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REVIEW

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Enabling regulatory policy globally will promote realization of the potential of animal biotechnology

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Abstract

Animal biotechnologies have the potential to improve the sustainability and security of our global food systems. Government regulatory authorities are responsible for ensuring the safety of food their citizens consume, whether it is produced via conventional breeding methods or biotechnologies. While some countries have implemented animal biotechnology oversight policies, many countries have yet to develop theirs. Historically, regulatory approvals were required before products of biotechnology could enter the marketplace, and the high cost of the approval process limited the number and types of animal and plant products that sought approval. Only one biotech animal in the world that was developed for food production has reached the market under a GMO or rDNA approval process. The advent of genome editing techniques has revolutionized the scientific approach to introducing changes into DNA sequences and how biotechnology can be used to enhance agricultural breeding. Regulatory dialogs about biotechnology also have changed as a result of these new technologies. Regulatory agencies have begun to respond to these scientific advances, and a growing number of countries are looking to modernize regulatory approaches for these products, based on risk (or lack thereof) and similarity to organisms that could be produced via conventional breeding methods. Advances in animal biotechnology, especially genome editing, can accelerate the incorporation of valued phenotypes in animals, including enhanced yield, disease resistance, resilience to changing climate, and improved animal welfare, as well as food qualities valued by consumers. For animals with these biotechnology-introduced traits to enter agricultural production and reach consumers, clear risk-proportionate regulatory approaches must be in place, and to facilitate international trade of animal products, regulatory processes need to be aligned and compatible. Effective scientific public communication is crucial to build public trust in precision animal biotechnology and risk-proportionate regulatory approaches. An international workshop on regulatory approaches for animal biotechnology was convened in 2022 with 27 countries represented. We synthesize here technical progress, development of regulatory policy, and strategies for engagement with diverse publics on animal biotechnology reported in the workshop. Our goal is to encourage development and implementation of risk-proportionate regulatory approaches and policies in a global context.

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Introduction

All animal breeding has the overall goal of changing the DNA makeup of offspring to express more desirable phenotypes. From a scientific standpoint, the term "genetically modified" is very broad and can refer to a wide range of animal breeding techniques, including mass selection, genomic selection, genetic engineering, and genome editing. Definitions regarding biotechnology can be confusing, especially as some terms can be used to mean different things. In this paper, two general types of techniques are focused upon, genetic engineering (GE) and genome editing (GnEd). Genetic engineering is defined as modifying an organism's genome with introduction of recombinant DNA (rDNA) in a random location in the genome to produce a desired phenotype, resulting in what many call genetically modified organisms (GMOs). Genome editing (GnEd) is defined as a targeted, precise modification of an organism's genome to produce desired phenotype (Wray-Cahen et al. 2022). It is also possible to modify gene expression to produce a desired phenotype through changes which are not heritable. Both genetic engineering and GnEd techniques can be used to create cisgenic (within-species) or transgenic (between-species) modifications, and so the distinction between "GMO" and "non-GMO" cannot be drawn solely on the basis of technique, but rather on the resulting animal and its genotype. From a regulatory standpoint, most countries with policies for the regulation of products of biotechnology have specific GMO laws in place and legal definitions of "GMO".

Application of animal biotechnology to livestock builds upon classical animal breeding practices to advance genetic gains, for example to improve yield, agricultural sustainability, or animal welfare. Yet, as described below, biotechnology, especially animal biotechnology, has proven controversial in some sectors of society. Regulation and regulatory uncertainty have negatively impacted the commercial application of animal biotechnology.

The authors of this manuscript were members of a committee that organized and held an international regulatory workshop that brought together regulators, researchers, developers, animal breeders, and other stakeholders from 27 countries to explore the development, evolving regulatory oversight, and nascent commercialization of animal biotechnology. Our aim in writing this review is to synthesize and share key findings with the broader agricultural community. While presentations all appear on our workshop website (https://www.isaaa.org/kc/proceedings/animalbiotechnology/2022-09-12-4th-intl-workshop/default.asp), in this paper we synthesize across the presentations and discussions. FAO (2022) recently presented a discussion of GnEd and its implications for alleviating hunger, human health, food safety, the environment, animal welfare, socioeconomic effects and distribution of benefits. Our review and synthesis of the workshop topics focuses on issues pertinent to animal biotechnology and associated regulatory approaches taken by different countries, as well as scientific advances.

Animal biotechnologies in the pipeline

Genome editors—zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR-Cas9)—allow researchers and developers to make targeted changes from as small as a single base pair to large templated insertions (Lillico 2022). After the genome editor makes a break in a DNA strand at the targeted genomic sequence, the desired edit is mediated by the animal's own DNA repair mechanisms, i.e., homology-directed recombination, non-homologous end-joining, or microhomology-mediated end-joining (Yeh et al. 2019). Genome editors are much more efficient than previous technologies, with higher transformation frequencies and lower frequencies of off-target mutations than classical gene transfer techniques. Following proof-of-principle in bacterial and model animal systems, genome editing is now being implemented in livestock. Research published over the past decade has demonstrated direct zygote editing in mammals, fishes, chickens, and insects in various applications. Genome editing can result in DNA changes that could be achieved, albeit more slowly, via conventional breeding and also can yield phenotypes not otherwise possible. The advantage over conventional breeding is that GnEd allows rapid incorporation of specific traits of interest without introduction of unwanted traits that are unavoidably incorporated when conventional breeding methods are used; these unwanted or negative traits can take decades to remove via backcrossing and selection. GnEd is being used to increase resistance to animal disease, enhance nutritional value and safety, improve animal welfare, and reduce ecological impacts.

The economic impact of animal disease affects every link in the food supply chain from farm to processing to

market to the consumer (Pendell 2022). Economic losses are not limited to the direct loss of livestock, and extend to indirect losses (e.g., loss of tax revenues), mitigation expenditures (response and cleanup) and market impacts (rises in commodity process and cost). A foot-and-mouth disease outbreak in the central United States could lead to losses from $US 16–140 billion (Pendell et al. 2015). Outbreaks of highly pathogenic avian influenza have had dramatic economic effects, causing losses of animals due to disease and necessary depopulation, shortages at the marketplace and soaring consumer prices (Ramos et al. 2017; Economic Research Service 2023). An illustrative example is provided by porcine reproductive and respiratory syndrome (PRRS), a viral disease (Tait-Burkard 2022). Suckling piglets infected with the virus have diarrhea, severe respiratory distress, and up to 100% mortality; pregnant sows experience abortion or death of fetuses in utero. This viral infection incapacitates the immune system, leaving animals vulnerable to secondary infections with bacterial and viral pathogens. Pathogenesis of PRRS involves binding of the virus to the CD163 receptor on the host cell, presenting a molecular target for GnEd. Pigs subject to excision of domain 5 of the *cd163* gene via GnEd (Burkard et al. 2017) showed heightened resistance to PRRS (Burkard et al. 2018). Production of PRRS-resistant pigs would improve animal welfare, decrease secondary infections and associated potential antibiotic use, and reduce viral shedding that could infect other pig production operations.

GnEd can be applied to adjust the composition of animal products to enhance nutritional value and food safety by introducing or increasing beneficial nutrients or by removing constituents to which some people are allergic (Tizard 2022a, b). For example, reducing the omega-6 to omega-3 (n-6/n-3) fatty acid ratio reduces the availability of inflammatory precursors and offers health benefits to both animals producing the food and consumers of that product. The enzyme fat-1 converts n-6 polyunsaturated fatty acids (n-6 PUFAs) to n-3 polyunsaturated fatty acids (n-3 PUFAs). Adding the nematode *C. elegans fat-1* gene to pigs using classical gene transfer reduced the n-6/n-3 PUFA ratio (Lai et al. 2006). Subsequently, You et al. (2021) used GnEd to introduce *fat-1* into porcine fetal fibroblasts and somatic cell nuclear transfer-based cloning to generate whole animals with reduced n-6/n-3 PUFA ratios. Among key allergens in animal food products are finfish and shellfish allergens, α- galactose-α-1,3-galactose in meat, β-lactoglobulin in cow milk, and egg-white and yolk allergens in eggs; all have been addressed in GnEd and genetic engineering animal research (Tizard 2022a, b). The spread of ticks, such as the Lone Star tick *Amblyomma americanum*, has resulted in a rise of people with α-gal syndrome, a serious, potentially life-threatening allergic reaction, in response to consumption of red meat, medical treatment components derived from certain animals (e.g., gel caps), or transplantation of tissues (e.g., pig heart valves). Revivicor knocked out the galactosyltransferase α-1,3 gene using classical gene transfer to direct insertional mutagenesis and generated GalSafe™ pigs that do not express galactose-α-1,3-galactose glycosyl groups on glycoproteins in their tissues. Although the primary intended use (and reason that the animals were developed) was for xenotransplantation into human patients, Revivicor also applied for and received food-use approval in the United States (U.S Food and Drug Administration-Center for Veterinary Medicine, 2020). A major food allergen produced by livestock is bovine β-lactoglobulin (BLG) in cow's milk. Wei et al. (2018) and Recombinetics worked together to target the *BLG* gene, achieving homozygous deletion using TALENs and yielding milk free of the BLG protein. Of the four key allergens in the egg white of chickens, one survives heat treatment (cooking) and food processing. A team at CSIRO (Commonwealth Scientific and Industrial Research Organisation, an Australian government agency responsible for scientific research) is using GnEd to produce low-allergen eggs (unpublished data; Tizard 2022a, b), holding promise for eggs produced for food and also for use in vaccine production.

In the egg-production industry, current sex-sorting methods involve the hatching, physical sexing and culling of approximately 7 billion male chicks each year. Application of GnEd by a group at CSIRO has led to a system for sex sorting at the point of lay (Cooper 2022), achieved by incorporating a detectable marker on the male sex-determining Z chromosome of the ZW chromosome-bearing female parent (Fig. 1). Carrying a marked Z*W genotype, when she is mated with a normal ZZ male, her sons would have a Z*Z genotype and express the detectable marker in the embryonic blastoderm at point of lay, while her ZW daughters would carry no foreign marker DNA and therefore exhibit no detectable signal. An automated detection system would identify and separate the point-of-lay eggs containing male or female embryos. Male eggs carrying the marker gene would be removed and could be processed and used for other purposes. Female eggs, deemed null-segregant (i.e. not carrying any foreign DNA), would be incubated to hatch layer hens. To incorporate the technology into the breeding structure of the layer industry, the marker system must be integrated into one of four pedigree lines bred to generate the grandparent lines for the four-line cross routinely used to produce production layer hens. Selection against the male-specific marker would be implemented at the terminal cross used to produce commercial egg layer hens. Because this

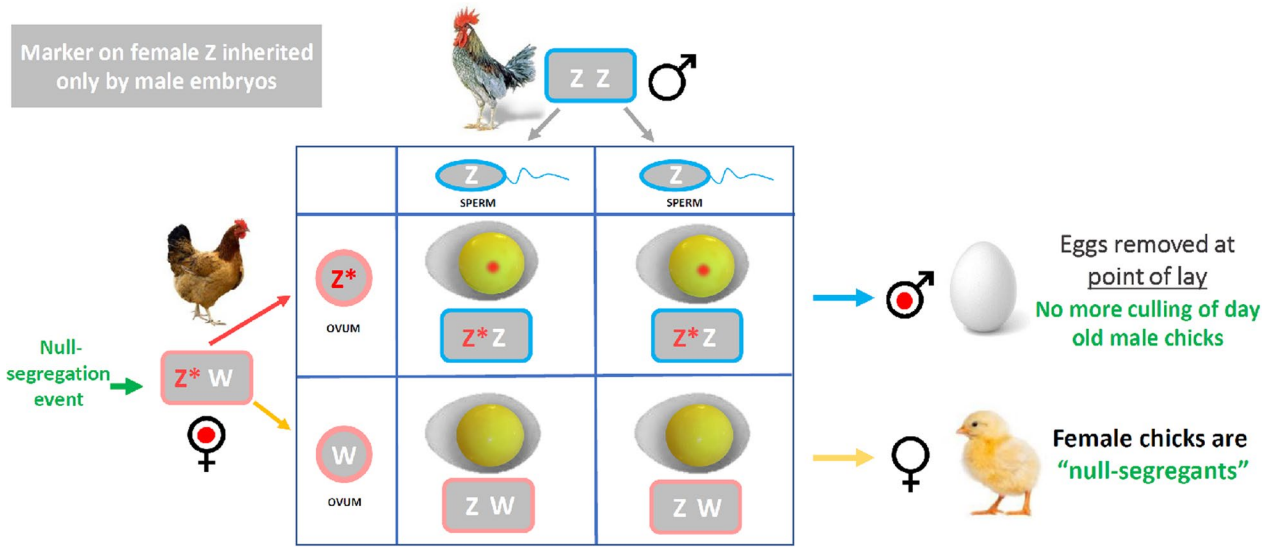


Fig. 1 A GnEd-mediated scheme for sex-marking and sorting of chicken eggs at time-of-lay (Tizard 2022a, b)

system would produce null-segregant hens, no genetic marker would be present in the production line or in the eggs thereafter produced (Cooper 2022). The approach addresses decades-long industry issues of welfare and the ethics of culling males. This technology has broader benefits in reducing the carbon footprint of production, reducing the number of eggs incubated for 22 days to hatch by half (that is, ~ 7 billion fewer eggs in incubators annually), avoiding the generation of ~ 7 billion male chicks and yielding ~ 7 billion eggs with the potential to generate other products.

Heat stress is an issue facing cattle production in the tropics, subtropics, and regions affected by climate change. Different breeds of Criollo cattle in the Caribbean basin have evolved different adaptations, i.e., different mutations of the prolactin receptor (PRLR), leading to the dominant SLICK coat trait. Puerto Rican Holstein cattle exhibiting the SLICK trait yield 1500 pounds more milk per 305 days and have a calving interval that is about 30 days shorter than those lacking the trait. Acceligen has developed a breeding platform for tropical dairy cattle utilizing GnEd of embryonic stem cells and regeneration of whole animals using cloning technology (Sonstegard 2022). The approach has been used to produce thermotolerant Thamani Holstein dairy cattle and Angus beef cattle. In the first GnEd application involving multiple genes, Acceligen aims to produce thermal-tolerant SLICK cattle that are also trypanosome resilient by editing the ferredoxin 2 (*fdx2*) and dehydrogenase/ reductase 4 (*dhrs4*) genes.

Agriculturally important insects might also be subject to biotechnological intervention. Oxitec has developed a platform for producing mosquitoes, ticks, flies, beetles, and moths that carry a self-limiting gene (Abreu 2022). Past work has developed reproductively confined mosquitos, including *Aedes aegypti* and *A. albopictus* (the vectors of dengue and zika) and *Anopheles stephensi* (malaria); as well as a variety of crop pests, including fall armyworm (which affects over 80 crops), soybean looper, Mediterranean fruit fly (citrus), diamondback moth (*Brassica* crops) and pink bollworm (cotton). A similar approach for Asian blue tick, a major parasite and disease vector for cattle, is under development.

Farmed fishes can escape from conventional aquaculture facilities and may pose ecological impacts upon receiving ecosystems and genetic impacts upon wild populations with which they interbreed. Reproductive confinement addresses these concerns and protects the investment of a breeder in a selective breeding program. Lines of research have been opened into reproductive confinement of several key aquaculture species (Wargelius 2022). For example, germ cell-free Atlantic salmon have been produced by using GnEd to knock out dead end (*dnd*), the gene encoding a factor required for the survival of germ cells necessary for fish to be able to breed. These fish remained immature and did not undergo puberty. Because fish lacking germ cells cannot be used for breeding, a rescue strategy was developed for producing germ cells in *dnd*-knockout fish, suggesting the possibility of large-scale production of germ cell-free Atlantic salmon offspring.

Much of the research effort on GnEd animals occurs in China (Li 2022). The Meishan breed of pigs has excellent reproductive ability and good meat quality, but its lean

meat yield is poor. GnEd of the myostatin (*mstn*) gene was used to mimic the natural mutation in exon 3 of the *mstn* gene of Belgian blue cattle (Qian et al. 2015). A 53% increase in lean meat percentage and 76% decrease of fat percentage was observed in homozygotes, with improved feed efficiency in genome-edited pigs and no significant effect on reproductive performance of sows. *Mstn* gene knockout also made the pork more tender (measured as shear force to break up the meat) and more healthful (higher PUFA content). *Mstn*-edited Luxi yellow cattle and Mongolian cattle have been bred. The *mstn* gene knockout significantly improved body weight (18.6%), daily weight gain (19%), slaughter percentage (11%), carcass weight (13.6%) and meat production (20%) relative to control cattle, with higher lean meat percentage and more healthful meat. Outbreaks of disease cause an estimated US $100 billion in economic losses to China's animal husbandry sector every year. Swine epidemics in China include African swine fever, PRRS, and diarrhea. There have been many attempts to create new germplasm resistant to African swine fever by GnEd technology in China, but there has not yet been any successful report. GnEd technology was used to create bovine tuberculosis-resistant dairy cattle (Y. Zhang, Northwest A&F University, pers. comm.). GnEd was used to increase the copy number of the bovine *NRAMP1* gene; an *in-vivo* challenge test showed that resistance of the GnEd cattle to the bovine tuberculosis bacterium was increased by more than 60% compared with the control group. Li (2022) suggested that approximately 20 GnEd livestock lines are currently undergoing safety evaluation, which is monitored by the Chinese Ministry of Agriculture.

New techniques are being developed to improve germline transfer using surrogate sires when artificial insemination is a challenge. Spermatogonial stem cell transplantation (SSCT) is an experimental technique for transfer of the germline from donor to recipient males, which could be useful for dissemination of desirable genetics in food animal populations, as well as biomedical research and preservation of endangered species. Recipient males must be devoid of their endogenous germline but possess normal testicular architecture and somatic cell function to support donor stem-cell engraftment and spermatogenesis. Male mice, pigs, goats, and cattle harboring knockout alleles of the *NANOS2* gene generated by GnEd had testes that were germline-ablated but otherwise were structurally normal (Ciccarelli et al. 2020). In adult pigs and goats, SSCT with donor spermatogonial stem cells showed sustained donor-derived spermatogenesis. These developments represent a major step toward use of surrogate sires for dissemination of improved germplasm in livestock species. The technology could give breeders in remote regions better access to the genetic material of elite animals from other parts of the world, and allow precision breeding in animals such as goats for which artificial insemination proves problematic. Using the approach, *NANOS2*-null indigenous thermotolerant bulls (such as Nelore cattle) could serve as recipients for SSC from European thermosensitive males (such as Angus cattle) to be used for natural mating in beef farms located in regions where heat stress is an issue (Camargo et al. 2022, Fig. 2). Primordial germ cell-like cells were induced to differentiate into functional sperm or oocytes when transferred in vivo or induced using chemical and growth factors in vitro (Hikabe et al. 2016; Ishikura et al. 2016; Zhou et al. 2016; Yao et al. 2022), suggesting future developments for livestock (Lillico 2022).

A number of challenges face implementation of GnEd techniques for practical animal breeding (Lillico 2022). Genome editing remains a technically demanding set of procedures, and many interested parties lack access to infrastructure and technical expertise. In response, some specialized laboratories contemplate offering GnEd of livestock as a service (Oatley 2022). The efficiency of GnEd is suitable for making edited genetic lines for scientific research, but is yet low for producing large cohorts of animals with identical edits (Lillico 2022). While GnEd is suitable for purposefully changing a targeted DNA sequence, causative single nucleotide polymorphisms are rare; thus, there is a need to achieve better understanding of the relation between particular variants of genes and valued phenotypes. Further, many valued phenotypes are the result of expression of polygenic traits.

Animal biotechnology and international development

Speakers also discussed international implications of animal biotechnology. For hundreds of millions of families across sub-Saharan Africa and South Asia, livestock are the most valuable household asset and an irreplaceable source of a nutritious diet, especially for children. The Bill and Melinda Gates Foundation invests in tools and technologies that target the specific needs of farmers in these regions. The Foundation is exploring proof-of-concept for gene editing in poultry (Browning 2022), together with the International Livestock Research Institute and the University of Edinburgh. While no commercial application of GnEd in poultry has yet occurred, traits and genes that could be targeted have been identified that affect feather color and structure, which impacts control of body temperature. There are many opportunities for improving livestock production under tropical conditions and thereby making animal-sourced food more affordable. The Foundation's main investment is in developing genomic selection methods to select the best breeding animals and further developing dissemination

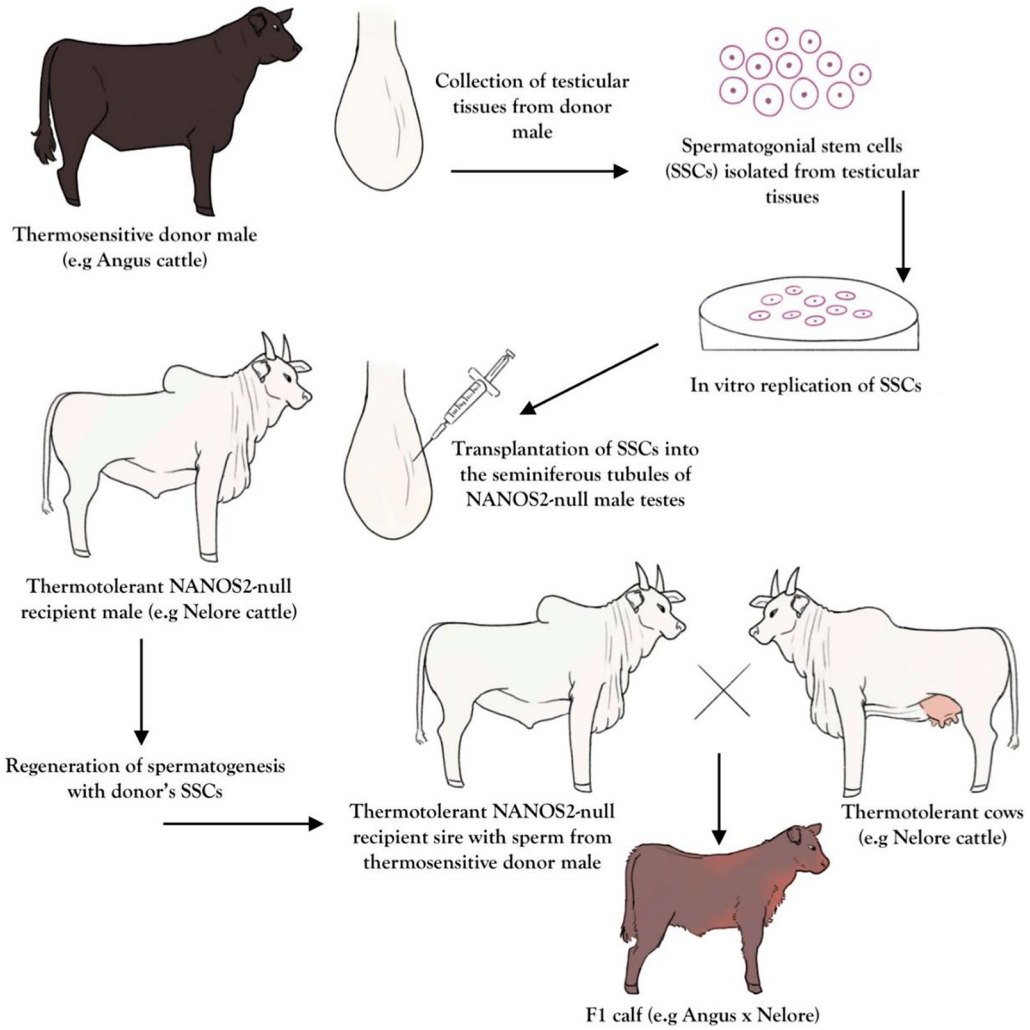


Fig. 2 Transplantation of spermatogonial stem cell (SCC) from a thermosensitive breed (e.g. Angus) to the testes of a NANOS2-null thermotolerant breed (e.g. Nelore). SCCs collected from Angus are isolated and expanded in vitro and then transplanted into the seminiferous tubules of NANOS2-null surrogate Nelore male. Beef farmers in the tropics can use surrogate Nelore sires to breed Nelore cows in order to produce Angus x Nelore F1 calves (Camargo et al. 2022)

pathways to achieve impact. GnEd offers opportunities to produce better-adapted cattle, making a productive breed like Holstein thermotolerant or increasing the productivity of a thermotolerant breed like Gir (Sonstegard 2022). Moreover, it is also possible to introgress disease resistance or tolerance traits like trypanotolerance from the West African N'Dama breed into a susceptible breed. The Foundation is working with Acceligen to apply multiplex gene editing in Holstein and Gir cattle. The first calf with three gene edits was born in June 2022, seeking to realize thermotolerance with the SLICK phenotype and tolerance to trypanosomiasis. The end result will be 16 breeding animals, both males and females, that will produce semen and embryos. The Foundation has also engaged with the National Dairy Research Institute and the University of Missouri to start gene editing in Indian buffalo. Goals are to produce hypoallergenic milk through knockout of the beta-lactoglobulin gene and increased milk yield and higher fat content using GnEd for natural variants of the *dgat1* (diacylglycerol O-acyltransferase (1) and *abcg2* (ATP binding cassette subfamily G member (2) genes. Beyond technical criteria for deciding whether to use the GnEd approach, there are non-technical criteria, including safety for humans, animals, and the environment; likelihood of regulatory approval; value for farmers and food production; and access and affordability for farmers in lower- and middle-income countries.

Poultry production in Africa is based upon a limited number of breeds, and within-breed genetic diversity is declining. Genetic variation is the basis for breeding to increase productivity and resistance to diseases and heat stress. Among options for conservation of poultry biodiversity, biobanking of primordial germ cells (PGCs) and chimera production offer advantages over exclusive reliance on living gene banks. The approach is being implemented at the Centre for Tropical Livestock Genetics and Health (Ethiopia) and the International Livestock Research Institute (Kenya), in partnership with other international collaborators. The Center holds 497 somatic and 240 gonadal cell lines which are intended for use to restore poultry biodiversity and disseminate elite lines using *DDX4* (DEAD box helicase 4) knockout and iCaspase-9 surrogate technologies in which sterile male and female chicken eggs are implanted with reproductive cells from donor birds and the resulting chickens are mated together to produce chicks of the donor breed. The goal is to restore these local breeds, providing communities their conserved elite breeds and thereby improve livelihoods. The Tropical Poultry Genetic Solutions model is to promote rapid scaling-up in distribution of elite adapted local lines to support emergence of the local poultry industry.

Broader implications of animal biotechnology

Additional issues frequently arise during dialogs regarding animal agriculture, including animal welfare, ethics, international development, access and benefits sharing, and conservation of biological diversity. These issues are broader than animal biotechnology, and also apply to production of conventionally bred animals. It is fitting, however, that these issues be considered as countries develop their regulatory systems and new products of animal biotechnology are created and marketed It is also important to consider communications around animal biotechnology and its regulation, realizing that socioeconomic issues may be of greater interest to the public than the science behind the technology. These topics were discussed in the workshop within the context of animal biotechnology.

Animal welfare

Speakers also noted that, while we rarely consider it explicitly, we have a "contract" with domesticated animals, to produce them in ways that maintain their welfare. As considered by Zanella (2022), applications of animal biotechnology can affect animal welfare in both positive or negative ways. Genome editing can provide the opportunity to improve farm animal welfare by allowing major changes to animal production practices that are considered necessary by farmers within existing production settings and potentially dictated by present markets, but which may have a negative impact on farm animal welfare. GnEd offers the potential to eliminate the need for production practices that may influence an animal's wellbeing. Disbudding or dehorning is often practiced in cattle production to reduce risk to other cattle and to producers, but can be painful to calves so treated; GnEd can be applied to yield polled (hornless) cattle (Sonstegard 2022), improving animal welfare. In routine production male pigs are castrated, to reduce boar taint in pork products, which imposes pain and can negatively affect animal welfare. Immunocastration via GnEd (Telugu 2020) can eliminate the need for castration, positively affecting animal welfare. In some animal production systems, such as dairy cattle and layer chickens, one sex is more highly valued such as female dairy cows and the other is of lesser or, as in the case of male laying chickens, no value. This can lead to an economic and ethical burden of producing and culling animals, such as male chicks that can't lay eggs. While sexed semen has become available for some animal species, it is not widely adapted in less-developed countries. As noted above, a system for sexing point-of-lay chicken eggs to avoid day-old male culling has been developed (Tizard 2022a, b). Douglas et al. (2021) recently demonstrated a GnEd strategy that produces male- or female-only litters of mice with 100% efficiency. The approach ultimately may prove applicable to other vertebrates, leading to welfare improvements for agriculture.

One concern when using GnEd technologies is posed by unintended genetic modifications (off-targets), which may generate mutations in untargeted loci that could result in an unwanted or a harmful phenotype. The methods to detect off-targets can be grouped in two approaches: biased (relying on detection of expected off-targets sites identified by predictive algorithms) and unbiased (relying on analysis of whole-genome sequencing (WGS) to detect unexpected off-target sites of mutation) methods. The detection rates for off-target modifications in animals varies among studies. In mice, for instance, Schaefer et al. (2017) reported a number of unexpected off-targets that could be detected only by WGS analysis, while other studies (Willi et al. 2018; Dong et al. 2019; Ayabe et al. 2019) reported that off-target mutation induced by Cas9 can be minimal and may be indistinguishable from the background rate of de novo mutation detected by WGS. The analysis of off-target sites in a GnEd product has been requested by regulatory agencies as a way to avoid unwanted mutations, but such mutations could also occur naturally, and hence their significance may be questionable. For some regulatory approach, off-target effects may not be negligible and their importance analyzed case-by-case; for researchers,

the chances of relevant off-targets will be rare if the sgRNAs are designed to achieve high specificity and used in an experiment that is well designed in terms of delivery procedure, cell type and cycle phases, nuclease and sgRNA concentration. On the industry side, detection of potential off-targets is necessary, and their presence should not be an obstacle for applications of GnEd products when risks are appropriately analyzed and do not impose a threat for animal health and welfare.

Ethical issues

The Nuffield Council on Bioethics (2021) report on genome editing and farmed animal breeding: social and ethical issues proposed that the introduction of new technologies into animal production should align with public and animal interests. The issue of whether classical genetic engineering and GnEd pose the same or different ethical issues was discussed in the workshop by Chan (2022). She noted that mainstream ethical arguments have tended to focus upon who benefits from animal biotechnology and who is in control of it. Key issues center upon societal effects, including the effects of patents and access to the technology and its products (see discussion below), including who controls commercial application of the technology, i.e., whether it is corporations or governments. An additional key set of issues focus upon risk and the "natural". Counter-arguments note that "natural" is not necessarily safer, and ask what to do in the absence of evidence of harm. It was pointed out that just because people may be uncomfortable with something, does not necessarily make it morally wrong. It was noted that if every action that is not traditional is taken as wrong, then nothing would ever be done the first time; further, the risk of not doing something also should be weighed. Animal welfare was presented as a key moral issue pertaining to animal biotechnology. For example, while polled cattle (limiting risk of harm to the animals and their handlers) and PRRSv-resistant pigs (reducing animal suffering and loss) are ethically acceptable to most people, it is unlikely that most people would find it ethically acceptable to create animals that better tolerated poor conditions. Also, if an application improves food security, a moral argument could be made to apply it. Enhanced control of health among production animals poses fewer pathogen spillover events into wild populations of susceptible species, though there is an issue of how we should consider non-human interests in considerations of ethics. Justice for animals was presented as an underdeveloped idea, as was the concept of utilizing agricultural genome editing as a means to address problems of global justice. Dr. Chan posed the question as to rather than there being a moral reason to avoid genome editing, might we have a moral obligation to apply it in some cases?

Access and Benefits Sharing

Many countries have adopted or are in the process of adopting access and benefit-sharing (ABS) measures for genetic resources. The rationale for ABS is that benefit-sharing would compensate biodiverse countries for the costs of the conservation of the resources in situ and create incentives to maintain conservation efforts (Lawson 2012). The Convention on Biological Diversity and the Nagoya Protocol include ABS principles. The Nagoya Protocol is difficult to apply to plant genetic resources and application of ABS principles to animal genetic resources (Box 1) is even more complex. Resolution of ABS issues relating to animal biotechnology is yet before us.

Box 1. Access and benefits sharing The Convention on Biological Diversity (CBD) and the Nagoya Protocol (NP) on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization recognize the sovereign rights of countries over their natural resources. Parties to the CBD commit to facilitate access to genetic resources for environmentally sound uses by other Parties. Parties to the NP commit to take measures to ensure that technical knowledge is accessed with the involvement and informed consent of communities holding traditional knowledge. The benefits arising from research and development, applications, and commercialization must be fairly and equitably shared with the Party providing genetic resources and technical knowledge (CBD articles 1 and 15; NP articles 5, 6, and 7). Many countries have adopted or are in the process of adopting access and benefit-sharing (ABS) measures to compensate biodiverse countries for the costs of conservation of genetic resources (Lawson 2012). Each country must determine which resources they claim as protected by ABS. The Food and Agriculture Organization of the United Nations (UN) has guidance documents and hosts conferences of Parties to the CBD and NP where ABS issues are discussed.

Workshop participants noted that ABS policy is more aimed at plant and microbial than to animal genetic resources. It is unclear how ABS policies might affect research, development, and commercial use of animal biotechnology. An animal breeding program might be considered outside the scope of ABS. One researcher expressed the view that ABS impedes science, complicating permitting for field trials, access to species, and sharing of germplasm, thereby stifling generation of knowledge and realization of benefits. Under especially restrictive ABS regimes, it becomes impossible to take a biological sample from the source

country, complicating research that might benefit that country. While farmers in developing countries have livestock with useful variation and traditional knowledge, developers mostly in developed countries have the expertise and resources to apply animal biotechnology. While stakeholders may agree in principle to strive for ABS through the research and development process, ABS is challenging to implement in practice. For example, cattle expressing natural SLICK phenotypes are bred in many places, informing the GnEd work of a biotechnology company. What, then, does the developer owe cattle breeders in developing countries? As a practical matter, to whom should payment be made, especially if the variant arose spontaneously in multiple countries? The Bill and Melinda Gates Foundation, which funds animal biotechnology research and development, has a policy promoting access and benefit-sharing. Developers and end-users must negotiate agreements, which could involve royalty-free access to the animal biotechnology products developed with Foundation support. Tiambo (2022) presented a detailed overview of the application of ABS principles to chicken genetic resources used or produced by the Centre for Tropical Genetics and Health and its partners.

Resolution of ABS issues relating to animal biotechnology is yet before us. The Nagoya Protocol is relatively new, and not all countries have ratified it. Harmonization of ABS policies is an emerging issue, especially within regions with transboundary animal breeds. One participant proposed creation of a working group to discuss implementation of the Nagoya Protocol from the viewpoint of animal biotechnology.

GnEd and conservation

In addition to agricultural applications, speakers discussed potential opportunities for conservation applications. As many as one million species may be at risk of extinction globally, with that risk being especially acute for island endemic species. Among the threats to be addressed, invasive rodents impact 88% of endangered island vertebrates. A mode of biocontrol for invasive rodents using GnEd and gene drives has been proposed (Saah 2022). A gene drive is a natural or synthetic genetic element that spreads through a population at a rate greater than Mendelian expectation. Gierus et al. (2022) proposed using a natural murine gene drive to suppress island populations of the invasive house mouse. A consortium of non-governmental organizations and university and public-sector researchers has been organized to test the approach in a defensible manner. Guiding principles adopted by the Genetic Biocontrol of Invasive Rodents group, a consortium of nine universities, government agencies, and non-governmental organizations, are to proceed cautiously, with deliberate step-wise methods and measurable outcomes; active engagement with the research community, regulators, communities and other stakeholders; commitment to biosafety, existing regulations, and protocols; use of and development of best practices; operation in countries with appropriate regulatory capacity; and transparency with research, assessments, findings, and conclusions (Saah 2022).

Biotechnology regulatory approaches

Overview

Regulatory frameworks are a critical component of bringing animal biotechnology innovations to market. Regulatory approaches need to be science-based, risk-proportionate, defensible, and credible to the public to establish trust in the safety and marketability of animals and their products developed with biotechnology. If regulatory processes are to encourage innovation, they must also be timely, predictable, and transparent. Workshop participants provided a variety of perspectives on this topic, covering key components of regulatory oversight such as applicability of international agreements, development of national regulatory paradigms, multinational harmonization efforts, managing regulatory complexity, economic consequences of regulation, and regulatory engagement with developers.

Many countries have biotechnology regulatory systems in place that cover animals (Fig. 3). Most of these countries have GMO laws and the processes were developed to regulate the products of genetic engineering, i.e., to regulate the incorporation of rDNA or transgenes into plants, animals, and other organisms. Some countries have experience making regulatory decisions for animals under these systems; however, this experience is overwhelmingly focused on non-agricultural, primarily biomedical, applications. To date, only two genetically engineered animals have been approved for food use globally. Though the reason for this is complex, regulation does have a significant role given the time and expense to developers. The advent of GnEd has caused many countries to take a fresh look at their regulatory paradigms to ensure that they are fit for purpose. This scrutiny is driven by several factors, among them: the precision and relative ease of GnEd is expected to increase the number of applications put forward for review, applicants are expected to be more diverse and may not have the resources required for traditional transgene-focused regulatory assessments, and many GnEd applications are expected to be cisgenic in nature and therefore present a different risk profile relative to transgenic products. There is a developing global consensus that maintaining a single, transgene-tailored

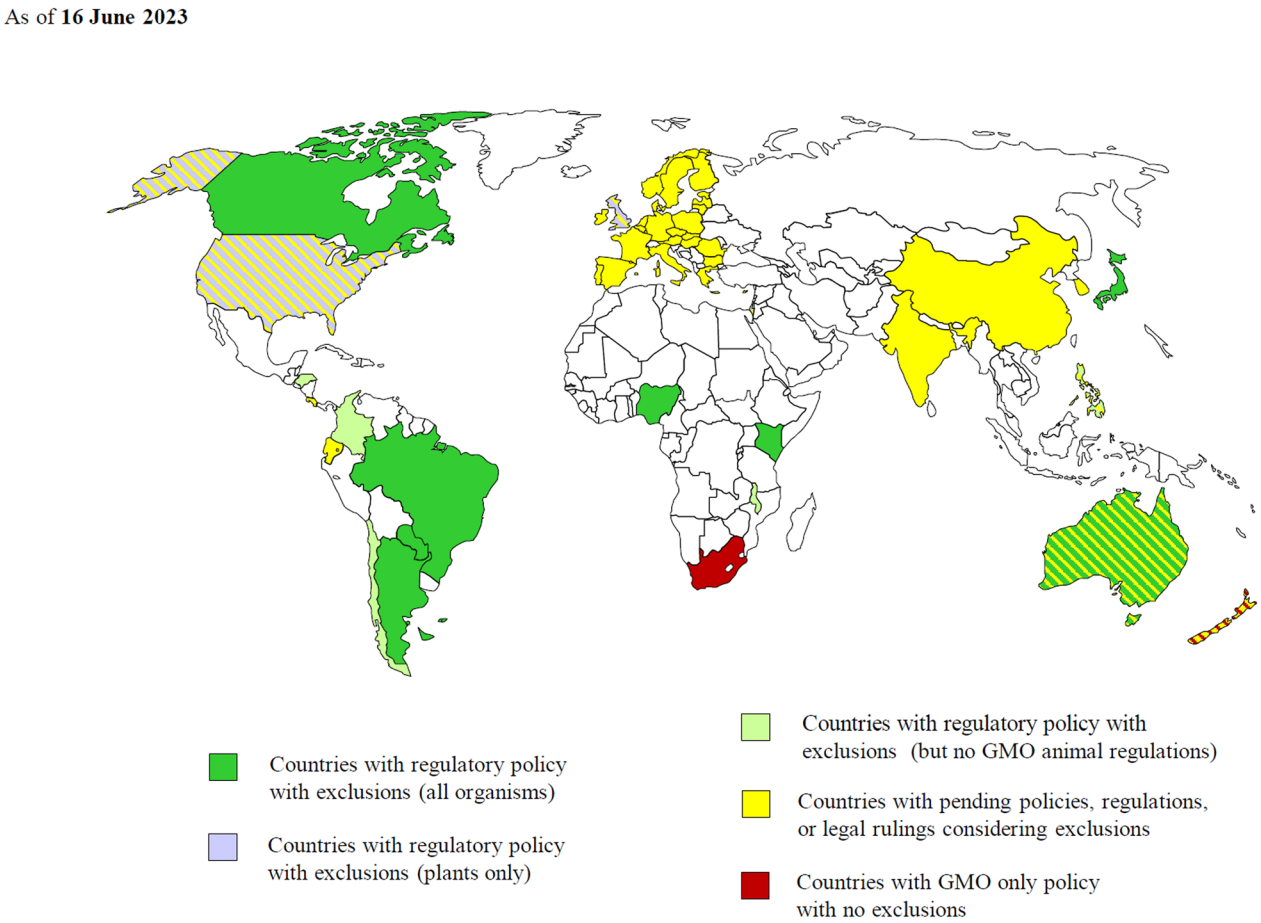


Fig. 3 Countries that show progress in development of policies for oversight of GnEd agricultural animals

approach to all applications of GnEd will hamper innovation by limiting the number of applications put forward for review, the diversity of developers, and adoption of innovations by farmers and producers.

Though GnEd has spurred policy and regulation reconsideration, it is important to note that, to date, countries have not developed separate regulatory systems for "genetically engineered" or "genetically modified" animals versus "GnEd" animals. For the most part, by both convention and legal definition, regulatory systems with GMO laws view "genetically engineered" or "genetically modified" animals as incorporating rDNA and therefore transgenic. The complexity and resource intensity of rDNA techniques, which largely precluded their use for making cisgenic alterations, supported this perspective. Though popular discourse around GnEd often uses the term as synonymous with the making of cisgenic changes, regulatory consideration recognizes that the tools of GnEd can be used to make both transgenic and cisgenic changes. Framing a regulatory distinction between transgenic and cisgenic changes can also be limiting and challenging for a variety of reasons including blurred scientific delineations of speciation, interspecies sexual compatibility, and translation of scientific definitions of cisgenesis into a reasonable and practicable legal definition. Therefore, consensus has developed around the concept of exploring new regulatory paradigms for animals with genetic alterations produced through biotechnology that could have been achieved through conventional breeding or are found in nature. This distinction is generally considered to cover the range of changes covered by the SDN-1 and SDN-2 designations, and in most cases cisgenic SDN-3 changes as well (Box 4). Because the line between SDN-2 and SDN-3 may not be distinct, regulatory systems seem not to be using these terms, e.g., Australia's Office of the Gene Technology Regulator uses SDN-1, but not SDN2 and SDN-3 in their regulatory oversight.

In most cases, countries have determined that their existing regulatory frameworks—sometimes with small modifications—can allow a different approach for genetic changes that could have been achieved through

conventional breeding and moreover that such an approach is consistent with international agreements that bear on biotechnology regulation. The approach may be a determination, after review, that the alteration does not fall under the scope of biotechnology or GMO regulation and therefore resulting animals and their products should be treated as conventional. Alternatively, the approach may require a regulatory assessment and decision as to the safety of the alteration, but still treat the resulting animals as conventional. Regardless, regulatory systems are tending to find that review or assessment of these types of alterations can be streamlined compared to those required for "genetic engineering" or "genetic modification" and still be scientifically and publicly defensible. Crucially, countries that have actively considered their regulatory systems in the light of GnEd, or that have already made regulatory determinations about GnEd animals, have declined to impose product labeling requirements on the produce of GnEd animals and their progeny with genetic alterations that could have been introduced through conventional breeding. One notable exception to this global trend is the European Union, which determined that any genetic change, whether transgenic or cisgenic, falls under the scope of its "Genetically Modified Organism" legislation. However, the European Commission has stated that this means that the legislation is not fit for purpose in light of GnEd and that it will therefore consider revision for animals; it is already considering revision for plant applications.

International agreements and conventions

Among the key conceptual documents underlying international biotechnology regulatory approaches is the Codex Alimentarius (Maggi 2022a). While its scope covers food safety in general, it includes the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals (FAO and WHO 2008), which addresses the safety and nutritional qualities of foods from GE animals that have a history of safe use as sources of food. The approach is to identify and characterize intended and unintended effects, and to evaluate their biological significance to assess the safety of novel traits. The safety assessment identifies similarities and differences between the new food and its conventional counterpart. The objective of the risk assessment is to determine whether the GE animal and foods derived from it are as safe and nutritious as those from its conventionally bred counterpart. Case-by-case assessment would address unintended effects that could result from random insertion of DNA sequences into the animal genome, which may disrupt or silence existing genes, activate silent genes, or modify the expression of existing genes. Risk analysis involves use of a stepwise approach and decision-making by weight of evidence.

A second major source of regulatory guidance comes from the Cartagena Protocol on Biosafety, developed in 2000 under the impetus of the Convention on Biological Diversity (CBD, http://bch.cbd.int/protocol). The CBD was signed by most countries following the United Nations Conference on Environment and Development in 1992 (Rocha 2022a). The objectives of the CBD included sustainable use of biodiversity and sharing of benefits from genetic resources, including access and technology transfer. Biotechnology is considered essential to attain CBD objectives (Articles 16 and 19), with a need to establish and maintain biosafety systems (Articles 8 and 19). The Cartagena Protocol, put into force in 2003, (1) provided definitions of "Living Modified Organism" (LMO) and "modern biotechnology", (2) established procedures for transboundary movement of LMOs in the absence of national regulations, (3) recognized the need for agreed principles and methodology for risk assessment, and (4) proposed the creation of the Biosafety Clearinghouse as a mechanism for promoting effective biosafety practice. We note, however, that the Clearinghouse has not been utilized by regulatory agencies for animals as it has been for plants. The GalSafe pig and the AquAdvantage salmon are listed, but the information about risk assessment and decisions is missing, as the companies have entered information, but not the regulatory agencies. To coordinate development and implementation of biotechnology oversight policy, regular meetings of the Conference of the Parties (COP) and Meeting of the Parties (MOP) review implementation, discuss topics related to risk assessment, communication, capacity building, financial mechanism, and emerging issues. In the context of our review of animal biotechnology oversight in the advent of GnEd, we note that GnEd products are not always considered LMOs as defined by the Cartegena Protocol; i.e., they do not always fit the definition of bearing a "new combination of genetic material". In the context of GMO regulations based on the Cartagena Protocol definition, a new combination of genetic material is understood as a stable insertion in the genome of one or more genes or DNA sequences that could not be obtained by conventional breeding or are not found in nature. Hence, some countries (e.g., Japan, Brazil, Argentina) have found that particular GnEd animals are not LMOs and may proceed to commercial production without the need for additional GMO authorization processes.

Approval of a product for marketing within a country suggests that the product may enter international trade, an arena governed by international trade obligations, most notably, the World Trade Organization (WTO)

Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures (Devine 2022). Key principles of the Agreement are that regulation governing trade should be non-discriminatory and not be a disguised barrier to trade (Articles 2.3 and 5.1). Regulation should be based on assessments of the risks to human, animal or plant life or health, taking into account assessment techniques developed by relevant international organizations (Article 5.1). Members should ensure that such measures do not restrict trade more than required to achieve the appropriate level of sanitary or phytosanitary protection, accounting for technical and economic feasibility (Article 5.6). Sanitary or phytosanitary measures should be consistent with relevant provisions of the Agreement and the 1994 General Agreement on Tariffs and Trade (Article 3.2).

In practice, the safe trade of animals and animal products requires extensive negotiation which needs to start prior or concurrently with negotiations regarding biotechnology-derived animals. Within this context, Maxwell (2022) discussed the mission of the U.S. Department of Agriculture Animal and Plant Health Inspection Services, International Services (APHIS-IS) unit. International trade of live animals and genetic material raises concern about the associated risks of transmitting animal pests and diseases, registration of animal breeds in terms of defined zootechnical traits, and importation of the most suitable individuals to promote genetic gains to the animal populations in the receiving country. Hence, agencies such as APHIS-IS develop import and export protocols, exchange information regarding the health status of animal breeds in importing and exporting countries, define import requirements and controls regarding diseases of economic importance, and develop a health certificate model. In addition to national laws in the United States and other countries, a code of international standards has been put forward by the World Organization for Animal Health (https://www.woah.org/en/home/).

Safety assessment

Biotechnology is perceived by some as posing not only benefits, but also risks, which should be assessed and managed so that benefits are realized and harms minimized or eliminated. Risk is the likelihood of harm resulting from an activity, and risk assessment is the process of risk identification and characterization. Problem formulation is a formal scoping process for conducting risk assessments (Roberts 2022). It incorporates available knowledge and experience, as well as mandated protection goals, laws and regulations, helps one plan and explain a risk assessment, and provides a record of the judgements and decisions that are inherent in the assessment process. As noted in Box 2, risk assessment is relevant to animal biotechnology in several contexts.

Within many countries, authority for oversight of the safety of animal biotechnology rests with different ministries or agencies within ministries, which complicates timely and effective oversight. For example, in Brazil, four ministries are involved, the ministries of the environment; agriculture, livestock and supply; health; and science, technology, and innovation. Brazil presents a case study of effective inter-ministry coordination (Dagli 2022) by CTNBio (Comissão Técnica Nacional de Biosegurança, or the National Technical Commission for Biosafety, http://www.ctnbio.gov.br/) (Box 3). CTNBio evaluates laboratories, researchers, projects, planned releases in the environment, and commercial releases. Decisions are decided upon following robust, science-based, transparent, and case-by-case technical assessments. This model of organization and inter-agency operation has served well for 17 years.

Box 2. Application of risk analysis to animal production and animal biotechnology Principles of risk analysis can be applied to animal production, whether for conventionally bred or biotechnology-derived animals. Gomes (2022) presented a case study of application of risk analysis to 68 dairy farms in Brazil, applying a tool developed by Bickett-Weddle (2009) to study risk perception, risk assessment, risk management, and risk communication. Principles of risk analysis are widely applied to manage food safety at slaughter. For example, the U.S. Food Safety and Inspection Service (FSIS) ensures food safety at slaughter through robust inspection procedures (Stumps 2022). These policies and procedures would be in place for ensuring the safety of products of animal biotechnology.

The principles of risk analysis can be applied to assess the safety of foods derived from animal biotechnology (Nutti 2022). Those principles are presented in Codex Alimentarius (https://www.fao.org/fao-who-codexalimentarius/en/), which is widely used as reference guidelines for countries. Codex calls for consideration of: a) health status of the recombinant-DNA animal, b) expressed substances (non-nucleic acid substances), c) compositional analyses of key components, d) food storage and processing, and e) intended nutritional modification. Critically in our context, the guidelines for the assessment of recombinant-DNA animals might not apply to GnEd techniques, and so national or regional oversight policy should be considered. Countries differ in their approaches to regulatory oversight of food safety assessment of products derived from GnEd animals,

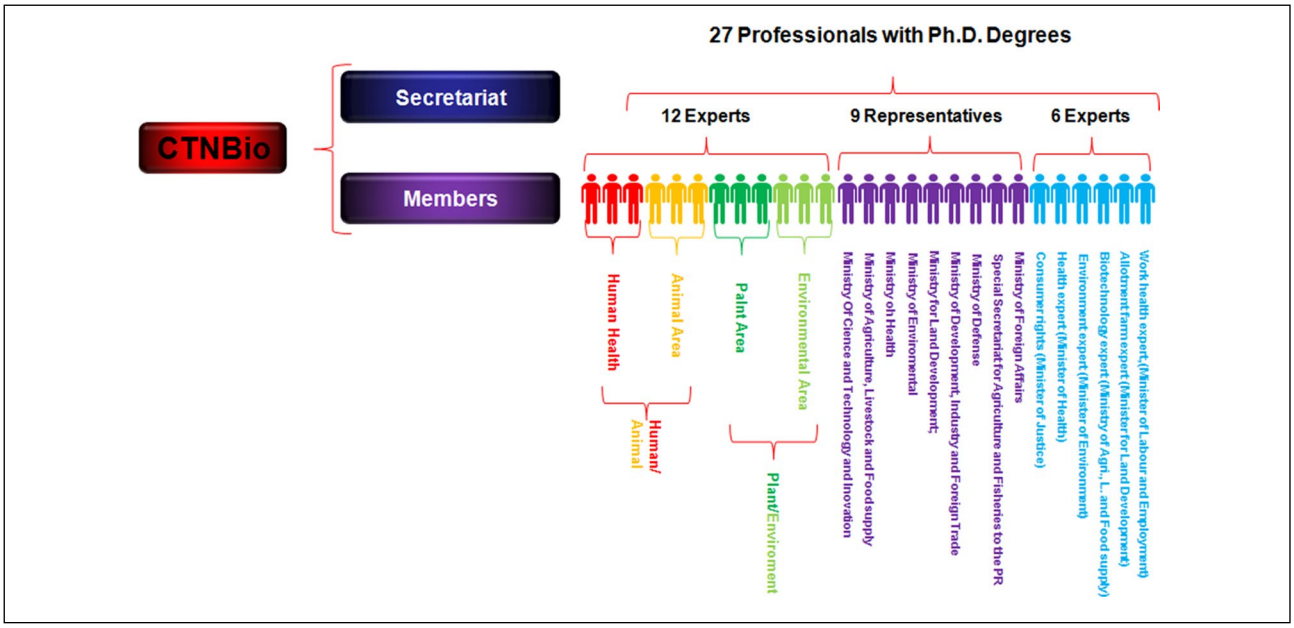
with some countries considering them GM, some not, and some not yet having implemented a policy.

Environmental issues posed by animal biotechnology can be assessed and managed within the formal risk analysis framework (Hallerman 2022). Risk assessment proceeds as a sequence of steps: (1) Identification of potential harms resulting from exposure to the biotechnology-derived animal; (2) Identification of the hazard that might lead to realization of harms, in this context, the biotechnology animal; (3) Assessing the likelihood the biotechnology animal would escape and establish or negatively impact the receiving environment; and (4) Assessment of the probability of harm being realized upon exposure to the hazardous agent. A chain of causality must be realized for harm to result, e.g.: escape of the organism, its establishment in the receiving ecosystem, its predation upon a population of interest, and decline of that prey population of focal interest. The probability of harm being realized is the product of each of these causal stages being realized. Risk, then, is the probability of exposure times the likelihood of harm becoming realized given exposure. Risk assessment may be quantitative (yielding a discrete decimal probability of harm becoming realized) or it may be qualitative (low, medium, or high risk), based on expert or stakeholder opinion; qualitative assessment is often sufficient to characterize and manage environmental risk. In most regulatory contexts, only marginal risk would prove acceptable. Risk can be managed by minimizing the probability of exposure via geographic, physical, and reproductive confinement. Some questions relevant to risk assessment may be answered by literature review, expert opinion, or laboratory experiments as opposed to full-scale field experiments. Only relevant questions should trigger new experiments. Mandating studies disproportionate to risk increases cost and discourages development and use of innovations.

Case studies were considered during the workshop. It was suggested that SLICK cattle posed no greater ecological risk than conventional cattle production. On the other hand, it was suggested that production of *mstn*-knockout Nile tilapia, because of the invasiveness of the species, needed effective confinement of both conventional and biotechnology-derived animals.

Box 3. Managing regulatory complexity: the Brazilian experience Noting that the products of animal biotechnology are becoming more complex, regulators can draw upon experience from plant biotechnology, food safety of conventional products, and animal breeding and whole-genome sequencing to more effectively assess and oversee the products of biotechnology (National Academies of Sciences, Engineering, and Medicine 2017). Fernando (2022) discussed how CTNBio, the Brazilian biotechnology oversight body, applies the concept of familiarity to oversight of biotechnology. In a consultation letter, CTNBio asks questions to assess whether the product (i.e., the offspring, lineage or final product) falls within the legal definition of GMO. This developer is asked for proof of the absence of recombinant DNA/RNA molecules, whether the product is commercially approved in other countries, whether the product applies the principle of gene drive that may allow the phenotypic change to spread throughout the population of the recipient organism, and how the possibility of unintentional, off-target effects of the product was assessed. If, under Normative Resolution Nº 16, CTNBio determines that the product is a GMO, then oversight follows the Biosecurity Act and determinations are subject to ordinary rules. If CTNBio determines that the product is not a GMO, the Biosecurity Act does not apply and oversight follows regular non-GMO regulations. Recent products approved as non-GMO include semen from myostatin knockout cattle, GnEd SLICK Angus cattle, and myostatin knock-out Nile tilapia.

Regulatory oversight of the development and commercialization of animal biotechnology is often divided among multiple agencies, and effective coordination among them can prove challenging. Dagli (2022) explained in detail how Brazil coordinates biotechnology oversight among the Ministry of the Environment; Ministry of Agriculture, Livestock and Supply; Ministry of Health; and Ministry of Science, Technology, Innovations and Communications. Brazil's Biosafety Law no 11.105 of 2005 provides safety norms and inspection mechanisms for activities with GMOs and their by-products. The same biosafety law applies to plants, microorganisms, vaccines, and animals. The Brazilian model of GMO regulation involves both process- and product-based regulation. The National Biosafety Technical Commission, referred to as CTNBio, coordinates the risk assessment aspect of biotechnology oversight (http://www.ctnbio.gov.br/). Its 54 members (27 members and 27 alternates) are all scientists holding a Ph.D. CTNBio includes 12 experts from outside of government, three each in human health, animal production, plant production, and environmental science), nine representatives of ministries, and six experts with appropriate expertise from the ministries of labor, land development, agriculture, environment, health and justice.



Box 3, Fig. 1. Organizational structure of CTNBio, Brazil's body for coordinating regulatory decision-making for the products of biotechnology (http://www.ctnbio.gov.br/).

Review of proposals for actions embodies science-based, case-by-case, robust and transparent technical assessment. All CTNBio actions require a simple majority of 14 votes from members. While CTNBio oversees risk assessment, other committees oversee other aspects of biotechnology oversight: CIBio, the Internal Biosafety Commission, maintenance of biosafety; OERF, registration and inspections functions; and CNBS, the National Biosafety Council, the national interest and socioeconomic factors.

Box 4. Three categories of site-directed nucleases (SDNs) SDN applications are often divided conceptually into three categories:

SDN-1 produces a double-stranded break in the host genome without addition of foreign DNA; host-mediated repair of this break can lead to a mutation or deletion, causing gene silencing, gene knock-out, or a change in the activity of a gene.

SDN-2 produces a double-stranded break, and a small nucleotide template is supplied that is complementary to the area of the break, which is used by the cell to repair the break. The template contains one to several small sequence changes in the genomic code, of which the DNA repair mechanism copies into the host genome, resulting in a mutation of the target gene. SDN-1 and SDN-2 mutations can be as specific as the editing of a single base.

SDN-3 induces a double-stranded break in the DNA, but is accompanied by a template containing a gene or other sequence of genetic material. The cell's DNA repair system utilizes this template to repair the break, resulting in the introduction of new genetic material.

Development of regulatory systems

Against this background, many countries have made progress toward developing systems for regulatory oversight of animal biotechnology. Notably, several countries have already approved commercialization of animal biotechnology products for food use and/or have made determinations that some GnEd animals are not GMOs and therefore are to be regulated as conventional animals.

Argentina.—Argentina initiated regulation of GMOs in 1991, in conjunction with the creation of the National Advisory Commission on Agricultural Biotechnology (CONABIA) (Murrone 2022). The first application was submitted in 2003 to initiate activities to develop GM animals for agricultural use, and the first field trial authorization was in 2005. The criteria applied by CONABIA are case-by-case study, based on technical scientific criteria, data quality, familiarity, and history of safe use. There are two types of evaluations: biosafety evaluation, which authorizes research activities and contained production, and environmental risk assessment, which is required for commercial authorization of the GMO. All incoming applications undergo a regulatory analysis by different regulatory authorities, which results in

three non-binding documents generated by CONABIA, SENASA (the National Food Safety and Quality Service) and MARKETS (the Undersecretary of Agricultural Markets—Directorate of Market Policies), which are submitted for consideration and possible final validation for the Undersecretary's approval. The regulatory framework was tested in 2022 to determine whether it allowed new uses and was found sufficient and effective to carry out not only GM insect-related, but also xenotransplantation activities. The Argentine regulatory framework for new breeding techniques (NBT)-derived products was the first of its kind globally (Goberna 2022). The regulation of products derived from NBT originated in 2015, making Argentina a leading country in creation of a regulatory framework for modern biotechnology techniques, providing advice and technical assistance to other countries regarding regulatory approaches. The analysis is oriented to the final product, rather than the technology applied to obtain it, and is the key criterion for determining the GM character of the subject of regulation. For this purpose, the regulatory system analyzes whether a new combination of genetic material is generated in the final genome of the organism. At the same time, developers can predict costs and product development times even at the design stage (Box 5).

Australia.—Australia's biotechnology oversight policy is based on legislation and implementing policies—the Gene Technology Act of 2000 and its regulations—that were crafted with that task in mind. The object of the Gene Technology Act is to protect the health and safety of people, and to protect the environment by identifying risks posed by gene technology and by managing those risks by regulating activities with GMOs. Under the gene technology regulatory scheme (Mitchell 2022), dealings with GMOs are prohibited unless authorized. The regulators of specific products interact with the Office of the Gene Technology Regulator (OGTR), a single decision-maker with monitoring and enforcement powers. OGTR practices independent, science-based assessment, with value placed on being transparent and consultative, with a public record of considerations of GMOs. The level of regulatory oversight depends upon the level of risk posed in a given context. There are exempt dealings, i.e., work with certain types of GMOs in containment; notifiable low-risk dealings (NLRDs) in containment are overseen by organizations doing the work. OGTR issues licenses with enforceable conditions for high-risk dealings in containment or for dealings outside of containment, for example research using GM animals in a PC2 (physical containment level 2) facility, GM cows in a PC2 large grazing animal facility, or contained work with gene drive-bearing GMOs. There have been 37 licenses for commercial release of GMOS issued since 2001, but none were for animals. Information on OGTR activities is publicized on the OGTR website (https://www.ogtr.gov.au/). The advent of GnEd led to modification of the Gene Technology Scheme. Organisms with SDN-1 modifications are not considered GMOs if they are produced using site-directed nucleases, no template was added to guide DNA repair, and there were no other changes as a result of application of gene technology. Template-guided SDN-2 and SDN-3 processes would be regarded as resulting in GMOs. Any work with organisms containing a gene drive needs a license. This review clarified that null-segregants (offspring which have not inherited manipulated genes or traits) are not GMOs. These amendments came into force in October 2019. A third review of the regulatory policy was undertaken independently of the OGTR, and its recommendations included maintaining the process-based regulatory trigger, amending key definitions to clarify the scope of regulations in light of ongoing technical advances, additional risk-tiering to facilitate the ability to adjust oversight to match the level of risk, and consideration of mechanisms to better respond to changes in science. Implementation is ongoing with further consultation on draft legislation expected in 2024. OGTR regulates GMOs by interacting with other agencies, avoiding duplicating regulation where another agency has oversight and aligning decision-making as much as possible. On food safety issues, Australia and New Zealand coordinate through Food Safety Australia New Zealand (FSANZ). The advent of new breeding techniques (NBTs) has led to development of Proposal P1055 to revise and update the GM food definitions in the Australia New Zealand Food Standards Code; a report including draft definitions is expected to be released for a public consultation in 2024 (https://www.foodstandards.gov.au/food-standards-code/proposals/p1055-definitions-for-gene-technology-and-new-breeding-techniques).

Brazil.—Brazilian Normative Resolution Number 16, issued by CTNBio (Box 3), determines that after a case-by-case analysis, a product generated by new breeding technologies, which include GnEd, should or should not be classified as a GMO (Camargo 2022). To have a product classified as non-GMO, the developers need to show that it is free of recombinant DNA/RNA or any DNA/RNA that is novel to the species and that there are no non-negligible unintended effects (e.g., non-negligible off-targets). CTNBio has made decisions about GnEd products following this normative resolution. As of July 2022, 27 genetically engineered products were classified as non-GMO by CTNBio, most of them microorganisms, but there also were a few crops (maize, soybean and sugarcane). For vertebrates, myostatin-knockout Nile tilapia with an increased growth rate and feed conversion, and

semen from one myostatin-knockout bull with increased muscle growth were classified as non-GMO. A bull and a heifer with mutations in the prolactin receptor to generate the SLICK hair phenotype also were considered non-GMO products. CTNBio's decisions mean that those products do not need to follow the GMO regulation; however, for commercialization, they still need the approval of the specific regulatory agency, i.e., environmental, animal or human health agencies, according to the product's category.

China.—China issued a simplified review guide for GnEd crops in January 2022, and a simplified review guide for GnEd animals has been drafted (Li 2022). Currently, GnEd animals are evaluated by five stages, the same as for transgenic animals and crops in China: experimental research, pilot trials, environmental release, production test, and issuance of a safety certificate.

Colombia.—The Instituto Colombiano Agropecuario (ICA), part of the National Science and Technology System in Columbia and attached to the Ministry of Agriculture and Rural Development, established normative requirements for authorization of LMOs exclusively for agricultural, livestock, fishing, commercial forest plantations, and agro-industrial uses through Decree 4525 in 2005 (Pinella Lopez 2022). Following application of the regulatory framework, 169 GM events (mainly corn, cotton and soybean) have been approved for animal consumption, and 41 for sowing (mainly corn, cotton, and flowers). In 2018 through Resolution 29,299, the ICA developed a procedure for processing applications for cultivars generated by using GnEd. By applying the procedure, GnEd waxy corn, bacterial blight-resistant rice, and mustard with an improved flavor profile were analyzed and considered as conventional. The remarkable opportunities that GnEd could bring to animals lead to Resolution 22,991 in 2022. This instrument, based on a clear definition of "novel combination of genetic material", established the procedure for processing applications for new plant and animal products obtained through "breeding innovation" to determine whether such products are considered LMOs or conventionally bred organisms.

Ethiopia.—In Proclamation No.655/2009, Ethiopia in 2009 ratified a highly restrictive biosafety law, which did not allow research or commercial use of GMOs. However, as scientific understanding and the economic importance of biotechnology products increased, in 2015 the government amended the biosafety law to allow research and commercialization of GMOs. Directives were approved issuing requirements for environmental risk assessment, transport and storage of GMOs. In the amended proclamation, however, some important terminologies—genetic engineering, GMO, and genome editing—were not defined. A National Biosafety Advisory Committee (NBAC) composed of a range of 15 experts was established in 2017 to provide scientific advice for the Environmental Protection Authority. For GnEd, it was agreed to have a guidelines document, leading to development of the Draft Guideline for the Regulation of Genome Editing Technology in Ethiopia covering animals, plants and microorganisms, which was submitted to the Environmental Protection Authority regulatory body for approval or comments (Dadi 2022). As of now, Ethiopia has no ongoing work on genome editing of animals.

Japan.—A Party to the Cartagena Protocol, in 2003 Japan adopted the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms implementing the Cartagena Protocol, legislation that is often referred to as Cartagena Act. Under this Act, the Japanese government has determined that some GnEd organisms should be considered LMOs, while others are not subject to Japan's Cartagena Act (Tsuda et al. 2019). GnEd end products derived by modifications of the SDN-1 type would not represent LMOs under the Japanese Cartagena Act. In 2021, the Japanese Ministry of Health, Labor and Welfare (MHLW) determined that two genome-edited fishes with increased edible muscle, myostatin-knockout red sea bream *Pagrus major* and leptin receptor-knockout tiger puffer *Takifugu rubripe*, are not LMOs and therefore are not subject to a requisite food safety review (MHLW 2021; Matsuo and Tachikawa 2022), clearing the way to their ongoing commercial sale.

Kenya.—The Kenyan parliament in 2008 passed into law the Biosafety Act, which led to the establishment of the National Biosafety Authority (NBA) to exercise general supervision and control over the transfer, handling, and use of GMOs. This includes regulation of research and commercial activities involving GMOs with a view to ensuring safety of human and animal health and provision of an adequate level of protection of the environment. To strengthen the biosafety framework and guide a transparent, science-based, and predictable decision-making process, Biosafety Regulations for Contained Use; Environmental Release; Import, Export and Transit; and Labeling were developed and published in 2011–2012. During the research phase, there is no difference in regulation of GM animals as compared to GM plants, except in consideration of the ability of animals to move and matters of animal welfare. Regulation of GnEd technologies is guided by Genome Editing guidelines published in 2022 that apply to animals, plants and microorganisms. Decision-making is based on the presence or absence of foreign genetic material in the GnEd animal. Regulatory

decisions have been made for GnEd crops and microorganisms, but not yet for animals (Muia 2022).

Paraguay.—Since 1997, Paraguay has been one of the top countries planting GM soybean. In 2012, the Comisión Nacional de Bioseguridad Agropecuaria y Forestal (CONBIO) was created (Decree 9699) to address, analyze, and recommend the introduction, field trials, release, and proposed uses of LMOs, mainly plants (Alvarez 2022). In addition, in 2019, the Ministry of Agriculture and Livestock approved Resolution MAG 565 setting out the pre-consultation process for products obtained through new breeding techniques. Although such a process could cover plant and animal developments, to date, no applications for the use of GnEd in animals have been submitted.

The Philippines.—The Philippines is the first Association of Southeast Asian Nations (ASEAN) member country to initiate a biotechnology regulatory system (Mingala 2022). The Philippine Biosafety Policy was signed in October 1990 as an Executive Order creating the National Committee on Biosafety of the Philippines. In 2006, the National Biosafety Framework (NBF) was established. A Joint Department Circular (JDC) for plant and plant products was signed by five government departments with relevant mandates and expertise in agriculture, science and technology, environment, health and public consultation. The Bureau of Animal Industry has recognized the need to establish guidelines for GM Animals and Animal Products. A similar JDC is drafted that is applicable to: (1) genetically-modified fishes and other aquatic resources, (2) domesticated animals and biological products used for animal husbandry or veterinary purposes, and (3) biological agents used for biocontrol derived from the use of modern biotechnology and containing novel combinations of genetic materials. GnEd products that do not contain novel combinations of genetic materials are not covered. In making biosafety decisions, the concerned agencies will be guided by the principles under the NBF: Standard of Precaution, Risk Assessment, Environmental and Health Risk Assessment, Socio-economic, Ethical and Cultural Considerations, and Access to Information. The draft has four classifications: (1) Research and Development of a Regulated Article under Contained Use, (2) Research and Development of a Regulated Article for Limited Release into the Environment, (3) Commercial Use of a Regulated Article under Containment, and (4) Commercial Use of a Regulated Article for General Release. The Bureau of Fisheries and Aquatic Resources or Bureau of Animal Industry will lead in assessing and issuing biosafety permits for aquatic and terrestrial animals, respectively. The developer of a regulated article may apply for deregulation, provided it will not pose greater risks to human and animal health and biodiversity than its conventional counterpart. The deregulation may be done: (1) *motu proprio* ("on one's own initiative"), or (2) through petition. If the latter, the applicant, among other requirements, shall state the factual basis why this regulation should not apply to the regulated article and published scientific literature relied upon by the petitioner.

United Kingdom.—Leaving the European Union has provided the United Kingdom the opportunity to adopt a more science-based and risk-proportionate approach to the regulation of what they term precision-bred organisms (PBOs). The Precision Breeding Bill, adopted in early 2023, introduces a new, simpler regulatory regime in England for precision-bred plants and animals to allow authorization and marketing of these organisms and derived human food and animal feed products (Georgescu and Povey 2022). Four key policy changes embodied in the Precision Breeding Act (https://www.legislation.gov.uk/ukpga/2023/6/contents/enacted) are: (1) removing precision-bred plants and animals from regulatory requirements applicable to GMOs (excluding those relating to microbes, organics and contained use), (2) introducing two notification systems for research and marketing purposes where breeders and researchers will need to notify the Department for Environment, Food and Rural Affairs (DEFRA) of precision-bred organisms, providing information to be collected to be published in a public register, (3) establish a new science-based authorization process for food and feed products developed using precision-bred organisms, and (4) establish a proportionate regulatory system for precision-bred animals to ensure that animal welfare is safeguarded. Georgescu and Povey (2022) provide details on the process of notification for a PBO and the process of applying for marketing or import of PBO-derived products. The Act will be brought into force by commencement regulations. Provisions relating to plants will be brought into force first. Provisions relating to animals will be brought into force later, once measures to safeguard animal welfare are put in place. With scientific advice that PBOs pose no greater risks than traditionally bred counterparts, labeling will be restricted to known health issues such as presence of allergens or significant nutritional or compositional changes.

United States.—The United States has taken a different approach to biotechnology regulation than other countries (Epstein 2023). Rather than creating new GMO laws, the United States implemented the Coordinated Framework for the Regulation of Biotechnology, which extends the scope of existing laws and regulatory authorities to establish oversight of biotechnology products (OSTP 1986). The Food and Drug Administration (FDA), an agency within the U.S. Department of Health and Human

Services (HHS), currently has jurisdiction over animals created via biotechnology. FDA regulates animal biotechnologies under their animal drug regulatory authority within the HHS/FDA Center for Veterinary Medicine, and in 2009 published Guidance for Industry (GFI) #187 identifying the heritable rDNA construct to be the regulated article (FDA-CVM 2009). The same regulatory process is used to approve animals for food and biopharma animals to be used for production of drugs or biologics. In 2017, FDA released a new draft GFI#187 (FDA-CVM 2017) that broadened its scope to include genome-edited animals, and now heritable "intentional genomic alterations" (IGAs) are identified as the regulated article. FDA makes its regulatory decisions on a case-by-case basis and may choose not to enforce a requirement, i.e., a decision to exercise Enforcement Discretion. If an Enforcement Discretion decision is made, the developer does not need to submit a drug approval application for the IGA. FDA intends to release new guidance to clarify their approval processes. FDA is proposing a tiered approach with three categories requiring either: (1) no review of data, (2) review of data prior to an enforcement discretion decision, or (3) full approval application (equivalent to a GMO approval). The first is a categorical enforcement discretion decision and only applies to non-food species laboratory animals, such as rats and mice, that are raised in contained and controlled laboratory conditions for research. The second has been used for research models of food species (pigs) and for aquarium pet fish, but has recently been expanded to allow enforcement discretion decisions for food animals, such as those that have DNA edits that can be demonstrated to already exist in conventionally-bred animals.

Regulatory cooperation.—Alignment of regulatory approaches among trade partners would minimize red tape and facilitate international trade in products of animal biotechnology. Some Latin American countries have undertaken efforts to achieve regulatory cooperation in oversight and trade of the products of agricultural biotechnology (Box 6).

Box 5. Economic impact of regulatory approach: the Argentine experience The Argentine regulatory system for agricultural biotechnology is recognized worldwide for being among the most experienced. Argentina was the first country that enacted regulatory criteria to determine whether organisms resulting from new breeding techniques (NBTs) are or are not to be regarded as GMOs (Garappa 2022). Argentina now has seven years of experience applying such criteria to cases involving GnEd plants, animals, and microorganisms of agricultural use. Whelan et al. (2020) explored the effects of such regulatory experience on economic innovation by comparing the cases of products derived from gene editing and other NBTs that had been presented to the regulatory system to cases of classical GMOs that have been deregulated in the country. The products analyzed can be either real (final) or hypothetical (under development). After several years of experience in NBT regulatory matters, 65 prior consultation instances (PCI) have been submitted for different organisms. The NBT regulation stimulated local innovation, allowing developers to vary the phenotypes sought in a diversity of organisms and products, including cattle: hypoallergenic milk, increased muscle mass, polled, and thermal tolerance (hypothetical products); horses: increased muscle mass (hypothetical product); fishes (Nile tilapia): improvement of fillet yield/double fillet (real product); and pigs; tissues for xenotransplantation (hypothetical product). Including products under development in the assessment process encourages research teams and developers to interact with the regulatory system. The results suggested that GnEd products will have different profiles and market release rates than did the first wave of GM products, with faster development from bench to market. Development of GnEd products is driven by a more diverse group of developers, led mostly by small and medium enterprises and public research institutions. The advantages of the Argentine regulatory framework are that it increases the availability of information, reduces uncertainty among both developers and users, facilitates the decision-making process and diffusion of innovation, and improves the predictability of regulatory costs for innovative products (Garappa 2022). Noting the number of developments and consultations carried out, Goberna et al. (2022) showed that the speed of innovation of these technologies was increasing, giving more opportunity to local developers who showed interest in generating products involving different species and phenotypes. Hence, the regulatory framework promotes harmonious development of biotechnologies, promoting scientific advances, societal perception of biotechnology, and greater public–private interaction (Garappa 2022).

Box 6. Regulatory cooperation and harmonization of criteria: The Latin American experience Latin America is a large, heterogeneous region that includes countries ranging from net food importers to leading world exporters, different regulatory authorities (ministries of agriculture, environment, health, etc.), and different levels of technological and regulatory progress (from none or incipient to fully consolidated).

The respective countries have developed different, effective regulatory approaches, and there is no one "best" approach. There are differences in regulatory philosophies, legal enabling authorities, and existing regulatory structures. Several countries in Latin America have developed their own GnEd regulatory approaches within a feasible and viable regulatory cooperation framework, which recognizes and accepts the specificity of each country and harmonizes criteria instead of policies. While creation of unified biotechnology policies for the region is not feasible, transition to a harmonized view through regulatory cooperation is possible, as demonstrated by some sub-regional initiatives (Rocha 2022b). For Latin American countries, the recognition, acceptance, and respect for regional heterogeneity play a major role in regulatory cooperation. On this basis, GnEd considerations have several common points. For example, countries agree that effective regulation must protect the public health, the environment, and allow the production and marketing of safe products. In general, they recognize that GnEd is an example of genetic manipulation that is different from transgenesis, and the key element for such a distinction is clarity on what an LMO and a new combination of genetic material are. In addition, a number of countries agree that GnEd products must be evaluated on a case-by-case basis, although this "case-by-case" is not interpreted as part of the risk assessment procedure suggested by the Cartagena Protocol on GMOs. The case-by-case consideration of GnEd products relates to the distinction between the transgenic or non-transgenic nature of the product, and there is consensus about there being no need for a new category of products. Certainly, there are certain issues to be considered, such as the fact that GnEd is a group of technologies with different names (gene editing, genome editing, new breeding techniques, plant breeding innovation, precision biotech, etc.), which can generate a nomenclatural problem under the regulatory point of view. In addition, although GnEd technologies are very precise, they are difficult to explain to the non-specialized public, which could be an issue for communication and training purposes.

Observing that 71 countries—including Argentina—have adopted cultivation of GM crops since 1996, Maggi (2022a, b) noted that Argentina's CONABIA and Brazil's CTNBio interact regularly in multinational forums regarding agricultural biotechnology (https://www.argentina.gob.ar/noticias/argentina-y-brasil-avanzan-en-la-cooperacion-bilateral-en-materia-de-agrobiotecnologia). In July 2022, Argentina and Brazil signed a Memorandum of Understanding aimed at strengthening collaboration in science, technology and innovation, particularly regarding biosafety of the products of modern biotechnology. This goal will be approached by promoting the exchange of scientific information related to biosafety and risk assessment, jointly establishing procedures that reduce costs and time and allow the establishment of common procedures, as well as analyzing the possibility of harmonizing norms for biosafety assessment and promoting exchange of information related to the regulatory approach regarding products derived from GnEd.

Developer—regulator perspective on regulation

Representatives of companies working to commercialize GnEd animals—including AquaBounty (March 2022a, b), Acceligen (Perez-O'Brien 2022a, b), Oxitec (Abreu 2022), and Genus (Nesbitt 2023)—discussed their experiences undergoing regulatory reviews in several countries. In parallel, regulators from several countries discussed their view of regulatory experiences with GnEd animals. It emerged that even though different countries have seemingly different regulatory processes, the overarching principles are similar. These include promoting safety for people and animals, safety for the modified animal, safety for the environment, and assessing the validity/efficacy of the claim made for the properties of the biotechnology animal. For most countries, the same process applies to modified plants, microorganisms and animals. Also identified as important to an effective dialog was continuing consultation and communication between developer and regulator before and during development, as well as submission of quality data and clear documentation.

Discussion among developers and regulators showed that both sectors seek to realize efficient regulatory processes for products. Since the products of animal biotechnology ultimately will enter international trade, compatibility among national oversight systems will prove critical to commercial success. Because public acceptance of such products will prove important, well-designed outreach will be needed. Some leading companies have already conducted consumer opinion surveys. Results show that consumers are highly supportive of products that promote animal health and welfare and are relatively less supportive of traits that enhance environmental sustainability.

Building upon classical selective breeding

It is important to put applications of biotechnology into biological and conventional breeding contexts. Nature creates millions of new genetic variants every reproductive cycle. Traditional animal breeding relies upon selection for performance-increasing variants. However, many

genomic variants are lost during breeding, and if beneficial variants are found, it requires many years to incorporate them into the production population. The process of introgressing alleles enhancing expression of a particular trait into a production line may lead to loss of genetic gain in other important traits. Breeding companies are interested in GnEd because it can reproduce useful variation in elite germplasm quickly by introducing naturally occurring variants (Rice 2022). It should be noted that in many cases, genetic changes that can be introduced by GnEd have already occurred or could occur naturally. Introgression of naturally occurring genetic variants into a livestock population can be accomplished by crossbreeding, although the approach also introgresses undesired background variation that can lead to loss of genetic progress for other important traits. In contrast, GnEd can directly introduce naturally occurring genetic variants into elite germplasm (Rice 2022).

Based on principles of quantitative genetics, classical selective breeding has exploited selection for the additive component of genetic variance to improve targeted traits and combinations of traits (Gianola 2022). While livestock breeding programs through the 1980s emphasized production traits, current programs also target animal health and welfare, product quality, and production efficiency traits, as well as reduction of environmental impact and retention of genetic diversity (Granados 2022). For example, breeding goals for pigs include not only such core traits as average daily gain and feed conversion, but also disease resistance or resilience and lessened antibiotic usage, heat tolerance, nutrient utilization, welfare, behavior, and meat quality (Paustian 2022); further, functional annotation of the porcine genome (Pan et al. 2021) contributes to trait discovery and more rapid genetic progress. Recent livestock breeding has incorporated selection for molecular markers linked to quantitative trait loci and genome-enabled selection (Meuwissen et al. 2001), increasing the rate of genetic gain, as exemplified by dairy cattle in the United States (Wiggans et al. 2017) and Brazil (Boison et al. 2017; Verardo et al. 2021; Silva 2022) and zebu beef cattle in Brazil (Chiaia et al. 2017; Brito Lopes et al. 2020; Baldi 2022). Genomic approaches are being applied to identify variants promoting adaptation or increasing performance of livestock under specific environmental conditions (Ortega 2022). For example, genomic methods are being applied to better understand mechanisms underlying fertility, conception and embryo quality and survival to improve fertility traits of cattle. Future challenges include integration of multiple -omics and big data into animal breeding and application of genomic selection in crossbreeding (Baldi 2022).

Animal biotechnology builds upon genetic progress achieved through classical selective breeding and can contribute to further genetic progress by addressing some of the limitations of classical methods. Artificial insemination and embryo transfer facilitate the diffusion of the genetics of high-performance animals (Baruselli 2022), although the rate of adoption differs among world regions (Thibier and Wagner 2002). Techniques have been developed to retrieve oocytes from genetically superior females before puberty or even before birth for in-vitro embryo production, dramatically shortening generation interval and increasing the rate of genetic gain (Baruselli et al. 2018). Critically, as noted above, animal biotechnology provides opportunities to improve particular traits—such as disease resistance, animal welfare, and reproductive confinement)—that are not possible with or can be achieved more rapidly than is possible through conventional breeding; 85% of EFFAB (European Forum of Farm Animal Breeders) and FABR-TP (Farm Animal Breeding and Reproduction Technology Platform) members consider GnEd a viable approach for improving animal health, and 45% for improving animal welfare (Granados 2022).

Hendrix Genetics is involved in genetic improvement of layer and broiler chickens, turkeys, swine, and aquaculture species globally; hence its approach to selective breeding and animal biotechnology is of broad interest. Focusing on chickens, Veninga (2022) noted that breeding goals have changed from production traits only in the 1960s to also include product quality, health and welfare and sustainability traits today. For example, layers are now being selected for persistency, livability and eggshell quality. To improve livability, Hendix is experimenting with different bird densities, light intensities, and maintenance of intact beaks to identify social "families" with good production as parents of future generations. Across species, genomic information is being added into evaluations of breeding value. Hendrix is not currently using GnEd in their products, but regards it as a promising technology. Its application in the future requires careful evaluation. The company is actively following developments in this research area, including disease resistance in salmonids and sex detection in eggs of layer chickens (Tizard 2022a, b). Interest in GnEd would not be on production traits, but on animal welfare, disease resistance, genetic security (reproductive confinement) and human health-related traits. The issue of whether to adopt GnEd technology will turn on whether it is legal, ethical, and acceptable to society. Elements of the ethical framework include the benefit of the application, the impact on the animal, and any alternative approach to realize the same benefit.

The aquaculture sector faces issues of genetic improvement of 20 major species and production in systems adjacent to ecosystems where wild relatives occur. Tinch (2022) described the work of the Center for Aquaculture Technologies to improve production, efficiency and sustainability of the aquaculture industry, including application of selective breeding, genomic selection, GnEd, and biosecurity. GnEd provides the opportunity to address traits, such as sterility, sex determination, and disease resistance, not amenable to classical methods. Company policy is that GnEd farm animals should be sterile, posing the issue of how to propagate them, leading to the need for fertile, surrogate broodstock. The company is collaborating with breeders and producers to ultimately deploy their technologies.

Writing in Foreign Affairs, Bill Gates (2018) offered the view that "Used responsibly, gene editing holds the potential to save millions of lives and empower millions of people to lift themselves out of poverty. It would be a tragedy to pass up the opportunity." Access to genetically improved animals derived from selective breeding or biotechnology remains an issue for smallholder farmers, who produce up to 35% of the world's and 80% of sub-Saharan Africa's food (Tsigadi 2022). Past genetic improvement efforts have proven problematic for reasons including incompatibility of introduced genotypes with producers' breeding objectives, management practices and environmental conditions and lack of a comprehensive approach to design simple but effective breeding strategies instead of adapting complex breeding programs that require complex logistics and technologies. Appropriate lessons must be drawn and strategies implemented to further adoption of improved genetics, whether derived from selective breeding or biotechnology. Tsigadi (2022) described the approach of Farmers Choice, Ltd., to integrate dissemination of semen from improved pig lines into production in Kenya, in concert with adoption of better production, processing, and distribution systems, an integrated approach that may inform similar efforts in other systems dominated by smallholder farmers.

Getting biotechnology-derived solutions to farmers and consumers

Breakout groups for researchers, developers, and representatives of the animal production industry considered the opportunities and identified the challenges they face as they try to bring a product to farmers and consumers.

Groups recognized the considerable investment necessary to develop GnEd animals. For private-sector developers, investors must see a clear path to the market, with strong prospects for revenue. Government and non-profit funding may prove important, especially in the early stages of the R&D process or for traits and animals that may not realize high profit. For example, initial research with transgenic salmon began at Memorial University of Newfoundland, and the province of Prince Edward's Island and the Canadian government were supportive of AquaBounty in its early development. Australia supports CSIRO's translational research to realize a pathway from concept to application for commercial production. Funding from the U.S. Department of Agriculture has supported GnEd and genetic engineering research efforts. Genus is currently working with public research institutions in the US and UK. The first food products from GnEd animals marketed resulted from the Regional Fish Institute's partnerships with universities in Japan. Public–private partnerships have been crucial to the development of many or most animal biotechnology traits.

Groups also recognized the long timelines necessary to develop GnEd animals and to multiply them to the numbers needed for the commercial production scale. Livestock breeding companies noted that they have that capacity and already do that for conventionally bred animals. Different animal production sectors (e.g., swine, cattle, poultry, fish, etc.) have different breeding structures and commercialization paradigms, which will affect the time needed to bring GnEd lines to commercial production.

All groups agreed that an enabling regulatory environment is critical to successful commercialization of GnEd animal products. This point is supported by the regulatory experiences that led to commercialization of the genetically modified AquAdvantage salmon, as well as GnEd red bream, pufferfish, and SLICK cattle. The recent decisions to regard the GnEd fishes as not being GM is illustrative. Unlike the GM crops that Japanese consumers had been opposed to, these fishes (and a GnEd tomato that received similar regulatory treatment in Japan) were domestically developed products. They were niche products and not intended for export, therefore not facing potential trade issues. Rich in healthy fatty acids, the products are associated with a healthy food claim, hence a consumer benefit. In these cases, the Japanese regulators asked only for molecular characterization of the products prior to making a decision that they were not GM and could enter the market without additional GMO regulatory authorizations.

Some developers expressed in the group discussions the opinion that the market must first be ready for GnEd animal products. Generally, there were two key groups of stakeholders identified; farmers and consumers. Farmers want their farms to be financially viable and need assurance that they will not face barriers for the product in

the value chain. Consumers want products that have real value to them. Although it was noted that AquaBounty does not fit well into this farmer and consumer model as the company has its own farms and doesn't sell directly to consumers, communication to stakeholders is important for product acceptance (an example of their communication and outreach is described later in the section on building public trust). Participants recognized that it is important to frame conversations with these stakeholders thoughtfully and, as considered below, barriers to trust will have to be addressed. The animal producers noted that there are many kinds of farmers, and only some can choose the particular animal lines produced—it depends upon the animal production sector and the market. Some participants expressed concerns that the big buyers at the wholesale level—integrated, multinational corporations—set market conditions in some sectors and may limit the ability to produce GnEd animals. However, there is some demand driving back through the value chain from farmers to wholesalers to be free to produce whatever lines they find appropriate, which could include GnEd lines.

The group discussed the Issue of labeling genetically modified and GnEd products is regarded by developers as a barrier to successful commercialization. Some thought that labeling can confuse the consumer, who often perceives a label as a warning. It was proposed that labeling needs to be construed as information, for example, as the label "raised without antibiotics" is to that segment of the consumer market.

Import–export markets and trade agreements may impact the commercialization of GnEd animal products. It was asked whether Brazil, for example, would put its international market at risk to produce GnEd cattle? Depending upon the regulatory regime, GnEd products might not be labeled; what if the market can't distinguish GnEd from conventional product? Consumer acceptance of genetically altered products requires trust: trust in the developer, trust in the regulatory system, and trust in the supply chain. The group thought that developers, regulators, and the supply chain must be open with consumers about the nature of the products they are buying and enable them to avoid those products if they so choose. Failure to engender trust could result in failure of a specific product, or of greater concern, failure of a technology. Some countries may be upset about the import of GnEd products, but only if they can detect the product. The group wondered if this is the product of a null segregant (e.g., sex-marked layer eggs), would this be an issue or concern? There may be limits relative to a company's ability to trace a product. For GnEd cattle, the SLICK gene occurs naturally in some populations, and SLICK2 occurs in Brazilian cattle, which complicates the detection issue.

Building public trust in animal biotechnology

Participants agreed that public trust in the safety of GnEd food products and in regulatory processes and determinations will prove critical to the commercial success of GnEd animal products Arujanan (2022). Among issues that could be barriers to public trust, GnEd is a new technology that is difficult for many prospective consumers to understand at a technical level. In addition, some consumers express anti-corporatism and lack of trust in people paid by a company or in research done by a company. Panel speakers emphasized that communicating contentious issues that attract highly polarized views should not just convey scientific data. Scientists often are seen as impersonal experts giving out only scientific data obtained from their laboratories, who often do not share the same concerns and lifestyles as their audience, which sets them apart from the audience. In science communication, stories should be based on real people, how technology transformed lives, and socioeconomic impact. Panelists indicated that a first step to connecting with an audience is finding common ground and shared values, such as conservation of the environment, farmer rights, and food safety, among others. There is no single approach to communicating science, as "the public" is heterogenous and their concerns, fears, background, religion, values, cultures, and levels of knowledge vary across a wide spectrum (Van Eenennaam 2022). Each public sector will require explanations and examples that are relevant and appealing to them.

Panelists noted that a key aspect of engaging a public effectively is defining a compelling narrative, taking a storytelling approach. One example shared was from AquaBounty. AquaBounty has an operation in Atlantic Canada and their local employees can relate to others in the community that they are now able to find work locally because new technology made aquaculture viable there. AquaBounty Technologies, Inc. (https://www.aquabounty.com), the first company to gain approval for a GE food animal in the United States and Canada (FDA 2015, ECCC 2016), began harvesting from U.S. farms and selling AquAdvantage Salmon (AAS) products in the United States in 2021. In preparation, AquaBounty conducted in-person and online consumer research to guide company communications and product positioning (March 2022a, b). AquaBounty identified five key product attributes motivating consumers—availability, affordability, fresh, safety, and taste—and focuses product messaging on those attributes. Key findings on consumer attitudes specific to AAS were: 53% were neutral to positive on first impression of GMOs, 60% neutral to very likely to purchase products regularly if labeled as GMO, 70+% neutral to very likely to purchase products regularly if labeled with the USDA Bioengineered Disclosure

symbol, 81% neutral to positive about the AquaBounty story and AAS benefits, and 70% willing to purchase and try AAS at least once. Although all AAS product is being sold only to food service customers, the first results are positive. The company is selling all the fish it harvests at standard market prices, is adding new customers, and customers are not getting GMO-related pushback from their own customers.

A second aspect of public engagement discussed is having trusted voices speaking. Academics, regulators, and farmers enjoy high levels of public trust; hence, it is advisable to get them to engage in outreach. In particular, it is critical to have farmers tell big seedstock producers and regulators about their need to have access to traits that can be provided by GnEd. Industry associations need to talk to regulators and opinion leaders about what is important to them. NGOs friendly to GnEd should become involved. Effective use of social media could prove important, especially to show the value of an innovation, for example, use of GnEd to promote animal welfare, e.g., sex-marked layer eggs and PRRSv-resistant pigs.

Animal biotechnology is not well received by all segments of society, and workshop participants discussed how to handle negative publicity. Not saying anything in the face of negative publicity is a mistake; rather, biotechnology companies should monitor key forums and respond as they see appropriate. In particular, it is important to build relations with influencers—science journalists, chefs, and nutritionists—and to let them express support. It is not necessary to respond to all social media posts; sometimes it is best to let threads of discussion simply die. It is important for outreach efforts to include the young, especially college students, who have open minds and are tomorrow's leaders.

Effective marketing will prove critical to successful commercialization of GnEd animal products. Third-party certification or branding might be part of the marketing approach, perhaps by means of a QR code to retrieve more information on a product. Key values to be addressed would include sustainability and ethics. For example, sex-marking of layer chickens offers the opportunity to eliminate the practice of male chick-culling, recover high-value food materials, and reduce the carbon footprint of food production.

Governments often play a major role in how the public views new technology. Therefore, communications strategies should be carefully tailored based on the audience, goals, risk of harm being realized, and level of concern (Bodnar 2022). Communication may be divided into: (1) science communication—inspiring interest, enjoyment, or understanding of science, (2) risk communication—providing information about risks (or safety), (3) crisis communication—providing needed information when risk and concern are high, and (4) concern management—encouraging calm when risk is low, but concern is high.

Looking to the future

Breakout groups at the workshop considered the potential for animal biotechnology to address regional needs (Box 7). Differences were noted both within and between regions, and especially between developed and developing countries. Common themes included the need for capacity-building for R&D and regulatory oversight; effective communication among the academic, private and government sectors; and the desire for greater regional and international coordination of biotechnology policy.

Clear progress is being made by researchers and breeders to generate GE or GnEd agricultural animals for the benefit of the animals themselves, the production industry, and consumers. The rapid pace of technical developments challenges regulatory systems around the world. Regulators in some jurisdictions have been implementing regulatory reforms, and regulators in some world regions have been coming together to seek commonality of purpose and process, promoting synchrony, alignment, and compatibility among regulatory approaches among trade partners. This workshop bringing together researchers, developers, breeders, and regulators, promoted critical discussion at the interface of science and regulation to inform and enable agricultural applications of animal biotechnology to reach farmers and eventually the global marketplace. To pave the road to commercialization, researchers, developers, and breeders must engage more actively with policy makers and regulators so that these government officials can better understand the animal breeding systems in which these new technologies would be used, the controls already in place for conventionally bred animals, and the utility of GE and GnEd animals. Enabling, risk-scaled regulatory oversight is needed. Education and informal interaction among developers, regulators, and the public will prove important in realizing successful production of biotechnology animals. Further engagement across these sectors and among different countries is needed to facilitate safe agricultural applications of new technologies reaching breeders, farmers, and consumers and promoting international trade.

Box 7. Potential for animal biotechnology in developing countries

Animal biotechnology has the potential to improve animal agriculture in developing countries. While there are rich biodiversity, young scientists, and opportunities for useful innovation in developing

countries, capacity-building is needed in terms of infrastructure, legislation and policies, collaboration, and mentorship of individuals; hence, uptake of biotechnology has been slow. This is partly attributable to lack of science-based information regarding biotechnology among various stakeholders, including policymakers, regulators, and the general public. There is a need for capacity-building to design and implement effective biotechnology communication. To build capacity in the research sector, support for graduate studies and fellowship programs, international collaboration, and expert exchange would promote continuing development of innovative technologies. For the regulatory sector, opportunities for hands-on training by expert consultants, regulator visits, and interaction with regulators in advanced countries would build capacity. There is a need for regional coordination and cooperation among developing nations in policy making, sharing of risk assessments and establishment of mechanisms for data transportability to reduce unnecessary duplication of resources. Future development of animal biotechnology requires recognition of heterogeneity within regions in terms of economic orientation (i.e. whether an agricultural importing or exporting country), scientific research and technological development capacity (from incipient to very advanced), governmental policy orientation (from very restrictive to very favorable), and institutional framework (from incipient and inexpert to very robust and experienced). Countries within regions regarded establishing compatible regulatory approaches for animal biotechnology important. It is desirable for some and necessary for others to explore the possibility of generating a regional regulatory framework for animal biotechnology that considers the range of current technologies and is based on specific, common, and agreed-upon technical criteria to overcome possible technical and market limitations.

Some countries of Latin America have years of experience in animal breeding, based on suitable natural resources, good animal genetics, and traceability capabilities. Moreover, they have competent officials in regulatory agencies and established institutions in animal health and biotechnology. In addition, they are party to international agreements, platforms, and initiatives, and are willing to cooperate and provide or receive training. In addition to establishing regulatory frameworks, it will be necessary to strengthen capacity building, training, and financing. Regulatory cooperation, international organizations, and regional initiatives could be useful platforms (Rocha 2022).

Some developed countries (e.g., the United States, Canada, and Japan) have brought forward animal biotechnology products to the market, but have limited prospects for larger-scale commercialization because of differing domestic commercialization and regulation practices, small markets, or complex or restrictive approval processes of trading partners, factors that dampen development of animal biotechnology. In the EU, where environmental and animal welfare issues are foremost, regulation is in place, but there is low consumer acceptance and strong anti-GMO groups. Thus, animal biotechnology development must be considered on a country-by-country basis.

Wherever they are located, researchers need to be familiar with national regulations, and regulators should be knowledgeable about the science behind new technologies. Frequent consultations between the researchers and regulators could facilitate the approval of biotechnology products. Participants recommended that a regulatory framework on biosafety be established in each country to enable biotechnology research in the laboratory, on-farm field tests, and eventual commercialization.

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Availability of data and materials
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Declarations

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Not applicable.

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Authors L.S.C., M.T., and M.W. are employed by public research organizations or private companies developing genome-edited animals intended for commercial production.

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