Breeding in an Era of Genome Editing

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Glossary

Genome editing – the manipulation of the genetic material of an animal by deleting, replacing or inserting a DNA sequence.
Genome editors – molecular tools capable of making a double stranded break in the DNA sequence, including TALENs, ZFNs and CRISPR-Cas.
Selective breeding – making breeding decisions based on parents with desirable traits and/or genetics to produce offspring with desirable traits.
Cross breeding – crossing of two breeds of animals within the same species, often to combine desirable traits from each breed.
Quantitative Trait Loci (QTL) – regions of the genome at which genetic variation is associated with a particular quantitative trait.
Trait linked alleles – a genetic variant or loci linked to a trait of interest (e.g., underlying a QTL)
Introgression-by-editing – identifying naturally occurring genetic variation in one breed or population and establishing it in another using genome editing.
Creation of de novo alleles – creation of novel alleles to accelerate genetic progress that would not have occurred due to naturally occurring genetic variation.
Surrogate sires – males that are fertile but have had their germline ablated using genome editing, can also be termed 'surrogate hosts' and in aquaculture breeding are referred to as 'surrogate broodstock'.
New reproductive technologies – technologies aimed at facilitating reproduction in breeding programs, including artificial insemination and embryo transfer.

Definition of the subject

Genome editing is, by definition, the manipulation of the genetic material of an animal by deleting, replacing or inserting a DNA sequence. There are three types of 'genome editors', ZFNs, TALENs and CRISPR-Cas, which are molecular tools or 'nucleases' that each have a similar ability to introduce double strand DNA breaks in an animal's genome at a target site. The double strand DNA breaks stimulate endogenous cellular DNA repair which allows DNA sequences to be precisely modified or introduced into the genome (1). This powerful technology allows animal breeders to specifically and efficiently alter an animals DNA to introduce beneficial genetic variation (2). As such genome editing technologies offer exciting opportunities for breeding fitter, healthier, more productive and sustainable farmed animals (3).

Introduction

What is genome editing?

When genome editing is applied in animal breeding it is typically with the aim of 'improving' a given trait or characteristic. This could be by providing resistance to disease (4), enhancing a production related trait such as muscling (5), or for improving welfare (6). For example, in 2016, genome editing was used to produce 'hornless' or 'polled' cattle, removing the necessity for physical dehorning and providing the potential to improve the welfare of millions of dairy cattle (6). The 'polled' cattle were created using genome editors called

transcription activator-like effector nucleases (TALENs) (6). Two additional types of genome editing technology are also used, Zinc Finger Nucleases (ZFNs) and perhaps most famously, clustered regularly interspaced short palindromic repeat (CRISPR)–Cas-associated nucleases.

The above genome editing technologies each work on the same principle. Gene editors introduce a double strand break at a target location in the genomic sequence which is subsequently corrected by endogenous repair mechanisms (1). There are two ways in which double strand breaks can be repaired: i) through non-homologous end joining (NHEJ), resulting in small insertions and/or deletions (INDELs) which can disrupt gene function and ii) through homology-driven repair (HDR), in the presence of a homologous DNA repair template, resulting in gene-editing events (1). The gene-editing process can allow existing DNA sequences to be precisely modified and/or new DNA sequences to be introduced into the genome of farmed animals.

Examples of genome editing in farmed animal species

Genome editing tools have been successfully applied to produce edited farmed animals including pigs (7), cattle (8), sheep (9), goats (9), chickens (10) and aquaculture species, including salmonids and catfish (11) for a range of health, welfare and production traits (Table 1).

Table 1: Examples of genome editing in farmed animal species to improve five different categories of trait.

Category	Trait	Species	Editing target	References
Health	PRRSV resistance	Pig	*CD163*	(12–15)
	ASFV resilience	Pig	*RELA*	(16–18)
	IPN resistance	Atlantic Salmon	*nae1*	(19)
	Bovine tuberculosis resilience	Cattle	*SLC11A1 (NRAMP1)*	(20)
Welfare	Polledness (Hornlessness)	Cattle	P_C *POLLED*	(6)
	Heat tolerance (Coat color)	Cattle	*PMEL*	(21)
	Heat tolerance ('Slick' coat)	Cattle	*PRLR*	(22)
Reproduction	Sterility/surrogate broodstock	Atlantic Salmon	*dnd*	(23,24)
	Sterility/surrogate sires	Pig	*NANOS2*	(25,26)
		Goat		(26)
		Cattle		(26)
	Sterility/surrogate hosts	Chicken	*DDX4 (Vasa)*	(27,28)
Appearance	Plumage color (Dominant white)	Chicken	*PMEL17*	(29)
	Feather type (Frizzled)	Chicken	*KRT75*	(29)
Production	Enhanced muscle growth	Cattle	*MSTN (GDF8)*	(30)
		Sheep		(30–33)
		Goat		(34,35)

		Pig		(36–38)
		Red Sea Bream		(39)
		Channel Catfish		(40)
	Hair fibre length	Goat	*FGF5*	(41)

Many genome editing studies have targeted genes involved in production traits, such the myostatin (*MSTN*) gene that is involved in muscle growth development in sheep, goats, cattle and pigs e.g., (30,35,36) (Table 1). These traits are particularly attractive editing targets for application in animal breeding programs due to their potential to enhance genetic gain. Production traits however are often highly polygenic making identifying suitable targets for editing difficult. Targeting health traits for genome editing studies has considerable reward, because tools in a breeding program that mitigate disease concomitantly improve health, welfare and productivity (4). For example, Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most costly infectious disease affecting pig production globally (42). PRRS causes huge losses annually to the pig production sectors in Europe and the United States. A sow infected by PRRS will abort or give birth to stillborn piglets at great welfare cost to the sow and great economic cost to the producer (42). Piglets infected with PRRS have a very high mortality rate (~80%) and suffer severe respiratory distress. PRRS is a disease caused by a respiratory virus that infects pigs via the scavenger receptor gene *CD163* which is expressed by macrophages (43). Genome editing has been used successfully to produce pigs that are resistant to PRRS virus (PRRSV) either by rendering the gene *CD163* non-functional (14,15) or by preventing the pigs from correctly producing the part of the *CD163* protein required for the PRRSV to establish an infection (13). These genome edited pigs have been shown to be resistant to PRRSV but otherwise healthy (13,14). Use of such genome edited animals in pig breeding programs could substantially reduce economic losses globally that are related to PRRS (3,14).

Another example, related directly to improving the welfare of farmed animals is the generation of cattle that are 'hornless' using genome editing, as mentioned above. The processes of disbudding in young cattle to prevent horn development, and dehorning in mature cattle, are undertaken to reduce the risk of injury to the cattle themselves, each other, their handlers and equipment (44). In the USA 80% of dairy farmers practice routine dehorning of dairy cattle (45). Despite the benefits, dehorning practices represent an animal-welfare concern (46) and calves show long term sensitivity to pain post disbudding (47). The frequency of cattle that are naturally hornless (termed 'polled') is greater in beef than in dairy cattle (48). Considerable research into polledness in cattle revealed that one of two alleles must be causal (49,50). Carlson et al. (2016) used TALENs to introduce the P^C *POLLED* allele from beef cattle into the genome of bovine embryo fibroblasts from four lines of dairy cattle (6). These were cloned using somatic-cell transfer, resulting in full-term pregnancies for three of the four lines. Five live calves were produced; however, only two were viable and went on to survive beyond day 60. All five calves were determined to have a likely polled phenotype at birth, and the two surviving calves were confirmed to be polled. This study confirmed the causality of the P^C *POLLED* allele, and presented genome editing as a viable potential approach for reducing physical dehorning in dairy cattle without a loss of productivity (6). A similar approach could be applied in other species where it is possible to select for polledness and where horns can be detrimental to welfare e.g., in Merino sheep (51).

Genome editing as an alternative to selective breeding

The productivity and resilience of farmed animals has in recent decades been transformed by selective breeding and genomic selection. These processes though very

effective take many generations to achieve. Production of farmed animals needs to be a dynamic process, evolving to flexibly meet future challenges such as climate change and disease outbreaks coupled with societal expectations to improve welfare and reduce the use of antimicrobials (4,52). In circumstances where a desirable genetic trait, e.g., for disease resistance or improved welfare, can be identified in a breeding population, then selection for the trait through selective breeding can be achieved. Selective breeding for some traits such as disease resistance has however proven difficult. Outbreaks of disease, for example, are often sporadic and resistant/resilient animals can be hard to identify within a breeding population (4). Genome editing offers the potential to move flexibly and quickly to introduce resistance alleles and overcome these challenges (4).

Selective breeding can also be restricted by genetic linkage and the available genetic variation within a breed (2). Genome editing can overcome this by introducing novel genetic variation, not present in the gene pool of high value breeding animals, that is predicted to result in improved genetic gain (53). Another potential obstacle to selective breeding is that when an allelic variant associated with a desirable trait is present at a much lower frequency it may prove difficult to incorporate effective selection into a breeding program without having to rely on only a small number of founder sires. This introduces the risk of inbreeding and related longer-term productivity loss. Taking polledness in dairy cattle as an example, increasing demand for polled dairy bulls, whose genetics were disseminated via artificial insemination (AI), resulted in increasing numbers of polled Holstein AI bulls globally (54). However, initially due to the limited number of polled founder AI bulls, polled Holstein individuals displayed lower than average breeding values and a higher average kinship than horned individuals (55). Subsequent selective breeding of polled bulls aimed to remedy this, minimizing inbreeding by reducing reliance on a small number of founder sires, leading to an observed increase in the breeding values of polled AI bulls (54,55). In this scenario genome editing would provide an alternative approach allowing the direct introgression of the beneficial allele, in this case the P_C *POLLED* allele, into the offspring of genetically diverse, highly productive dairy bulls, minimizing inbreeding and any deleterious effects on breeding goals in a much shorter space of time.

Genome editing as an alternative to crossbreeding

Crossbreeding can be highly effective to introduce desirable traits from one breed into another (56). Indigenous breeds of farmed animals often exhibit desirable traits that are highly adapted to local and regional environments, and represent important genetic resources for crossbreeding (57). This is particularly true in tropical agri-systems (58). Indigenous breeds though often very robust, which is important particularly in the face of the pressures associated with climate change, unlike production breeds have not been selected over generations for high productivity. As such desirable robustness traits from indigenous breeds are unlikely to be introduced into highly productive populations of breeding animals by standard crossbreeding. Doing so would result in a reduction in productivity, that would compromise many years of improvements in productivity using selective breeding and genomic selection. Genome editing can overcome this by introducing genetic polymorphisms from indigenous breeds, that are not present in highly productive breeding animals, without resulting in a reduction in productivity (53). For example, genome wide analysis has revealed SNPs associated with trypanotolerance in the N'Dama, an indigenous central African breed of cattle (59,60). This variation was not present in susceptible Boran cattle or highly productive commercial dairy cattle breeds (59,60). Crossbreeding of the susceptible Boran with the tolerant N'Dama has been successful with superior trypanotolerance reported in F1 animals (61). However, crossbreeding of indigenous cattle with highly productive dairy cattle sires in East Africa has been shown to result in a reduction in milk yield (62). As such genome editing

could provide an alternative to crossbreeding and introduce the genetic variation associated with trypanotolerance from the N'Dama into highly productive commercial dairy cattle without disrupting existing breeding goals for high productivity.

Genome editing, also has the advantage that is can be applied to modify a single trait in different breeds of farmed animals that are adapted to specific environments and purposes, without compromising the beneficial traits that they already exhibit (8,63). Conserving beneficial traits in breeding populations of indigenous breeds is particularly important in tropical agri-systems where certain traits, such as tolerance to extreme environmental pressures, are essential for the survival of the animals and their small holder farmers (58,62). Careful application of genome editing technology in the context of the local environment allows for breed-specific traits not to be disrupted, while specific traits are improved. This helps to preserve breed diversity because specific traits can be introduced into locally adapted breeds without the need for crossbreeding (8,63). It is also minimally disruptive at a local level as no additional genetic material is transferred between breeds largely eliminating the deleterious side effects of selective sweeps and conserving local genetic diversity (8,63).

In some situations, crossbreeding is simply just not biologically feasible such as when a desirable trait is observed in one species and not in another. In this scenario genome editing can provide an alternative to crossbreeding. One example of this is the resilience to African Swine Fever Virus (ASFV) observed in wild species of pig, such as warthogs, while domestic pigs exhibit high morbidity and mortality (18,64). Introducing the genetics underlying resilience to ASFV is not possible via crossbreeding as warthogs and domestic pigs are too far apart genetically (65). Instead, comparative genomics can be used to identify the functional differences underlying resilience or susceptibility to ASFV in the two species e.g., (17,18). Genome editing can then be performed to substitute immune modulatory alleles associated with resilience to ASFV from warthog into domestic pigs (16,17).

Genome editing research of relevance to application in farmed animal breeding programs

Detection and utilization of causative variants at QTLs

Genome editing applied in commercial breeding programs offers considerable opportunity for improvement of the sustainability and efficiency of farmed animal production. There are three main categories of genome editing research of relevance to application in farmed animal breeding programs. The first category is detection and utilization of causative variants at Quantitative Trait Loci (QTLs) affecting production traits segregating within farmed animal populations. Computer simulations have demonstrated that using genome editing for favorable trait-linked alleles at multiple QTLs within a breeding program can accelerate genetic gain (66). However, a major challenge is the successful identification of the causative variation that underlies QTLs of interest, particularly those that have a small effect (67,68). To illustrate the scale of this task a recent estimate suggested that, 2932, 856, and 609 genomic regions, representing potential QTL for disease susceptibility, have been identified in cattle, chickens and pigs, respectively (69). Some studies have achieved success and have identified promising candidate loci underlying QTLs, and more are being discovered all the time, facilitated by high density SNP data, whole genome sequencing, genome editing and functional genomics (67,68,70). For example, recently using gene editing technology the nedd-8 activating enzyme gene (nae1) was identified as the gene that underlies the major QTL for genetic resistance to Infectious Pancreatic Necrosis (IPN) virus in Atlantic Salmon (19). IPN is an important viral disease in salmonids and breeding for resistance to IPN has been one of the greatest success stories in the history of fish breeding (71). However, despite targeted breeding programs outbreaks still occur and new variants have recently emerged (71). The identification of the putative causative resistance gene in Atlantic Salmon, combined with

advances in genome editing technology in aquaculture (11), have given rise to new opportunities for cross-species comparison and transfer of the genetic mechanisms of disease resistance. One example of this is 'introgression-by-editing' (11) of DNA sequence templates corresponding to salmon resistance alleles into other economically important farmed fish species such as trout (19).

Introgression-by-editing of desirable alleles

'Introgression-by-editing', is the second category of genome editing research of relevance to application in farmed animal breeding programs. This category relies on identifying beneficial genetic variation in one breed or population and establishing it in another. The key point being that the variation being edited could have occurred naturally by chance in both breeds to populations, had there been sufficient time, opportunity and selective pressure. 'Introgression-by-editing' of favourable alleles from other populations, breeds or species into a closed breeding population can be performed without the negative consequences of introgression by traditional breeding methods, such as selective breeding or crossbreeding, including linkage drag (53). This approach has been suggested as a means to reduce the impact of heat stress in dairy cattle via introgression of the *SLICK* mutation from Senepol cattle into dairy cattle (72,73). The *SLICK* mutation is a frameshift mutation in the prolactin receptor gene (*PRLR*) gene found in Senepol cattle that causes a relatively hairless appearance and increased thermal tolerance (74,75). *SLICK* is of interest to cattle breeding because it could be introgressed from indigenous cattle breeds into populations of production cattle to improve thermal tolerance (72,74,75).

In another example, Mueller et al. (2021) compared using gene editing versus conventional breeding to introgress the *POLLED* allele into tropically adapted Australian Brahman beef cattle, which are naturally predominantly horned (76). Their study demonstrated that due to the limited number of polled Australian Brahman bulls, a strong selection pressure on the polled trait would be required to increase the number of polled animals in the population to sufficient numbers (76). The scenarios they modelled demonstrated how genome editing could be used as a tool to accelerate introgression of homozygous polled sires with high-genetic-merit into the Australian Brahman population and mitigate the trade-off of slower genetic gain associated with decreasing the frequency of the *HORNED* allele frequency when conventional breeding was used (76).

'Introgression-by-editing' in this way could also be used to combine desirable traits for sustainable breeding and production. Wiltshire horn sheep, for example, are considered to be a very sustainable breed because they have a good carcass and naturally shed their wool, vastly reducing the costs to the farmer that are associated with shearing, whilst having good production value (77). The horns that are characteristic of the Wiltshire breed are however difficult to manage and polled animals would be desirable from a welfare and management perspective. As for the Australian Brahman example above the frequency of naturally polled Wiltshire rams is likely to be very low and as such strong selection would be required to introgress the polledness trait by selective breeding. Genome editing would considerably accelerate this process. However, the genetic control of polledness in sheep has so far proved more complex than first expected. A 1.78Kb insertion in the 3'UTR region of the *RXFP2* gene on chromosome 10 has been identified which is strongly associated with polledness in GWAS (78). The insertion upstream of *RXFP2* does not however segregate in the same way across all breeds (79) and the causative SNP for polledness has yet to be identified (51). As such genome editing for polledness in sheep is likely to be more complex to achieve than in cattle.

Creation of de novo alleles with favourable effects on target traits

The third category of genome editing research of relevance to application in farmed animal breeding programs is the creation of *de novo* alleles to accelerate genetic progress (11). These *de novo* alleles unlike in the 'introgression-by-editing' example above, which harnesses naturally occurring genetic variation, would not arise naturally. *De novo* alleles with favorable effects on target traits can be identified based on *a priori* knowledge of the biology of the trait of interest or through genome-wide high throughput functional screening approaches using cell lines (11). An example of the former is the creation of sterility in Atlantic Salmon by modification of the *dnd* allele. Escape of the genetics of farmed Atlantic salmon into wild populations is a major environmental and sustainability concern for salmon aquaculture (24). Through studying the genetics of germ cell function the *dnd* gene was found to encode the dead end protein which is involved in germ cell formation in Atlantic Salmon (23). Knocking out the *dnd* gene ablates germ cells producing sterility (23). However, rendering a whole population sterile, including high value breeding animals, is not sustainable in a breeding program either. To remedy this Güralp et al. (2020) used genome editing with CRISPR-Cas9 to modify alleles in the *dnd* gene, in the first step of a procedure to generate sterility in production stocks of salmon while preserving the fertility of the breeding nucleus (24). In this example, creation of *de novo* alleles by gene editing provided a tool to protect the genetic integrity of wild salmon populations, increasing the environmental safety and sustainability of salmon farming (24).

Another example of the creation of *de novo* alleles using genome editing, are the PRRSV resistant pigs described above, resulting in a viable animal missing an entire *CD163* receptor (14,15) or that have a modified *CD163* receptor missing a protein domain (13). This example is very unlikely to have arisen naturally given the functional importance of the *CD163* gene in the innate immune response (80). Similarly , the substitution of immune modulatory alleles from the warthog into the domestic pig with the goal of improving resilience to ASFV could also not occur naturally (16). Creation of *de novo* alleles using genome editing has also be applied to enhance production traits. For example, the *MSTN* mutation found in Belgian Blue cattle, that causes double-muscling, has been introduced into Duroc pigs using CRISPR editing (37). The ability to efficiently achieve inter-species allele introgression in one generation, by the creation of *de novo* alleles, opens unprecedented opportunities for farmed animal breeding and basic research. As our comparative understanding of the fundamental biology underlying resistance to disease and other traits across species increases so will the number of potential beneficial alleles and the opportunities available.

Applying genome editing in breeding programs

Promotion of Alleles by Genome Editing (PAGE)

The categories of research described above allow targeted genome editing with the potential for direct commercial application, to enhance genetic gain for target traits. Seamless integration with existing selective breeding programs and genomic selection strategies will be necessary if genome editing is to be adopted for trait improvement. Several concepts to achieve this have been proposed, based on computer simulation (53). Promotion of alleles by genome editing (PAGE) (66), for example, offers the opportunity to use genome editing to move genetic variation between individuals in a population much more freely than by selective breeding. This enables individual alleles with large effects to be moved independently of other alleles to maximize genetic gain. More recently the concept of removal of alleles by gene editing (RAGE), has been proposed to reduce the frequency of deleterious alleles within a breeding program (81). Reducing deleterious load in this way could improve fitness traits, with subsequent benefits for animal welfare, sustainability and profitability.

New reproductive technologies

At present, due to a mix of legislative and logistical barriers, application of genome editing technology is largely limited to the research and biotechnology sectors. To apply genome editing in breeding programs several logistical challenges would need to be overcome. For example, if genome editing is performed via somatic cell nuclear transfer, or zygote microinjection expert technicians are required to perform these tasks with specialized equipment. This makes the process complex, inefficient and too costly to be performed routinely on farm or on a large scale (2). To overcome this barrier new reproductive technologies that simplify the delivery of genome editors into the reproductive cells of farmed animals are required (2). Recently new cutting-edge reproductive technologies have emerged, including zygote electorporation. McFarlane et al. (2019) suggest a pipeline where for on-farm settings, donor female animals would be super-ovulated and oocytes collected for in vitro fertilization (2). This process would be no different to conventional embryo transfer programs that are run on farm. After fertilization, the zygotes would undergo electroporation to introduce the genome editors. The genome edited embryos would then be matured in vitro and the success of the editing of each zygote confirmed by portable biopsy sequencing. Embryos in which genome editing had been validated would then be transferred into recipient females to give birth to genetically superior animals. This approach, if scaled up effectively, could provide animal breeders with the tools required to deliver genome editors directly to reproductive cells and allow genome editing to take place on-farm (2).

Using surrogate sires to disseminate 'elite' genetics

Another innovative solution to applying genome editing in breeding programs is surrogate sire technology. Surrogate sire technology allows the creation of males that lack their own germline cells, but have transplanted spermatogonial stem cells from other donor males (25,26,82). In effect surrogate sires are rendered genetically sterile by CRISPR-Cas9 editing of the *NANOS2* gene (25). Once rendered genetically sterile donor derived stem cells are transplanted into the gonads of the surrogate sire (25). As their gonads are structurally normal the surrogate sire is able to support regeneration of spermatogenesis and effectively becomes a vehicle for the dissemination of the donors sperm (82). The donor would mostly likely be a high value animal whose genetics with the help of the surrogate sire can be disseminated to a large number of offspring. These males can disseminate the genetics of the donor by natural breeding, operating effectively as a mobile insemination unit for 'elite' genetic material. To date male mice, pigs, goats and cattle harbouring knock-out alleles of the *NANOS2* gene, generated by CRISPR-Cas editing, have been shown to have testes that are germline ablated but otherwise structurally normal (26). In goats, mice and pigs sustained donor-derived spermatogenesis from transplantation with allogenic donor stem cells has been achieved (26). In mammals only in mice so far has attainment of natural fertility been observed post transplantation (26).

Germline ablated surrogate sire animals represent a major advancement in realizing the enormous potential of surrogate sires as a tools for dissemination of genetics within animal breeding programs (82). Surrogate broodstock, created in a similar way by editing the *dnd1* gene, also represent a considerable opportunity to accelerate genetic gain in aquaculture breeding, providing the potential, for example, to tailor fish populations to specific environments (83). In chicken sterile surrogate hosts have been created by knocking out the gene *DDX4* (27). The introduction of donor genome edited primordial germ cells carrying a beneficial allele into the sterile male and female host embryos produces adult chicken that harbour only exogenous germ cells (29). Subsequent direct mating of the surrogate hosts, termed Sire Dam Surrogate (SDS) mating, recreates the donor chicken breed

carrying the edited allele in a single generation (29). Using this method Ballantyne et al. (2021) were able to introgress and validate two feather trait alleles, Dominant white and Frizzle into two pure chicken breeds. SDS surrogate host technology in chicken provides the opportunity to make precise genetic changes in chickens allowing for functional validation of genetic variants associated with climate adaptation and disease resilience, and facilitating the transfer of beneficial alleles between breeds (29).

Surrogate sires and other current and future reproductive technologies will soon have the potential to be implemented on farm and facilitate commercial-scale dissemination of genome-edited animals (2). While the integration of genome editing into breeding programs provides an important opportunity for trait improvement, to achieve it in practice will require innovative solutions. For example, how will genome edited farmed animal genetics be disseminated effectively within a breeding program? Gottardo et al. (2019) modelled a strategy to exploit surrogate sire technology in livestock breeding programs (84). They hypothesised that in a commercial breeding program a single 'elite' male donor animal could produce huge numbers of progeny. The results of their simulations showed that using surrogate sire technology would significantly increase the genetic merit of commercial sires, by as much as 6.5 to 9.2 years-worth of genetic gain in comparison to a conventional commercial breeding program (84). However, they noted that the use of only one or a handful of 'elite' donor animals to generate the production animals in the breeding program would be very different to current practice. This would therefore introduce risks, including of the potential for high levels of inbreeding, which would need to be closely monitored and mitigated accordingly (84). Their study also identified two major bottlenecks, the time required and the ability to identify 'elite' donor animals, and the time taken to produce the surrogate sires themselves. The cost of applying surrogate sire technology in a breeding program are also still very high although these may in time decrease if the technology were to be adopted widely. Other considerations they identified included the ratio of existing reproductive rates of males in comparison to those enabled by surrogate sire technology, the time and cost associated with performing progeny tests and the levels of accuracy that can be obtained by genomic prediction (84). Practical implementation of a surrogate sires strategy in animal breeding programs would need to account for these considerations.

A key aspect of surrogate sire males is that while they lack their own endogenous germline they are otherwise physiologically normal (82). In breeding systems across the globe where the outward characteristics of an animal are important to meet societal expectations or for adaptation to local farming systems this is particularly useful (62). In Sub-Saharan Africa, for example, surrogate sire technology could be used to provide indigenous germline ablated cattle or small ruminants that fitted seamlessly into the local infrastructure whilst at the same time disseminating the genetics of 'elite' animals with higher production potential or better disease resilience. In Sub-Saharan Africa the existing infrastructure is not set up to easily deliver new reproductive technologies such as artificial insemination, particularly in small ruminants (62). In a small number of countries community based breeding programs have been established for small ruminants that enable local farmers to improve the genetics of their animals through selective breeding (85). Using surrogate sires to distribute 'elite' genetics would cause minimum disruption to the local infrastructure but allow for uptake of animals with improved genetics via these community based breeding programs.

Genomic evaluation of genome edited animals

Modelling studies have revealed how the use of genome editing to introduce beneficial alleles into animal breeding programs could maintain or even accelerate the rate of genetic gain accomplished by selective breeding programs (53,66). Using genome editing to accelerate genetic gain is a faster and more efficient approach to the lengthy process of

naturally introgressing beneficial alleles from one breed into another over long periods of time using conventional breeding methods (8). Genome editing also provides the potential to eliminate mutations leading to obviously deleterious phenotypes from breeding programs (63,81). Genomic evaluation strategies will need to be adjusted for genome edited animals, and their successful integration within breeding programs will rely on careful monitoring of genetic progress.

To provide data to guide emerging regulatory frameworks and benefit future applications of genome editing in farmed animals, Young et al. (2020) (86) set up a breeding experiment to investigate whether the *POLLED* genome edit from one of the hornless dairy bulls (6) was inherited by his offspring. They also measured whether there were any unique phenotypic or genotypic changes in those offspring. By crossing one genome-edited dairy bull, homozygous for the dominant P_C *POLLED* allele, with horned cows (pp) they obtained six heterozygous (P_Cp) polled calves. The calves had no horns and were healthy and phenotypically normal (86). The performance of these offspring and subsequent generations would ideally now be evaluated and measured against existing breeding values for dairy sires. Certainly the scenarios modelled by Mueller et al. (2021) demonstrated that genome editing could be used as a tool to accelerate introgression of homozygous polled sires with high-genetic-merit into a predominantly horned population of cattle (76). Using genome editing in combination with genomic evaluation of polled sires would mitigate the trade-off of slower genetic gain associated with decreasing the frequency of the *HORNED* allele when conventional breeding is used (76). Large-scale cattle breeding programs are complex, and it is likely that continuous selection, using genomic evaluation, would be required even if genome editing could improve one or a few beneficial traits such as polledness (87). The same would be true for the large and complex breeding programs for pigs when improving resistance to PRRSV by editing the *CD163* gene (88).

Breeding programs for farmed animals are continually evolving and breeding goals and selection strategies need to be flexible to accommodate new traits when novel genetic variants are identified (87), or when challenges arise e.g., from disease or effects of climate. Genome editing can help maintain this flexibility but monitoring of genetic progress in the breeding program would need to be continually evaluated using genomic and phenotypic evaluation (87) (Figure 1). The infrastructure, production system and environment would also need to be considered as a driving factor in the success of integrating genome editing in a breeding program with breeding goals shifting flexibly to accommodate changes (Figure 1).

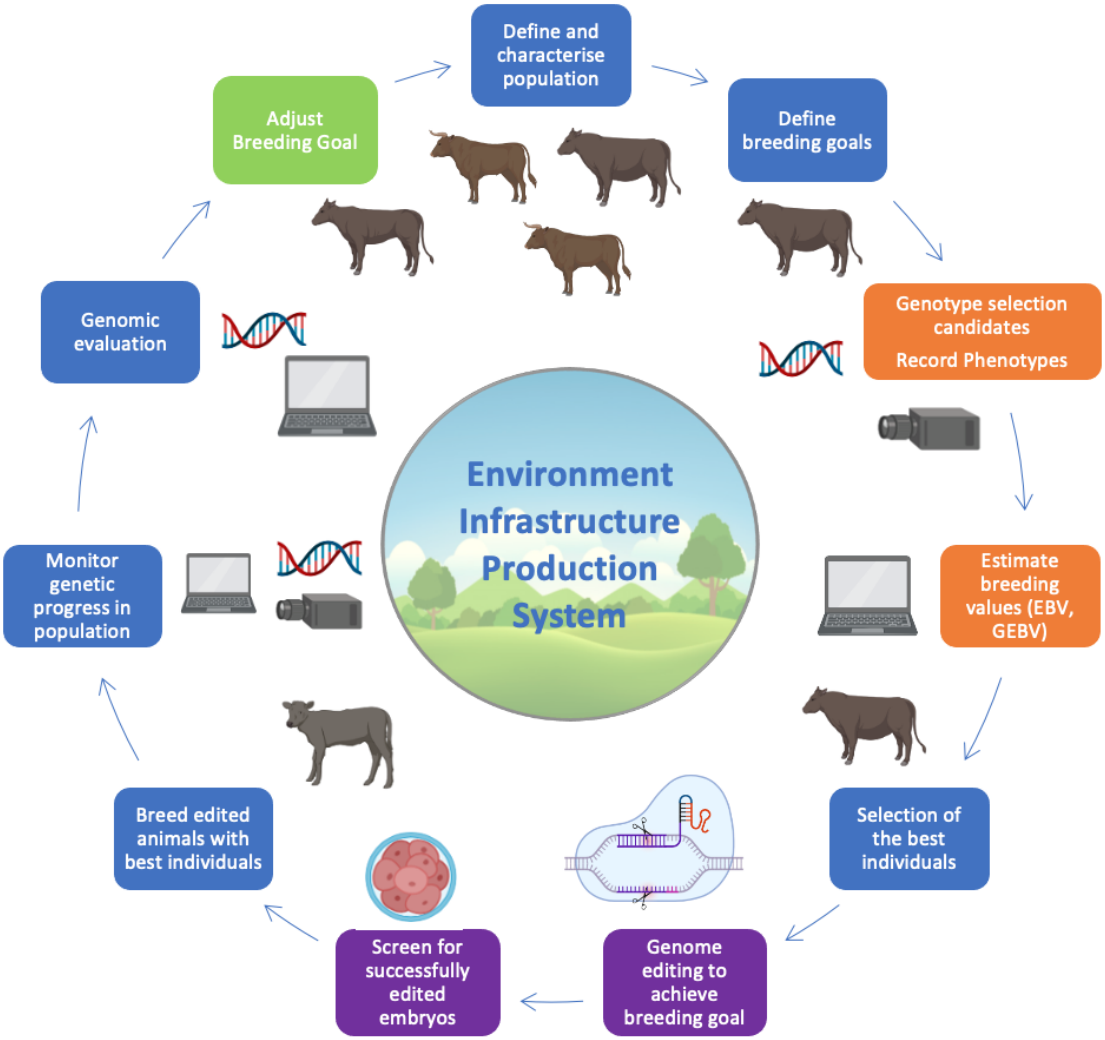

Figure 1: Schematic representing how cattle edited for the polledness trait might be integrated and evaluated in a breeding program. In any breeding program all components of the process will be linked to the environment, production system and infrastructure in which the animals are being bred. In this example the breeding goal might be adjusted to incorporate a leaner more sustainable carcass type in addition to polledness. The colors represent the four key stages of the process. Adapted from Eriksson et al. 2018 (87)***.***

Considerations for including genome editing in breeding programs

Careful integration of genome-editing technologies into breeding programs is essential to ensure continuous genetic improvement whilst conserving existing genetic diversity (11). Another key consideration is the potential for pleiotropic effects associated with targeted edits, particularly when *de novo* alleles are created and utilized. Extensive genetic and phenotypic testing of edited animals may be required to exclude the possibility of deleterious pleiotropic effects, such as those described in humans (e.g., (89)). Similarly, where novel genetics are created using genome editing that would not have arisen naturally as is the case with the PRRSV resistant pigs (12) thorough phenotypic characterization of the edited animals must be performed. This is because deleting all or a region of a functional protein could lead to a loss of (systemic) biological function (4). It will also be important to consider when applying genome editing in a breeding program if a gene or variant of interest is located within a locus that has been actively selected. This could indicate whether a

potential target is associated with known production traits. This approach has been taken for PRRSV-resistant pigs, with evaluation as to whether the *CD163* gene locus has been selected for in pig breeding programs (88).

Ethical and regulatory considerations

The use of genome editing in farmed animal breeding also has important societal, economic, and political implications (90,91). The potential of genome editing in animals raises questions related to product safety, animal health and welfare, and the most appropriate ways to meet societal challenges such as feeding a rapidly growing human population. In their 2021 report the Nuffield Council on Bioethics listed in their recommendations that "responsible breeding was key to the adoption of genome editing technology stating that commercial breeders of farmed animals should adopt an explicit and recognised set of breeding standards, with independent oversight" (91). They indicate that "these standards should seek to ensure that animals are not bred to enhance traits merely so that they may better endure conditions of poor welfare, or in ways that reduce their capacity to enjoy life". In addition, they recommend that incentives for responsible breeding be put in place so that "ways to encourage responsible breeding and the use of responsibly bred animals should be explored, for example through incentive payments to farmers associated with the use of animals with desirable characteristics." In the 2021 Nuffield report it is also stated that development and adoption of genome editing technology should be informed by public views and that regular review of policy and regulation is essential (91). Others have indicated that approval processes and regulatory guidelines for genome edited food animals are currently lengthy and complex and require simplifying before genome editing can be widely adopted to meet the challenges to food production of coming decades (92,93). The disparate regulatory approaches being proposed for genome editing in food animals globally gives rise to some uncertainty as to whether this potentially hugely valuable breeding tool will ultimately be permitted to serve as a complementary approach to efficient and sustainable genetic improvement programs for animal breeding (52,93). Whether animal breeders will be able to employ genome editing in genetic improvement programs for farmed animals will depend largely upon global decisions around the public perception, regulatory framework and governance of genome editing for food animals (8).

Future Directions

Genome editing technologies will undoubtedly have a significant role in the future of animal breeding programs. Their application is currently limited to modifying a single gene or a variant with a large effect; however, the majority of production relevant traits involve multiple genes. Traits that are important in future sustainable farmed animal breeding programs, such as improved feed efficiency, reduced methane emission and improved health and welfare appear to be highly polygenic, and as such will require multiple edits. Multiplexing technologies that allow for polygenic traits to be altered in a single step are under development and will become available for farmed animals in the future (94). These improvements in editing technologies will be required to enable multiple edits in elite breeding animals within a breeding nucleus to target multiple traits or multiple causative alleles for the same trait (3). Introducing edits into multiple elite animals, into a breeding program, will be required to avoid genetic bottlenecks and editing of different breeds and lines will be essential to maintain genetic diversity, and enable structured cross-breeding (52). Efficient means of evaluating breeding values when genome edited animals and their offspring are included in a breeding program will be essential. Efficient and scalable means to trace genome edited animals and their progeny in a breeding program will also be required,

and the further development of whole genome sequencing and other 'omics approaches should help to facilitate this (95). Molecular characterization of genome edited animals and their progeny will also likely need to be expanded in any regulatory framework to detect any genomic irregularities, including off-target effects, un-intended on-target effects and effects on genome regulation (95).

In conjunction with well-managed efficient breeding programs genome editing for trait improvement provides a significant opportunity for improving farmed animal health, productivity and welfare (52), particularly in the face of the challenges, from disease and climate change, that are facing global food production in coming decades (96). Public and regulatory perception are very important in the future adoption and application of genome editing in farmed animal breeding programs (91). As research and development relating to genome editing in farmed animals rapidly develops, dialogue surrounding the regulatory framework including a wide diversity of stakeholders is necessary to inform its potential application in commercial breeding programs. It is likely that the utilization of genetic variation that could have occurred naturally, as opposed to the creation of *de novo* alleles, in genome editing strategies may be viewed more favorably from a regulatory and societal perspective. If this is the case the distinction between the two strategies will become increasingly important. While legislative hurdles still exist in many countries genome editing has the potential to allow animal breeders to improve health, welfare, sustainability and efficiency, maximizing genetic gain and contributing to a sustainable future for farmed animal production.

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