

# Developments in genetic modification of cattle and implications for regulation, safety and traceability

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**Abstract** Genetic modification techniques, in particular novel gene editing technologies, hold the yet unfulfilled promise of altering genetic traits in farm animals more efficiently than by crossbreeding, allowing for a more rapid development of new cattle breeds with distinct traits. Gene editing technologies allow for the directed alteration of specific traits and thereby have the potential to enhance, for instance, disease resilience, production yield and the production of desired substances in milk. The potential implications of these technological advancements, which are often combined with animal cloning methods, are discussed both for animal health and for consumer safety, also with consideration of available methods for the detection and identification of the related products in the food supply chain. Finally, an overview is provided of current regulatory approaches in the EU and major countries exporting beef to the EU, for products from animals bred through established practices as well as modern biotechnologies.

**Keywords** cattle, food safety, gene editing, genetic modification, GMO detection, regulation

## 1 Introduction

A growing and increasingly affluent world population is increasing the demand for wholesome food products including animal products such as bovine meat and milk. This demand moves cattle breeders toward developing livestock with novel or changed genetic traits in order to increase disease resilience, animal welfare or meat and milk production and quality. Established selective breeding programs have set the standard for the development of top-producing livestock breeds. Biotechnological methods of genetic modification, including transgenesis and more recently genome editing technologies, allow for the enhancement of bovine and other livestock through specific genetic alterations<sup>[1]</sup> but have not yet found commercial applications. These technologies may be employed to enhance beneficial traits and boost production yield. This is the case for the fast-growing AquAdvantage salmon, being the only transgenic animal commercialized to date, into which a constitutively expressed growth hormone transgene has been introduced<sup>[2]</sup>.

Different potentially useful traits have been identified as candidate targets for genetic modification to improve herd genetics, such as increasing muscle growth or modulating disease resilience. Care must be taken, however, to ensure that the genetic alterations do not negatively impact on animal health and welfare. Also, animal products resulting from genetic modification should be demonstrably safe for human consumption.

Current developments in gene editing have made genetic modification of food-producing organisms, <sup>1</sup>including animals, more efficient and less time consuming. This paper provides an overview of recent developments in genetic modification of cattle from the humpless *Bos taurus* subsp. *taurus* and humped *B. taurus* subsp. *indicus* (Zebu) subspecies, and the likelihood of food products derived from

genetically modified (GM) cattle entering the market in the near future. Furthermore, regulation of genetic engineering in major beef-exporting countries are analyzed together with current safety assessment strategies for food products from GM cattle. It is important to note that genetic modification will be discussed from a scientific point of view which includes technologies such as gene editing. Certain regulatory regimes, however, may not always regard minor genetic alterations as GM based on similarities with natural genetic variation. This will also be highlighted in our description of how the different countries address the issues of transgenesis and gene editing in animals. Finally, issues related to the detection and traceability of (un)authorized food products from GM cattle, including gene-edited breeds, are discussed.

## 2 Development of cattle breeding

The history of cattle breeding goes back thousands of years. In modern times, cattle breeding programs show various trends in the methods used to apply genetics and the choice of targets. Traits are chosen to fulfil several criteria such as:

- be useful for the development of products of economic value or reduce costs,
- show enough variability and heritability among different animals, and
- be clearly distinguishable and measurable<sup>[3]</sup>.

In the late 1800s and early 1900s, dairy cattle breeding associations initially focused on increasing production. Selection of animals was facilitated by record keeping of the milk production traits in registries of animals used for breeding purposes, and by standardized and accessible tests for a range of traits<sup>[3]</sup>. In addition to productivity, physiologic and product quality traits, some of which are also predictors of production, were also included in the classification schemes. The number of these parameters has increased over time. A more recent selection tool for production and health traits is the estimated breeding value (EBV), based on the information from the pedigree, the individual animal, and its progeny. In EBVs, genetic factors have been disentangled from environmental and other confounding factors<sup>[4]</sup>. A more recent development is genomic selection, which involves the inclusion of genomics data into breeding programs, supplementing existing genetic approaches. These developments have revolutionized the sector. Advantages include, among others, time savings as progeny testing would normally require seven or more years, and a greater number of genetic markers beyond the individual ones (such as for milk casein) previously used<sup>[5,6]</sup>.

Other historical developments that have advanced cattle breeding include the introduction of various assisted reproduction technologies, most notably:

- artificial insemination (AI) with sperm collected from elite donors,
- embryo transfer (ET) between donor and recipient allowing for an increased number of offspring from a selected cow, and
- *in vitro* fertilization (IVF), which involves the culture of oocytes obtained from a selected cow followed by addition of capacitated sperm from a donor.

IVF, in particular, has been increasingly used in recent years and is expected to further grow in importance as modern genomics programs allow for early selection, creating the need for obtaining embryos from heifers and calves that are too young for superovulation and embryo transfer<sup>[7]</sup>. The practice of superovulation helps ensure the production of high numbers of embryos for embryo transfer in older dams. This also requires synchronization of reproductive cycles in the embryo donor and recipient animals<sup>[7-9]</sup>. Another important development in breeding is the possibility of determining the gender of the fetus, either by sexing the sperm of the donor bull (into male or female) or to measure the DNA of preimplantation ET embryos or fetal DNA circulating in the blood of pregnant cows<sup>[10]</sup>.

## 3 Developments in genetic modification of cattle

Major developments in animal biotechnology and a better understanding of genetic traits that influence factors such as animal health, growth and reproduction have contributed to the improvement of animal breeding strategies. This holds the potential to make livestock more resilient to disease and simultaneously increase animal health and welfare as well as yield. Genetic modification in cattle, for instance, has led to the development of experimental breeds with enhanced traits, such as special milk composition<sup>[11,12]</sup>,

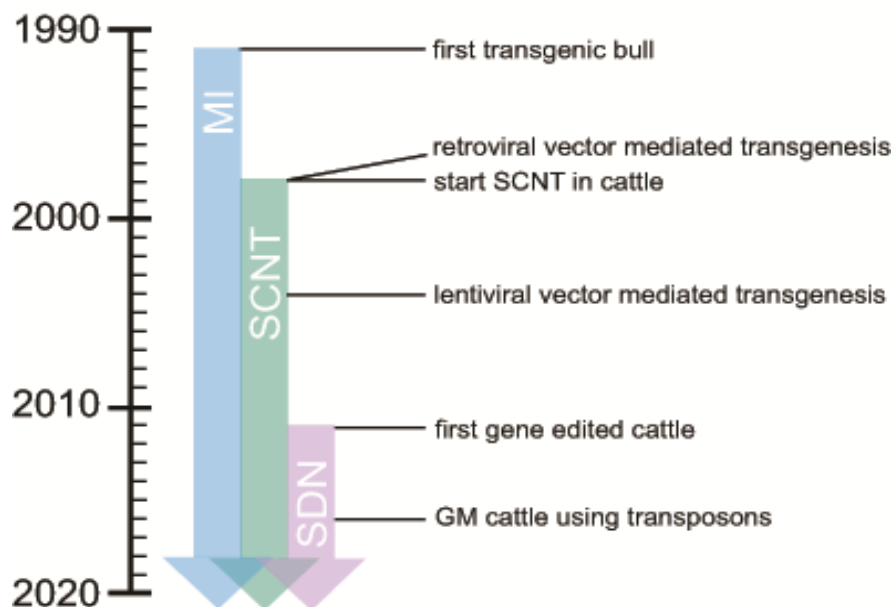
improved disease resilience<sup>[13]</sup> or increased muscling<sup>[14]</sup>. A detailed list of transgenic or gene-edited cattle breeds is shown in Table 1, which includes breeds with genetic modifications to benefit agriculture, and bioreactor cattle for the production of biopharmaceuticals as well as transgenic animals developed in proof-of-concept fundamental research. To our knowledge, none of these have been commercialized to date.

**Table 1** Examples of transgenic and gene-edited cattle

Trait	Method	Trait	Reference	
Milk composition and human	Transgenesis using microinjection	Introduction of gene encoding human lactoferrin	[15]	
Protein production	Transgenesis using microinjection	Introduction of gene encoding human $\alpha$ -lactalbumin	[16,17]	
	Transgenesis in somatic cells, SCNT	Introduction of gene encoding human bile salt-stimulated lipase	[18]	
	Transgenesis in somatic cells, SCNT	Introduction of gene encoding human immunoglobulin	[19,20]	
	Transgenesis in somatic cells, SCNT	Introduction of additional gene copies encoding bovine $\alpha$ - & $\kappa$ -casein	[21]	
	Transgenesis in somatic cells, SCNT	Introduction of gene encoding human lysozyme	[22]	
	Gene editing using zinc finger nucleases, NHEJ repair, SCNT	Disruption of $\beta$ -lactoglobulin gene	[12]	
	Transgenesis in somatic cells, SCNT	Introduction of gene encoding humanized <i>Caenorhabditis elegans</i> n-3 fatty acid desaturase	[11]	
	Transgenesis in somatic cells, SCNT	Introduction of gene encoding human $\beta$ -defensin-3	[23]	
Disease resilience	Transgenesis in somatic cells using TALENs & SCNT	Introduction of gene encoding <i>Sulfolobus solfataricus</i> lactase	[24]	
	- Mastitis	Transgenesis in somatic cells, SCNT	Introduction of gene encoding <i>Staphylococcus simulans</i> lysostaphin	[25]
	- Bovine spongiform encephalopathy	Transgenesis and embryonic cloning	Disruption of prion protein via integration of knockout vectors	[26]
	- Mannheimia hemolytica leukotoxin	Gene editing and homology-directed repair, SCNT	Gene-edited <i>CD18</i> , substitution of a glutamine for a glycine codon in its signal peptide	[27]
	- Bovine tuberculosis	Transgenesis in somatic cells using TALENs & SCNT	Introduction of mouse nuclear body protein encoding gene <i>SPI10</i>	[28]
		Transgenesis in somatic cells using Cas9n & SCNT	Introduction of additional genes encoding solute carrier family gene <i>NRAMP1</i>	[13]
	Hornlessness	Gene editing and homology-directed repair, SCNT	Introduction of bovine P <sub>c</sub> <i>POLLED</i> allele, resulting in hornless phenotype	[29]
	Thermotolerance	Gene editing using TALENs, SCNT	Introduction of the <i>SLICK</i> locus for improved thermotolerance	[30,31]

Increased muscle growth	Gene editing using TALEN mRNA	Introduction of small deletions in the myostatin ( <i>GDF8</i> ) gene by use of gene editing	[14]
Other fundamental research			
-Reverse transcribed gene transfer	Transgenesis via pronuclear injection of retroviral vector DNA	Integration of hepatitis B surface antigen gene using a retroviral vector	[32]
-Marker assisted selection	Transgenesis via transfection of somatic cells with retroviral vector DNA, SCNT	Integration of a $\beta$ -galactosidase-neomycin fusion gene driven by cytomegalovirus promoter	[33]
-Lentiviral infection	Transgenesis in somatic cells using lentiviral vectors, SCNT	Integration of eGFP using lentiviral vector	[34]
-Transposon integration	Transgenesis via microinjection of transposon DNA	Sleeping Beauty and Piggybac transposons used to deliver sequences containing fluorescent protein genes	[35]
-Targeted integration	Transgenesis in somatic cells using TALENs & SCNT	Integration of eGFP in Rosa26 "safe locus"	[36]

An overview of the technological developments and milestones in the genetic modification of cattle is shown in Fig. 1. Notably, the first transgenic bull, Herman, dates back to 1991 and marks the beginning of the era of genetic modification in cattle<sup>[15]</sup>. Herman was obtained by the use of pronuclear microinjection of recombinant DNA into bovine zygotes, at that time an effective method for transfecting mammalian cells. The DNA integration frequency and survival rate of microinjected embryos have been shown to be two important factors affecting the efficiency of this method in cattle<sup>[37,38]</sup>. Given the low efficiency of this method to produce live GM cattle, it is most likely that studies in subsequent years focused on increasing the survival rate of microinjected bovine zygotes<sup>[39,40]</sup>.



**Fig. 1** Timeline of technological developments and milestones used to obtain GM cattle. MI, pronuclear microinjection; SCNT, somatic cell nuclear transfer; SDN, site-directed endonucleases. Important milestones are summarized on the right.

In 1998 the first GM cattle were obtained by use of somatic cell nuclear transfer (SCNT) performed with nuclei of genetically altered somatic cells<sup>[33]</sup>. SCNT can be used to obtain transgenic animals when the genome of the donor cells has been genetically modified to contain the desired traits<sup>[41]</sup>. Furthermore, SCNT is considered to have several advantages over microinjection, including (1) the genetic modification applied to donor cells can be verified before nuclear transfer and (2) targeted integration of DNA by

homologous recombination is possible *in vitro*<sup>[42]</sup>. Other developments in genetic modification of cattle are the use of viral vectors in combination with SCNT or pronuclear injection, and perivitelline space injection of viruses or genetic constructs into fertilized bovine eggs before transferring them to dams<sup>[32,34]</sup>.

As shown in Fig. 1, technological developments over the years to develop GM cattle indicate that recent research has its main focus on the use of new gene editing technologies, including zinc finger nucleases, transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats with associated protein 9 (CRISPR-Cas9), increasingly in combination with other techniques such as SCNT. Examples of gene editing in cattle are hornless and thermotolerant breeds developed by Recombinetics, Inc. in South America<sup>[29,30]</sup>. Hornless dairy cattle were obtained by introgression of the Pc *POLLED* allele responsible for hornlessness in bovine embryo fibroblasts by making use of TALENs and homology-directed repair. The gene-edited fibroblast cell lines were subsequently used to produce bovine embryos *in vitro*, by use of SCNT, and transferred to recipient dams. In a similar fashion, thermotolerant Angus cattle were obtained by introduction of the *SLICK* hair locus, responsible for less dense hair and increased thermal transpiration<sup>[30,43]</sup>. It is important to note that recent analysis of the genome of the hornless cattle developed by Carlson et al. revealed, in addition to the desired introduction of the Pc *POLLED* locus, an unintended duplication of Pc *POLLED* and chromosomal integration of the full-length repair template plasmid backbone<sup>[44,45]</sup>. These unintended plasmid integrations were also inherited by offspring of the gene-edited bulls<sup>[45]</sup>. No phenotypic effects other than hornlessness were evident in the gene-edited cattle.

Modifications of traits that have a beneficial impact on animal health and productivity will be likely candidates for commercialization. In particular, traits which improve animal health and welfare when altered using animal biotechnology may gain wider public acceptance since these are not only economically beneficial but also may prevent animal suffering. Many studies have been dedicated to the prevention of disease in cattle, for example protection against mastitis through the production of antimicrobial compounds such as lactoferrin, lysostaphin, and lysozyme<sup>[25]</sup>, prevention of bovine spongiform encephalopathy through mutation of the implicated PrP proteins<sup>[26]</sup> as well as resistance to *Mannheimia hemolytica*<sup>[27]</sup> and to bovine tuberculosis<sup>[13]</sup>. If it is demonstrated that these genetic alterations yield more disease resilient animals then these are likely candidates for future commercialization.

To our knowledge, the only gene-edited cattle that are likely candidates for market release in the near future are thermotolerant *SLICK* cattle<sup>[30,43]</sup> which are (currently) bred in Brazil. These gene-edited cattle are regarded as non-GM animals under Normative Resolution #16 in Brazil and may therefore be released for commercialization following a case-by-case assessment by the National Biosafety Technical Commission (CTNbio)<sup>[46]</sup>.

In addition to food production, cattle are also attractive for biopharmaceutical production because of several factors including (1) the large amounts of milk that can be produced daily containing the desired biochemical, (2) the scalability of production, (3) the limited number of animals needed to address the global demand for products such as growth hormone, (4) the post-translational modifications in a mammalian host, (5) the availability of existing technology to milk the animals and process the milk collected, (6) the safety of the matrix (milk) used, and (7) the economics of production compared to cell culture, for example. Owing to the high levels of containment and identity preservation required under national guidelines for working with recombinant DNA organisms and animals in particular, the likelihood of food products originating from these transgenic bioreactor cattle or from cattle used in fundamental research entering the food chain is small, but it will require dedicated traceability systems to safeguard the food chain in this respect.

## 4 Regulation of transgenic and gene-edited cattle

For genetic modification in livestock, for instance through transgenesis and more recently via genome editing technologies, cloning technologies are commonly employed, in particular SCNT, to aid in the development of the genetically altered animals. Application of the SCNT procedure, however, has its downsides as it increases the occurrence of placental as well as fetal abnormalities and thereby places a significant burden on animal health and welfare. It is important to note that other artificial breeding technologies, such as methods utilizing *in vitro* produced embryos, also increase the frequency of these anomalies occurring, albeit to a lesser extent than SCNT<sup>[47,48]</sup>. Alternative strategies, such as gene editing in zygotes, provide an efficient methodology that avoids the need for SCNT and its associated developmental

defects<sup>[49,50]</sup>. The impact on animal health and welfare caused by the SCNT procedure is one of the reasons why governments have developed legislation and/or guidelines that cover the application of this technology<sup>[51]</sup>. Also, food products derived from cloned, transgenic and/or gene-edited livestock are likely to require regulatory approval as well as extensive safety assessments to ensure there is no adverse health risk for consumers of the products derived. **Table 2** presents a summary of the regulation of animal cloning and transgenesis as well as gene editing in the EU and the main countries exporting beef to the EU, according to Eurostat<sup>[63]</sup>. Canada is also a major beef-exporting country and is also included because a free-trade agreement, the Comprehensive Economic and Trade Agreement (CETA), was started with the EU in 2017 that allows Canadian farmers to profit from beef exports to the EU, but only if they comply with EU regulations as there is no harmonization under CETA.

## 4.1 Regulation in the European Union

Within the EU, food derived from animal clones comes from a novel breeding practice and is therefore deemed a novel food and regulated under the Novel Foods Regulation (EU) 2015/2283. Based on ethical and animal welfare considerations after advice from the European Group on Ethics in Science and New Technologies (EGE)<sup>[64]</sup> the European Commission decided to prohibit animal cloning until specific regulation is passed. Proposals for new legislation regarding the use of animal cloning for food production have been presented to the European Parliament, covering placing on the market of food products derived from animal clones<sup>[65]</sup>. The proposal calls for a ban in the EU on the release of these products on the market, based on the ethics and welfare concerns that were previously raised by the EGE as well as by the European Food Safety Authority (EFSA)<sup>[64,66]</sup>. The scope of this ban does not extend to products from healthy offspring of clones.

European regulation of GM animals falls under Directive 2001/18/EC for the environmental release of GM organisms (GMOs). In addition, placing on the market of food products derived from GMOs is regulated under Regulation (EC) No. 1829/2003. It is important to note that, according to a ruling by the Court of Justice of the European Union in July 2018 (Case C-528/16), organisms obtained by directed mutagenesis techniques (e.g., gene editing) are regarded as GMOs within the scope of Directive 2001/18/EC. Before release on the market, the EFSA Panel of experts on GMOs (GMO Panel) will perform a safety assessment of the GMO and derived food and feed products in question. This safety assessment by EFSA is required for approval, and will be further explained below. In addition, the European Commission's Directorate General for Public Health, Food Safety and Consumer Affairs recently consulted the EGE for general advice on ethical aspects of gene editing of animals.

## 4.2 Regulation in the USA

In contrast to the EU, livestock animal cloning is not prohibited in the USA. The US Food and Drug Administration (FDA) issued guidance and a risk management plan for industry for the use of animal clones<sup>[53,54]</sup>. Furthermore, to decrease the frequency and impact of anomalies associated with the cloning procedure, the FDA collaborated with the International Embryo Transfer Society and issued a manual on animal care standards to aid cloning practitioners<sup>[67]</sup>.

Regulation in the USA specifies that, according to article 201(g) of the Federal Food, Drug, and Cosmetic Act, the intentional alteration of animal genomes is deemed a new animal drug and therefore requires a New Animal Drug Application to be filed with and approved by the FDA. The altered genomic DNA is defined as the drug in the context of section 201(g) and refers to the intentionally altered region within the animal genome, through either targeted or random mutagenesis (e.g., transgenesis and gene editing)<sup>[55]</sup>. The FDA will perform a food safety assessment to evaluate whether food products derived from the GM animal are safe for human consumption. Furthermore, an environmental safety assessment, which complies with requirements of the National Environmental Policy Act, is performed by the FDA to evaluate the environmental impact of the genetically altered animal.

## 4.3 Regulation in Canada

In Canada any food product that is deemed to be novel or food products that contain a novel genetic trait, through either transgenesis or gene editing, will require a pre-market safety assessment. Health Canada will perform these safety evaluations according to the Food and Drugs Act on a case-by-case basis. Genetically altered animals will also require further evaluation according to the Canadian Environmental Protection

Act. Although animal cloning is permitted in Canada, a statement by Health Canada in 2003 indicates that products from animal clones, as well as their progeny, are deemed to be novel foods and therefore are subject to the Food and Drug Regulations (Novel Foods) and to a pre-market safety assessment<sup>[56]</sup>.

**Table 2** Regulation of animal cloning, transgenesis and gene editing

Country	Animal cloning	Transgenic livestock	Gene-edited livestock	Reference
EU member states	Prohibited, until specific regulations on animal cloning are in place	Requires approval according to EU Directive 2001/18/EC and Regulation (EC) No 1829/2003, safety assessment performed by EFSA GMO Panel	Requires approval according to EU Directive 2001/18/EC and Regulation (EC) No 1829/2003, safety assessment performed by EFSA GMO Panel	[52]
USA	Allowed, a risk management plan and guidance for industry have been issued by the FDA	Requires approval according to Federal FD&C Act, regulations for new animal drugs as stated in 2009 FDA Guidance for industry #187 (Draft guidance) and NEPA	Requires approval according to Federal FD&C Act, regulations for new animal drugs as stated in 2009 FDA Guidance for industry #187 (Draft guidance) and NEPA	[53–55]
Canada	Allowed, food products of cloned animals and clone progeny are considered “novel foods” and require pre-market safety assessments according to the regulations in Division 28, Part B, of the Food and Drug Regulations (Novel Foods)	Requires approval according to the Canadian Environmental Protection Act, 1999, the New Substances Notification Regulations (Organisms) and Food and Drugs Act	No specific policy on gene editing, may be considered “novel” and require case-by-case safety assessment by Health Canada	[56,57]
Argentina	Allowed	Requires approval according to animal biotechnology regulation, case-by-case assessment by CONABIA	Requires approval according to animal biotechnology regulation, case-by-case assessment by CONABIA	[58]
Brazil	Allowed, commercial animal cloning mostly in partnership with EMBRAPA, registration of cloned cattle at ABCZ	Requires approval according to animal biotechnology regulation, case-by-case assessment by CTNBio	Requires approval according to animal biotechnology regulation, case-by-case assessment by CTNBio, gene-edited animals lacking recombinant DNA are regarded non-GM according to Normative Resolution #16	[46]
Australia & New Zealand	Allowed, generally in confined research environment	Requires approval according to Gene Technology Act 2000, by OGTR	Requires approval according to Gene Technology Act 2000, by OGTR, gene editing techniques that do not introduce new genetic material are not regulated as GMOs	[59,60]
Uruguay	No specific legislation on animal cloning, animal biotechnology performed in research institutes such as Institut Pasteur in Montevideo and the Animal Reproduction Institute of Uruguay	No specific legislation on animal biotechnology, environmental release of GMOs and biosecurity is subject to prior authorization by competent authorities, as stated in article 23 of law N° 17,283 on the protection of the environment	No specific legislation on gene editing in animals, during a meeting of the CAS the minister of agriculture signed a declaration in favor of gene editing. Gene-edited animals may be subject to prior authorization according to law N° 17,283	[61,62]

Note: EFSA, European Food Safety Authority; FD&C Act, Food, Drug and Cosmetic Act; NEPA, National Environmental Policy Act; FDA, Food and Drug Administration; CONABIA, National Advisory Commission on Agricultural Biotechnology; EMBRAPA, Brazilian Agriculture and Livestock Research Enterprise; ABCZ, Brazilian Zebu Cattle Association; CTNBio, National Technical Biosafety Commission; OGTR, Office of the Gene Technology Regulator; CAS, Southern Agricultural Council.

## 4.4 Regulation in Argentina

In Argentina the National Food Safety and Quality Service (SENASA) is responsible for the assessment of novel food products entering the market. SENASA recognizes that clones of food-producing animals are mainly used for breeding purposes and are not intended to be sold as food. However, with the increasing trend in the development of animal clones, the likelihood of food products with a clone origin similarly increases. After examination of assessments on the safety of food products from animal clones, it was decided that there was no scientific reason to regulate commercialization<sup>[58]</sup>.

Before release on the Argentinean market, GMOs are required to be evaluated by the National Advisory Committee on Agricultural Biotechnology, which is a multidisciplinary advisory agency that assesses new GMO events on a case-by-case basis, considering impact on the environment as well as risks to human or animal health. Furthermore, SENASA is responsible for the evaluation of the biosafety of food products derived from GMOs for consumption by humans and animals<sup>[58]</sup>.

## 4.5 Regulation in Brazil

Animal cloning for commercial purposes is permitted in Brazil and is often done in partnership with the Brazilian Agriculture and Livestock Research Enterprise (EMBRAPA). However, there is mandatory registration of cloned production animals in the Genealogical Registry of Zebu Breeds. At present there is no regulation in place for cloned animals or products with a clone origin. However, a draft bill that is still before the Senate proposes to regulate the cloning of animals. It proposes, among other things, to make the Ministry of Agriculture, Livestock, and Food Supply responsible for the authorization of commercial sales and imports of cloned animals and their offspring for food purposes<sup>[46]</sup>.

Food-producing GM animals are subject to the approval of CTNbio on a case-by-case basis. Part of this approval is a full risk assessment and management of GMOs but certain modifications are exempt from regulation. According to Normative Resolution #16, new breeds developed using Precision Breeding Innovation, which includes New Breeding Technologies such as gene editing approaches, and that lack introduced recombinant DNA are exempt from GMO assessment.

## 4.6 Regulation in Australia and New Zealand

In Australia and New Zealand no specific regulation is in place for animal cloning but in Australia cloned animals are subject to animal welfare legislation as well as the Australian code of practice for the care and use of animals for scientific purposes<sup>[59]</sup>. Similarly, in New Zealand there is no specific regulation concerning animal cloning. New Zealand's Animal Welfare Act 1999 covers the holding of both farmed and experimental animals, including cloned animals. Furthermore, cloned animals in New Zealand need to be documented and are required to have a unique cloned animal ear tag.

The commercialization of GM animals in Australia requires approval from the Office of the Gene Technology Regulator (OGTR). A detailed risk assessment is performed on the environmental impact as well as health implications. For these assessments the OGTR consults with relevant authorities such as the States and Territories, local councils, the Department of Agriculture, the Australian Pesticides and Veterinary Medicines Authority as well as with the public. Furthermore, a biosafety evaluation of the GM animal food products is carried out by Food Standards Australia New Zealand (FSANZ). In a recent review of the Gene Technology Act it was concluded that genetically altered organisms obtained using techniques that do not introduce new genetic material are exempt from regulation. This includes site-directed nuclease techniques (e.g., CRISPR-Cas9) that create small changes, oligo-directed mutagenesis and some RNA interference methods<sup>[60,68]</sup>.

In New Zealand the release into the environment of living organisms that do not already exist in New Zealand, including GMOs, is regulated by the Hazardous Substances and New Organisms Act 1996. Approval by the Environmental Protection Authority of New Zealand (NZ EPA) is required prior to commercialization of a novel GMO. Regarding GM foods, a biosafety evaluation is conducted by FSANZ according to the Food Standards Code of Australia and New Zealand.



## 4.7 Regulation in Uruguay

In Uruguay, animal biotechnology (including cloning) is not specifically regulated<sup>[61]</sup>. However, the environmental release of GMOs is regulated in Law N° 17,283 on environmental protection which also states that, before release, authorization by competent authorities is required. Regulation and biosafety of GMOs mainly focuses on the genetic modification and environmental introduction of GM vegetables, as specified in Regulation N°65<sup>[69]</sup>. For commercialization of these GMOs, authorization is required from the National Biosafety Commission (GNBio). GNBio evaluates GMO applications on a case-by-case basis.

Together with the ministers of agriculture from Brazil, Paraguay, Chile and Argentina, Uruguay signed a declaration in favor of the application of gene editing at a ministerial meeting of the Southern Agricultural Council in 2018<sup>[70]</sup>. This declaration recognizes that current regulatory frameworks and safety standards for the commercialization of biotechnology products are sufficient for evaluation of gene editing derived products. In addition to this declaration, the government of Uruguay passed a law (Law N° 19,317) for the promotion of biotechnology. Biotechnological innovation and its application are regarded as being in the national interest and this new law is meant to boost the economy as well as sustainable development of the country.

## 5 Safety assessment of products derived from transgenic and gene-edited cattle

Similar to foods derived from GM plants and microorganisms, an internationally harmonized approach has been developed for the safety assessment of foods derived from GM animals. In 2008 the Codex Alimentarius Commission published guidelines toward this end<sup>[71]</sup>. This commission is an intergovernmental body of which many countries (188) and the EU are members. It was established through a collaboration between two UN organizations, namely the World Health Organization and the Food Agriculture Organization (FAO). Codex develops internationally harmonized standards, standard procedures and guidelines for the quality and safety of foods. These also serve as a reference in disputes over internationally traded food products and therefore should be implemented by member states into their own national food safety systems.

Central to the recommended safety assessment approach within these Codex guidelines for foods derived from GM animals is the comparison between the GM animal and a non-GM counterpart with a history of safe use in order to identify hazards that are new or that have been changed as a result of the genetic modification. The latter relates to both intended and unintended effects of the genetic modification. The document also recognizes that precise DNA targeting methods, such as homologous recombination, may reduce the occurrence of unintended effects, while molecular analytical tools may help identify changes at the level of gene transcription and translation. The safety assessment needs to include procedures to assess the relevance of any hazards found, following a weight-of-evidence approach as no single test will be fully predictive in this regard. However, the Codex guideline does not include considerations of animal welfare.

As regards the information to be provided on the materials and methods used for the modification, this includes the following items.

- General background information on the recipient animal such as history of food production, known adverse effects related to genotype and phenotype, potential toxicity and allergenicity, and reservoir for zoonotic pathogens.
- Information on the donor organism (not necessarily animals) such as pathogenicity, toxicity, allergenicity and history of use in food production.
- The genetic modification, including:
  - (1) the procedure for introducing DNA such as whether zoonotic pathogens, for example viruses, were used for the transfer including, for example, details on their host range, and
  - (2) the DNA construct used to transform the recipient animals, including the nature and function of the various elements.
- Procedures and techniques to obtain the first GM animal for example, microinjection with DNA or somatic cell nuclear transfer using transgenic nuclei. This also includes the breeding process using these founder animals to obtain food-producing GM animals.

- Characterization of the inserted DNA including the inserted elements and their orientation, and the possible presence of new open reading frames obtained by insertion. Also, the formation of newly expressed products such as transgenic proteins need to be described and where, when and at which levels these are expressed. Potential changes as compared with the construct used, such as rearrangements and mutations, need to be reported.

For the comparative assessment, a compositional analysis of key components is recommended of the GM animal versus its non-GM counterparts. This is to verify that no changes in the nutritional components have taken place that may negatively impact consumer health. To this end, specific husbandry conditions or environments and other factors such as physiologic cycles ideally need to be matched for the GM animal and its comparators, although this may not always be feasible. This also holds true for the selection of a counterpart that is as close as possible to the GM test animal.

The following details are recommended for the food safety assessment of the GM animals.

- The health of the GM animal. This is based on, *inter alia*, the practice of having only healthy animals used for food production to pursue the safety of derived products. These data need to include at least general health and performance parameters such as growth and reproduction.

- Possible toxicity or bioactivity of newly expressed non-nucleic products. For proteins, this would entail:

- (1) similarity of the primary structure (amino acid sequence) with those of toxic proteins as identified in bioinformatics-based comparisons,
- (2) stability to degradation by physiologic protein-degrading enzymes in model digestive systems, and
- (3) oral toxicity studies in laboratory animals, if needed, such as for new proteins that bear no similarity to other proteins that have already been consumed safely with foods.

- Potential allergenicity, that is the capacity to provoke allergic reactions. Similar to the assessment of toxicity, this entails a bioinformatics-guided comparison with known allergenic proteins and *in vitro* degradability by proteases. Animal models are not recommended and this may be related to the fact that these are still in development and non-validated for this purpose.

- Nutritional assessment of any intended nutritional modifications, also taking into account the consumption figures for animal-derived products in specific population groups.

- Other factors, such as the propensity to accumulate toxic chemicals and the recommended avoidance of the use of antibiotic resistance selection genes<sup>[71]</sup>.

Extending on this Codex document by Codex Alimentarius is the guidance document published by the EFSA Panel of experts on GM organisms (EFSA GMO Panel) jointly with that of the panel on animal health and welfare. This guidance document provides more detailed requirements for the various items and a number of additional items to consider as follows.

- The types of samples (edible tissues and products) to be taken from the different species for compositional analysis.

- More specifics on the statistical analysis, including a test of difference and possibly also an equivalence test (if reference animal data can be included). In addition, it is recognized that the testing may also involve additional comparators and different environments if the purpose of the modification is to substantially alter the nature of the GM animal or expand its potential cultivation environment.

- The possibility of conducting whole-food feeding studies not only in laboratory rodents but also in other species of interest such as livestock target species.

- Animal health and welfare studies: more details and an expanded set of possible parameters are provided, beyond growth and reproduction, such as immune function, welfare, and pre-birth parameters.

- Allergenicity studies in possible changes in the *intrinsic* allergen repertoire of the recipient animals<sup>[72]</sup>.

Particularly important are the health and welfare of the animal since this is also considered to be an essential indicator of potential safety issues for products derived from these animals, besides the well-being of the animal itself. Similar to what is done for the comparison of compositional characteristics, information on the bandwidth of health and welfare-related characteristics in normal populations of non-

GM animal breeds serves as background information on what is considered permissible for new GM breeds. Reproductive parameters in particular are considered to be important indicators of the health and physiology of the animals. Moreover, monitoring the vaccination response may provide insights into the immune function of the animal. Other critical indicators of health issues such as feed intake, performance, and disease incidence, may likewise be included in a comparative safety assessment. These parameters are to be monitored throughout the various life stages of the GM animals and their comparators, also including the prenatal stages and birthweights, for example. In addition, testing will successively go through the laboratory, experimental farm and field trial stages, with increasing numbers of animals and the possibility of testing animals under real-life conditions. This way, the experimental design and statistical procedures can be applied so that they will specifically assess the impacts of the genetic modification, even when they occur at low frequency<sup>[72]</sup>.

The assessment of animal welfare will generally focus on the ability of the animal to display its natural behavior and development, and it is important to use multiple indicators in the comparative analysis as the absence of an effect on one indicator does not necessarily imply that the welfare is good. Decisions on which indicators to use should be decided on a case-by-case basis and can be guided by preliminary tests conducted during the laboratory phase. A range of different conditions that the animals may encounter during commercial practice should be covered such as climate, housing and management, and disease-causing agents. If negative effects are noted at an early stage, a decision needs to be made as to whether it is still permissible to proceed with testing to the next stage<sup>[72]</sup>.

In summary, the comparative approach commonly applied to GM plants is also applicable to animals, although specific circumstances apply, making it more difficult, for example, to perform comparative analyses in multiple locations with large sets of reference animals.

## 6 Detection and traceability of products derived from transgenic and gene-edited cattle

In cattle breeding there is a long-standing practice of animal identification and recording, primarily for breeding purposes and to maintain pedigree details. These systems have been further developed over the last century and have seen further global standardization in recent years. In 2016 the FAO published their guidance on the development of integrated multipurpose animal recording systems to ensure global compatibility of animal registration systems<sup>[73]</sup>. In these guidelines, however, the application of cloning or modern biotechnological techniques and related traceability aspects are not covered because regulations related to these aspects differ among countries.

There are currently no cost-effective methods to screen for or identify (products derived from) cloned animals<sup>[51,74]</sup>. Traceability, when required, will depend primarily on documentary control. However, more openness and standardization in supply chains will be required for this strategy to be effective that may include products from cloned animals or their progeny.

The situation regarding GM animals is different. If GM animals are to be notified for market approval in the EU, applicants must provide an analytical method that will be able to specifically identify the animal line for which authorization is sought. While this may not be the case in other jurisdictions, the anticipated market introduction of AquAdvantage salmon in North America, where it already received market approval<sup>[2]</sup>, shows the need for such detection methods among private sector parties, including producers of salmon labeled as “certified non-GMO”<sup>[75]</sup>. Notably, the GeneScan subsidiary of the EuroFins company has developed a DNA detection method for this purpose<sup>[76]</sup>. In such cases where methods are available it will be feasible to set up screening strategies that will be able to detect derived food products.

This will not be so straightforward for the more recent category of gene-edited animals that may or may not be considered to be GM animals under the respective regulatory regimes. First of all, as the modifications may be minor it may not be possible to distinguish these genetic alterations from similar changes in animal genomes that may occur naturally over time. Secondly, as these animals may not be subject to approval systems in different parts of the world, the details of the genetic modifications may not be shared in common databases, thus severely hampering the options of effective traceability of the respective animal lines and breeds and related products in other countries where the traceability would basically be a requirement. This aspect will require further attention in the years to come, with a focus on those products that may require further assessment from a safety point of view.

## 7 Concluding remarks

Although experimental transgenic cattle have been around since the 1990s, none has yet been commercialized for food and feed production. The recent emergence of gene editing techniques may in the near future change the landscape of cattle biotechnology, as there will be more options to alter specific traits. The targeting of gene edits toward key influential genetic sequences will be facilitated by the vast amount of genomic, production and breeding data that are collected systematically for commercial dairy and beef cattle. These techniques are generally applied in strategies that include cloning steps (such as SCNT). Products of cloned animals are regulated in only some parts of the world, and this hampers effective traceability strategies as there are no methods available that can reliably distinguish products from cloned animals from similar non-cloned products.

Also, in the case of GM animals, regulations are becoming divergent, with some countries regulating all gene-edited animals as GMOs, whereas others exempt those with minor modifications. Regarding the safety of the products derived from these transgenic or gene-edited cattle, here again, regulatory requirements may differ under different legislation, but in general the health and welfare of the resulting animals will be considered important indicators of potential safety issues for the derived products, as is the case for non-GM products.

Internationally harmonized guidelines for assessing the safety of foods derived from transgenic animals have been developed by Codex Alimentarius, but these do not include aspects of animal welfare. Other organizations such as EFSA in Europe explicitly include aspects of animal health and welfare in the pre-market assessment of GM animals for food and feed purposes. In practice, gene-edited animals will pose challenges in relation to aspects of traceability in the food chain. So far, to our knowledge, no GM bovine, either transgenic or gene-edited, has reached the market, though developments indicate this may happen soon on a limited scale, such as in South America. When gene editing of livestock becomes more of a standard globally, it may need further consideration of how to effectively monitor the animal production chain for new, edited traits that may not have been assessed for aspects of safety for humans and the environment, in addition to aspects of animal welfare. Available knowledge of the respective host elite breeds will form a good basis for effective monitoring in this respect.

**Acknowledgements** This work was supported by the Ministry of Agriculture, Nature and Food Quality of the Netherlands (WOT-02-002-001/002).

**Compliance with ethics guidelines** Jan Pieter van der Berg, Gijs A. Kleter, Evy Battaglia, Martien A. M. Groenen, and Esther J. Kok declare that they have no conflicts of interest or financial conflicts to disclose.

This article is a review and does not contain any studies with human or animal subjects performed by any of the authors.

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