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Genome Editing and the Future of Farming

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Genome editing to the rescue: sustainably feeding 10 billion global human population

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COMPETING INTERESTS

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ABSTRACT

Modern animal breeding strategies based on population genetics, molecular tools, artificial insemination, embryo transfer and related technologies have contributed to significant increases in the performance of domestic animals, and are the basis for a regular supply of high quality animal-derived food at acceptable prices. However, the current strategy of marker-assisted selection and breeding of animals to introduce novel traits over multiple generations is too pedestrian in responding to unprecedented challenges such as climate change, global pandemics and feeding an anticipated 33% increase in global population in the next three decades. Here, we propose site-specific genome editing technologies as a basis for "directed" or "rational selection" of agricultural traits. The animal science community envisions genome

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editing as an essential tool in addressing critical priorities for global food security and environmental sustainability, and seeks additional funding to support the development and implementation of these technologies for maximum societal benefit.

RATIONALE FOR GENOME EDITING

It is predicted that by 2050, the current 7 billion world population will grow by another 2.6 billion (http://www.fao.org/fileadmin/templates/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf), particularly in developing countries and in urban areas. The demand for food is expected to require at least a 70% increase in meat production. The vast majority of arable land around the world is already under production, with land use being further restricted by urbanization, production of biofuels and climate change. Production practices related to animal welfare such as castration and dehorning are often vilified and continue to influence public conscience. Finally, risks of global pandemics affecting animals, such as foot-and-mouth disease or zoonotic diseases that affect both the humans and animals alike (e.g. influenza), are One Health challenges that need to be tackled. Addressing these pressing challenges requires dramatic approaches including replacement of existing alleles and transfer of alleles between individuals, lines, breeds and even species.

In the past 50 years, average milk output per dairy cow in the United States has more than doubled, but fertility in dairy cattle as a measure of daughter pregnancy rate has declined by 30% (Figure1A), with associated high incidence of metabolic imbalance, mastitis and lameness (1). Sustained selection pressure on a singular production trait has created similar bottlenecks in other agricultural animals. As an alternative, selection based on the genomic breeding value (GBV) is increasingly being used in livestock selection schemes for being precise, economical and less time consuming. However, the utility of GBV is limited if the economically important traits are closely linked to undesirable traits and segregate as a unit (called haplotype), thus preventing the elimination of undesirable traits and associated loss of desired genetic or

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economic value (Figure1B). Additionally, even with GBV based selection, introducing novel alleles or traits for creating a new phenotype in a population is painstakingly slow because of crossing over (meiotic recombination) during gametogenesis and subsequent mixing of the genomes following fertilization. At a minimum, 5-6 generations of backcrossing are required to introduce the desired phenotype into an existing breed. In cattle, it translates to 30 years (2) for achieving 30% gain in genetic value. Consequently, new “next-generation” animal breeding technologies are needed to enable animal breeders to take advantage of independent introduction and transmission of desirable traits.

DIRECTED SELECTION USING GENOME EDITING

Site-specific nucleases (SSN) that generate a double stranded break (DSB) at the target locus and allow for precise alteration of traits or alleles while preserving genome integrity of the high value individual (Figure1C) provide an exciting new avenue for rapidly and effectively addressing animal industry needs such as improving animal adaptability and well-being, production capacity and efficiency; decrease or eliminate of genetic abnormalities; and increase disease resistance and resilience, thereby providing on-demand solutions. There are two broad classes of SSN consisting of either an engineered DNA-binding domain (DBD) fused to a nuclease, such as ZFNs (Zinc Finger Nucleases)(3) and TALENs (Transcription Activator-Like Effector Nucleases)(4); or an RNA-guided nuclease system, the CRISPRs (Clustered Regulated Interspaced Short Repeats)(5). The engineered DSBs in the genome undergoes repair by an error-prone non-homologous end joining (NHEJ) mechanism enabling the efficient generation of knock-out alleles in livestock species (6), or if accompanied by a donor-targeting vector with homology to the ends flanking the DSB allows for knock-ins, or point mutations in somatic cells for generating precisely modified animals via somatic cell nuclear transfer (SCNT)(7). These editing tools have already been used to accomplish gene deletions or knock-outs by direct injections of TALENs and CRISPRs into embryos of large animal species (6). Multiple groups around the world are working towards achieving gene targeting by injection of SSN and targeting vectors directly into the embryos, as was achieved in mice (8). This may be critical in light of EU countries seeking a ban on using cloned animals and products thereof for food.

Moreover, the precise genome editing tools are already providing a much needed stimulus for applications beyond animal agriculture, such as generating models of human disease, xenotransplantation research, as bio-reactors for the production of pharmaceutically active compounds, environmental remediation and for regenerative medicine research. Areas where related biotechnologies have already shown promise have been extensively reviewed, and are shown in Table-I.

A PROPOSAL FOR FUTURE DEVELOPMENT

First and foremost, a distinction needs to be made between genome editing technologies that utilize SSN to precisely alter 1 or 2 nucleotides as compared to transgenics, where exogenous or foreign genes are incorporated, and have been negatively received in some parts of the world. Active decoupling of editing and transgenics is critical in the short term to accelerate progress in the field for generating "acceptable" food animals. One strategy to garner public acceptance will be to focus on animal welfare, human health and nutrition, and sustainability projects, e.g. disease resistance, heart health, malnutrition, adaptation to climate change that are either not possible or would be prohibitively expensive using conventional methodologies. Internationally, mainly triggered by the more advanced applications in plants, discussion about whether and how to regulate these new technologies is intensifying with some regulators indicating that the introduction of precise mutations may not require regulatory oversight (9). It can be argued that far more random mutations arise from meiotic crossover events de novo during breeding that are of much greater prevalence and are not regulated, than those following the precise editing with the SSN. However, in the race to fast-track genomic selection and generating "superior" animals, restraint should be exercised in preventing the scenario of "Jurassic Park full of harmful genes" (10). One of the legitimate concerns with the use of SSN is the generation of off-target mutations. To a varying degree, all SSN have the potential for binding at sites resembling the actual target site and generating cuts at off-target sites, potentially generating novel, unintended mutations. This undercuts the unique advantage of using these tools for generating precise modifications in the genome. Next-generation SSN- use catalytically inactive CRISPRs with hybrid FOK1 nucleases of TALENs and are expected to offset these concerns as the off-target events would have to happen in close proximity, which can potentially be avoided by rational selection of target sequences (11). In the United States, the FDA has signaled intentions towards introducing new regulations for overseeing genome editing technologies.

Proactive steps should be taken by the animal scientists, regulators and industry stakeholders to address current constraints in the acceptance and approval of genomic technologies in food animal systems that have been demonstrated to be safe and beneficial to society. Opportunities to seek international consensus and collaboration should be increased to maximize the potential advances. Funding support is specifically required to translate the development of the SSN and associated technologies from the laboratory to industry through demonstrable and practical projects in animal agriculture. Equally important is the need for public education and extension. There is a further need to develop centres of excellence around species of interest, where technologies and tools can be developed, vetted with industry, regulators and society, and transferred to industry. Finally, resources for coordination to initiate workshops/conferences, e.g. OECD Co-operative Research Program conference sponsorship, and public education initiatives will need to be further encouraged.

In summary, the opportunity that the new SSN technologies offer must be rigorously tested and actively supported by the scientific community. The topics outlined in this manuscript are essential for food animal production to meet the needs of anticipated global population growth. There is a finite period of time until 2050 arrives and ignoring the ramifications of that inevitability, and ignoring promising technologies is irresponsible for future generations; and even unethical to accept the risks of ‘doing nothing’. We are facing unprecedented global challenges that need global thinking and global action. These efforts must cut across funding agencies and international borders. A concerted effort should be made to foster collaborative efforts among scientists around the globe, to work together to meet global challenges.

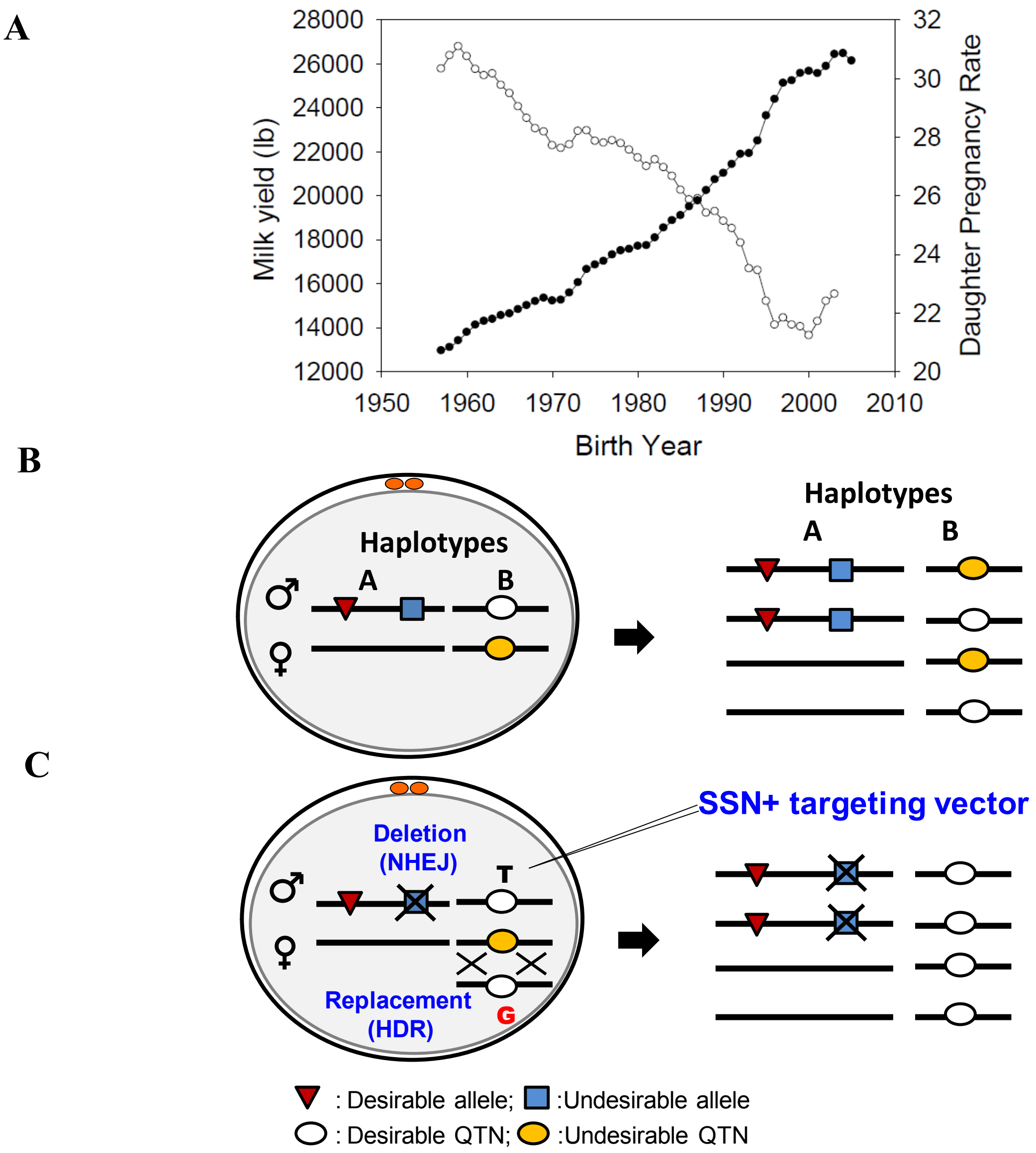

Figure 1.Solutions to constraints of animal breeding by the use of Site-specific nucleases (SSN). **A**) Trends in daughter pregnancy rates (°) and milk yield (•) for US Holsteins (Data from USDA-ARS Animal Improvement Programs Laboratory). **B**) The current breeding programs are based on selection of desired haplotypes. As illustrated in the figure using two haplotypes, "Haplotype A" consists of two alleles, one desirable and another undesirable. The two alleles within haplotype A segregate as a unit, and therefore selection for a desired allele (▼)also accompanies with an undesirable allele (■). Likewise, "Haplotype B" coincides with a desirable (○) and an undesirable (🟡) quantitative trait nucleotides (QTN). Selection of this individual

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based on desirable alleles and QTNs will generate four different combinations of gametes, potentially complicating and affecting genetic gains. **C**) However, with the use of SSN, non desirable alleles within haplotypes can be eliminated by SSN-mediated deletion, and/or beneficial QTN be introduced. If the selections are performed in somatic cells followed by nuclear transfer or even more desirably if performed in embryos, they can advance genetic selection in one generation.

Table 1. Application of transgenic technologies aimed at the improvement of agricultural production characteristics.

Introduced modification	**Application**	**Species**	**Reference**
Meat production			
Insulin-like growth factor 1	Increased meat production	Pig	Pursel *et al.* 1999
Human and porcine growth hormone releasing factor	Increased meat production	Pig	Pursel *et al.* 1990 Draghia-Akli *et al.* 1999
Human growth hormone releasing factor	Increased meat production	Sheep	Rexroad *et al*.1989
Bovine, human and porcine growth hormone	Increased meat production	Pig	Pursel *et al.* 1989, 1990 Nottle *et al*. 1999
Ovine growth hormone	Increased meat production	Sheep	Ward and Brown 1989 Adams *et al.* 2002
Fat-1 transgene	Elevated omega-3 fatty acids- heart healthy pork	Pig	Lai, L *et al.* 2006
Milk production			

Bovine α -lactalbumin	Increased milk yield and piglet survival	Pig	Wheeler *et al.* 2001
Bovine b- and k-casein	Improved milk composition	Cattle	Brophy *et al*. 2003
Biofarming	Recombinant human antithrombin (ATryn)	Goat	Schmidt, C 2006
	Recombinant human C1 esterase inhibitor (Ruconest)	Rabbit	van Veen, HA *et al*, 2012
Nutriceuticals	lysozyme and lactoferrin	Goats Cows	Maga *et al.* 2006b Van Berkel *et al.* 2002
Fiber production			
Ovine insulin-like growth factor 1	Improved wool production	Sheep	Damak *et al*. 1996
Ovine growth hormone	Improved wool production	Sheep	Adams *et al*. 2002
Ovine keratin intermediate filament	Improved wool processing and wearing properties	Sheep	Bawden *et al*. 1998
Bacterial serine transacetylase and O-acetylserinesulfhydrylase	Improved wool production	Sheep	Ward 2000
Feed conversion			
Bacterial Bacterialisocitratelyase and malate synthase	Increased glucose supply	Sheep	Ward 2000

Human glucose transporter 1 and rat hexokinase II	Improved glucose utilization	Fish	Krasnov *et al.* 1999
Adaptation to new habitat			
Piscine antifreeze protein	Fish farming in colder waters	Fish	Hew *et al*. 1999 Wang *et al.* 1995
Disease resistance / food safety			
S. simulans lysostaphin	Mastitis resistance	Cattle	Wall *et al.* 2005
Human lysozyme	Food spoilage	Goat	Maga *et al.* 2006b
Prion-gene knockout	Resistance to spongioformencephalopathies	Cattle Sheep Goat	Kuroiwa, Y *et al.* 2004 Denning, *et al.* 2001 Yu, G *et al.* 2006

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