Status of Genome Editing in Food Animals

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@BioBeef

BLOG: https://biobee.facyulty.ucdavis.edu/
http://animalscience.ucdavis.edu/animalbiotech
Breeders have selected for desired changes to our food and companion animal populations.
Plant and animal breeders have perhaps the most compelling sustainability story of all time.
Improvement in efficiencies have been associated with inflection points enabled by new breeding methods.

US Dairy Cattle Inventory 1944; 1964 – 2019

Stocks Down (Million head; blue, left) vs. Milk Production Up (Million Tonnes; red, right)

USDA FAS Beef and Veal Production - Selected Countries Summary -
The GHG emissions associated with a glass of milk in the US today is $\frac{1}{3}$ the 1944 value.

Gene editing could be the next inflection point

“We have ways now to use gene editing to separately modify fruit size, weight, the branches that make flowers, and the amount of flowers, as well as the architecture of a plant from a compact bush to one that keeps on growing.”

Gene editing allows the introduction of double-stranded breaks at a specific sequence in the genome.
Many animal applications are disease resistance and welfare traits with no foreign DNA

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>TRAIT</th>
<th>TRAIT/GOAL</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATTLE</td>
<td>Beta-lactoglobulin gene knockout</td>
<td>Elimination of milk allergen</td>
<td>Silence gene</td>
</tr>
<tr>
<td></td>
<td>Prion protein (PRNP) knockout</td>
<td>Resistance to BSE (mad cow disease)</td>
<td>Silence gene</td>
</tr>
<tr>
<td></td>
<td>CD18 gene edit</td>
<td>Resistance to BRD (bovine respiratory disease)</td>
<td>Silence gene</td>
</tr>
<tr>
<td></td>
<td>Intraspecies POLLED allele substitution</td>
<td>No horns/welfare trait</td>
<td>Between breed allele swap</td>
</tr>
<tr>
<td></td>
<td>Intraspecies SLICK allele substitution</td>
<td>Heat tolerance</td>
<td>Between breed allele swap</td>
</tr>
<tr>
<td>GOAT</td>
<td>Prion protein gene knockout</td>
<td>Elimination of prion protein</td>
<td>Silence gene</td>
</tr>
<tr>
<td></td>
<td>Beta-lactoglobulin gene knockout</td>
<td>Elimination of milk allergen</td>
<td>Silence gene</td>
</tr>
<tr>
<td>PIG</td>
<td>CD163 gene knockout</td>
<td>PRRS Virus Resistance</td>
<td>Silence gene</td>
</tr>
<tr>
<td></td>
<td>RELA allele substitution</td>
<td>African Swine Fever Resistance</td>
<td>Interspecies allele swap</td>
</tr>
<tr>
<td></td>
<td>Knockout of sexual maturity pathway</td>
<td>No need for castration/welfare trait</td>
<td>Silence gene</td>
</tr>
<tr>
<td></td>
<td>Inactivate germline development pathway</td>
<td>Germline complementation with elite genetics</td>
<td>Silence gene</td>
</tr>
<tr>
<td>SHEEP</td>
<td>Scrapie resistance PrP allele substitution</td>
<td>Scrapie resistance</td>
<td>Between breed allele swap</td>
</tr>
<tr>
<td></td>
<td>FGF5 gene knockout</td>
<td>Increased wool length &amp; yield</td>
<td>Silence gene</td>
</tr>
<tr>
<td>CHICKEN</td>
<td>Inactivate genes required for virus infection</td>
<td>Avian influenza (bird flu) resistance</td>
<td>Silence gene</td>
</tr>
<tr>
<td></td>
<td>Identify eggs with male chickens before hatch</td>
<td>All female chicks for egg industry/welfare trait</td>
<td>Marker gene</td>
</tr>
</tbody>
</table>
Gene editing to produce Porcine Reproductive and Respiratory Syndrome (PRRS) virus resistant pigs


University of Missouri, USA
Gene editing to produce African Swine Fever resistant pigs

China’s African Swine Fever Outbreak Likely Caused by Imports From Russia

BY FRANK FANG, EPOCH TIMES
Updated: August 27, 2018


1. Scientists take a fertilised egg from a pig, then snip out the normal version of an immune gene called RELA.

2. RELA gene replaced with a version carried by African pigs, such as a warthog.

3. Embryos transferred back to mother pig using standard IVF procedures.
Gene editing to produce Tuberculosis resistant cattle

Genetic improvement (permanent, cumulative) as a solution to animal disease rather than antibiotics/chemicals
Gene Edited Polled Calves
Naturally-occurring bovine allele at polled locus

Gene Edited Polled Calves

Naturally-occurring bovine allele at polled gene

10 base pairs (p)

212 base pairs (P)

POLLED GENE
March 28th, 2018 USDA statement
No additional regulatory requirements if plants could otherwise have been developed through traditional breeding
January 18th, 2017 FDA draft guidance 187

Plans to regulate all “intentional alterations” in animals as new animal drugs

Unapproved new animal drugs cannot enter the food chain

There are more than 86.5 million genomic alterations between different breeds of cattle.

Am I regulated?

FDA CVM

Were modern molecular techniques used to intentionally introduce alterations (including nucleotide insertions, substitutions, or deletions) into the animal genome?

No → Not subject to new animal drug regulation

Yes → Subject to regulation as a new animal drug

USDA APHIS

This variety could not have been developed through traditional breeding techniques, or it is a plant pest or was developed using a plant pest

False → Not subject to regulation as a genetically engineered organism

True → Subject to regulation as a genetically engineered organism

Regulation of New Breeding Techniques (NBTs) 2015
Argentina


MINISTRY OF SCIENCE, TECHNOLOGY, INNOVATIONS AND COMMUNICATIONS

TECHNICAL OPINION No. 6125/2018

Process n°: 01259.0455811 / 2018-08
Applicant: AgroPartners Consulting
CNPJ: 24.742.277 / 0001-58
Address: Teresina Street, 57, Itu-SP. CEP 13301-400.
Subject: Consultation on the application of Normative Resolution 16 in animal products developed with innovative precision improvement techniques - TIMP
Meeting: 216th CTNBio Ordinary Meeting, held on October 10, 2018.
Decision: DEFERRED

CTNBio, after examination of the Consultation on the application of Normative Resolution 16 in animal products developed with innovative techniques of precision improvement - TIMP, concluded by deferral, in accordance with the terms of this Technical Opinion.

Within the scope of the powers established in Law 11,105 / 05 and its decree 5.591 / 05, the Commission concluded that this application complies with CTNBio standards and relevant legislation aimed at ensuring biosafety of the environment, agriculture, human and animal health.

Summary: The applicant query CTNBio about the product (bovine semen), produced from an animal (bull) generated by the application of innovative techniques set Accuracy Improvement (TIMPs), which includes the group of the New Enhancement Technology (NBTs) in light of the provisions of Law 11,105 of March 24, 2005 and of Normative Resolution No. 16 of January 15, 2018.

TECHNICAL BACKGROUND

The company AgroPartners Consulting consults with CTNBio about the product (bovine semen), produced from an animal (bull) generated with the application of a set of Innovative Techniques of Improvement of Precision (TIMPs), which integrates the group of New Technologies of (NBTs) in light of the provisions of Law 11.105 of 24 March 2005 on whether or not to be classified as a Genetically Modified Organism (GMO).

The purpose of the present consultation is to enable the use of semen of an animal (known as "Buri"), of a dairy breed and without horns (owl), thanks to the genetics of the region which determines horn formation in bovine animals in order to develop naturally occurring animals through crosses with cows in Brazil and, consequently, to use the products obtained from their descendants (meat and milk) for human consumption.

"Buri" was developed by combining Innovative Precision Enhancement Techniques (TIMP) based on homology-directed repair gene editing (HDR) using transcription activator-like effector nucleases - transcription activator-like effector nucleases (TALENs) and embryonic cloning via somatic cell nuclear transfer (SCNT) from fibroblasts selected for being homozygous for the Celtic (Fce) owl allele (which naturally determines the characteristic absence of horns in cattle).
May 29, 2018
Canada has novel product based regulations

Recombinetics formed an alliance with Semex, a Canadian-based, farmer-owned cattle genetics organization to implement a precision breeding program to introduce hornless into elite dairy cattle genetics using genome editing.
2019
Australian Office Gene Technology Regulator

10 base pairs (p)

212 base pairs (P)

POLLED GENE

- not gene technology, not GMOs, not regulated
- gene technology, GMOs, regulated

Template-guided regulated, not deletions
European High Court rules all genome edits are “GMOs”

July 25, 2018

"Organisms obtained by mutagenesis are GMOs within the meaning of the GMO Directive, in so far as the techniques and methods of mutagenesis alter the genetic material of an organism in a way that does not occur naturally. It follows that those organisms come, in principle, within the scope of the GMO Directive and are subject to the obligations laid down by that directive.

The Court states, however, that it is apparent from the GMO Directive that it does not apply to organisms obtained by means of certain mutagenesis techniques, namely those which have conventionally been used in a number of applications and have a long safety record.” (defined as before 2001)
Would gene-edited animals with no foreign DNA be subject to additional regulations in this country?

<table>
<thead>
<tr>
<th>Country</th>
<th>Additional Regulations?</th>
<th>Basis of trigger/regulation?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>No</td>
<td>Novel (foreign) DNA sequence/transgene</td>
</tr>
<tr>
<td>Australia</td>
<td>Yes</td>
<td>Use of “long” template</td>
</tr>
<tr>
<td>Brazil</td>
<td>No</td>
<td>Novel (foreign) DNA sequence/transgene</td>
</tr>
<tr>
<td>Canada</td>
<td>No</td>
<td>Trait novelty (i.e. novel product risk)</td>
</tr>
<tr>
<td>European Union</td>
<td>Yes</td>
<td>Is a GMO if used a mutagenesis technique not in existence before 2001</td>
</tr>
<tr>
<td>Japan</td>
<td>No</td>
<td>Presence of foreign genes</td>
</tr>
<tr>
<td>New Zealand</td>
<td>Yes</td>
<td>Using of in vitro technique that modifies the genes/genetic material</td>
</tr>
<tr>
<td>United States</td>
<td>Yes</td>
<td>Intentional genomic alteration from use of modern molecular techniques</td>
</tr>
</tbody>
</table>
Conclusions

• Gene Editing offers an approach to precisely knock out undesirable traits and precisely introgress desirable traits in food animal breeding programs.

• It opens up new opportunities for animal breeders to address critical problems such as disease resistance, animal welfare and resilience, and product quality traits.

• Currently there are a patchwork of proposed regulatory approaches for the use of gene editing of food animal species which will potentially result in trade disruptions.

• Harmonizing the regulations associated with gene editing in food species is imperative to allow both plant and animal breeders access to gene editing tools to introduce useful sustainability traits like disease resistance, climate adaptability, and food quality attributes into global agricultural breeding programs.
Questions?

My laboratory receives public funding support from the National Institute of Food and Agriculture and the Biotechnology Risk Assessment Grant (BRAG) program, U.S. Department of Agriculture, under award numbers 2013-68004-20364, 2015-67015-23316, 2015-33522-24106 and 2017-33522-27097.