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Genetic Engineering of Livestock: The Opportunity Cost of Regulatory Delay

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Abstract

Genetically engineered (GE) livestock were first reported in 1985, and yet only a single GE food animal, the fast-growing AquAdvantage salmon, has been commercialized. There are myriad interconnected reasons for the slow progress in this once-promising field, including technical issues, the structure of livestock industries, lack of public research funding and investment, regulatory obstacles, and concern about public opinion. This review focuses on GE livestock that have been produced and documents the difficulties that researchers and developers have encountered en route. Additionally, the costs associated with delayed commercialization of GE livestock were modeled using three case studies: GE mastitis-resistant dairy cattle, genome-edited porcine reproductive and respiratory syndrome virus–resistant pigs, and the AquAdvantage salmon. Delays of 5 or 10 years in the commercialization of GE livestock beyond the normative 10-year GE product evaluation period were associated with billions of dollars in opportunity costs and reduced global food security.

INTRODUCTION

Genetically engineered (GE) crops have been commercialized for more than 22 years, and in 2018 alone they were grown on 191.7 million hectares by 17 million farmers in 26 countries. However, only a single GE food animal has ever been commercialized. The dawn of a new decade, 35 years after the generation of the first transgenic food animals (1), offers an opportunity to examine learnings and perspectives from the past. Early reviews detailed some of the technical issues associated with the production of GE livestock, including low rates of transgene integration, mosaicism, unpredictable expression patterns due to the random locations of introgressions, and the expense associated with the production of large transgenic food animals. Almost without exception, these papers finished with optimistic projections regarding future developments and expected applications. The first review, written in 1985 prior to the publication detailing the production of the first transgenic livestock, concluded,

> Clearly, gene transfer and recombinant livestock offer a means to alter the fundamental genetic makeup of livestock to a greater extent, in a few decades, than may have been achieved in the entire past history of the science of livestock genetics. Those of us involved in this research look forward to the challenge and promise of this exciting new technology. (2, p. 36)

Some authors foresaw potential regulatory and social license issues as far back as 1987; for instance, Simons & Land (3, p. 249) predicted, "The application of the technology will, however, depend on the establishment of a suitable social framework. The new gene is neither a drug nor an infectious agent and falls outside the legislation for either." Their feeling was that genetic improvement and transgenics were a more natural approach to achieve desired outcomes as compared to direct management practices as detailed:

> The basic advantages of genetic improvement over direct manipulation of stock are strengthened by current moves against the use of hormones in commerce and the progressively strengthening preference for natural products. Transgenic practice could enhance these advantages by increasing the rate at which the characteristics of stock could be changed to meet the requirements of the community. (3, p. 249)

A 1990 review lamented the absence of mapped genomes in domestic animals but suggested that if this hurdle were surmounted, livestock breeding would change drastically before the turn of the century:

> Presently there is a poor understanding of the genes influencing animal growth, efficiency of growth, environmental adaptation, meat, milk or egg composition or animal disease resistance. Their identification will come from badly needed efforts to map the genome of domestic animals. These and other new technologies promise to change livestock breeding drastically in the next decade. (4, p. 3)

The progress that has been made since that time in sequencing livestock genomes is remarkable.

In 1987, Dr. Jim Womack (5, p. 68), a well-known bovine geneticist, optimistically forecast,

> Mapping of over 200 polymorphic loci in any of our livestock species in the near future is a formidable task. If, however, we could direct the spacing of markers, complete coverage of the bovine genome could be accomplished with only 75–80 markers, with no two adjacent markers more than 40 cM apart.

It is incredible that 22 years after this paper was published, this same author coauthored the paper reporting the sequencing of the entire bovine genome (6), and a decade later the number of

sequenced bovine genomes would grow to more than 2,700 animals (7). Such developments were enabled by massive investments in sequencing technologies and large consortiums that worked collectively to take on the audacious goal of sequencing the genomes of many food animal species [chicken (8), pig (9), goat (10), sheep (11), and salmon (12)]. These data have been used to implement genomic selection (13) on a very large scale in pigs, sheep, and cattle (14), with millions of animals having been genotyped for this purpose to date (15). Since the cattle genome has been sequenced, enabling genomic-based selection of young elite bulls, the rate of genetic improvement in the dairy industry has doubled (16).

Even before genomic sequence data became available, Georges & Andersson (17, p. 918) predicted, "Transgenic tools are indeed likely to become a key component for testing hypotheses with regard to function and regulation of underlying genes uncovered by genomic approaches." That was written in 1996, the year Dolly the infamous first cloned mammal was reported (18), which opened the way to cloning livestock from GE somatic cells, coincident with the promulgation of the US Coordinated Framework for the Regulation of Biotechnology (19). That same year, Wall (20) reported that of the 289 published papers in PubMed found when searching for the term "transgenic livestock," 24% of the publications were review articles. That percentage has not changed much, with that same search today returning approximately 30% review articles, with anywhere from 9 to 41 review articles published each year for the last 20 years. To date only one single transgenic food animal (21) has been commercialized in 35 years, few transgenic livestock are used to test gene function and regulation (outside of xenotransplantation studies), and almost a third of the publications in the field are reviews rather than original research articles. What happened to the once-promising field of GE livestock?

TECHNICAL ISSUES

Production of the first transgenic livestock built on recombinant DNA (rDNA) technologies, pronuclear microinjection techniques, and advanced reproductive techniques like superovulation and embryo transfer. This first paper in 1985 reported that the transgenic rDNA integration rates were low, 10.4% in pigs and 1.3% in sheep, an issue that has remained problematic for livestock engineers (1). Large animal researchers face several other issues in producing transgenic livestock, including the fact that the zygote of livestock species is opaque, making (pro)nuclear microinjection more difficult than in mice, as well as low rates of random transgene integration, mosaicism in transgenic embryos, long generation intervals, expensive animal care and maintenance costs, and the inability to isolate embryonic stem cells (ESCs) from most livestock species.

Some have tried precomplexing DNA with sperm to introduce exogenous DNA into the oocyte via sperm-mediated gene transfer. Despite some successes, this approach has not proven reproducible (22). Others used oncoretroviruses, which dramatically increased the efficiency of founder production, but transgene silencing was an issue (23). Although somatic cell nuclear transfer cloning of GE cells provided a solution to some of these problems, the low efficiency of producing live and healthy cloned offspring has limited the utility of this approach to produce GE livestock (24). Many review articles have been published about the technical challenges associated with producing GE livestock. This review does not reiterate these technical issues but rather focuses on some of the other barriers to widespread adoption of GE food animals.

Figure 1 summarizes the major milestones in producing GE livestock. It is sobering to consider the substantial scientific progress that has been made in the production and utility of millions of transgenic mice, and even in producing transgenic pigs for biomedical research, as compared with transgenic livestock, for which commercialized applications can be counted on one hand. One reason for this is the relatively small scientific community that has been working

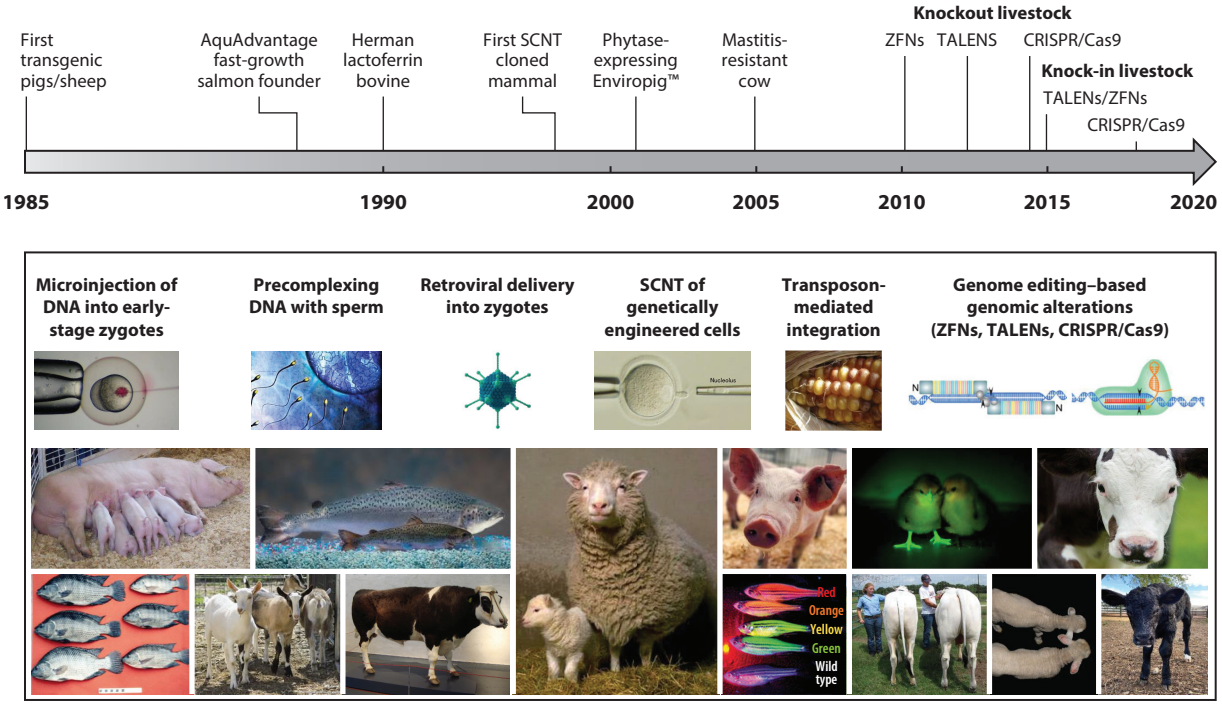

Figure 1

An abbreviated schematic history of 35 years of genetically engineered livestock featuring some of the well-known celebrities of the field. Abbreviations: CRISPR/Cas9, clustered regularly interspaced short palindromic repeat targeted by Cas 9 nuclease; SCNT, somatic cell nuclear transfer; TALEN, transcription activator-like effector nuclease; ZFN, zinc-finger nuclease.

in this field, owing in part to a paucity of both public sector and private funding, especially as compared with basic and applied biomedical research. It is also true that this research is very expensive. In 1992, the cost of producing a single founder transgenic pig was estimated at $25,000, and producing a single functional transgenic calf cost more than $500,000 (25). The high cost of producing transgenic livestock has been, and continues to be, a major factor limiting those interested in exploring the potential of this technology.

EXTANT APPLICATIONS

The decades-old promise of the multiple applications of transgenic livestock (**Table 1**) has not yet been realized. The publication dates of much of the research in **Table 1** are rather discomforting, given that many date back more than 30 years, and the vast majority of this research never moved beyond the laboratory.

Recent developments, including the isolation of ESCs from some livestock species (73, 74) and the advent of genome editing, may open a new chapter in animal genetic improvement. Genome editing involves the use of site-directed nucleases to introduce a double-stranded break at a targeted, predetermined location in the genome. The double-stranded break can be repaired by the cell's natural DNA repair mechanism (nonhomologous end joining), often resulting in single-nucleotide changes, and small (1–2-nucleotide) deletions or insertions at the DNA cut site. In this case, although the location of the cut site is targeted, the exact change that occurs when the DNA

Table 1 Listing of transgenic food animals for agricultural applications and the intended trait outcome, and date of publication

Species	Transgene	Origin	Trait/goal	Year	Reference(s)
Cattle	Lysozyme (*LYZ*), lactotransferrin (*LTF*)	Human	Milk composition, animal health, mastitis resistance	2002, 2011	26, 27
	Prion protein (*PRNP*)	Knockout	Animal health	2007	28
	β-, κ-Casein (*CSN2*, *CSN3*)	Bovine	Milk composition	2003	29
	Omega-3 fatty acid desaturase fat-1 (*fat-1*)	Nematode	Milk composition	2012	30
	β-Casein (*CSN2*) miRNA	Bovine	Milk composition	2012	31
	Lysostaphin (*lss*)	Bacterial	Mastitis resistance	2005	32
	Sp110 nuclear body protein (*Sp110*)	Murine	Bovine tuberculosis resistance	2015	33
	Myostatin (*MSTN*) shRNA	Knockout	Increased muscle yield	2012	34
Chicken	Subgroup A avian leukosis virus envelope glycoprotein	Viral	Disease resistance	1989	35
	Influenza A virus polymerase shRNA	Viral knockout	Disease resistance	2011	36
	β-Galactosidase (*lacZ*)	Bacterial	Animal health	2003	37
Carp	Growth hormone (*GH1*)	Mouse-human	Growth rate	2005	38
	Lactotransferrin (*LTF*)	Human	Disease resistance	2004	39
Catfish	Cecropin-B (*CecB*)	Insect	Disease resistance	2002	40
Goat	Lysozyme (*LYZ*)	Human-bovine	Animal health	2006	41
	Stearoyl-CoA desaturase (*Scd*)	Bovine-rat	Milk composition	2004	42
	Lactoferrin (*LTF*)	Human	Prophylactic treatment	2008	43
	Defensin β 103A (*DEFB103A*)	Human	Milk composition	2013	44
	Myostatin (*MSTN*) shRNA	Knockout	Increased muscle yield	2013	45
	Prion protein (*PRNP*) shRNA	Knockout	Animal health	2006	46
	Omega-3 fatty acid desaturase fat-1 (*fat-1*)	Nematode	Milk composition	2018	47
Pig	Phytase (*appA*)	Murine-*Escherichia coli*	Feed uptake, decreased phosphorus in manure	2001	48
	Growth hormone (*GH1*), growth hormone–releasing hormone (*GHRH*), insulin-like growth factor 1 (*IGF1*)	Human/murine-porcine/human	Growth rate	1989, 1990, 1999	49–51
	SKI proto-oncogene (*SKI*)	Chicken	Muscle development	1992	52
	Lysozyme (*LYZ*)	Human	Piglet survival	2011	53
	Delta(12)-fatty-acid desaturase FAD2-like (*LOC110785100*)	Spinach	Meat composition	2004	54
	Omega-3 fatty acid desaturase fat-1 (*fat-1*)	Nematode	Meat composition	2006	55
	α-Lactalbumin (*LALBA*)	Bovine	Piglet survival	2001	56
	MX dynamin-like GTPase 1 (*Mx1*)	Human-murine	Disease influenza resistance	1992	57

(*Continued*)

Table 1 (*Continued*)

Species	Transgene	Origin	Trait/goal	Year	Reference(s)
	Foot-and-mouth disease virus (FMDV) antiviral small hairpin RNAs (shRNAs)	FMDV Knockout	FMDV resistance	2015	58
	Uncoupling protein 1 (*Ucp1*)	Murine	Enhanced thermoregulation	2017	59
	Classical swine fever virus (CSFV) antiviral small hairpin RNAs (shRNAs)	CSFV knockout	CSF resistance	2018	60
Sheep	Growth hormone (*GH*), growth hormone–releasing hormone (*GHRH*)	Murine/ovine-ovine/bovine/human	Growth rate	1989, 1998	61, 62
	Insulin-like growth factor 1 (*IGF1*), keratin intermediate filament type II (*KRT2.10*)	Murine-ovine, ovine	Wool growth	1996, 1998	63, 64
	Visna-maedi virus envelope protein (*env*), rev protein (*rev*)	Viral	Disease resistance	1994	65
	Omega-3 fatty acid desaturase fat-1 (*fat-1*)	Nematode	Meat composition	2013	66
	Prion protein (*PRNP*)	Knockout	Animal health	2001	67
	Immunoglobulin α and κ chains against phosphorylcholine	Murine	Disease influenza resistance	1991	68
Salmon	Growth hormone 1 (*gh1*)	Piscine	Growth rate	1992	21
	Lysozyme (*lyz2*)	Piscine	Animal health	2011	69
	Liver-type antifreeze protein (*wfAFP-6*)	Piscine	Cold tolerance	1999	70
Tilapia	Growth hormone 1 (*gh1*)	Piscine	Growth rate	1998	71
Trout	Follistatin (*fst*)	Piscine	Muscle development	2009	72

is repaired is random, and so several different outcomes representing minor sequence changes are possible. Alternatively, repairs can be directed to introduce, delete, or replace a series of letters in the genetic code using a nucleic acid template. This essentially enables the introduction of known, desired alleles or haplotypes via homology-directed repair. Genome editing enables alteration of animal genomes without necessarily incorporating transgenic genetic material. To date, somatic cell nuclear transfer of edited somatic cells, especially homology-directed repair donor-template directed alterations, has been the primary method to produce livestock carrying nuclease-mediated genetic changes in their genomes (75). Genome editing offers an efficient approach to introduce useful genetic variation into livestock breeding programs through targeted inactivation of gene function and/or through allele introgression without the undesired linkage drag.

Researchers have already produced several genome-edited farm animals for biomedical research applications, as well as for agricultural purposes (76). The latter group includes animals carrying no novel DNA sequences, for example, porcine reproductive and respiratory syndrome (PRRS) virus–resistant *CD163* knockout pigs (77–80); myostatin knockout sheep, goats, and cattle with increased lean muscle yield (81–85); and *FGF5* knockout sheep with increased wool length and yield (86). There are intraspecies allele substitutions, e.g., hornless dairy cows owing to substitution at the *POLLED* locus (87), and intraspecies gene insertions, also known as cisgenics, e.g., cows with increased resistance to tuberculosis owing to knock-in of the bovine *SLC11A1*

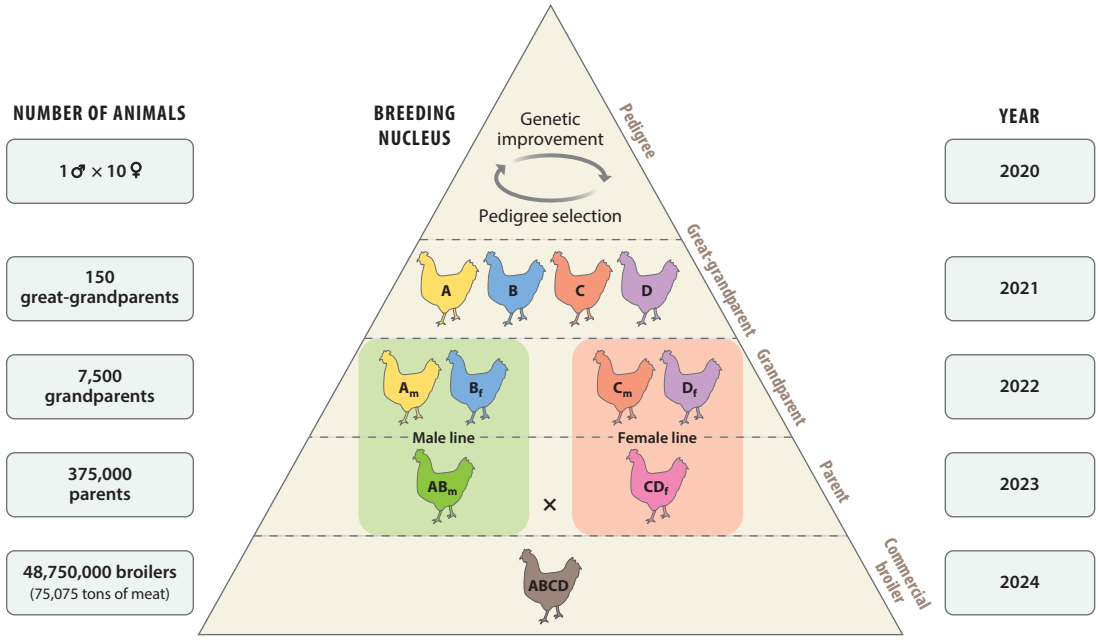

Figure 2

A typical modern broiler chicken breeding program, represented as a pyramid in which each level represents a generation. On the left is the approximate number of chickens produced in each generation. On the right is the approximate timeline to move genetics from the top of the broiler-breeding pyramid to the consumer (92).

(*NRAMP1*) gene (88). There are also examples of genome-edited animals harboring foreign or transgenic DNA from another species, such as pigs with interspecies allele substitutions [e.g., domestic pigs (*Sus scrofa*) carrying a *RELA* ortholog allele from the African warthog (*Phacochoerus africanus*), with the objective of improved resistance to African swine fever (ASF) (89), although ultimately ineffectual (90), and pigs resistant to classical swine fever virus carrying antiviral small hairpin RNAs (60)]. Not surprisingly, the traits that have been targeted with gene editing to date (animal health and well-being, product quality, and yield) are common to the breeding objectives of traditional selection programs. As with earlier genetic engineering approaches, whether breeders will be able to employ genome editing in commercial farm animal genetic improvement programs will very much depend upon global decisions around regulatory frameworks and governance.

INDUSTRY STRUCTURE

The livestock breeding sector is quite distinct from the plant breeding sector (91). Some of this is due to biological differences between the two kingdoms, such as the mode of reproduction (e.g., plants can self-pollinate) and the number of progeny per reproduction cycle. A major difference between animal and plant breeding has been that animal breeders focus selection on elite animals in the breeding nucleus at the top of the breeding pyramid (**Figure 2**), with the end product being millions of genetically distinct individuals developed primarily by multiplication of elite genetics through outcrossing.

Plant breeders, in contrast, focus on developing a recognizable plant variety, often protected by plant breeders' rights. When it comes to GE traits, a single transgenic plant transformation

event can be introgressed into different cultivars to produce genetically distinct varieties. In animal breeding, multiple independent founder events in elite animals in the breeding nucleus would be required to avoid inbreeding. The transgenic trait would then be transmitted down the breeding pyramid through multipliers, a process that can take decades depending upon the generation interval of the species.

Livestock breeding also varies considerably among the different animal agriculture industries (14). Genetic improvements have been fastest in those industries that have a highly structured breeding sector (e.g., pig and poultry). There, a small number of animal breeding companies control the genetics of these vertically integrated industries. For example, more than 90% of global poultry breeding stock is managed by three companies selling to a worldwide market (93). These species have high reproductive rates and relatively short generation intervals, and this allows incremental improvements in efficiency to be multiplied across many animals. This directly influences the level of investment that can likely be directed toward developing genetically improved founders in the breeding nucleus.

Genetic improvement in pastoral animal protein industries that have less vertical integration (e.g., beef and sheep) is typically coordinated through breed associations. These organizations were historically the keepers of herd books to trace the pedigree of selected animals. It has been difficult to develop a single, industry-wide breeding objective that is economically rational for all sectors in these industries because producers value production traits, whereas other sectors value feeder and processer traits. In the absence of vertical integration, breeding goals tend to focus on the individual producers' financial interests. This, combined with long generation intervals, low reproductive rates, and limited utilization of reproductive technologies like artificial insemination, means that the overall rate of genetic improvement has been slower in these pastoral industries. It is difficult to obtain a return on investment from the high costs associated with developing GE founder animals for these less vertically integrated, low-margin industries.

FUNDING ISSUES

The lack of progress on GE animal agricultural applications can also be attributed in part to a scarcity of both public and private funding sources, as well as the absence of a clear path to market. These two factors are directly related. Although initially there was public funding for animal biotechnology research in the 1980s and 1990s (94), between 1999 and 2012 fewer than 0.1% of the research grants from the US Department of Agriculture (USDA) went to work on GE food animals (95). In the United States, this was in part due to the fact that research in transgenic food animals was not accepted. As detailed by Tizard et al. (94, p. 582),

> For almost a decade starting in the mid-2000s, the annual USDA request for grant applications included the text "applications whose primary aim is to improve the efficiency in the production of clones or transgenic animals through manipulation of the nucleus will no longer be accepted by the Animal Genome program."

In the absence of a clear and predictable path to market, there has been little support or market pull for transgenic animals from the livestock breeding sector. There were high hopes for increased research funding and industry interest when genome-editing techniques came onto the scene (96). And indeed, there has been some public sector funding from the USDA in the United States, and in other countries, most notably China (97), specifically targeted to improving genome-editing efficiencies in livestock.

REGULATORY ISSUES

When AquaBounty sought to commercialize its fast-growing GE fish expressing a Pacific salmon *gh1* transgene (21) in the mid-1990s, there was no official regulatory pathway in place. According to former CEO of AquaBounty technologies Dr. Ron Stotish (98, pp. 913–14),

> AquaBounty consulted FDA [US Food and Drug Administration] and other government agencies in hopes of identifying a regulatory process that could be employed to review and approve the AquAdvantage salmon for food use in the United States. AquaBounty established an Investigational New Animal Drug file with the Center for Veterinary Medicine [CVM] in 1995, well in advance of any clear regulatory paradigm. Between 1995 and 2009, the sponsor [AquaBounty] conducted a variety of GLP studies aimed at meeting what was hoped to be the eventual regulatory requirement for an application of this nature. Although there was informal consultation and communication between the sponsor and CVM staff during this time, it was not until 2009 that CVM released Guidance Document 187, codifying requirements for consideration of an application for a transgenic animal.

This 2009 Guidance Document 187 was entitled "Guidance for Industry on Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs" (99). The Federal Food, Drug, and Cosmetic Act (FD&C Act) defines a drug as an "article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals" and "articles (other than food) intended to affect the structure or any function of the body of man or other animals." A "New Animal Drug includes a drug intended for use in animals that is not generally recognized as safe and effective for use under the conditions prescribed, recommended, or suggested in the drug's labeling, and that has not been used to a material extent or for a material time."

Using this definition, the FDA considered the "regulated" article to be "the rDNA construct in a GE animal that is intended to affect the structure or function of the body of the GE animal, regardless of the intended use of products that may be produced by the GE animal." In that 2009 guidance, the FDA defined GE animals as animals with both heritable and nonheritable rDNA constructs. Classifying transgenes in animal genomes as drugs meant that each animal lineage derived from a separate transformation event (or series of transformation events) was considered to contain a distinct new animal drug subject to a separate new animal drug application (NADA). Additionally, and of relevance to food animals, the FD&C Act mandates that a new animal drug may not be sold into interstate commerce unless it is the subject of an approved NADA. This determination meant that all GE animals, their offspring, and their food products (milk, meat, and eggs) were unsalable. The FDA exercised enforcement discretion for GE animals of nonfood species that are raised and used in contained and controlled conditions, such as GE laboratory animals used in research institutions. In other words, researchers working with literally millions of GE laboratory animals do not have to go through the investigational new animal drug (INAD) paperwork requirements to conduct their research.

That left the small group of mostly public sector agricultural researchers working with food animals facing the INAD requirements needed to get a NADA approved if any of their work was to be commercialized. These requirements include a seven-step regulatory process in which the agency examines the safety of the rDNA construct to the animal, the safety of food from the animal, and any environmental impacts posed (collectively the "safety" issues), as well as the extent to which the performance claims made for the animal are met ("efficacy"). Molecular characterization of the rDNA construct determines whether it contains DNA sequences from viruses or other organisms that could pose health risks to the GE animal or to those eating the animal. Molecular characterization of the GE animal lineage determines whether the rDNA construct is stably inherited over multiple generations. Phenotypic characterization assesses whether the GE animals

are healthy, whether they reach developmental milestones as non-GE animals do, and whether they exhibit abnormalities. A durability assessment reviews the sponsor's plan to ensure that future GE animals of this line will be equivalent to those examined in the preapproval review. If the GE animal is intended as a source of food, the FDA assesses whether the composition of edible tissues differs and whether its products pose more allergenicity risk than non-GE counterparts.

According to Guidance 187, all investigational GE animals, their littermates, and surrogate dams and their products were by definition deemed unsafe and were required to be disposed of by "incineration, burial, or composting." Dr. Matt Wheeler at the University of Illinois has been working on transgenic pigs for more than 25 years. His work has included expressing the bovine gene encoding lactalbumin in the milk of transgenic pigs (100), which enhances lactation performance and preweaning piglet growth rates (101). Throughout his research, transgenic swine were required to be separated from nontransgenic animals to avoid the potential for "horizontal gene transfer" from housing, mating, gestation, lactation, and suckling. A recent paper (102) documented the absence of any horizontal gene transfer between GE pigs and non-GE pigs. Dr. Wheeler has incinerated thousands of transgenic pigs and their littermates and surrogate dams as unapproved new animal drugs during the course of his research, and estimates the added costs of being under INAD requirements to be approximately $2 million (Matthew Wheeler, personal correspondence, cited with permission). Fortunately, academic research institutions and small companies are exempt from additionally paying the recurring annual INAD review fee, which can cost thousands of dollars.

COMMERCIALIZED GENETICALLY ENGINEERED ANIMALS

There have been three approvals for therapeutic human proteins produced by transgenic animals. These include goats producing ATryn1® [antithrombin-III (SERPINC1)], approved to treat hereditary antithrombin deficiency by the European Commission in 2006 and by the FDA in 2009 (103); rabbits producing Ruconest™ [Rhucin® outside the European Union (SERPING1)] (104), approved to treat hereditary angioedema in 2014 (105); and chickens producing Kanuma™ [lipase A, lysosomal acid type (LIPA)] in their eggs, approved in 2015 for the treatment of patients diagnosed with lysosomal acid lipase deficiency (106).

Perhaps the most visually apparent, and in any event, the world's most widely available, commercialized transgenic animal is the fluorescent aquarium GloFish®. These GE designer tropical fish, first produced by a laboratory in Singapore (107) with the long-term idea of detecting water toxins, were licensed to Yorktown Technologies in 2003. They are marketed to aquarists in the United States, where they are now sold in every state in the nation, as well as throughout Canada.

In the United States, GloFish® have been marketed under "enforcement discretion," which avoids some of the complexities of a full NADA process but still requires a FDA risk assessment for each line (i.e., fish derived from a GE insertion event in a founder animal) of GloFish® marketed. Among other things, the assessment considers human health risks, ecological health risks, and risks to the fish themselves. According to the FDA website in 2003, with regard to the risk assessment of the first GloFish® line,

> FDA chose to exercise enforcement discretion for a GE aquarium fish that fluoresces in the dark. FDA made this decision in part because the fish (Zebra danio) is not a species used for food, and in part because the agency was able to determine that it did not pose any additional environmental risks compared with conventional Zebra danios. (Zebra danios are unable to survive outside the very warm waters of the tropics, which effectively limits the ability of an escaped or released fish to affect the U.S. environment.)

Figure 3

Genetically engineered GloFish® are sold in all 50 US states and Canada. Photo provided by GloFish.com; reproduced with permission.

Subsequent lines were also reviewed under enforcement discretion, but no associated public statements were made with their market release. Although some authors raised environmental and ethical concerns about this decision, particularly in the years immediately after GloFish® first became available for sale (108–110), these concerns have waned over time.

The sale of GloFish® is restricted in other jurisdictions, including Europe, Australia, and Singapore (108). There are a total of four species of transgenic fish [zebrafish (*Zebra danio*), tetra (*Gymnocorymbus ternetzi*), tiger barb (*Puntius tetrazona*), and rainbow shark (*Epalzeorhynchos frenatum*)] in six fluorescent colors (**Figure 3**). GloFish® sales represent approximately 15% of US aquarium fish sales. Yorktown Technologies sold GloFish® to a consumer goods company for approximately $50 million in cash plus incentives in 2017 (111). The success of this product suggests that consumers are willing to purchase transgenic animals, at least as aquarium pets. With regard to the public acceptance of transgenic animals, Alan Blake, CEO and Cofounder of Yorktown Technologies, stated at the 2015 Transgenic Research Conference (112) that consumers will purchase a product that they desire, irrespective of the breeding method that was used to produce it. In his words, "It is not about the process [of genetic engineering], it is about the product."

APPROVAL OF THE FIRST GENETICALLY ENGINEERED FOOD ANIMAL APPLICATION

According to AquaBounty,

> By mid 2010, [AquaBounty] had concluded all the necessary research, submitted all required regulatory studies, and received the results of the CVM reviews indicating satisfaction with the submitted data in addressing all requirements for approval. The Center convened a Veterinary Medicine Advisory Committee [VMAC] to review the results of the CVM assessment and conclusions on September 20, 2010. (98, p. 914)

During the course of this meeting, the VMAC members discussed the strengths and weaknesses of the data (113). Ultimately, the consensus document of the VMAC, charged with providing advice and recommendations to the FDA, found (*a*) that there was "no evidence in the data to conclude that the introduction of the construct was unsafe to the animal"; (*b*) that the studies selected to evaluate whether or not there was a reasonable certainty of no harm from consumption of foods derived from AquAdvantage salmon were "overall appropriate and a large number of test results

established similarities and equivalence between AquAdvantage Salmon and Atlantic salmon"; and (*c*) that the AquAdvantage salmon did grow faster than their conventional counterparts (114).

The potential environmental impacts from AquAdvantage salmon production were mitigated by the proposed conditions of use, which limits production to FDA-approved, physically contained freshwater culture facilities. The eyed-egg production site is located on Prince Edward Island (PEI) in Canada, and the grow-out of market fish was proposed to occur in Panama with multiple biologically (all female, triploid), physically (land-based tanks with fencing/screening), and geographically (hydroelectric dams with no fish passage, thermal-lethal downstream temperatures) redundant containment measures. The VMAC concluded that although they "recognized that the risk of escape from either facility could never be zero, the multiple barriers to escape at both the PEI and Panama facilities were extensive." Little did the company know that there would be a further five-year delay following the VMAC meeting until the product was ultimately approved in November 2015, and then an additional four years before the product could be imported into the United States.

The FDA ultimately approved AquAdvantage salmon for sale in November 2015. But an obscure rider was attached to a budget bill by Alaska Senator Lisa Murkowski in December of that same year, effectively blocking the FDA from allowing GE salmon into the United States. In the meantime, Canadian regulatory authorities approved the fish, and five tons were sold there in 2016 without being labeled as such because Canada has no law that requires labeling of GE seafood. On March 8, 2019, the FDA finally deactivated this import alert pertaining to the GE salmon. Since that time, AquaBounty has registered an additional secure, land-based grow-out facility in Indiana. The company estimated it has spent $8.8 million on regulatory activities to date, including $6.0 million in regulatory approval costs through approval in 2015, $1.6 million (and continuing) in legal fees in defense of the regulatory approval, $0.5 million in legal fees in defense of congressional actions, and $0.7 million in regulatory compliance costs (~$200,000/year for ongoing monitoring and reporting, including the testing of every batch of eggs), not to mention the $20 million spent on maintaining the fish while the regulatory process was ongoing from 1995 through 2015 (David Frank, AquaBounty, personal communication, Jan. 2020). Because the sole product of AquaBounty was the AquAdvantage GE salmon, absent an approval, there was no way for this small US company to obtain any revenue to offset their ongoing research and development costs during the regulatory process.

MEANWHILE. . .

While the AquAdvantage salmon was awaiting regulatory approval, salmon breeders were conventionally selecting for fast-growing salmon. The genetic gain for growth rate in salmon has been estimated at 10–15% per generation (115). Farmed salmon have been exposed to ≥12 generations of domestication and were the first fish species to be subject to a systematic family-based selective breeding program, which began in 1975 (116). Studies on farmed salmon in the ninth and tenth generation of selection showed their size relative to wild fish was 2.9:1 under standard hatchery conditions and 3.5:1 under hatchery conditions in which growth was restricted through chronic stress (117). In other words, selective breeding programs have produced genetically distinct lines of fast-growing salmon, and since the 1970s, tens of millions of these fertile farmed salmon have escaped into the wild (118). Glover estimated that over three to four decades, introgression of farmed salmon into Norwegian wild salmon populations ranged from 0% to 47% per population, with a median of 9.1% (119). Presumably, these fast-growing salmon pose the same environmental risks as the AquAdvantage salmon, although the latter were associated with decades of delay and millions of dollars in new animal drug regulatory approval costs, must be raised as sterile triploid females in land-based containment tanks, and are subject to ongoing regulatory compliance costs.

REGULATION OF GENOME EDITING IN ANIMALS

In early 2017, the FDA released an updated draft of their Guidance for Industry 187 and changed the title to "Regulation of Intentionally Altered Genomic DNA in Animals" (120). This guidance proposes to regulate all food animals whose genomes have been intentionally altered using modern molecular technologies, including genome-editing technologies, as new animal drugs. This includes many of the same nucleotide substitutions, insertions, and deletions that could be obtained using conventional breeding. No longer is it the presence of a transgenic rDNA construct that triggers mandatory premarket FDA regulatory oversight prior to commercial release, but rather it is the presence of any "intentionally altered genomic DNA" in an animal's genome that initiates oversight.

This runs against the wording of the 1986 Coordinated Framework, which indicated that regulatory review was required only for organisms deliberately formed to contain an intergeneric (i.e., transgenic) combination of genetic material (121, 122). It also runs counter to both the Office of Science and Technology Policy Federal Register notice stating that regulatory oversight "should not turn on the fact that an organism has been modified by a particular process" (122) and the USDA approach to the regulation of genome-edited food plants (123). It also counters decisions being made by other regulatory agencies in several countries around the world (124).

Mandating premarket regulatory approval for deletions, mutations, and the conversion of one wild-type allele to another wild-type allele in the same species (cisgenic) that could have been obtained using conventional breeding is difficult to justify given the known genetic variation that exists in livestock genomes (125). Similarly, in 2018, the European Court of Justice issued a ruling (126) stating that organisms obtained by directed mutagenesis techniques (genome editing) are to be regarded as genetically modified organisms (GMOs). According to this ruling, genome-edited animals would therefore also be regulated as GMOs in Europe, with implications on global trade. Several academic scientists have argued that the trigger for regulatory review should be novel product hazards/risks, if any, weighed against the resulting benefits (127–129).

To date, only a single company, a large global animal genetics company, has announced plans to commercialize a genome-edited food animal. In 2015, the University of Missouri signed an exclusive global licensing deal for potential future commercialization of PRRS virus–resistant pigs (79) with United Kingdom–based Genus plc. This company has also entered into a strategic collaboration with Beijing Capital Agribusiness Co. Ltd., a leading Chinese animal protein genetics business, to research, develop, register, and market elite pigs in China that are resistant to the PRRS virus.

COSTS OF DELAYING INTRODUCTION OF GE TECHNOLOGIES

Many GE plant varieties have been commercialized, generating a vast literature on the economics of plant biotechnology that is relevant to animal biotechnology. A 2014 meta-analysis showed that the adoption of GE varieties increased crop yields, reduced pesticide use, and improved farmer well-being and that the relative gain was more significant in developing countries (130). The use of GE crops was also associated with a reduction in greenhouse gas emissions by reducing both the amount of land required to produce a given amount of product and the use of tillage to control weeds. A survey that same year suggested that the adoption of biotechnology reduced the price of soybean and cotton feedstocks by 20–30% and the price of corn by 5–10% (131). The livestock sector and, indirectly, consumers benefit from less expensive feedstock. Much of the benefit from regulations that allow GE crops to reach the market has been realized in developing countries, to the advantage of global food security.

Zilberman et al. (132) introduced a method to assess the economics of delaying the introduction of GE technologies owing to concerns about their unintended effects. Regulations that delay the introduction of the technology by just one year slow the adoption rate and are associated with substantial opportunity costs. In the case of golden rice, which could provide a source of vitamin A in deficient diets to help prevent blindness, a one-year delay was calculated to result in 1.1–5 million eyesight years lost (depending on the extent to which the technology was adopted), which translated to a cost of $300 million–$1.2 billion dollars for each year of regulatory delay. The cumulative cost of 10 years of regulatory delay was calculated to be between $10 and $30 billion dollars. Another paper estimated the regulatory cost delay for the introduction of GE plants targeting disease-affected subsistence crops in Africa, including bananas in Uganda, cowpea in West Africa, and corn in Kenya (133). They found that one year of regulatory delay resulted in an increase in stunting and malnourishment and estimated that it translated to a cost of $33–$46 million dollars in Nigeria alone (133). These values are the baseline against which the value of the information gained during the course of that one year of delay must be compared (134). Similar analyses can help to assess the cost of delaying the commercialization of GE technologies in animal agriculture.

Although some review papers have alluded to the unspoken opportunity costs of delaying the environmental and welfare benefits that could have resulted from the adoption of GE animals (94, 96, 135, 136), there are no formal economic evaluations in the peer-reviewed literature. We consider this a major gap in the literature. We therefore performed a simple economic analysis to estimate the costs of delaying or precluding the adoption of three extant GE livestock examples. The analysis presented here is not from a refereed publication, as is customary in Annual Review journals. Rather, the calculations in this section present original research using economic models widely used in the literature, as well as publicly available data. Our aim is to provide examples of how this topic might be addressed and encourage the initiation of a new line of research. Throughout the analysis, we highlight the role of assumptions on the results presented and indicate the next logical steps that would be required to augment this line of economic research.

We estimated the cost of delaying or precluding the adoption of three examples of GE livestock: the GE mastitis-resistant cow first reported in 2005 (32), the genome-edited PRRS virus–resistant *CD163* knockout pigs first reported in 2016 (79), and the fast-growing AquAdvantage transgenic salmon first reported in 1992 (21). In the case of the first two examples, we have existing estimates (137–142) of the costs of these two diseases, which were used to estimate the cost of delaying the diffusion of the adoption of disease-resistant stock. In the case of the fast-growing salmon, we factored in data indicating that this GE fish would decrease feed consumption by 25% and time to reach market weight by 40% (143), and we assumed that if allowed to come to market it would increase farmed salmon supply (144). The annual value of this increase was then calculated to enable an estimation of the cost of delayed adoption.

Estimates of the 30-year net present value (NPV) of regulatory delay of the two disease-resistant livestock case studies, one historical and one current, were calculated using a simple economic model (see **Supplemental Appendix A**) widely used in the literature; see Altson et al. (145) for a review and Zilberman et al. (132) and Wesseler et al. (133) for applications. The analyses were based on three points in time: (*a*) the year the technology appeared in the peer-reviewed literature, (*b*) the year in which the technology was potentially available in the market, and (*c*) the year in which the technology started to be adopted. We recognize that there is a gap between when an innovation is reported and when it becomes available for use. The normative time required for the regulatory evaluation of a GE product is approximately 10 years (146). We distinguish this from genome-edited products containing no novel DNA (e.g., knockouts), which can be commercialized immediately in several countries. For the PRRS virus–resistant pigs, we therefore assumed a

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4-year period from first publication in 2016 to potential commercial availability in 2020. Results would change with these assumptions; an earlier adoption would lead to greater benefits from adoption.

We built four scenarios to identify the cost of banning, or delaying, the approval of the technology: (*a*) There is no diffusion (status quo/banning the technology), (*b*) diffusion starts when commercially available (10 years for transgenic products; 4 years for genome-edited products), (*c*) diffusion is delayed 5 years, and (*d*) diffusion is delayed 10 years. Adoption is the use of the technology by an individual, whereas diffusion is the rate of adoption, i.e., the share of the potential users that adopted the technology. A diffusion process takes time, and in this model we assumed it would take 15 years. This is a conservative measure. Genomic selection, first introduced into the dairy industry in 2009, was adopted more rapidly. After only 7 years, more than half of all artificial insemination matings in the United States were made to genomically tested young bulls (147). We modeled two uptake scenarios: 50% and 100% (full) adoption at the end of the diffusion period. All cost estimates are presented in US dollars. A scenario in which adoption is less than 50%, as suggested by Zilberman et al. (132), for example, assuming that only 20% of the population adopts the new technology, would lead to lower benefits from the diffusion of the technology.

Case Study One: Mastitis-Resistant GE Cow

Mastitis is a disease of the mammary gland and is estimated to cost the global dairy industry $19.7–$32 billion annually (148). Mastitis is categorized as either clinical or subclinical, and a case of clinical mastitis may lead to an economic loss in the first 30 days of lactation that ranges between the direct cost of $128 and a total cost of $444 (142). *Staphylococcus aureus* is a bacteria that causes chronic mastitis and represents 10–12% of all clinical mastitis infections (149). *S. aureus* is a problematic cause of mastitis because of its pathogenicity, contagiousness, persistence in the cow environment, colonization of skin or mucosal epithelia, and poor cure rates associated with current therapies (150). Culture results from 27,000 milk samples showed 10% of cows were infected with *S. aureus* (151). The GE cow developed in 2005 expresses a protein, lysostaphin, to which *S. aureus* is particularly sensitive (32).

There is significant literature on the cost of mastitis that distinguishes the cost to the farmers from the social cost. The private costs include loss of production, therapeutics, contaminated milk, veterinary services, and higher rates of culling. Yearly direct private costs per cow were estimated in 2016 to include diagnostics ($10), therapeutics ($36), nonsalable milk ($25), veterinary services ($4), labor ($21), and death loss ($32). Indirect costs included future milk production loss ($125), premature culling and replacement loss ($182), and future reproductive loss ($9). The longer-term indirect costs represent 71% of the total cost per case of mastitis (142). The externalities or social costs are those associated with resistance to antibiotics, animal welfare concerns, and extra greenhouse gas emissions in the production of milk. A 2019 survey estimated the average cost of mastitis to be $147 per cow per year (138). Using this estimate and the fact that in 2018 there were more than 265 million dairy cows in the world (152), and assuming that the cost of *S. aureus* mastitis is equivalent to 10% of the cost of all causes of mastitis (10% of $147), the global annual cost associated with *S. aureus* mastitis can be approximated at $3.9 billion.

Assuming that regulatory evaluations take 10 years (146), cows with this trait could have been commercialized in 2015. Therefore, the costs associated with *S. aureus* mastitis for the 30 years from 2015 through 2044 were examined. To be conservative, a subset of countries (European Union countries and the United States) with improved dairy genetics was used to estimate the cost of delaying the introduction of GE *S. aureus* mastitis–resistant cows. In 2018, in the European

Table 2 Benefit of the diffusion of the new disease-resistant animal and the cost of delaying its approval for commercial use for 5 or 10 years in the United States and the European Union[a]

Year of adoption[b]	Cost ($ billion)[c]		Benefit of diffusion ($ billion)[d]				Cost of delay ($ billion)[e]			
	0% adoption		50% adoption		100% adoption		50% adoption		100% adoption	
	US	EU	US	EU	US	EU	US	EU	US	EU
***Staphylococcus aureus* mastitis–resistant cow**										
Never	3.03	7.40	-	-	-	-	-	-	-	-
2015			0.93	2.26	1.86	4.53	-	-	-	-
2020			0.64	1.57	1.28	3.13	0.29	0.7	0.57	1.39
2025			0.41	0.99	0.82	1.99	0.52	1.27	1.04	2.54
PRRS virus–resistant *CD163* knockout pigs										
Never	11.92	28.86	-	-	-	-	-	-	-	-
2020			3.65	8.83	7.30	17.66	-	-	-	-
2025			2.53	6.11	5.05	12.22	1.12	2.72	2.25	5.44
2030			1.60	3.88	3.20	7.76	2.05	4.95	4.09	9.90

[a]All numbers are calculated considering a 30-year time frame. For the case of the mastitis-resistant cow, the time period is 2015–2044. For the case of the porcine reproductive and respiratory syndrome (PRRS) virus–resistant pig, the time period is 2020–2049.
[b]Year of adoption reflects the year in which diffusion started.
[c]Cost reflects the net present value (NPV) of the costs associated with the disease in the absence of diffusion, which can also be interpreted as the gross value of full adoption.
[d]Benefit is measured as the difference between the NPV cost and the NPV cost associated with treating the disease under different timelines of approval and adoption of a genetically engineered disease–resistant animal.
[e]Cost of delay is measured as the difference between the benefit from diffusion starting when the technology is commercially available, as compared to an additional 5- or 10-year delay in commercial availability.

Union and United States, there were approximately 33 million dairy cows, 23 and 9.4 million cows, respectively (152). The annual cost of *S. aureus* mastitis in these countries can therefore be estimated to be approximately $485 million ($14.70 multiplied by 33 million cows).

The NPV of the costs associated with *S. aureus* mastitis from 2015 to 2044 without the adoption of a solution was $10.43 billion for the United States and European Union (**Table 2**). This implies that a full adoption in 2015 of *S. aureus* mastitis–resistant cows would have led to savings of this amount. If the technology were introduced in 2015, the losses associated with *S. aureus* mastitis for these countries would amount to $7.24 and $4.05 billion under the 50% and 100% adoption rate scenarios, respectively. Diffusion of the technology starting in 2015 would therefore be associated with a savings or NPV of benefits of $3.19 ($0.93 + $2.26) and $6.39 ($1.86 + $4.53) billion for the two scenarios, respectively.

Diffusion starting at 2020, implying a delay of 5 years, would have led to a NPV of costs associated with *S. aureus* mastitis in the United States and European Union jointly of $8.22 and $6.02 billion under the 50% and 100% diffusion scenarios, respectively. Diffusion starting in 2020 would lead to a NPV of benefits of $2.2 and $4.4 billion under the two adoption rate scenarios. The cost of delaying 5 years is then calculated as the difference in the benefits (see Equation A2 in **Supplemental Appendix A**). The cost of a 5-year regulatory delay can therefore be calculated as $0.99 and $1.96 billion, respectively, for the two scenarios. Likewise, the cost of delaying 10 years, meaning that adoption would not begin until 2025, would be $1.79 and $3.58 billion respectively, for the two scenarios. These estimates will change as assumptions in the model are modified. In this analysis, we assume that the technology would be adopted in the same year in different countries. That will depend on the technology transfer rate and timing. A delay on transferring the technology to another country, or a partial transfer, would lead to a lower benefit to that country.

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This scenario is conservative, as it ignores dairy producers beyond the United States and European Union who would also be likely to adopt the technology. If we expand the model to include other major producers, this cost of delay is much higher. Dairies with improved genetic lines are also likely to be widespread in Russia (6.7 million cows), New Zealand (5 million cows), mainland China (5.5 million cows), Brazil (16.3 million cows), and Turkey (6.4 million cows). If the target population of the technology includes these countries, in addition to the United States and European Union, then the potential target population increases to 72.4 million cows. The NPV of costs associated with *S. aureus* mastitis for all of these countries is $23.3 billion over the period from 2015 to 2044, which can also be interpreted as the gross value of full adoption starting in 2015.

Even though cow productivity, disease incidence, and treatment costs are different in other countries as compared with the United States and European Union, for the sake of comparison, we assumed that the same costs of treatment would apply to Brazil, China, Russia, New Zealand, and Turkey. We find that the present value of the cost associated with *S. aureus* mastitis is $12.86 billion for these countries. The NPV of the benefits of diffusion of a *S. aureus* mastitis–resistant cow in 2015 for these selected countries would be $3.94 and $7.87 billion under the 50% and 100% adoption scenarios, respectively. The cost of delaying 5 years would amount to $1.21 and $2.42 billion, respectively. Even this scenario does not consider the biggest dairy producer, India (52.8 million dairy cows and 44.8 million buffalo), or the entire African continent (67.5 million cows and 1.6 million buffalo), or more rapid rates of diffusion. These results would also change with different interest rates. This simple analysis can be expanded to incorporate consumers, international trade, and technology spillovers [see Alston et al. (145)].

Case Study Two: Porcine Reproductive and Respiratory Syndrome Virus–Resistant Pig

PRRS is one of the costliest pig diseases globally. Annual production losses in breeding- and growing-pig herds from PRRS were estimated to be approximately $663 million in the United States alone (139). European countries were surveyed in 2013, and calculations revealed that PRRS virus infections had cost more than $1.6 billion (137). Research groups at the University of Missouri (79), at the Roslin Institute in Scotland (77), and in China (80) reported their production of a genome-edited, PRRS virus–resistant *CD163* knockout pig in 2016, 2017, and 2018, respectively. There is no foreign transgene or rDNA present in these knockout pigs, meaning that they do not fit the classical definition of a GE animal. They would not be considered a GMO in many South American countries [e.g., Argentina (124)] or in Australia. However, in the European Union and United States, they would be regulated as GMOs or drugs, respectively. To calculate the costs associated with the delay of adopting PRRS virus–resistant pigs, we assumed that the technology could have been commercially available in 2020. This enables an estimate of the opportunity cost associated with regulating genome-edited animals as new animal drugs or GMOs. Four scenarios were compared: (*a*) no adoption (status quo/banning the technology), (*b*) diffusion starting at the present (in 2020), and diffusion starting with (*c*) a 5-year delay (in 2025) and (*d*) a 10-year delay (in 2030), for both the United States and the European Union. Again, we modeled two adoption diffusion rates such that either 50% or 100% of the herd were PRRS virus–resistant pigs after 15 years of diffusion. We are not aware of any peer-reviewed literature that estimates the likely rate and extent of adoption of genome-edited disease-resistant animals, and this would be an interesting subject for future research.

The NPV of the costs associated with PRRS from 2020 to 2049 without the approval of a solution was $11.92 and $28.86 billion for the United States and the European Union, making a

total of almost $40.8 billion (**Table 2**). If diffusion began in 2020 and reached 50% of the herd in 15 years, this cost could be reduced to $28.3 billion for both the United States and European Union, and $15.8 billion under the 100% adoption scenario. In other words, the benefit from full adoption would be $12.48 and $24.96 billion for the United States and European Union under the two diffusion scenarios, respectively (**Table 2**).

The proposed regulatory approach in the United States and the European Union is likely to delay the adoption of this technology. Assuming a delay of 10 years, and adoption beginning in 2030, then the NPV of costs associated with PRRS would be $10.32 and $8.72 billion in the United States and $24.98 and $21.11 billion in the European Union under the scenarios of 50% and 100% adoption, respectively. Total benefits under these two scenarios would be reduced to $5.48 and $10.96 billion, respectively. The total cost of delaying adoption of this technology by 10 years would therefore be $7 and $13.99 billion given 50% and 100% adoption, respectively. Put another way, delaying the commercial availability of PRRS virus–resistant pigs by 10 years in the United States and European Union would be associated with opportunity costs as high as $14 billion.

These numbers would be even higher if diffusion in China and other countries were included in the model. In 2018, mainland China had more than 442 million pigs, 45% of all live pigs in the world, whereas the United States had only ∼75 million (152). The losses associated with PRSS and its treatment are therefore substantially bigger in China. To estimate the potential benefits of adopting PRSS virus–resistant pigs in China, it was assumed that the cost of PRRS per pig in China was approximately half of the cost in the United States, approximately $5 per pig. A higher cost would generate greater losses and a greater benefit under the adoption scenarios. The NPV cost associated with PRRS in China would therefore be $39.74 billion over the period from 2020 to 2049. Diffusion of PRRS virus–resistant pigs under 50% and 100% adoption scenarios starting in 2020 would reduce the NPV costs to $27.58 and $15.43 billion, respectively. Delaying the adoption of PRRS virus–resistant pigs in China by 10 years would therefore be associated with opportunity costs of $6.82 and $13.64 billion, respectively, for the two scenarios. These case study estimates are based on a partial assessment of benefits lost under specific assumptions. A faster adoption diffusion, an interest rate higher than 4% per year, and/or smaller costs per pig would result in smaller losses. However, these analyses shed light on the opportunity cost of delaying the introduction of GE disease-resistant animals. As in the analysis of *S. aureus* mastitis, this model could be extended to incorporate consumers and trade, as discussed by Alston et al. (145), which is an important topic for future research.

It is worth noting that another viral disease, ASF, has recently had a huge impact on global pork production. This highly contagious and deadly disease spread from Africa into Europe (153) and was first reported in China in August 2018. It has since spread into every province, and Chinese government statistics in October 2019 showed a decline in swine inventory of more than 40% from a year earlier. Pork is the most consumed meat for China's population of 1.4 billion, and its $118-billion pork market dominates global sales. Before the ASF epidemic, China's pork output was 55 million metric tons (MMT), double that of the European Union and almost five times that of US production. That has since dropped sharply and will likely decline to 36 MMT in 2020 (154). Pork prices in China began to rise in 2019 to more than twice the previous-year levels in the second half of 2019, prior to the emergence of the severe acute respiratory syndrome coronavirus 2 (155).

Perhaps unsurprisingly, researchers globally have been working for many years on approaches to develop ASF-resistant pigs (89). Classical swine fever virus–resistant pigs were produced in 2018 by using genome editing to insert small hairpin interfering RNAs that targeted parts of the virus genome (60). It goes without saying that the global annual cost associated with ASF runs into the tens of billions of dollars. Therefore, the economics of potentially delaying the introduction of

ASF-resistant pigs, if they are successfully developed, would be orders of magnitude higher than the two disease-resistance case studies examined in this article, to say nothing of the food security implications.

Case Study Three: AquAdvantage Salmon

Atlantic salmon is a major farmed cold-water fish, and its production exceeded 2.248 MMT in 2016 (144). US imports of salmon totaled 339,000 MT in 2016, worth more than $3 billion (156). The vast majority of that came from imported farmed Atlantic salmon raised in floating sea cages and flown in from Canada, Chile, Norway, and Scotland. Escapes of farmed salmon from ocean cages and infectious disease outbreaks such as infectious salmon anemia, a viral disease of Atlantic salmon, are associated with significant environmental and economic impacts. The alternative of land-based closed systems would mitigate a significant proportion of these risks to the global salmon farming industry, with implications for global food security. The carbon footprint of salmon produced in land-based closed systems is less than half of that of salmon produced in conventional fish farms in Norway and delivered to the United States by air (157).

As discussed previously, the lone example of a transgenic animal that has been commercialized for food is the fast-growing AquAdvantage Atlantic salmon. In 1989, Canadian public sector scientists produced this line by microinjecting fertilized Atlantic salmon (*Salmo salar*) eggs with an rDNA construct composed of the growth hormone gene from Chinook salmon (*Oncorhynchus tshawytscha*) under the control of a promoter isolated from an antifreeze gene of the ocean pout (*Zoarces americanus*) (21). The resulting Atlantic salmon with enhanced growth characteristics was licensed by AquaBounty Technologies from Memorial University and Toronto Children's Hospital in Canada. Individuals carrying this transgene reach market weight considerably (40%) faster than nontransgenic Atlantic salmon and require 25% less total feed to produce the same fish biomass (143).

We calculated the total opportunity cost for regulatory delays involving AquAdvantage salmon by using a simple methodology widely used in the literature to estimate gains from research (145) (see **Supplemental Appendix B**). Briefly, a simple partial equilibrium model was used to calculate the losses from delaying diffusion of this technology into the world market of salmon. The costs associated with delaying the adoption of this GE fish from 2002, 10 years after publication in 1992 (21), to 2020 was estimated at more than $25.5 billion. This number may change depending upon assumptions regarding elasticities, adoption, and the effect of the technology on the salmon supply. For instance, this cost estimate ranged from $17.5 to $36.5 billion under different assumptions for elasticities. This is an initial analysis, and future research should consider the spatial heterogeneity and quality differences in the salmon market, as well as potential for introduction in new locations.

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These examples illustrate high opportunity costs associated with delaying, or indefinitely shelving, GE livestock applications. Further research is needed to refine these estimates and to assess the distributional impacts between producer and consumers, and among different regions of the world. It is also important to assess to what extent the more vulnerable are paying a disproportionate share of the costs resulting from the underuse of these technologies.

CONCLUSIONS

Although scientific progress regarding genetic engineering and genome editing food animals has been ongoing for the past 35 years, albeit slowly, in part owing to technical difficulties and industry structure, regulatory issues have functioned to delay and forestall timely approvals to produce and market new GE animals. There are real opportunity costs associated with delaying the introduction of GE technologies. Animal diseases like mastitis in dairy cows and PRRS in

pigs are associated with billions of dollars in losses annually. The examples provided in this article estimate the costs associated with delaying approval for commercial use for 5 or 10 years in the United States and European Union. These costs are the baseline against which the value, if any, of such delays must be weighed. Delaying or banning diffusion has consequences when the status quo is associated with high levels of animal disease, or with continuing the use of less sustainable production systems. This is especially true when considering zoonotic diseases, or diseases like ASF and their potentially huge impact on the global food security of animal protein sources. There is a pressing need to consider both the costs and benefits in regulatory evaluations of new technologies, and to consider the very real opportunity costs associated with delaying the adoption of beneficial genetic innovations.

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