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CURRENT STATE OF GENOME EDITING AND WHAT IT MEANS TO BEEF PRODUCERS

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Introduction

Genome editing involves using a site directed nuclease (e.g., Zinc finger nuclease, TALENS, or CRISPR/Cas9) to cut the DNA double-helix and introduce a double-stranded break (**DSB**) at a targeted, specific sequence in the genomic DNA. It is effectively a sophisticated pair of molecular scissors. The DSBs are then repaired by machinery in the cells using one of two mechanisms. One method is non-homologous end joining (**NHEJ**) where the two broken ends are brought alongside each other and are glued back together. This method is error-prone and often results in small insertions and deletions (**indels**) at the target cleavage site due to inevitable mistakes in the repair process. These errors alter the nuclease target site and prevent further cleavage events and often disable or knockout gene function. An alternative repair mechanism is homology-directed repair (**HDR**) using a homologous nucleic acid repair template. The repair template can be designed with desired modifications between regions of homology that match up with either side of the DSB. This can be used to introduce a range of genome edits, from point mutations to whole-gene insertions.

Genome editing presents an approach to introduce targeted modifications into existing genes and regulatory elements within a breed or species, without necessarily introducing foreign DNA, potentially avoiding concerns regarding transgenesis (sometime called GMOs). It offers a new opportunity to accelerate the rate of genetic gain in livestock by precisely introducing useful extant genetic variants into structured livestock breeding programs. These variants may repair genetic defects, inactivate or knockout undesired genes, or involve the movement of beneficial alleles and haplotypes between breeds in the absence of linkage drag (unwanted genes introduced along with the beneficial gene during backcrossing).

Introduction of Editing Components into the Genome

Genome editing reagents can be delivered into target cells via physical methods or through the employment of vectors (viral or non-viral). Genome edited mammalian livestock have predominantly been produced using physical methods which include electroporation of somatic cells (typically fetal fibroblasts) and microinjection, or more recently electroporation, of zygotes (one-cell embryo). Electroporation uses high-voltage pulses to induce transient pore formation in the cell membrane. These pores allow the flow of genome editing components from the suspension liquid into the cell cytoplasm (Lin and Van Eenennaam, 2021). Although electroporation has traditionally been used to edit cultured cell lines, it is also effective on zygotes (Chen et al., 2016).

For a long time, cytoplasmic microinjection (**CPI**) has been the go-to technique for delivering genome editing components directly into livestock zygotes. Electroporation has only recently begun to show its potential for this purpose with effective introduction of indel mutations into zygotes of cattle (Wei et al., 2018a, Miao et al., 2019, Namula et al., 2019, Camargo et al., 2020). Unlike CPI, where a needle is used to deliver genome editing reagents into zygotes individually, electroporation allows the manipulation of zygotes en masse, reducing the time and expertise required (Figure 1).

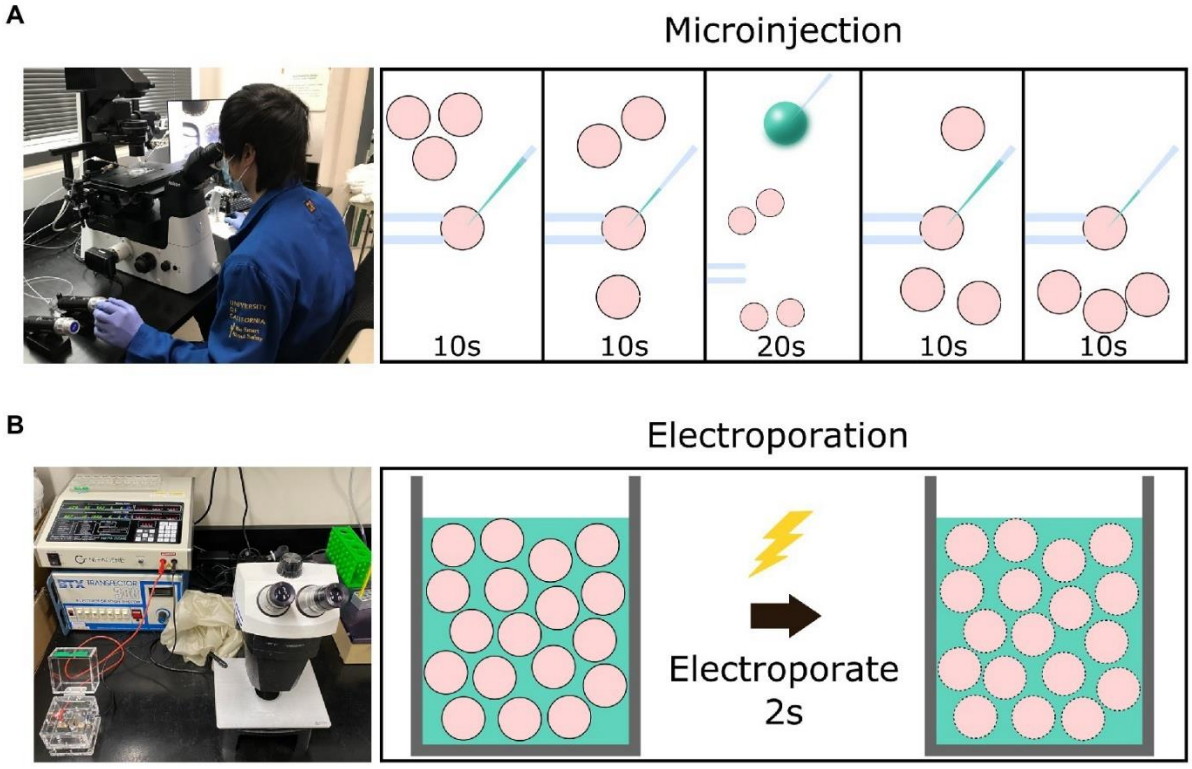

Figure 1. Graphical schematic of a comparison between setup and time necessary for the microinjection vs. electroporation of embryos. (A) The equipment necessary for the microinjection of embryos and the workflow involved to introduce editing reagents (green) into four presumptive zygotes (pink) using a holding needle (left) to stabilize the zygote before introducing the injection needle (right). (B) The equipment necessary for the electroporation of embryos and the workflow involved to introduce editing reagents into 30–100 presumptive zygotes via a cuvette. Image from Lin and Van Eenennaam (2021).

Introducing genome editing reagents directly into zygotes using both methods has been a successful approach to achieve targeted knockouts in embryos. However, issues still exist. Firstly mosaicism, where two or more genetically different sets of cells exist in an animal, is a common problem that can reduce the efficiency of producing a line of knockout animals if the germ line (i.e. cells that will produce sperm and eggs) is not genome edited. Second, inserting new genes is much more difficult than targeted knockouts. Targeted whole-gene insertions rely on using the HDR pathway of repair which tends to only be active in dividing cells. As such it is difficult to achieve gene knock-ins in zygotes.

Genome Editing in Cattle Genetic Improvement

In animal breeding programs, germline transmission is the ultimate goal because edits must be passed on to the next generation to achieve genetic improvement. In mammalian livestock species, genome editing can be performed either in somatic cells and the edited cell line subsequently cloned by somatic cell nuclear transfer (**SCNT**), or in developing zygotes. Most targeted gene knockouts in mammalian livestock, and a few targeted gene insertions, have been achieved by editing in cell culture, followed by SCNT (Tan et al., 2016). The use of SCNT to derive embryos from edited cells greatly reduces the efficiency of the method due to the low rate of birth of healthy cloned animals, particularly in cattle (Akagi et al., 2013, Keefer, 2015).

Delivery of genome editing components into the zygote avoids the shortcomings of SCNT, but has the drawback of significant rates of mosaicism when the editing event occurs at a multinuclear/multicellular stage, and unknown editing success prior to the birth of the calf, unless the embryo is biopsied prior to transfer. For mosaic animals, a breeding strategy must be employed to obtain homozygous, non-mosaic animals (Figure 2). Genome editing of zygotes also has the advantage of producing a diversity of foundation animals as each zygote will produce a genetically distinct animal, as opposed to animals derived from a clonal cell line.

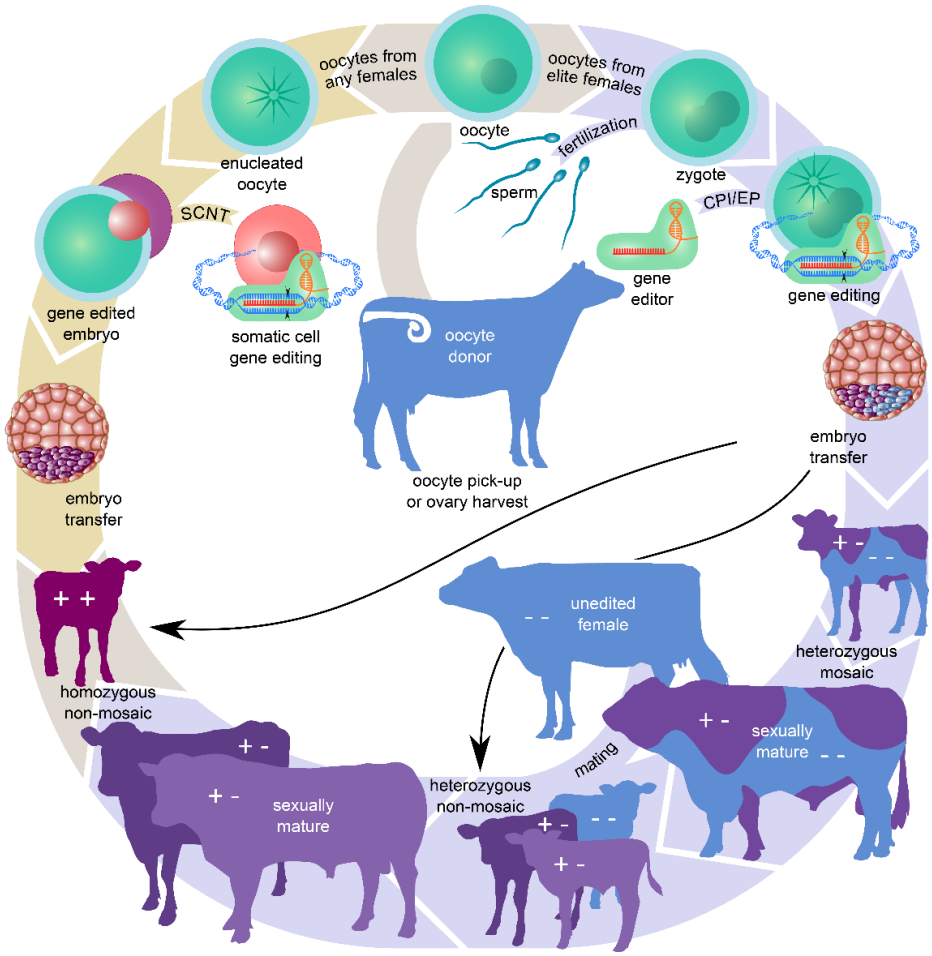

Figure 2. Steps for producing genome-edited livestock through somatic cell nuclear transfer (SCNT) or zygote editing. Schematic showing the steps involved to produce homozygous, non-mosaic livestock by either SCNT cloning of gene-edited and screened somatic cells (yellow arrows) or cytoplasmic injection CPI)/ electroporation (EP) of zygotes (purple arrows) with genome editing components. Image from Bishop and Van Eenennaam (2020).

Table 1. Publications using genome editing in cattle for agricultural applications. Modified from Mueller and Van Eenennaam (2022).

Trait category	Goal	Genome target and function	Reference
Animal health/ welfare	Prevent horn growth	Horn/Poll	Tan et al. (2013); Carlson et al. (2016)
	Disease resistance: mastitis	CSN2 (Beta-casein): milk protein gene	Liu et al. (2013)
			Liu et al. (2014)
	Disease resistance: tuberculosis	Intergenic region between SFTPA1 and MAT1A	Wu et al. (2015)
		Intergenic region between FSCN1 and ACTB	Gao et al. (2017)
	Disease resistance: bovine respiratory disease (BRD)	ITGB2 (integrin subunit beta 2): encodes the leukocyte signal peptide CD18	Shanthalingam et al. (2016)
	Disease resistance: bovine spongiform encephalopathy (BSE)	PRNP (prion protein): susceptibility to BSE	Bevacqua et al. (2016)
	Repair mutation: IARS syndrome	Isoleucyl-tRNA synthetase (IARS)	Ikeda et al. (2017); Ishino et al. (2018)
	Thermotolerance	PMEL (premelanosomal protein gene): coat color	Laible et al. (2020)
		PRLR (prolactin receptor): hair coat length	Rodriguez-Villamil et al. (2021)
Product yield or quality	Eliminate a milk allergen	PAEP (Beta-lactoglobulin): whey protein gene	Yu et al. (2011) Wei et al. (2015) Wei et al. (2018b)
		CSN2 (Beta-casein): milk protein gene	Su et al. (2018)
	Increase lean muscle yield	MSTN (myostatin): a negative regulator of muscle growth	Carlson et al. (2012) Luo et al. (2014) Proudfoot et al. (2015) Namula et al. (2019)
Reproduction and novel breeding schemes	Generate host for germ cell transfer	NANOS2 (Nanos C2HC-Type Zinc Finger 2): necessary for male germline development	Miao et al. (2019), Ciccarelli et al. (2020)
	All male offspring	Safe harbor loci, H11	Owen et al. (2021)

Genome editing research in cattle to date has focused primarily on monogenic (single gene) traits for animal health and welfare, or product yield and quality. There are also some applications that focus on reproduction and novel breeding schemes that may be of relevance to beef cattle breeding programs (Table 1).

It should be emphasized that many of the processes involved in genome editing livestock are time consuming, and at present inefficient. There are a large number of procedural steps and unpredictable biological variables including gamete collection and maturation, introduction of the editing reagents, cloning and transfer of embryos into synchronized recipients, all of which have their own limitations and constraints. Microinjection of zygotes that result in mosaic offspring, and then subsequently breeding to produce heterozygous and/or homozygous edited offspring is both time consuming and expensive when performed in large food animals. Many genome editing applications require homozygous modifications to ensure inheritance of one copy in the F1 generation, or for alleles with a recessive mode of inheritance. The complexity and inefficiencies associated with many of these processes makes the genome editing of livestock far from routine at the current time (Figure 3).

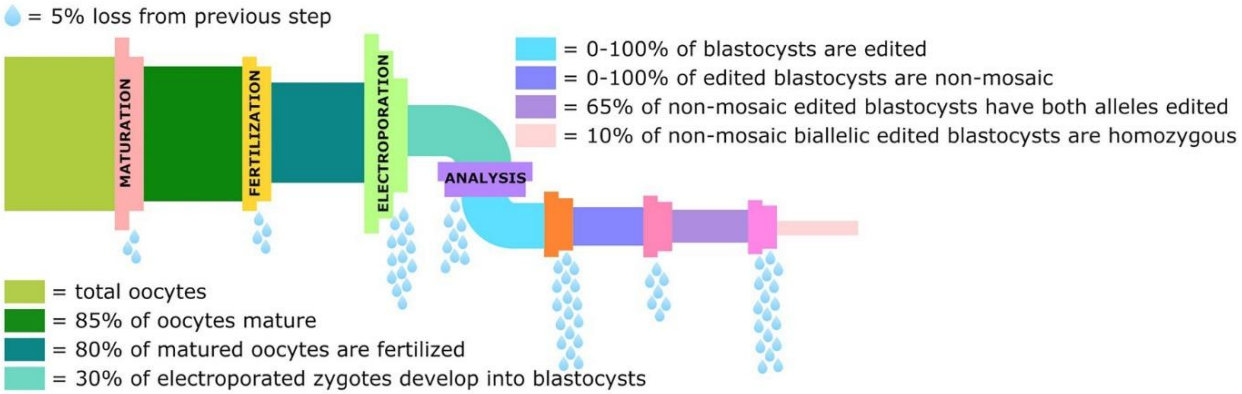

Figure 3. Graphical representation of the losses in the genome editing pipeline from collection of oocytes to the percentage of blastocysts that are non-mosaic homozygotes for the intended edit. Image from Lin and Van Eenennaam (2021).

It is perhaps not obvious to those outside of this field, but a source of bovine oocytes for in vitro maturation and fertilization has to be readily available to perform zygote editing, often obtained from ovaries collected at a local slaughter facility, unless specific female genetics is required, in which case ovum pick-up may be used. To produce viable mammalian offspring, it is also necessary to have a ready supply of synchronized recipient or surrogate cows. This is not an inexpensive undertaking in the case of large livestock species, and due to seasonal breeding and other climatic factors, it is almost impossible to conduct this work during certain times of the year.

Future Applications of Genome Editing

Skewing of sex ratios: In mammals, sex determination is typically dependent on the inheritance of the sex chromosomes, X and Y. Individuals with two X chromosomes are genetically female and individuals with one X chromosome and one Y chromosome are genetically male. Dairy farmers often use "X-sorted" semen in artificial insemination as it contains only sperm carrying an X chromosome and will result in all female calves.

It is actually only a single gene on the Y chromosome that determines whether an embryo develops as a male or female. This gene is known as the sex-determining region of the Y chromosome or

"SRY" for short. SRY expresses a protein in early embryogenesis that initiates male sexual differentiation by triggering a cascade of factors necessary for male gonadal development and shutting down formation of the female gonad.

In 2020 we generated a genome edited calf, Cosmo, who carries an extra copy of SRY on one of his non-sex chromosomes (Owen et al., 2021). Cosmo is expected to produce 75% male offspring: 50% of which will be XY males; 25% of which will be XX females; and 25% of which are expected to be XX individuals that appear male due to the inheritance of the chromosome 17 carrying the SRY gene. These XX males are not expected to produce viable sperm as that requires the expression products of additional genes located on the Y chromosome (Figure 4).

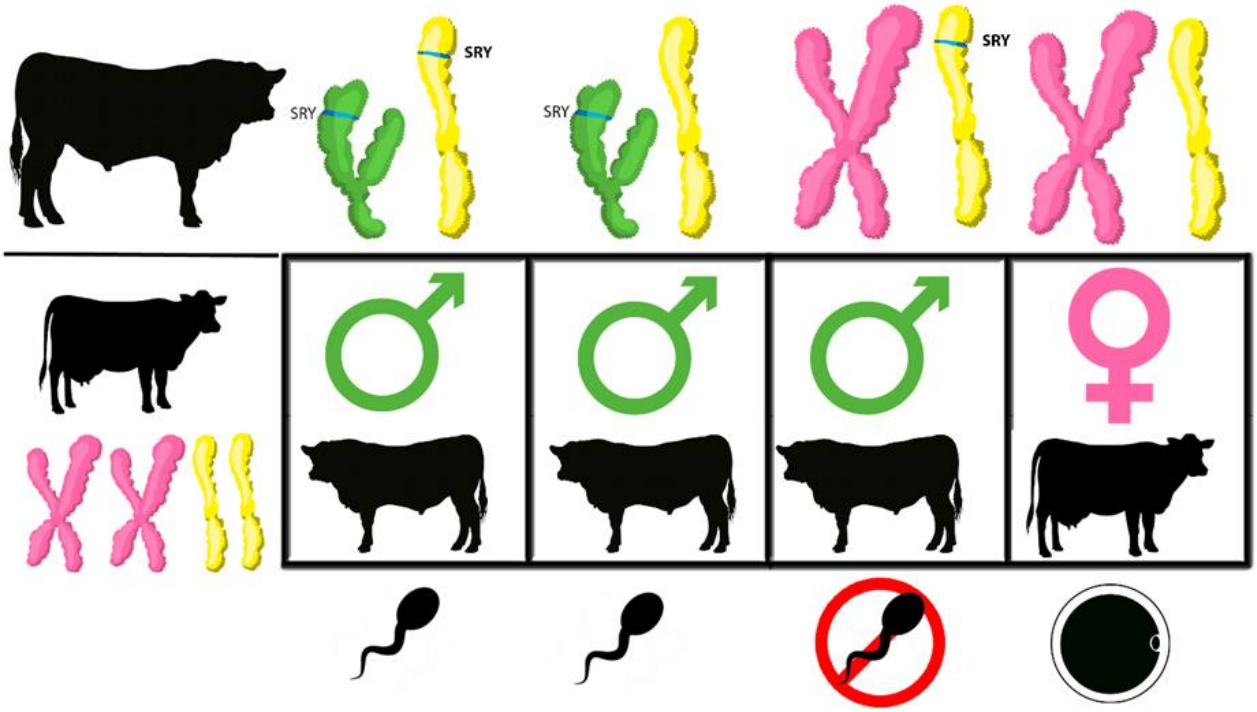

Figure 4. Cosmo will produce sperm carrying either an X (pink) or a Y (green) sex chromosome, and one copy of Chromosome 17. All Y-bearing sperm will produce a male calf, whereas only half of the X-bearing sperm will produce a female. The other half carrying the SRY gene on Chromosome 17 (yellow) are expected to produce a male-appearing XX individual. However, this animal would not be expected to be produce fertile sperm.

Bulls/cows carrying gametes belonging to a different animal: There is a lag in the genetic improvement between the elite nucleus seedstock sector and commercial animals. One way to decrease this lag would be to make germline copies of elite animals. This aim could be achieved through the use of surrogate sires (Gottardo et al., 2019) which would involve replacing the germline of inferior males (e.g. herd sires) with the germline of genetically elite males (e.g. AI sires) by introducing germ cells derived from the elite sires into the testes of the herd sires.

Recently, genome editing has been used to knock out genes necessary for an animal's own germ cell production (Ideta et al., 2016, Park et al., 2017, Taylor et al., 2017). These germline knockout

animals make ideal hosts for elite donor-derived germ cell production. In germline knockout mice, pigs and goats, transplantation of donor spermatogonial stem cells (Ciccarelli et al., 2020), or embryonic stem cells (Miura et al., 2021) resulted in donor-derived sperm production in the otherwise sterile testes. Additionally, donor-derived oocytes have been generated in the sterile ovaries of germline knockout heifers (Ideta et al., 2016).

It is anticipated that surrogate sire technology could be realized through germline complementation, which consists of using donor cells from one genetic background to complement or replace the germline of an otherwise sterile host of a different genetic background. Germline complementation requires two components: 1) a host that lacks its own germline, but otherwise has normal gonadal development (e.g., intact reproductive tract), and 2) donor cells that are capable of becoming gametes (Mueller and Van Eenennaam, 2022). The process of germline complementation (i.e., combining donor cells with a host) can occur at different stages of a host animal's development, depending on the donor cell source (Figure 5).

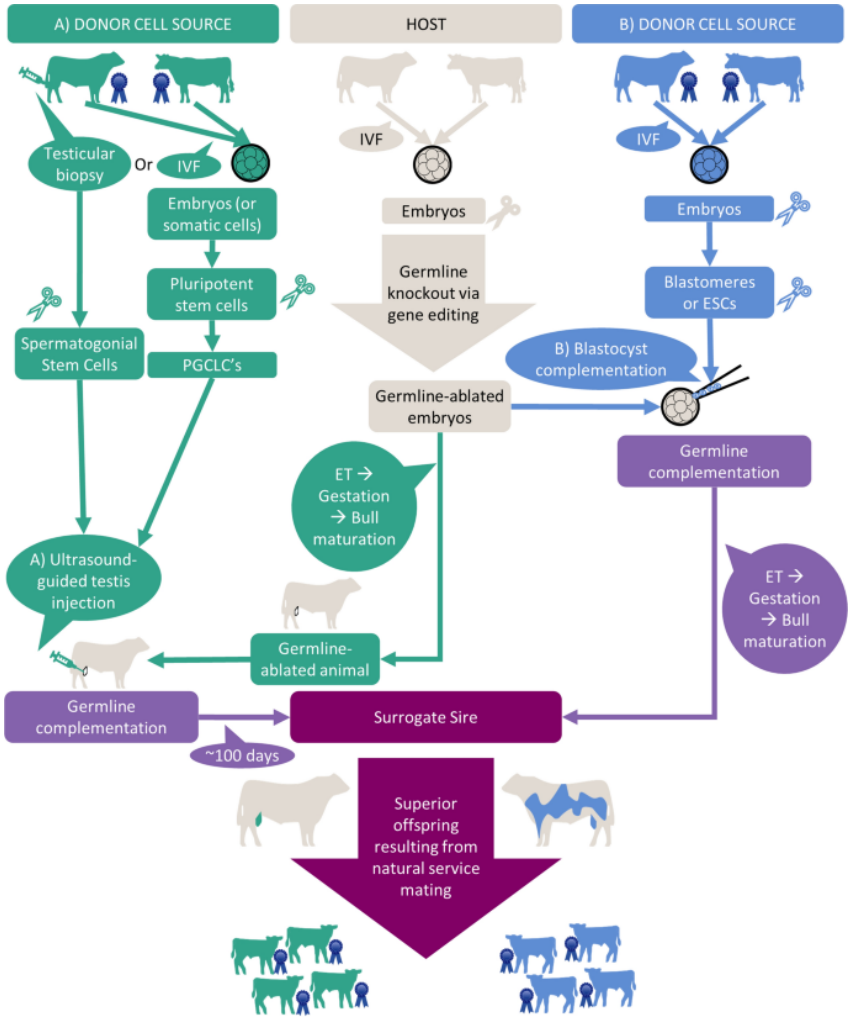

Figure 5. Schematic of potential surrogate sire production systems. Grey represents steps to generate the host animal. Green and blue represent potential alternative sources and steps for generating donor cells. Light purple represents the germline complementation steps and dark purple/maroon represents the resulting final surrogate sire product. Key differences are that in the green (A) path, germline complementation would take place in a live, juvenile or adult, animal and the host would be non-mosaic. In contrast, in the blue path (B), germline complementation would take place at the embryo stage and the resulting host could be mosaic. Blue ribbons represent elite genetics and scissors represent steps that require (solid) genome editing, or where genome editing could potentially be introduced (outline). PGCLC: primordial germ cell-like cells, ESC: embryonic stem cell. Image from Mueller and Van Eenennaam (2022).

In vitro breeding: New advances in vitro with germ cell and gamete development from mouse ESCs have led to recent interest in the potential for in vitro breeding in livestock (Goszczynski et al., 2018). The advantage of this proposed method would be that it could effectively remove the wait required for animals to reach sexual maturity prior to meiosis and conception. This has the potential to dramatically decrease the generation interval component of the breeders' equation. If both in vitro gametogenesis and fertilization could be successfully accomplished in a petri dish, this offers the possibility of maintaining an entire breeding population of large animals in a laboratory (Figure 6). Genome editing could be included at the ESC stage to introduce useful genetic variation in the selected cell line.

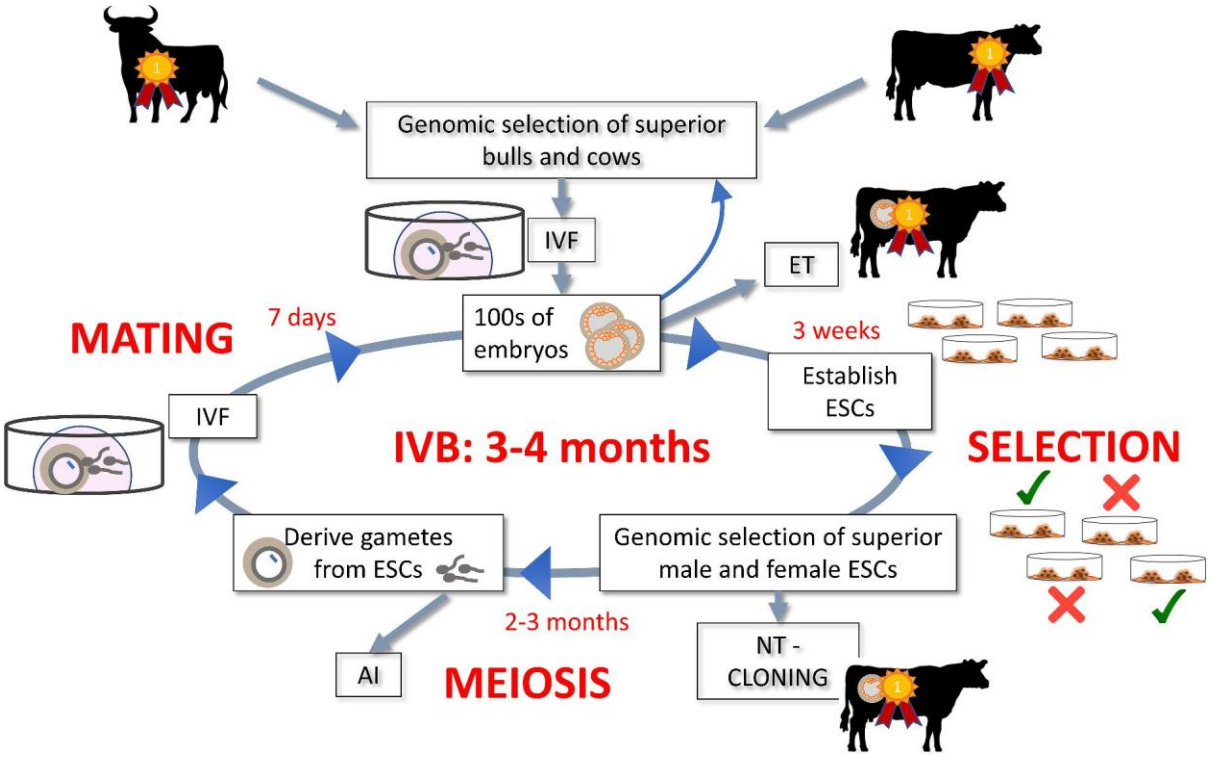

Figure 6. In vitro breeding (IVB). Diagram of the strategy, estimated times, and possible alternatives for its implementation in animal production systems. NT: nuclear transfer. IVF: In vitro fertilization**.** ESCs: Embryonic Stem Cells. Image from Goszczynski et al. (2018).

Regulations

As with earlier genetic engineering approaches, whether breeders will be able to employ genome editing in cattle genetic improvement programs will very much depend upon global decisions around regulatory frameworks and governance of genome editing for food animals Argentina was the first country to publish its proposed regulatory approach for genome editing and other new breeding techniques (Whelan and Lema, 2015). The Argentine approach is that if there is no "new combination of genetic material", and if the final product is free of "transgenes", then that product will not be subject to regulation as a genetically modified organism (GMO). No distinction is drawn between genome edited plants and animals which differs from the United States (Table 2).

Country / Region	Food / Crops	Animals	Avg Ag. Rating
Africa	5	5	5
Argentina	10	10	10
Australia	8	8	8
Brazil	10	10	10
Canada	8	8	8
Central America	6	6	6
Chile	10	1	5.5
China	5	5	5
Colombia	10	1	5.5
Ecuador	10	10	10
European Union	2	2	2
India	6	6	6
Israel	10	5	7.5
Japan	8	8	8
Mexico	1	1	1
New Zealand	4	4	4
Norway	6	6	6
Paraguay	10	10	10
Russia	5	5	5
South Korea	??	??	??
Switzerland	5	5	5
United Kingdom	2	2	2
Ukraine	1	1	1
Uruguay	6	6	6
United States	10	4	7

Regulation Status	Rating
Determined: No Unique Regulations*	10
Lightly Regulated	8
Proposed: No Unique Regulations†	6
Ongoing Research, Regulations In Development	5
Highly Regulated	4
Mostly Prohibited	2
Limited Research, No Clear Regulations	1
Prohibited	0

Lightly Regulated: Some or all types of genome editing are regulated more strictly than conventional agriculture, but not as strictly as transgenic GMOs.

***Determined**: No Unique Regulations: Gene-edited crops that do not incorporate DNA from another species are regulated as conventional plants with no additional restrictions.

†**Proposed**: No Unique Regulations: Decrees under consideration for gene-edited crops that do not incorporate DNA from another species would no require unique regulations beyond current what is imposed on conventional breeding.

Table 2. Agricultural Genome Editing Regulation Index (Genetic Literacy Project, 2021. Accessed July 25, 2022)

In 2020, the United States Department of Agriculture (USDA) published its SECURE (Sustainable, Ecological, Consistent, Uniform, Responsible, Efficient) rule which confirmed that the "USDA does not regulate or have any plans to regulate plants that could otherwise have been produced through traditional breeding techniques." However, the United States Food and Drug Administration (FDA) has taken a very different approach for edited animals, and in a 2017 draft guidance announced that "all intentional genomic alterations" (IGAs) in the genome of animals

would be regulated as new animal drugs (FDA, 2017; Maxmen, 2017). The guidance elaborates that each alteration would need to go through a mandatory premarket multigenerational safety and efficacy review, irrespective of whether that alteration already exists in the target species or could have been achieved using conventional breeding. It should be noted that only two genetically engineered animals for agricultural purposes (fast-growing AquAdvantage salmon, and GalSafe pig) have ever been approved using this regulatory approach, whereas numerous genetically engineered crops, and even a couple of genome edited crop varieties are commercially available.

Unapproved animal drugs are not allowed to enter the food or rendering chain, requiring incineration or burial following euthanasia of experimental gene-edited food animals. This added expense is inhibitory for genome editing research into food animal species. Typically, the income derived from marketing surplus animals, and the milk, meat and eggs produced by both university and USDA (e.g., MARC) herds and flocks, used in both research and teaching, is an integral offset to the sizable costs associated with large animal research. Categorizing all genome edits as drugs, irrespective of novelty, eliminates saleable products from edited livestock, and increases the costs associated with this research considerably. It also dramatically increases the developmental costs associated with commercializing genome edited livestock. A U.S. 2019 petition calling for regulations that are proportionate to unique product risks, and the harmonization of regulations for genome edited plants and animals was supported by hundreds of scientists (Van Eenennaam et al., 2019).

On January 19, 2021, the USDA announced the finalization of a Memorandum of Understanding (MOU) with the U.S. Department of Health and Human Services outlining regulatory responsibilities over certain animals developed using genetic engineering that are intended for agricultural purposes (such as human food, fiber, and labor). However, the FDA is opposed to losing their regulatory oversight of genetically engineered and genome edited livestock for food purposes. A public comment period on the USDA proposal closed in May 2021, and as of writing this paper it is unclear how this regulatory turf battle will play out.

It should be noted that in March 2022 the FDA announced it planned to exercise enforcement discretion for the marketing of products, including food, from two of Acceligen Inc.'s "PRLR-SLICK" genome-edited beef cattle and their offspring after determining that the intentional genomic alteration which in this specific case was prolactin receptor mutations did not raise any safety concerns (i.e., low-risk determination). This was first low-risk determination for enforcement discretion for an IGA in an animal for food use. What this means in practice is that, while developers are generally required to have an approved new animal drug application for IGAs in animals prior to marketing, on a case-by-case basis for those edits that are low risk, the FDA may not expect developers to seek approval of these IGAs. The data that was provided to the FDA to obtain this low risk determination is detailed in the risk assessment summary and included "genomic data and other information to FDA to demonstrate that the IGA contained in PRLR-SLICK cattle is the equivalent to naturally occurring mutations that occur in conventionally raised cattle with a history of safe use as a source of human food" (FDA, 2022).

According to this document, "Acceligen provided raw whole genome sequencing (WGS) data and their bioinformatics analysis for four edited calves and their unedited parents. This genotypic data was used to evaluate the actual sequences of the IGA in the animals' genomes and screen for the

presence of any unintended alterations that might pose an animal or food safety concern and risk to the environment. FDA independently analyzed the WGS data and confirmed Acceligen's conclusion that of the four calves born, three contained the IGA in exon 9 of the PRLR gene and one was determined to be unedited ("no-take"). [One of the edited animals subsequently died of bovine congestive heart failure; BCHF].

Acceligen's and FDA's analyses showed that the alleles containing the IGA contain premature stop codons that would result in truncated PRLR protein analogous to the naturally occurring slick mutations. Due to the specific process used to generate the IGA, the cattle containing the IGA were mosaic, meaning that in different cells/tissues of the cattle, different alleles may be present (these cattle could have several, including multiple distinct but equivalent IGA alleles as well as wild-type or otherwise non-IGA alleles). Acceligen included a disclaimer in the product label to describe that PRLR-SLICK cattle may have 2 or more genetically different sets of cells and, as a result, first-generation progeny may not all inherit the slick phenotype.

From the WGS data, there was evidence of unintended alterations in the cattle containing the IGA. FDA's analysis confirmed the unintended alterations reported by Acceligen and found evidence for a few additional unintended alterations, including a duplication located in a repetitive intergenic region and indels (short insertions and deletions) in intergenic regions. Except for the duplication, all unintended alterations are indels ranging from 1 to 11 DNA base pairs in length located in intronic or intergenic regions. The discrepancy between FDA's and Acceligen's analysis results are attributable to differences in bioinformatics analysis. None of the identified unintended alterations are expected to result in changes to protein expression based on their locations and available genome annotation. Based on the molecular characterization and animal health data, FDA determined these unintended alterations do not pose any safety concerns for the cattle or for humans consuming food from the cattle.

Based on the data and other information submitted by Acceligen, FDA determined that the IGA contained in PRLR-SLICK cattle and the cattle's associated products, including offspring, semen, embryos, and food products derived from them, pose low risk to people, animals, the food supply, and the environment. Therefore, FDA does not intend to object to Acceligen marketing the IGA in PRLR-SLICK cattle or marketing the cattle's associated products. FDA also does not intend to object to Acceligen introducing cattle containing the IGA into the food supply. The agency's decision is limited to the marketed products (for example, live PRLR-SLICK cattle, semen, embryos, and meat) derived from the existing two cattle containing the IGA for which FDA has reviewed data and their progeny. Additionally, FDA intends to treat facilities or farms that are engaged in standard agricultural practices for PRLR-SLICK cattle, including assisted reproduction techniques (such as embryo transfer) or raising them for food production, the same as facilities or farms that are engaged in these practices for cattle without IGAs."

Meanwhile in Europe, the Court of Justice of the European Union (ECJ) ruled in 2018 that gene-edited crops should be subject to the same stringent regulations as conventional GMOs (Callaway, 2018). This approach has effectively precluded the approval of the cultivation of most genetically engineered plant varieties in the EU, although not the importation of many varieties of genetically engineered feed, and will likely hinder both the use of genome editing by both plant and animal researchers in the EU, and the adoption of this technology in European agriculture

Considerations for Incorporation of Records from Animals Produced Using Advanced Reproductive or Molecular Biotechnologies into National Cattle Genetic Evaluations

(Text from Mueller and Van Eenennaam, 2022).

Currently, an important question is how to best accommodate animals produced using advanced reproductive and/or molecular biotechnology and their progeny into genetic evaluations. In the U.S., the majority of genetic evaluations for beef cattle are carried out by breed associations following the industry-standard Beef Improvement Federation (BIF) guidelines (BIF, 2022a; Van Eenennaam, 2019). U.S. dairy cattle genetic evaluations were previously performed by the U.S. Department of Agriculture-Agricultural Research Service-Animal Genomics and Improvement Laboratory (USDA-ARS-AGIL) and are currently performed by the Council of Dairy Cattle Breeding (CDCB). Additionally, the International Committee for Animal Recording (ICAR), which is an international Non-Governmental Organization (NGO), provides guidelines, standards, and certification for animal identification, animal recording, and animal evaluation.

Records from Animals Resulting from ART

For animals resulting from multiple ovulation embryo transfer (MOET), BIF recommends that all observations, or phenotypic information, for traits that do not have maternal effects be used in genetic evaluations and that observations "for traits that have maternal effects, be used in genetic evaluations as long as the recipient dams' ages (heifer, 1st parity, or multiparity) and approximate breed compositions are available" (BIF, 2022b). Additionally, "BIF recommends that embryo stage (1-9) and grade (1-3) and whether frozen, split, sexed, or genotyped be recorded and submitted to breed association or other recording organization" and that, "when sufficient information becomes available, genetic evaluation models for MOET calves include effects of fresh versus frozen and of biopsied (sexed and/or genotyped) or not" (BIF, 2022b). However, due to historic concerns of large offspring syndrome, BIF does not recommend to use phenotypic observations from animals resulting from in-vitro produced (IVP) embryos in genetic evaluations (BIF, 2022b; Thallman and Snider, 2021). Although, BIF does recommend that observations on all ET calves (i.e., resulting from MOET or IVP) be recorded and submitted to breed association or other recording organizations, along with the form of technology used and other pertinent details related to producing the ET calves (BIF, 2022b), so that this information could eventually be used in analyses that would enable the incorporation of records from IVP produced beef cattle to be included in future genetic evaluations (Thallman and Snider, 2021). In contrast, phenotypic observations from animals resulting from both MOET and IVP are included in dairy cattle genetic evaluations. For dairy animals known to be produced by ET (both MOET and IVP), production records (e.g., lactation records) are included in genetic evaluations, but fertility and calving data (e.g., stillbirth records) are excluded from genetic evaluations of those traits because they don't represent "normal" expressions of fertility (personal communication, John B. Cole).

Regarding animals resulting from SCNT, due to concerns of large offspring syndrome and abnormal clone syndrome, BIF recommends to not use phenotypic observations from these animals in genetic evaluations (BIF, 2022c, Thallman and Snider, 2021), but also recognizes that "there are instances where genetically identical animals are in the pedigree (i.e., identical twins and clones)." In these cases where genetically identical animals exist in the pedigree, BIF recommends that, "for purposes of routine genetic evaluation, each set of genetically identical

individuals is assigned a common identifier, so they have identical expected progeny differences (EPDs)," and recommends that, "they should also be assigned different permanent identification numbers" (BIF, 2022c). An EPD, which is the standard term used in the U.S. beef industry, is a predictor of the genetic merit of an animal's progeny and is equal to half of an animal's EBV. Data from clones is handled similarly for dairy genetic evaluations, where each clone receives a unique permanent identification number and an individual evaluation, but the same predicted transmitting ability (PTA) is distributed for all clones from the same donor (personal communication, John B. Cole). A PTA, which is the standard term used in the U.S. dairy industry, is a predictor of the genetic merit of an animal's progeny and is equal to half of an animal's EBV.

ICAR recommends that detailed data should be recorded at all steps of embryo production (e.g., embryo stage, embryo grade, and whether frozen, split, sexed, or genotyped) and this information should be submitted to breed association or other recording organizations. ICAR is working to develop standardized codes for identifying features of embryos (e.g., sex, SCNT, IVP, etc.). Additionally, ICAR advises having parentage verification for animals resulting from ET. (ICAR, 2017, ICAR, 2019).

Records from Animals Resulting from Genome Editing

Given that all genome edited animals are currently produced via SCNT or IVP, the phenotypic observations of the resulting animals would be recommended to be excluded from beef genetic evaluations, but could potentially be included in dairy genetic evaluations (BIF, 2022b; Thallman and Snider, 2021). ICAR recommends that "breed Associations should check the rules of their countries with regard to allowing genome edited animals in the herd book," and "if an animal has been genome edited it should be recorded against the animal when registered and should appear on the Zootechnical Certificate" (ICAR, 2019). Additionally, BIF has developed more detailed guidelines for what data should be required from genome edited animals for breed association registration (BIF, 2022d). Recently, two major beef breed associations, the American Angus Association (AAA) and the Red Angus Association of America (RAAA) adopted bylaws regarding the registration requirements for genome edited (also called IGA) founders (GEF) and descendants (GED) (AAA, 2021; RAAA, 2021). Moreover, in September of 2021 the RAAA was the first breed association to announce that "they will provide herdbook registry of Red Angus animals carrying genome edited traits for heat tolerance and coat color" (RAAA, 2021).

Moving forward, the GED will eventually enter genetic evaluations and the method for inclusion of these phenotypic records may differ depending on the type of trait affected by the genome editing (Thallman and Snider, 2021). Most genome editing targeting qualitative traits (e.g., horned/polled or coat color), would have no influence on genetic evaluations. In contrast, genome editing targeting quantitative traits (e.g., muscle yield or disease resistance) could have a major impact on the genetic evaluations of close relatives. Thallman and Snider (2021) state that "gene editing directly violates fundamental assumptions of traditional (non-genomic) genetic evaluation." However, they also point out that fortunately, it will likely be easier to accommodate genome editing in genomic evaluation models (e.g., Single Step), and that research will be needed to determine the best way to include these records in different genomic models (Thallman and Snider, 2021).

Records from Surrogate Sires

Based on the current proposed methods, surrogate sires will also be produced using IVP to generate the germline knockout host for germline complementation (Figure 5). Therefore, based on current BIF guidelines, phenotypic observations on surrogate sires would also be excluded from beef genetic evaluations (BIF, 2022b). However, phenotypes recorded on the somatic host are unrelated to the genetic merit of the donor germline, and therefore should not be included in the genetic merit estimate calculations associated with the donor. It should be noted that genome edited, homozygous *NANOS2* -/- knockout females are expected to be fertile, so when crossed with a genome edited, heterozygous *NANOS2* +/- knockout, fertile male this mating would be expected to produce 50% homozygous *NANOS2* -/- knockout, infertile male offspring, even in the absence of IVP or other ARTs (Park et al., 2017). Similar to animals resulting from ET, it will be useful to record as much information as possible on all contributing factors to the surrogate sire embryo (i.e., sire and dam of the host embryo, identification and genomic information of the germline donor source, ET recipient identification, and details on the production process). Regarding progeny of the surrogate sires, they should be genotyped to confirm inheritance of the germline donor's DNA. Once paternal inheritance is confirmed, then potentially those progenies could be handled similarly to those of clones (BIF, 2022c), where all offspring data is attributed to the original germline donor and the progeny would all share a common identifier, but also be assigned unique permanent identification.

Conclusions

Genome editing is a tool that is well-suited for modifying qualitative, single-gene traits at comparatively rapid rates and which could be used in conjunction with conventional selection approaches to address issues such as thermo tolerance, coat color, disease resistance, improved product yield or quality, and animal welfare traits. It could also be used to introduce traits that skew the sex ratio of offspring, and enable novel breeding schemes to accelerate the rate of genetic gain. The availability of this technology for use by industry likely hinges on the regulatory framework imposed, which varies dramatically by country. From a risk-based perspective, it makes little sense to regulate genome edited animals carrying the same allelic DNA at the targeted locus as conventionally bred animals differently, solely because the former was produced using genome editing. There is currently a lack of global agreement regarding the appropriate regulatory approach for genome editing technology, and approaches even differ between agricultural applications in the plant and animal kingdoms. The future of genome editing will likely be governed by the development of a fit-for-purpose, risk-based regulatory framework that fosters innovation, supports trade, and promotes public acceptance.

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