoot et al., 2015
Caprine zygotes	MSTN, FGF5	–	13% (double KO)	Yes (23% in mutants)	Cas9	mRNA/sgRNA	Cytoplasmic injection	Wang et al., 2015a
Chicken PGCs	OVM	> 90%	G1 from #372: 58% G1 from #376: 48%	No	Cas9	Plasmid	Transfection	Oishi et al., 2016
PKFs	CMAH, GTA1	–	0.5% ** (double KO)	N.D.	Cas9	mRNA	Sequential SCNTs of edited cell lines	Fischer et al., 2016
HDR (homology-directed repair)								
BEFs	POLLED introgression	–	7% (Day 70)	No	TALEN	mRNA, Oligo DNA	SCNT of edited cell lines	Carlson et al., 2016
GFFs	hLF insertion after BLG KO	–	40% (3 mo)	N.D.	TALEN	mRNA, pBLG-hLF-puro	SCNT of edited cell lines	Cui et al., 2015

*Genetically modified embryos per injected zygote (%). **Genetically modified offspring per injected embryo (%). N.D.: not determined. PKF: Porcine Kidney Fibroblast. PGC: Primordial Germ Cell. GFF: Goat Fetal Fibroblast. Bovine Embryo Fibroblasts. SCNT: Somatic Cell Nuclear Transfer.

Despite its ability to perform robust genetic modifications, some practical issues currently remain in genome editing. The one-step-generation approach may result in not only systemic genetic modification, but also mosaicism in which wild-type cells, including germ cells, coexist with genetically modified cells in the resultant organisms (Table 1). However, this is simply a technical issue that can be avoided by more carefully considering the injection methods (the timing or use of pronuclear injection) in addition to the dose and the form of the nucleases. Although the site-directed nucleases may fail to induce a biallelic modification in the resultant animals, thereby resulting in an individual animal with a monoallelic modification, this also represents a technical issue that may be surmounted by careful screening or by optimizing the conditions of genome editing (Table 1). More importantly, if the guiding molecule of nucleases is inappropriately designed and its specificity is insufficiently validated, then the artificial nucleases could create off-target mutations at unintended sites in the animal genome. Notably, two of the 17 reports on genome-edited animals described the occurrence of off-target mutations in the resultant sheep and goats (Crispo et al., 2015; Wang et al., 2015a) (Table 1). Although the absence of off-target mutations was confirmed by analyses in the modified animals in eight reports, the remaining reports did not address this issue (7/17; Table 1).

Off-target mutations may result in a silent mutation or a loss of function. However, other mutation could result in the formation of an aberrant form of protein that confers allergenicity in food consumption. Similar to the GM salmon, the use of genome editing in the food industry is new. Thus, in the USA, under sections 201(s) and 409 of the Federal Food, Drug, and Cos-

metic Act, any food products derived from genome-edited livestock would be considered to be a "food additive," which is subject to an FDA premarket review to examine whether the products can be generally recognized as safe (so-called **GRAS**; FDA, 2016b). However, FDA review is performed based on the opinions of qualified experts, and the opinions of the representatives of the public are not included. Moreover, some people will be likely to ask: "Do off-target mutations only affect food safety?"

Recent and Previous Discussions Surrounding Animal Biotechnology

What are the important norms regarding animal biotechnology? Religions may impact the development of animal strains using genetic engineering. Muslims and Jews avoid eating pork product. Cattle are sacred to Hindus. However, it is unlikely that religions will have a significant impact on animal biotechnology in secular nations.

In December 2015, a 2-d National Academies of Sciences, Engineering and Medicine (**NASEM**) workshop was held to consider the scientific and ethical implications of animal genome editing for research purposes (NASEM, 2015). In addition to the regulatory implications, the attendees argued the welfare of animals that undergo genome editing based on the principles of the 3Rs (replacement, reduction, and refinement). Subsequently, a news report appeared with a headline, "Panel tackles—and is tackled by—genome editing in animals" (Elizabeth, 2015). The report stated that it was difficult to conclude that the use of genome editing reduces the number of laboratory animals, replaces higher animals with lower animals, or refines animal welfare although genome editing is a robust form of genetic engineering that can be applied in a wide range of animal species. With regard to the relevant regulations, some attendees preferred different or increased regulations, some asserted that genome editing should be less strictly regulated, and some wished to maintain the current regulations. Thus, the report described the workshop as less conclusive (Elizabeth, 2015). Although the meeting offered a precious opportunity for considering the implications of animal genome editing, a more specific or different focus might have been useful when planning the workshop.

Some lessons can be learned from the history of animal cloning in the debates that stemmed from the birth of a cloned sheep, Dolly in 1996 (Campbell et al., 1996). At present, the agricultural use of cloning is not common. In the USA, some companies have used cloning, but primarily for breeding, not food production. Meanwhile, a Chinese company plans to produce 100,000 cattle embryos a year, initially for meat production (Phillips, 2015). In retrospect, the FDA had held a voluntary moratorium on livestock cloning for food production since 2001. In 2008, the FDA concluded, based on an investigation, that there were no discernable differences between cloned and wild-type cattle, swine, and goats and declared that products derived from cloned animals are safe (FDA, 2016a). However, citizen groups opposed the regulatory decision, questioning the long-term safety and expressing animal welfare and ethical concerns in relation to the high rates of abnormalities and mortality and the inevitable necessity of euthanasia in cloned animals (Martin and Pollack, 2008). In 2009, the Food Safety Commission of Japan also concluded that the food safety of cloned cattle and swine is equivalent to that of such animals raised by conventional breeding (Food Safety Commision of Japan, 2009). People expressed concerns similar to those expressed in the USA. Conversely, in 2015, the European Parliament took animal welfare and ethical concerns into account and voted to prohibit the cloning of all livestock, (Vogel, 2015). The proposed bans include the sale of cloned livestock and products derived from them.

With regard to the cloning of animals for agricultural purposes, the European Parliament considered animal welfare and ethical concerns, whereas the US and Japanese regulators did not: they focused on food safety based on the opinions of experts. Despite the different regulatory positions, the course of events in these jurisdictions suggests that it is important to consider the people's sense of ethics as well as animal welfare when considering biotechnology developments that are related to animals.

People's Sense of Ethics in Relation to Animal Cloning

For further considerations in relation to animal genome editing, it is worth gaining deeper insight into people's concerns over animal cloning because animal welfare is addressed by people, not the animals themselves. We analyzed 99 public comments regarding the results of an investigation on the food safety of cloned cattle and swine, which were submitted to the Japan Food Safety Commission in 2009 (Food Safety Commision of Japan, 2009; Figure 2a). We categorized the comments into six subcategories, some of which overlapped. Some people looked forward to food products derived from cloned animals (8%). However, most people were not satisfied with the results or conclusion of the investigation and showed distrust in the researchers or regulators (total 65%), suggesting that many people did not appreciate the regulators or researchers. Other comments included questions due to a lack of scientific knowledge (10%: e.g., mistaking cloned animals for GM animals), concerns over the welfare of cloned animals (9%: e.g., concerns about the high rates of mortality and abnormality in the resultant offspring), and insufficient communications (8%: e.g., suggesting the need to hold public meetings regarding food safety and animal welfare). These public attitudes suggest the need to sufficiently inform people of the pros and cons in relation to the technology, to hold more public dialogues, and to carefully consider animal welfare (Figure 1).

Food does not merely supply nutrition to sustain human lives; it also provides taste, pleasure, entertainment, and company. Ethics must be more carefully considered in the development of animal-related biotechnology. Although some might assert that livestock are, whether or not they have undergone a biotechnological process, just animals that are raised to produce commodities such as food, hides, and fiber for use by humans. Moreover, one might also assert that NHEJ in genome editing does not differ from conventional breeding due to the similarity to naturally occurring mutations as well as the absence of transgenes. Nonetheless, the welfare of genome-edited livestock is of great importance until such animals are used for agriculture, as illustrated by the previous and current debates surrounding the use of cloned animals. Greater efforts to address animal welfare might change people's attitude toward researchers and regulators and enhance the possibility of the social acceptance of products derived from genome-edited livestock. It may be useful to consider the Aristotelian concept of "telos": the essence and purpose of a creature (GM, cloned or genome-edited animals) in addition to the moral imperative of producing such animals (Rollin, 2003; Elizabeth and Ortiz, 2004).

Case Studies

Next, genome editing-mediated on-target genetic modification for an animal breeding program is discussed. This section considers the type of

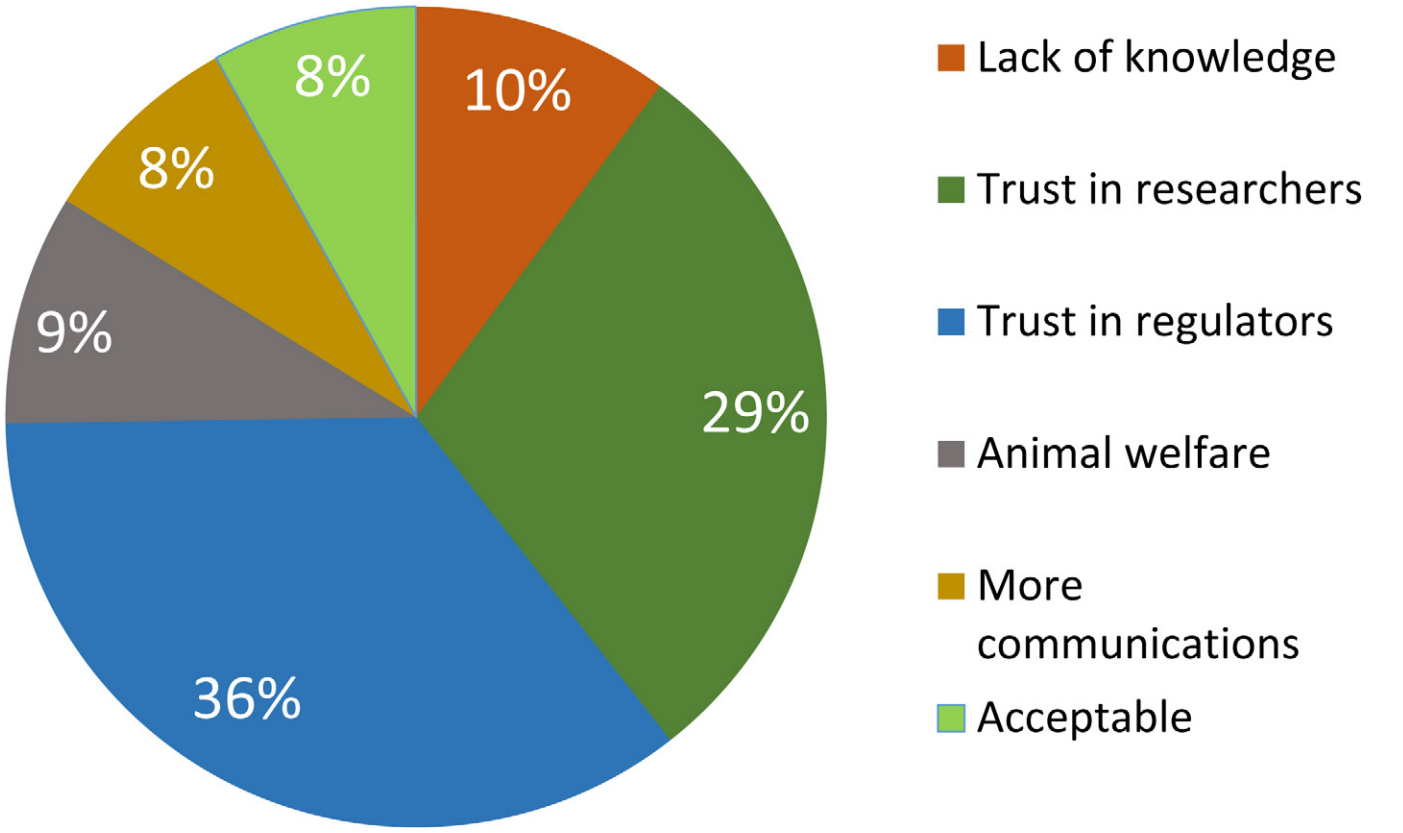

Figure 2a. An analysis of the public opinions regarding livestock bred by somatic cloning and their products. The public opinions were accepted from 12 Mar. to 10 Apr. 2009. Fifty-four people submitted 99 opinions to the Food Safety Commission via the internet, fax, and postal mail. Further details: http://www.fsc.go.jp/iken-bosyu/pc1_shinkaihatu_clone_210312.html (in Japanese).

animal breeding that can best satisfy concerns over the animal welfare and people's sense of ethics. Research reports on livestock genome editing, which were shown in Table 1, were selected and categorized into four purposes (Figure 2b). The implications of on-target mutations in each report are scrutinized in due considerations of the moral imperatives and "telos."

Genome editing for human health. The major causative antigen of egg allergy is ovalbumin and ovomucoid (Anet et al., 1985). Ovalbumin is readily denatured by heating, resulting in a reduction of the antigenicity. In contrast, heat treatments only cannot reduce the allergenicity of ovomucoid in egg whites. To date, the genetic modification in chickens has been delayed due to the difficulty in accessing and manipulating zygotes. Recently, a report demonstrated CRISPR/Cas9-mediated mutagenesis in chickens to disrupt an egg white allergen, the ovomucoid gene (*OVM*; Oishi et al., 2016). Primordial germ cells, in which *OVM* was disrupted via NHEJ, were transferred into recipient chicken embryos, resulting in the establishment of three germline chimeric roosters, all of which had donor-derived mutant-*OVM* spermatozoa. Subsequently, *OVM*-homozygous offspring mutant were produced by crossing the chicken mutants. This study shows the possibility of generating a chicken strain with low allergenicity.

However, egg white allergy usually only occurs in infants and young children (Sampson and McCaskill, 1985; Bock and Atkins, 1990). Moreover, it is unclear whether there is a compelling need of producing ovomucoid-deficient chickens because the heated and ovomucoid-depleted egg whites display less allergenic (Urisu et al., 1997). Moreover, egg substitutes are available for cooking and there are plenty of recipes without egg whites (The Asthma and Allergy Foundation of America, 2016). Furthermore, the eggs of the chickens that underwent the genome editing lost a major protein, which may be regarded as a loss of "essence in a creature."

Genome editing to improve productivity. As shown in Table 1, the knockout of *MSTN* has frequently been performed in animal genome editing. Other than cattle, *MSTN* knockout has been performed in sheep, goats, and pigs. *MSTN* encodes myostatin, which is exclusively observed in the skeletal muscles. The expression of *MSTN* is already active before birth. Because myostatin ordinarily regulates muscle growth to prevent excessive grow, *MSTN* knockout animals display an ultra-muscular physique (so-called, "double-muscling"; Lin et al., 2002). Some animals have naturally occurring *MSTN* mutations. For example, a breed of beef cattle from Belgium (the Belgian Blue) has lean muscle due to an *MSTN* mutation (McPherron and Lee, 1997). Thus, NHEJ-mediated MSTN mutagenesis is a conceivable line of breeding research that may improve the meat productivity of individual animals.

However, many ethical concerns can be expected arise by promoting double-muscling through genome editing (Treston, 2015). Difficult delivery abounds in Belgian Blue cattle because the active expression of *MSTN* starts in pregnancy and frequently necessitates Caesarean section. Belgian Blue calves can suffer from leg problems (due to their heavier weight), breathing complications, and enlarged tongues. Some people would consider that animals that are destined to acquire double-muscling through genome editing lose their "purpose as a creature."

Genome editing for animal health. Because farmed animals are raised in close proximity to each other, the outbreak of an infectious disease in a

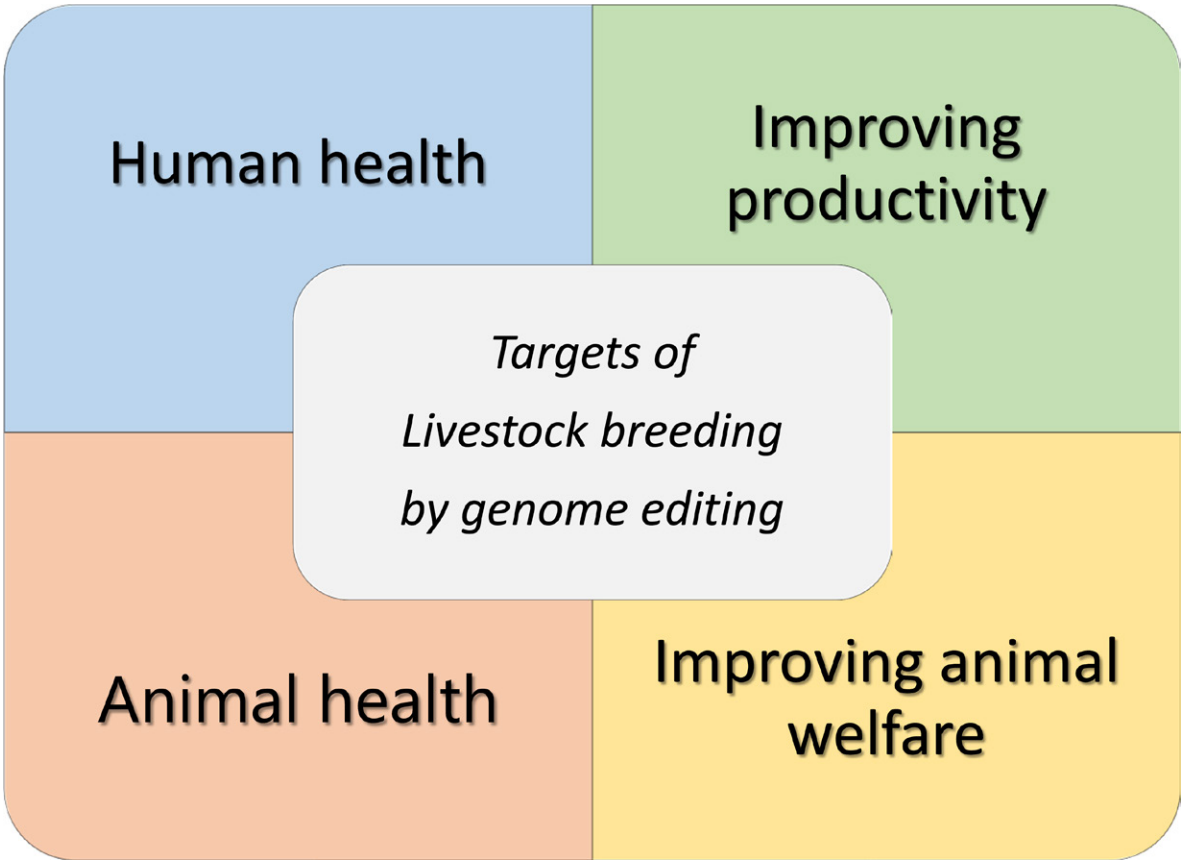

Figure 2b. The major agricultural purposes for the use of genome editing in livestock breeding. Recent reports on genome editing in livestock were selected from Table 1 and were categorized into four purposes.

barn would likely lead to disastrous consequences of reduced animal production or euthanasia for preventing the spread of infectious disease. Genome editing may serve infection control by providing animals with disease resistance. Recent studies on genome editing have described the generation of two breed of pig with mutations of the *CD163* and *RELA* (*p65*) genes, which confer tolerance for porcine reproductive and respiratory syndrome (PRRS) and African swine fever, respectively (Carlson et al., 2012; Whitworth et al., 2016). Of particular note, pigs that lacked a functional CD163 after NHEJ were resistant to a PRRS virus isolate, displaying no clinical signs (fever or respiratory signs) and remaining healthy for 35 d after infection.

Vaccines have been ineffective for preventing PRRS. If genome editing can truly contribute to the control of virus infections, the genetic modification can be considered to have improved animal health. One could rebut this type of genome editing by stating that gene disruption diminishes or changes the "telos" in pigs (Verhoog, 1992). However, given that livestock breeding is accepted in many countries and that animals that live in close proximity to other animals are vulnerable to virus outbreaks, a moral imperative may be recognized in this form of animal breeding. Although more investigations are still required to confirm that the NHEJ has no side effect on animal health, people might have a favorable view of the NHEJ as serving a "purpose in a creature." In humans, the case reports of the "Berlin patient" who benefitted from *CCR5* D32 mutation (Hutter et al., 2009) justified the world's first genome editing trial in which the *CCR5* in T cells was intentionally disrupted ex vivo to provide patients with the resistance to HIV infection (Tebas et al., 2014).

Genome editing to improve animal welfare. There has been an ongoing debate surrounding the dehorning of cattle. Although dehorning frequently uses invasive and laborious procedures such as disbudding and heat cauterization, it is performed worldwide to avoid causing injuries to other cattle and farm workers (Carroll et al., 2016). Thus, in addition to farmers, the public are concerned about the welfare of cattle that undergo painful dehorning. A recent study described the production of a hornless strain of dairy (Holstein) cattle by copying the *POLLED* of beef cattle (Angus) via HDR and somatic cloning (Carlson et al., 2016). The frequency of *POLLED* in Holstein cattle is much lower due to the small number of sires that produce commercially available *POLLED* semen. Therefore, this breeding could reduce the frequency of dehorning in the dairy industry, potentially enhancing the welfare of cattle.

However, people are likely to contemplate the implications of the visible change in the cattle. Thus, some consider this visible change to represent a loss of the "essence of a creature" through genome editing. One might assert that hornless cattle are generated to prevent injury to both farmers and other cattle. However, some would still view the use of genome editing in this regard as the initiation of "increasingly imbalanced distribution of power between humans and animals" (Schicktanz, 2006). In addition, the need for this animal genome editing would be questioned. There are alternatives: enriching the rearing environment to prevent accidents, the use of horn covers (Zen-Noh Livestock Co., 2016), and performing the dehorning of cattle under anesthesia. It appears that the moral imperative for animals is scant

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in this breeding program. As a result, it is unlikely that people would accept that the use of genome editing in this setting enhances animal welfare.

Taken together, the aforementioned arguments suggest that genome editing to prevent viral infections (for the purpose of animal health) may best satisfy the animal welfare concerns and would be most acceptable under people's sense of ethics. Thus, this type of breeding may be considered for a priority program for a research group or a research institute.

Rethinking Off-Target Mutations

Genome editing differs from older genetic engineering techniques that require the intracellular use of artificial nucleases that a researcher has designed. Some off-target mutations could be deleterious mutations that negatively affect animal health; this may lead to concerns over animal welfare. For example, missed off-target mutations could affect animal health if such unintended genetic changes lead to tumor formation due to mechanisms such as the disruption of a tumor suppressor gene. As the history of cloned animals suggests, the investigation of off-target mutations seems vital to the use of genome editing in livestock breeding from the viewpoint of animal welfare. Notably, the negative attitude of people toward GMOs is, in part, based on a lack of trust in researchers and regulators (Ishii and Araki, 2016). Thus, the further consideration of animal welfare by reducing the risk of off-target mutations might enhance people's trust and eventually foster the social acceptance of products from genome-edited livestock.

There are three main approaches by which off-target mutations may be detected: the sequencing of only potential off-target sites, whole-genome sequencing (**WGS**), and whole-exome sequencing (**WXS**). Although it is cost-effective to interrogate potential off-target sites that are deduced in silico from a target sequence, some would question its appropriateness for securing animal health. In contrast, WGS is a comprehensive approach that can be used to interrogate coding regions as well as the promotors and terminators that impact a gene's expression. However, it seems difficult to distinguish small off-target mutations from a single nucleotide polymorphism (SNP) or spontaneous mutations that occur during cell culture. Whole-exome sequencing, which analyzes all of the protein-coding regions (approximately 2.4% of the cattle genome: 64 Mb), might be an efficient method for ensuring animal safety because an off-target mutation in an exome is more likely to exert a serious influence on a protein function than in the remaining region. Nonetheless, there is currently no consensus regarding the means of assessing off-target mutations in genome-edited organisms (Joung, 2015). At present, it would be appropriate to investigate off-target mutations in animal embryos or somatic cells as deeply as possible, as a report on bovine genome editing demonstrated (Carlson et al., 2016).

Summary

Rapid advances in livestock genome editing research suggest that animal products will enter the market soon after their food safety is confirmed in a country. However, previous controversy over GM animals and animal cloning underscores the importance of people's sense of ethics as well as animal welfare (Figure 3).

The breeding of farm animals using genome editing should be performed after due considerations in relation to the ethical implications of animal genetic modification in society. Moreover, for animal welfare, developers should thoroughly investigate the occurrence of off-target mutations in the breeding of genome-edited animals. Regulators should interrogate developers about off-target mutations and promote public dialogues about livestock breeding using genome editing if they wish to enhance the public acceptance without any major disputes in society. Such farm animal products will never be accepted without consideration about both the practical and ethical aspects of animal genome editing.

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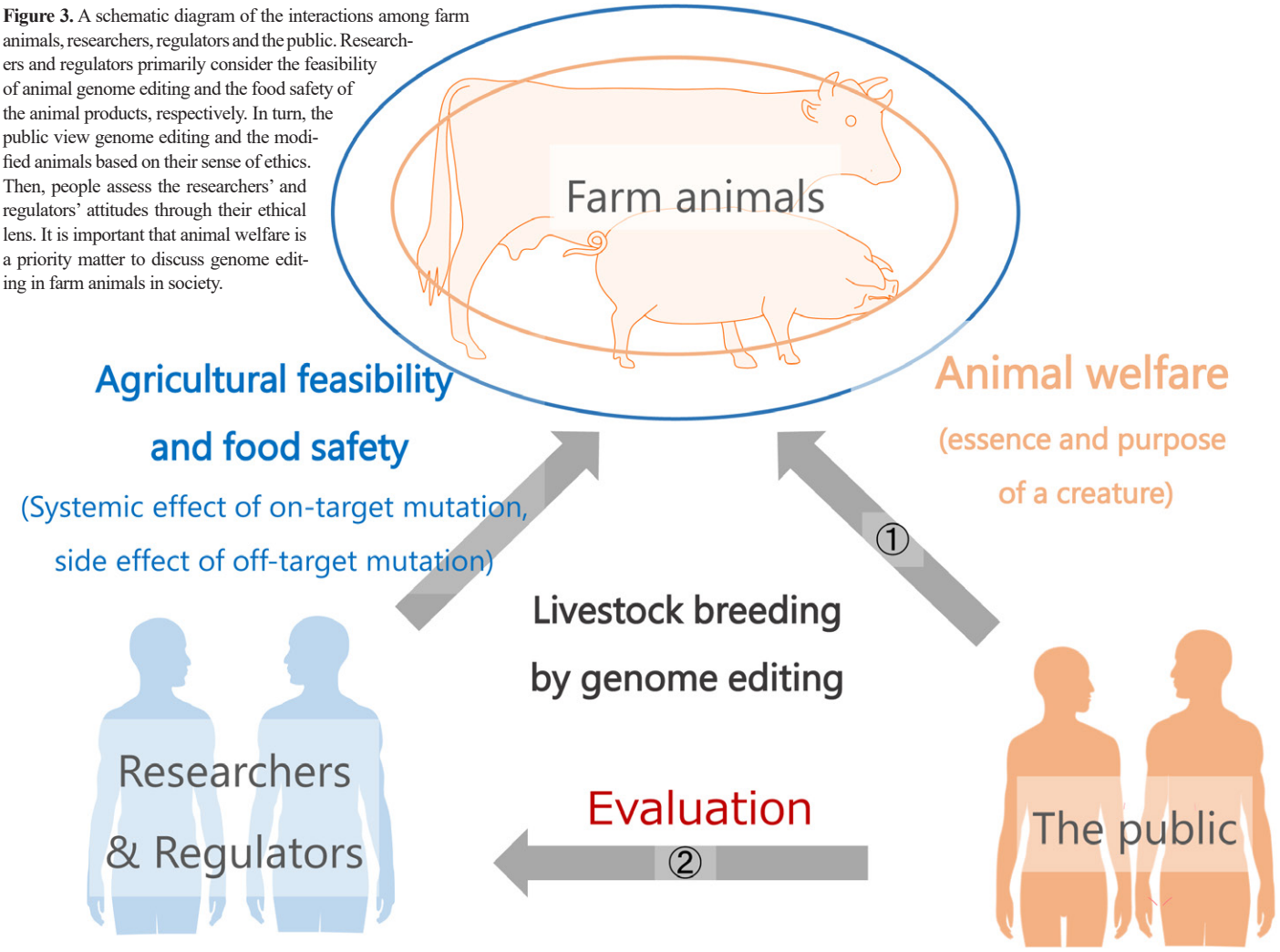

Figure 3. A schematic diagram of the interactions among farm animals, researchers, regulators and the public. Researchers and regulators primarily consider the feasibility of animal genome editing and the food safety of the animal products, respectively. In turn, the public view genome editing and the modified animals based on their sense of ethics. Then, people assess the researchers' and regulators' attitudes through their ethical lens. It is important that animal welfare is a priority matter to discuss genome editing in farm animals in society.

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