Gene Editing in Food Animals: Perspectives and Policy

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BLOG:https://biobeef.faculty.ucdavis.edu/

http://animalscience.ucdavis.edu/animalbiotech

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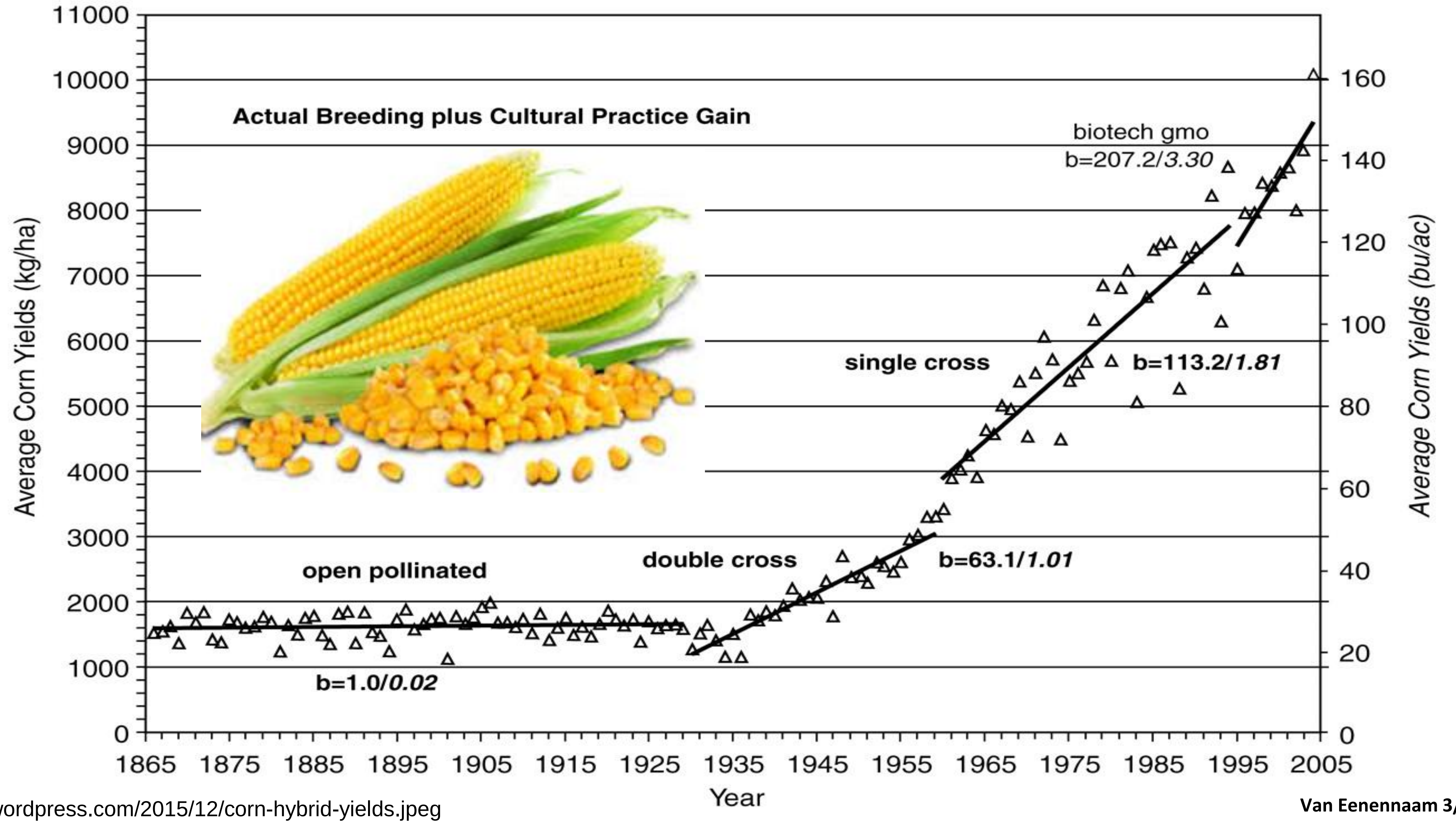
> 86.5 million genomic alterations (SNPs; Indels) between different breeds of cattle

1000 Bull Genomes Project: International consortium resequenced 2703 bulls of many different cattle breeds to 11x fold coverage

Hayes, B. J. & Daetwyler, H. D. 2018. 1000 Bull Genomes Project to Map Simple and Complex Genetic Traits in Cattle: Applications and Outcomes. Annual Review of Animal Biosciences 7:1.

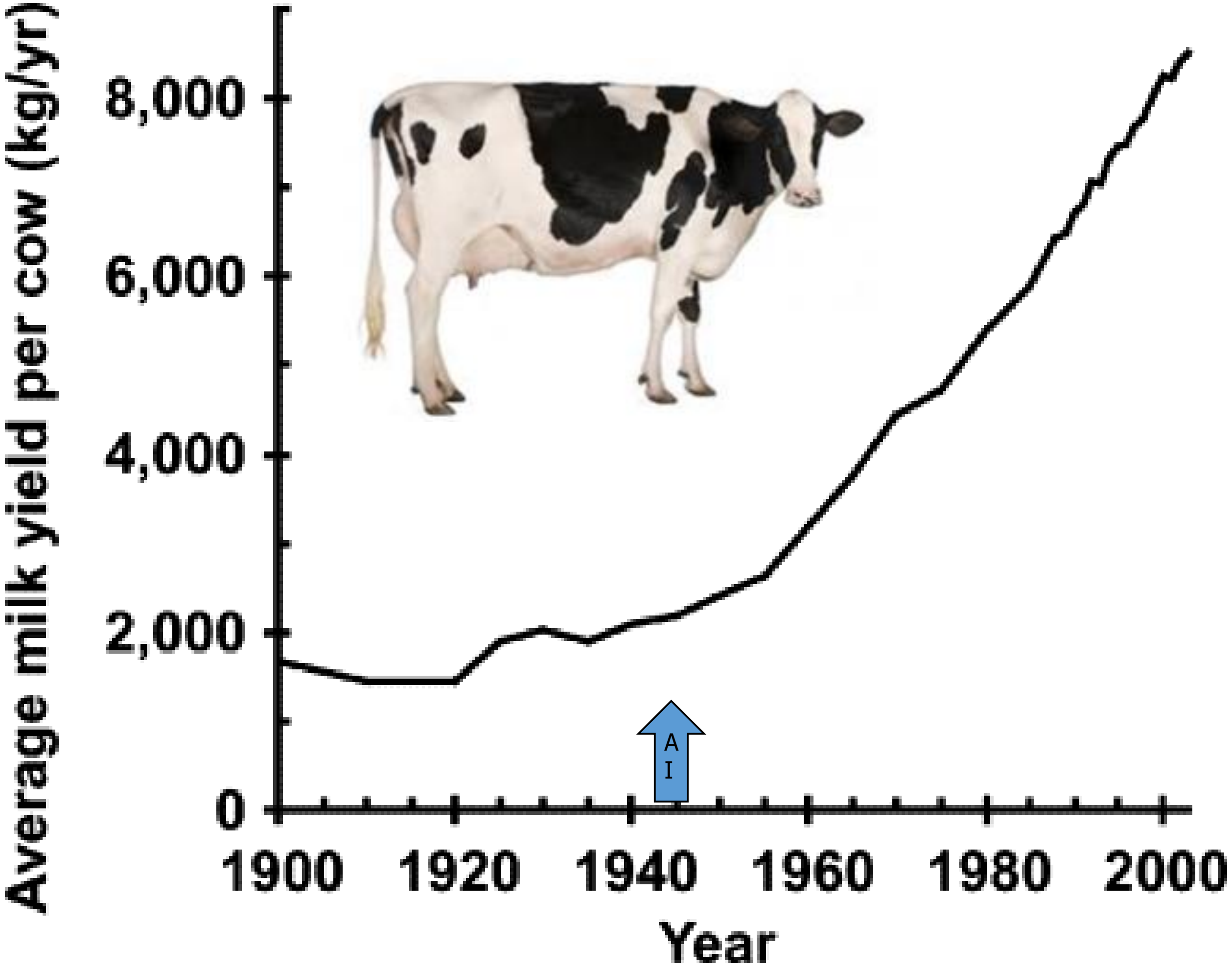
Breeders have selected for desired changes to our food and companion animal populations

Plant and animal breeders have perhaps the most compelling sustainability story of all time



https://grist.files.wordpress.com/2015/12/corn-hybrid-yields.jpeg

Improvement in efficiencies have been associated with inflection points enabled by new breeding methods



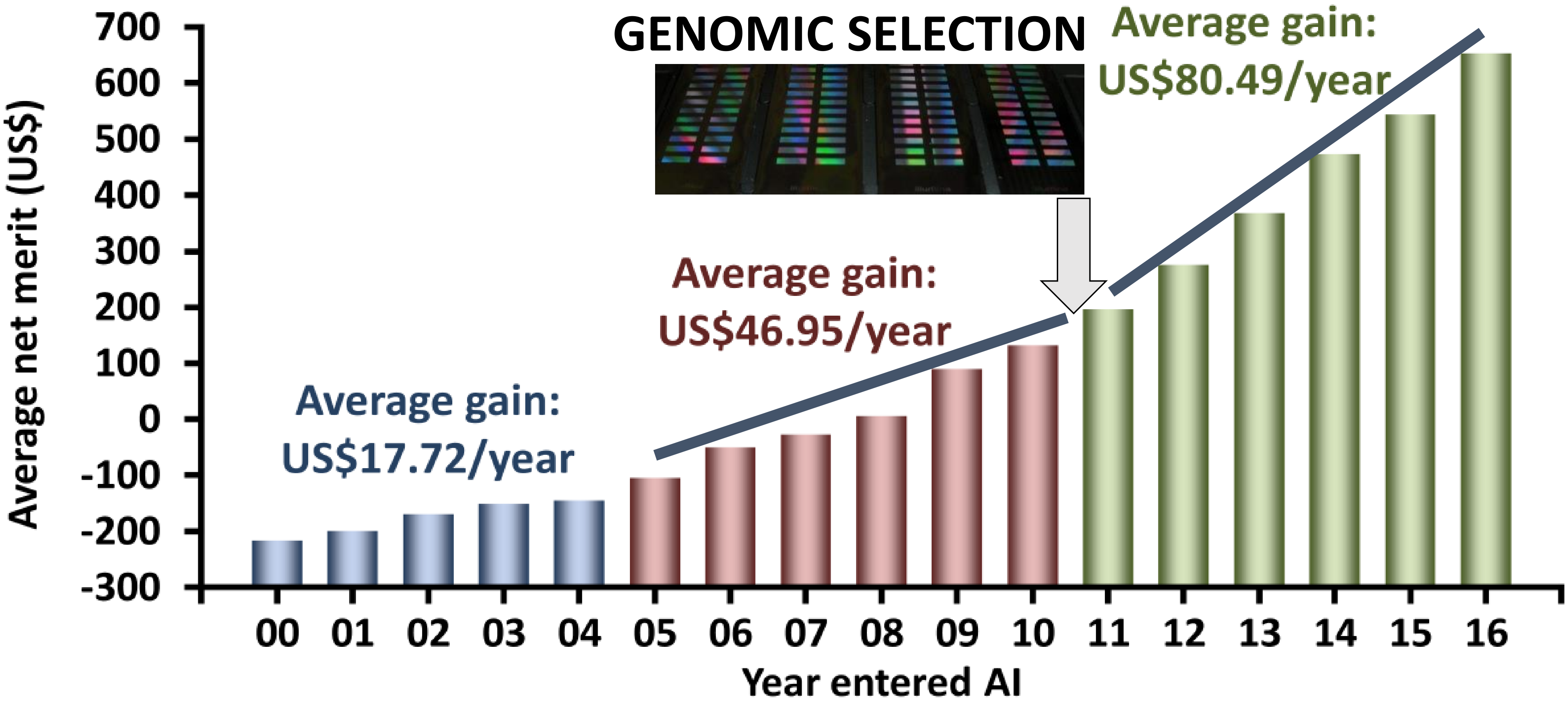
VandeHaar, M.J. and St-Pierre, N. (2006). Major Advances in Nutrition: Relevance to the Sustainability of the Dairy Industry. *Journal of Dairy Science* 89, 1280-1291.

The rate of genetic gain depends upon the four components of the breeders’ equation

$$\textbf{Genetic change per year} = \frac{\textbf{(Accuracy x Intensity x Genetic Variation)}}{\textbf{Generation Interval}}$$

Accuracy = how certain we are about an animal’s true genetic merit
Intensity of selection = fraction of animals selected as parents
Genetic variation = variation available in the population
Generation interval = time between generations

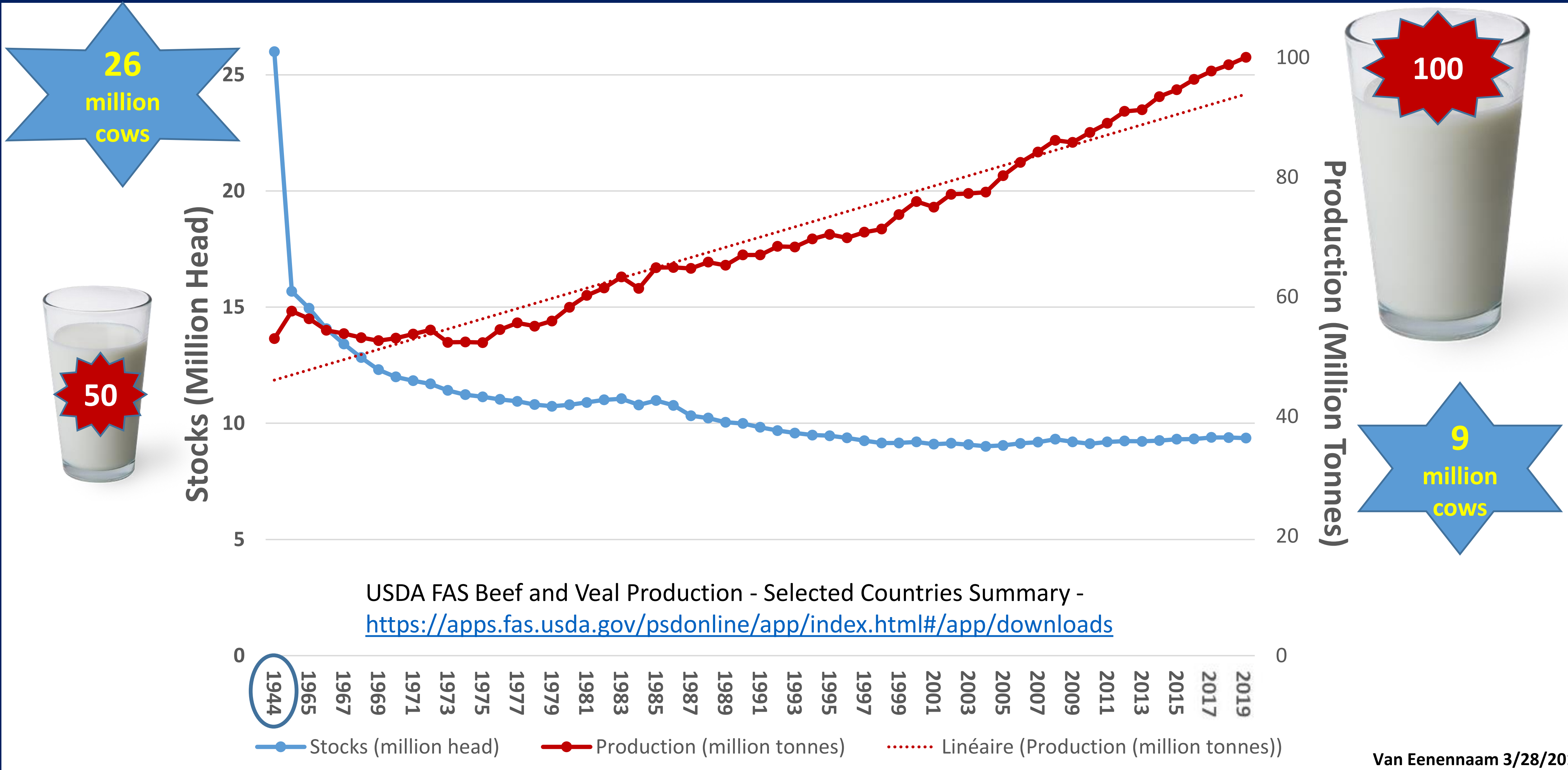
Rate of genetic gain doubled in marketed Holstein bulls since 50,000 (50K) SNP chip introduction in 2019



http://www.farms.com/news/two-million-genotypes-in-u-s-dairy-database-125448.aspx

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 - https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads

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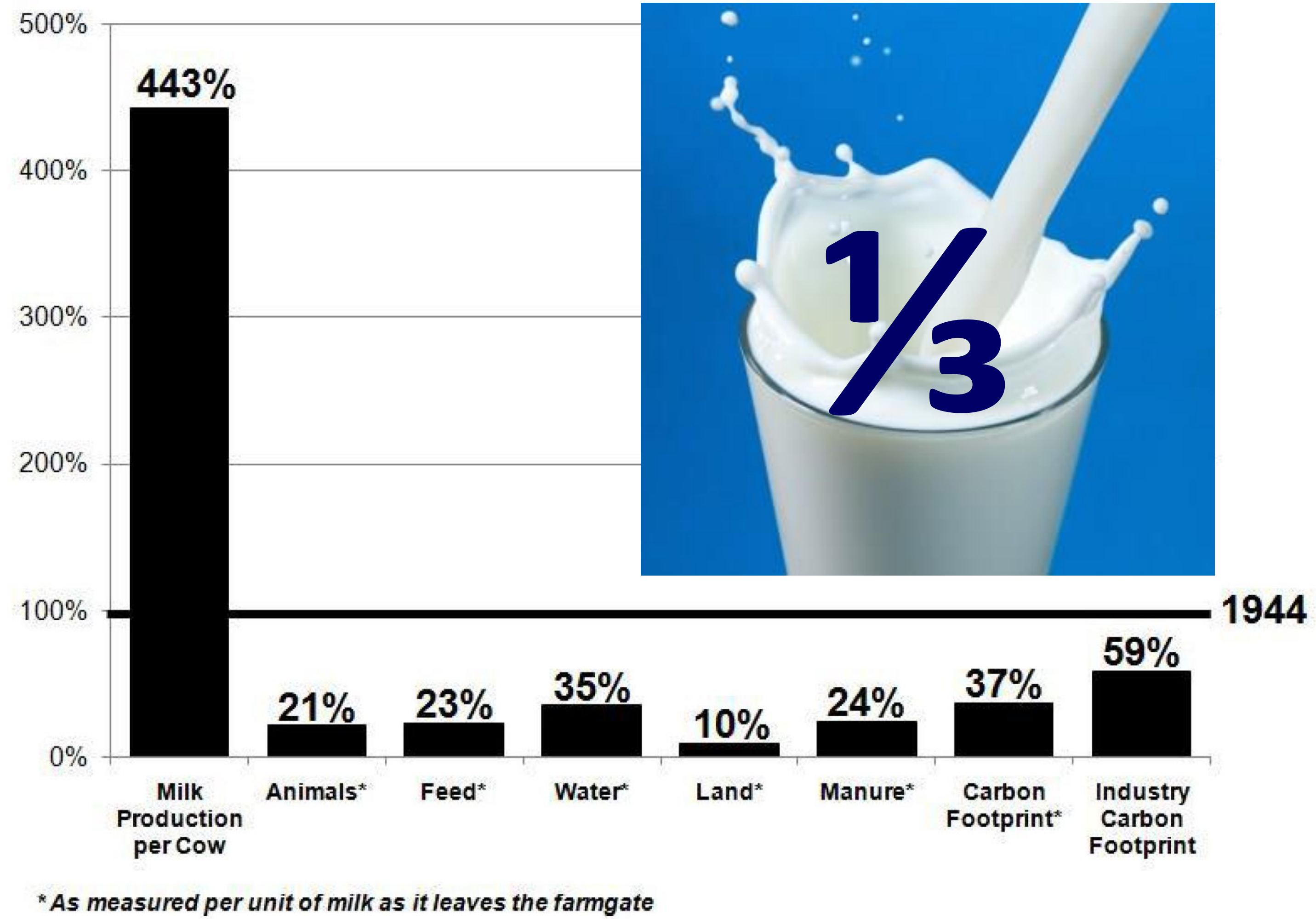
The GHG emissions associated with a glass of milk in the US today is ⅓ the 1944 value



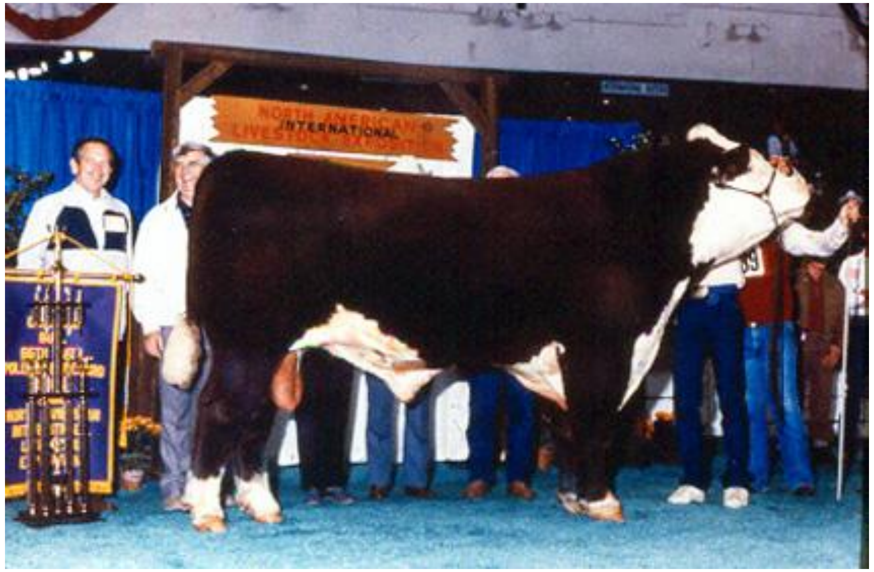
Capper, JL and DE Bauman, 2013. Annual Review of Animal Biosciences. 1 pp. 9.1–9.21

Beef Cattle Champions 1950s vs 1980s

1953. Grand Champion Angus Female

1950. Grand Champion Steer, weighing 1025 lbs

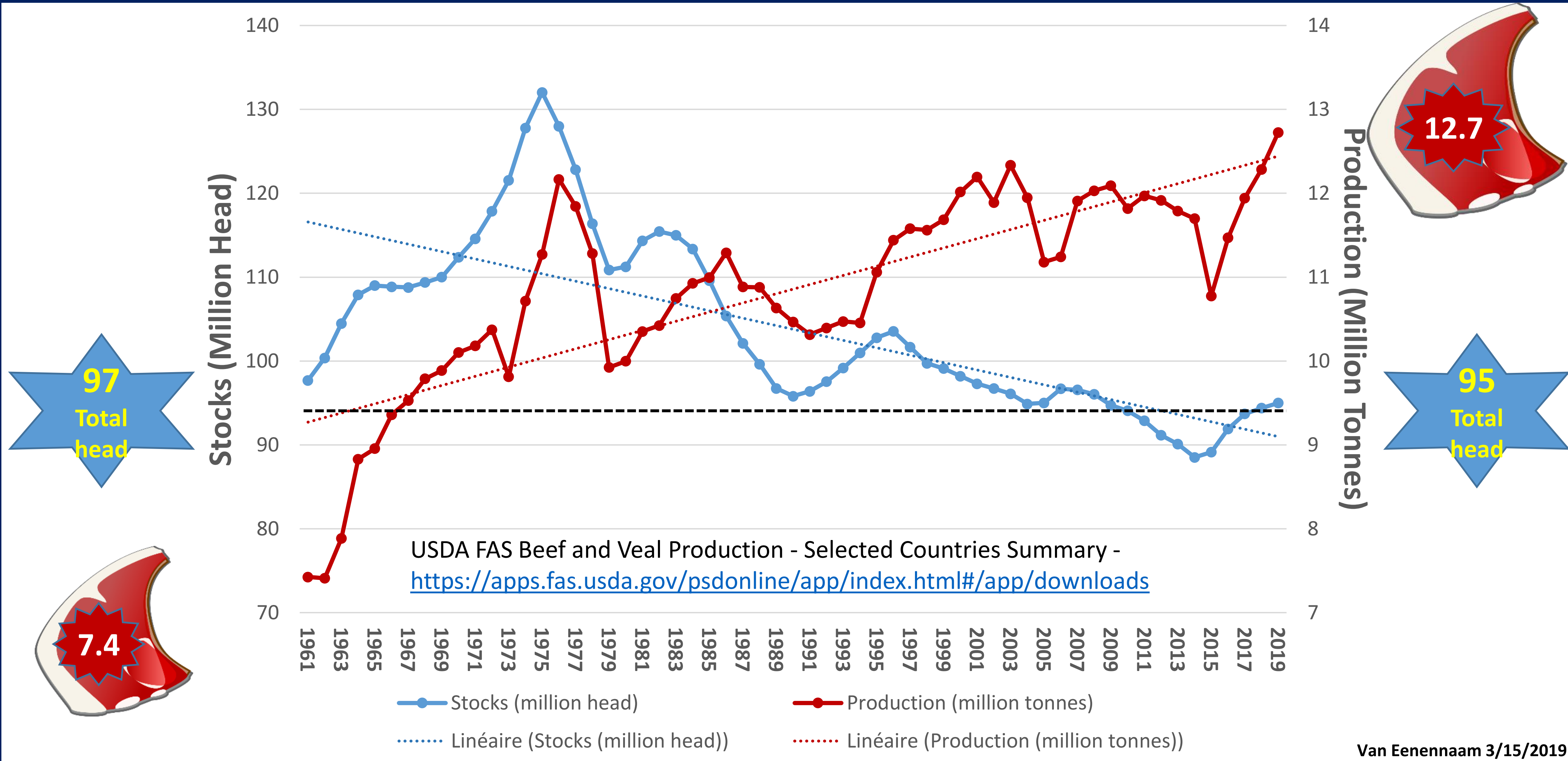
1986. Denver Champion weighing 2529 lbs



1988 Grand Champion Polled Hereford Show

US Cattle Inventory 1961 – 2019

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



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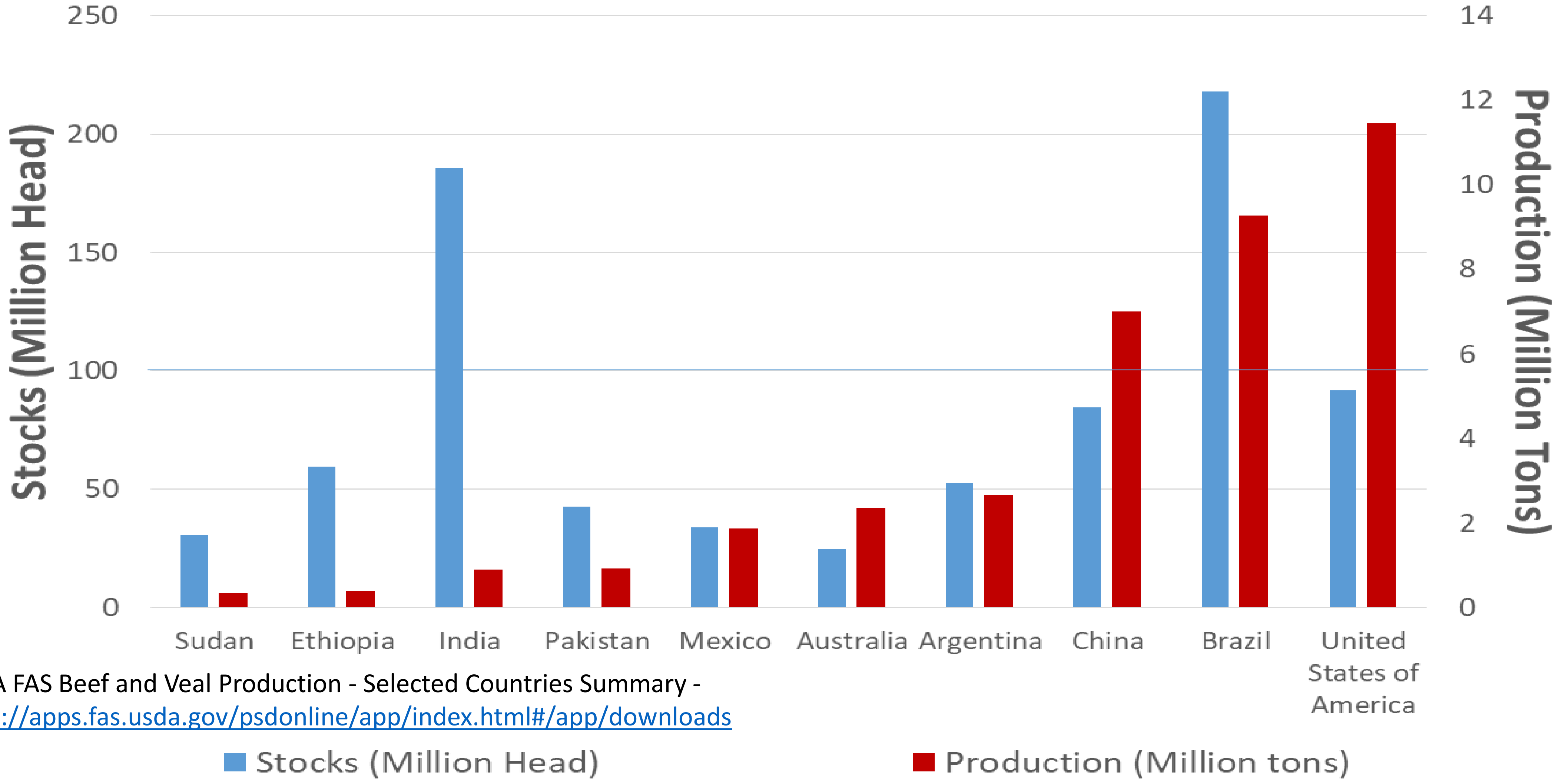
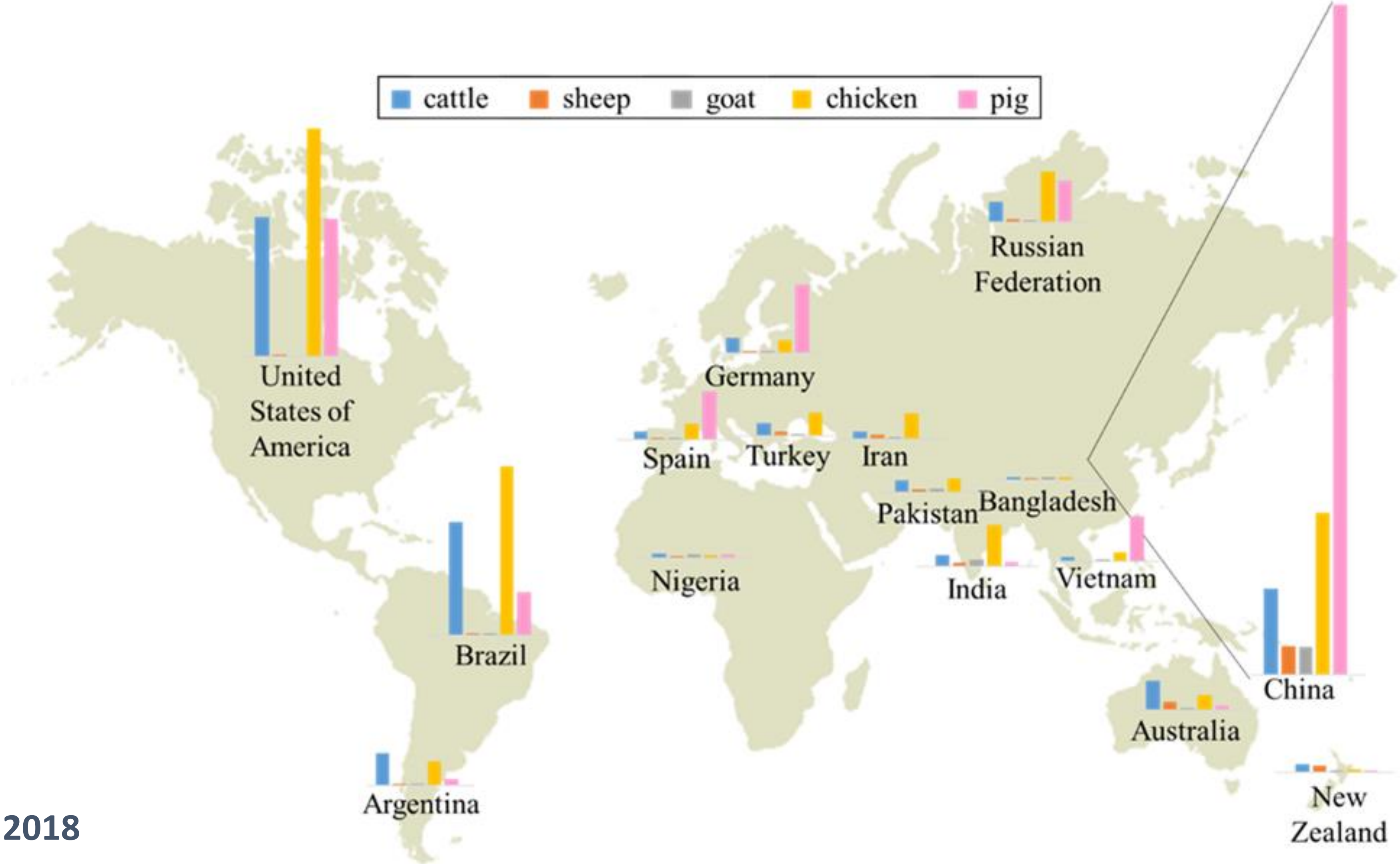
2016 Global Beef Production Numbers

Cattle numbers (Million Head; blue, left)
vs. Beef production (Million Tons; red, right)

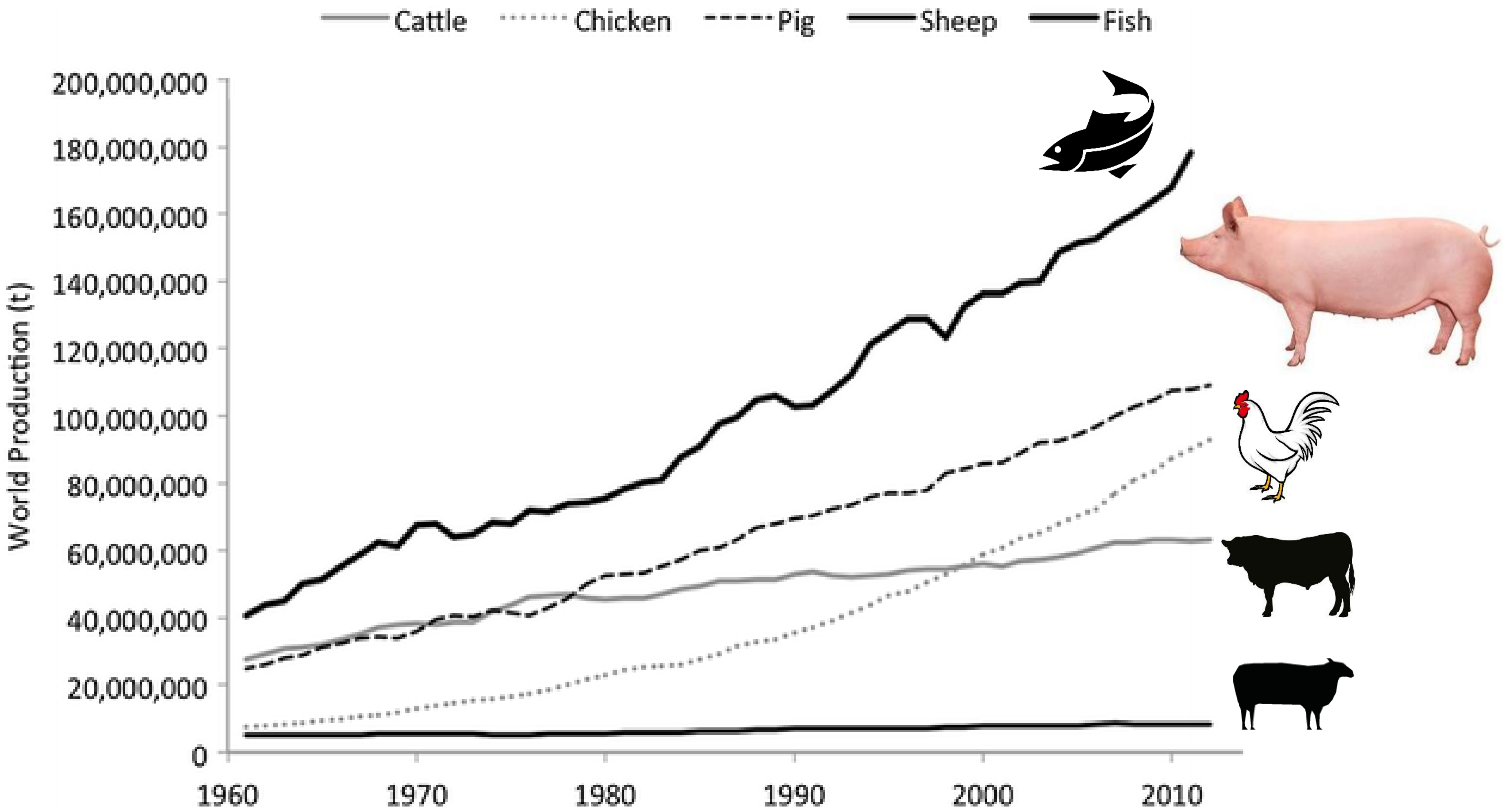


USDA FAS Beef and Veal Production - Selected Countries Summary -
https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads

Meat production by country in 2016: Top 5 producing countries for beef, chicken, pork, sheep and goat meat

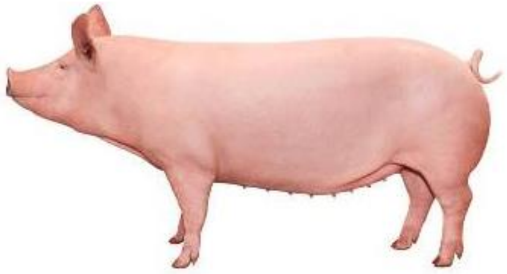


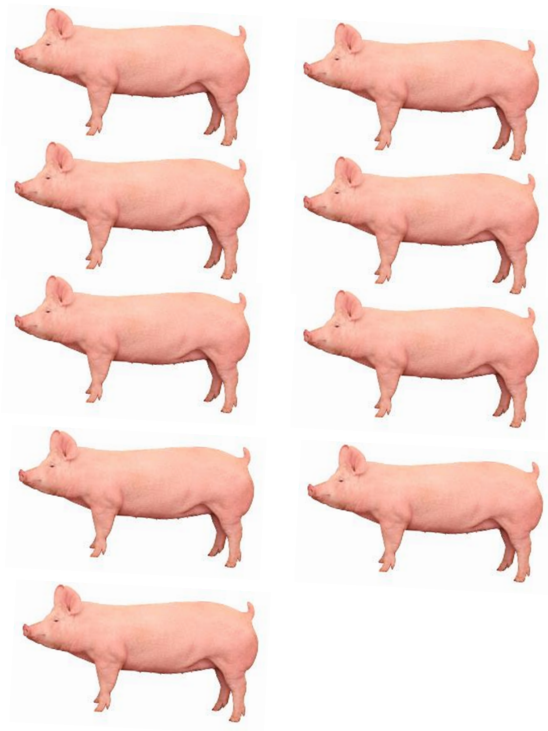
FAO, 2018



(Thornton, P.K. 2010 Livestock production: recent trends, future prospects. Philosophical Transactions of the Royal Society B: Biological Sciences 365:2853-2867).

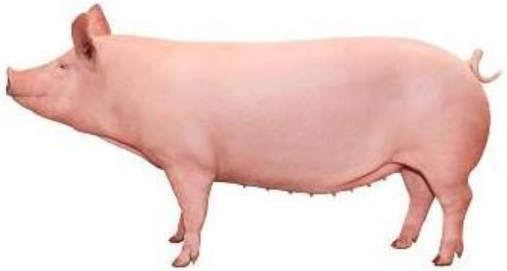
1980

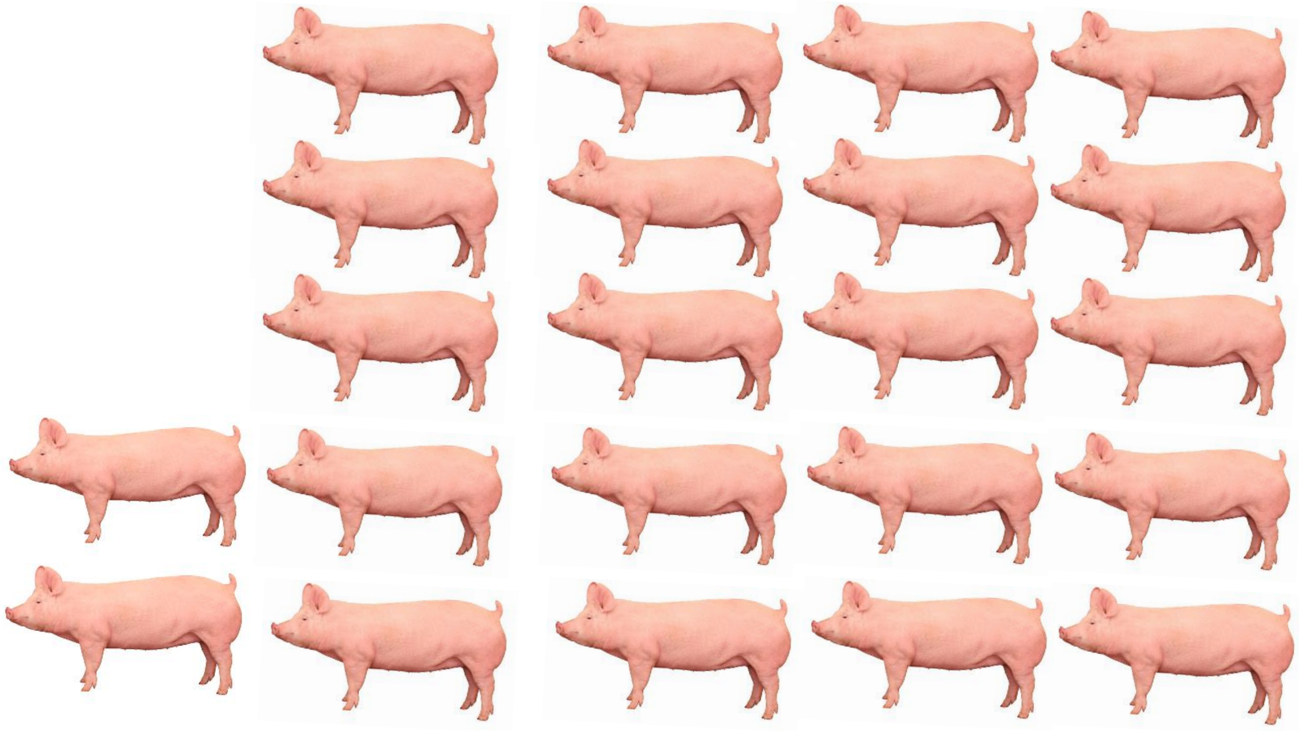




Trait	1980	2015
Feed conversion (feed/gain)	3.2	2.6
Lean meat/carcass (lb)	<80	>118
Pigs marketed/sow/year	9.2	22
Pork produced; lb/sow/year	1770	4200

2015

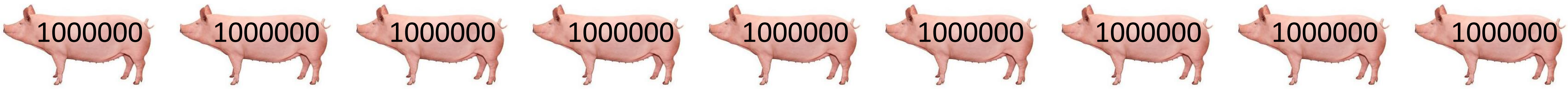




Tokach et al 2016 (Performance enhancing technologies in swine production, Animal Frontiers, doi:10.2527/af.2016-0039)

If not for pig genetic improvement in last 35 years…

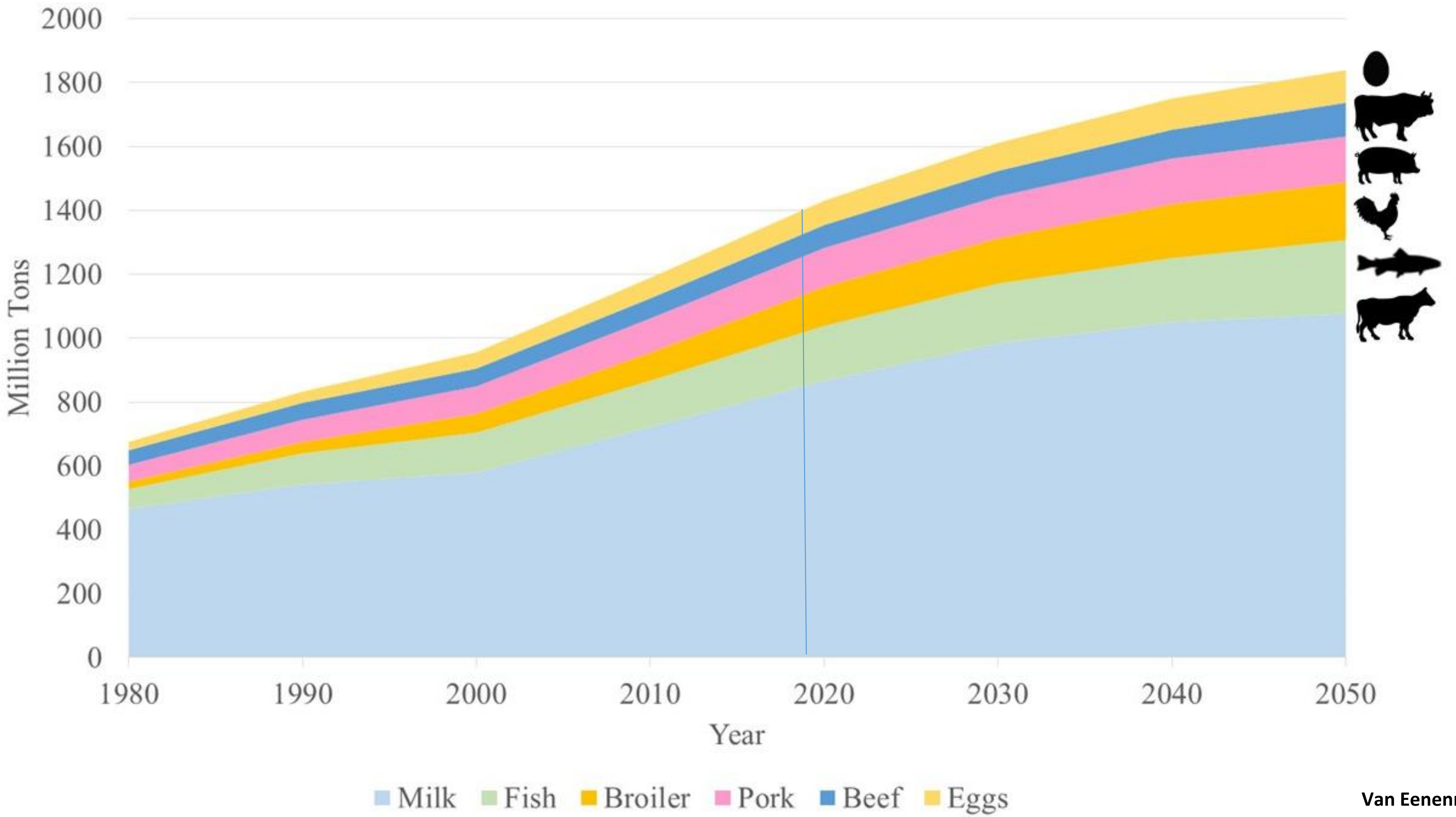
- Market pigs today require 4% less feed to produce a 17% heavier carcass than 1980
- This has allowed for a 28% increase in pork production with only a 10% increase in the annual number of animals harvested over the same time period.
- Combining increases in sow productivity & market weight, the average U.S. pig farms are producing **> 4,200 lb of live weight /sow/year**
- **Without these genetic improvements, it would take another 9 million sows (approximately 15 million in total) compared with today's 6 million sows to achieve current level of US pork production.**



Tokach et al 2016 (Performance enhancing technologies in swine production, Animal Frontiers, doi:10.2527/af.2016-0039)

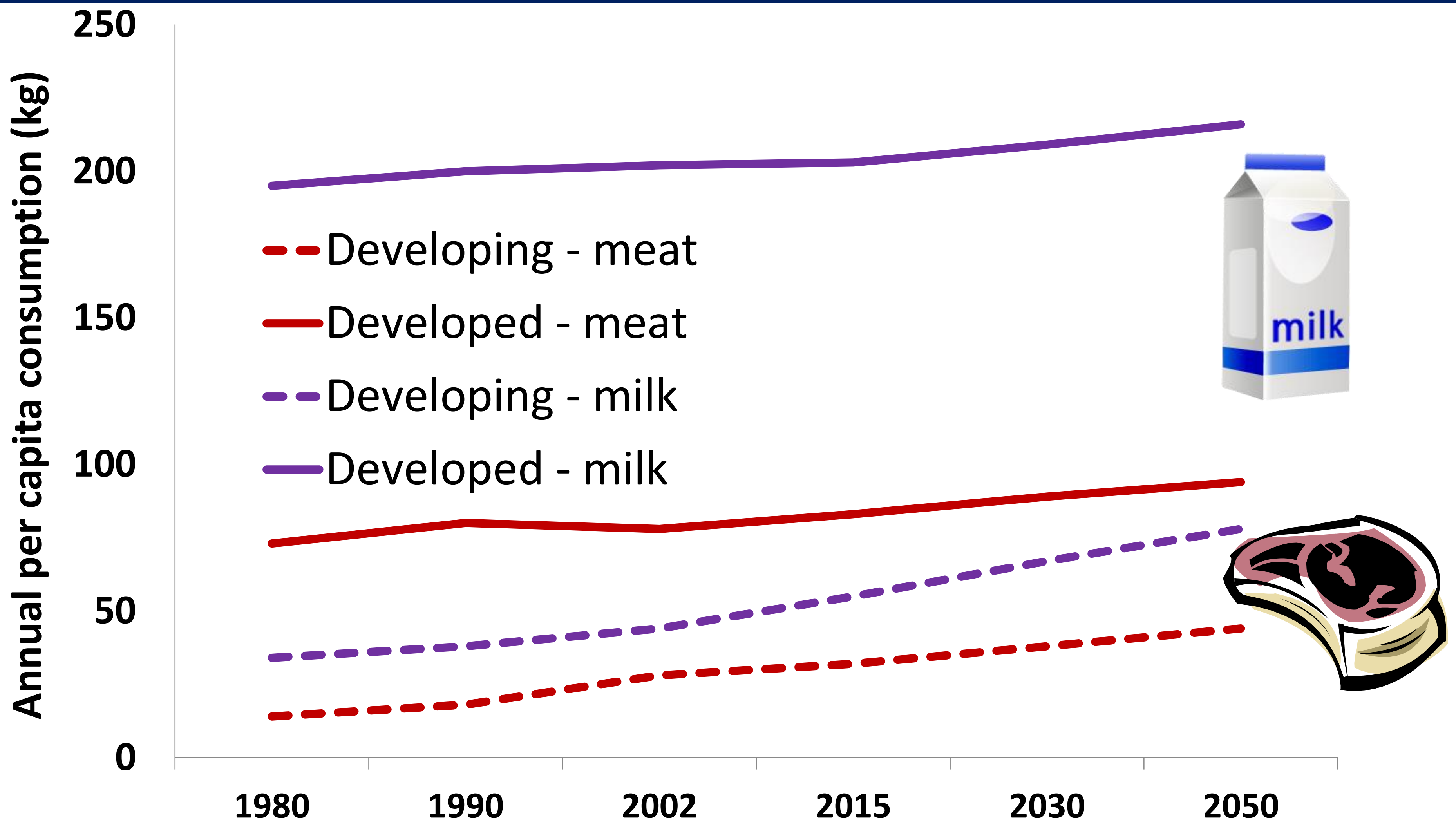
Egg, beef, pork, chicken, fish and milk production since 1980 and projected to 2050

(FAO 2018; Alexandratos and Bruinsma, 2012).



Past and projected trends in consumption of meat and milk in developing and developed countries

(Thornton, P.K. 2010 Livestock production: recent trends, future prospects. Philosophical Transactions of the Royal Society B: Biological Sciences 365:2853-2867).



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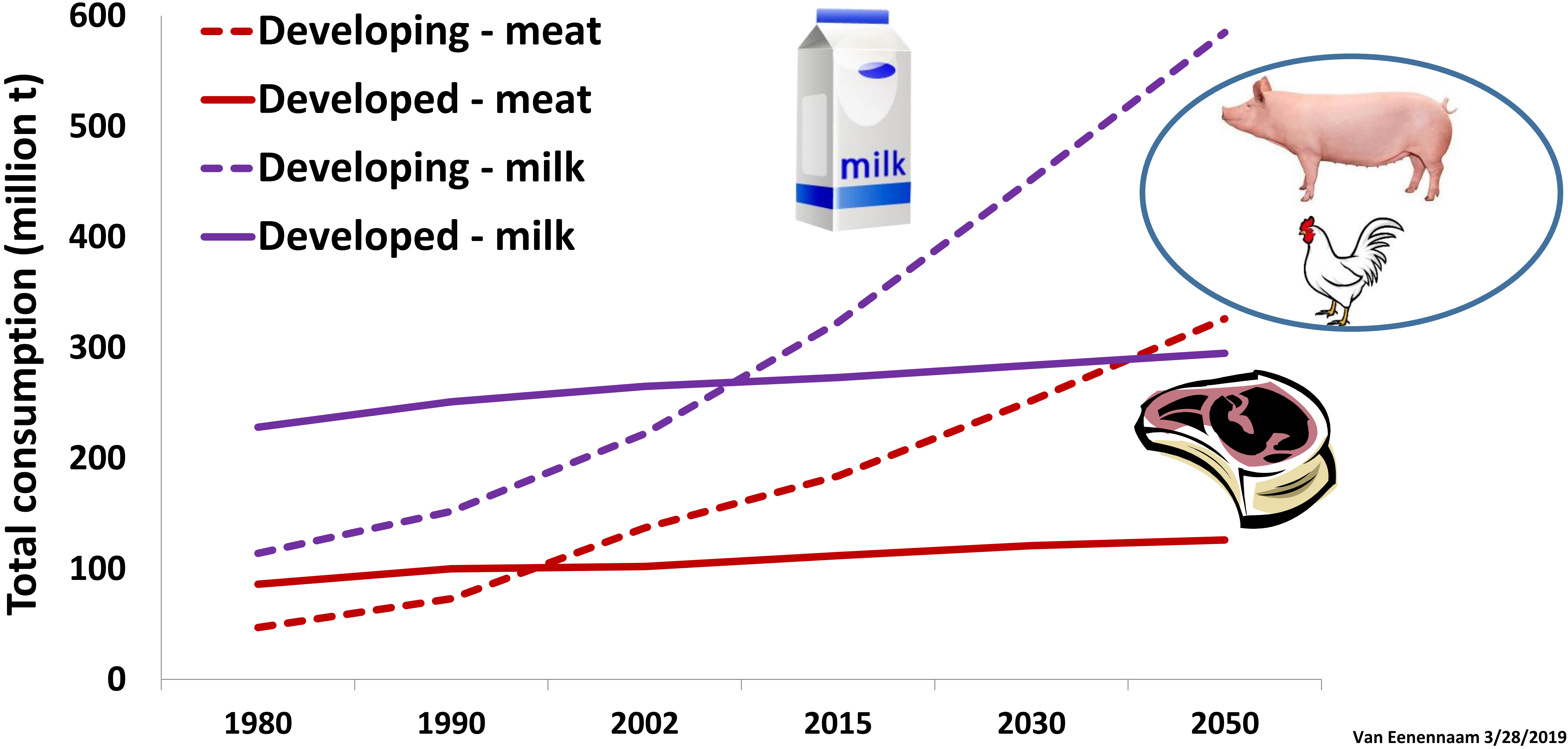


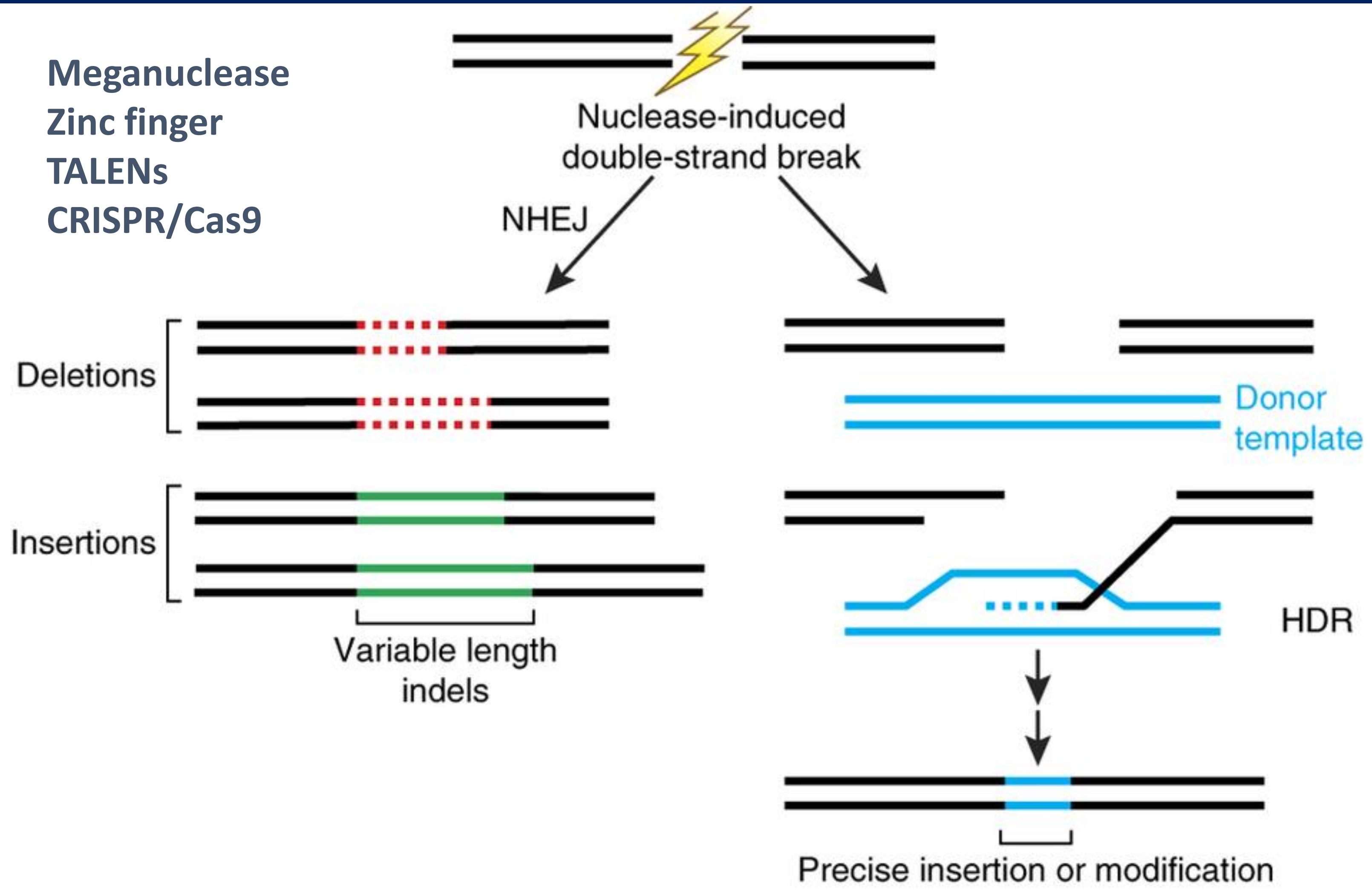
Gene editing could be the next inflection point

"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."

Rodríguez-Leal D, Lemmon ZH, Man J, Bartlett ME, Lippman ZB. **Engineering Quantitative Trait Variation for Crop Improvement by Genome Editing.** Cell. 2017 Oct 5;171(2):470-480.e8. doi: 10.1016/j.cell.2017.08.030. Epub 2017 Sep 14. PubMed PMID: 28919077.Cell.

Gene editing allows the introduction of double-stranded breaks at a specific sequence in the genome

Meganuclease
Zinc finger
TALENs
CRISPR/Cas9



Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotech* 2014;32:347-355.

Many animal applications are disease resistance and welfare traits with no foreign DNA

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

Gene editing to produce Porcine Reproductive and Respiratory Syndrome (PRRS) virus resistant pigs

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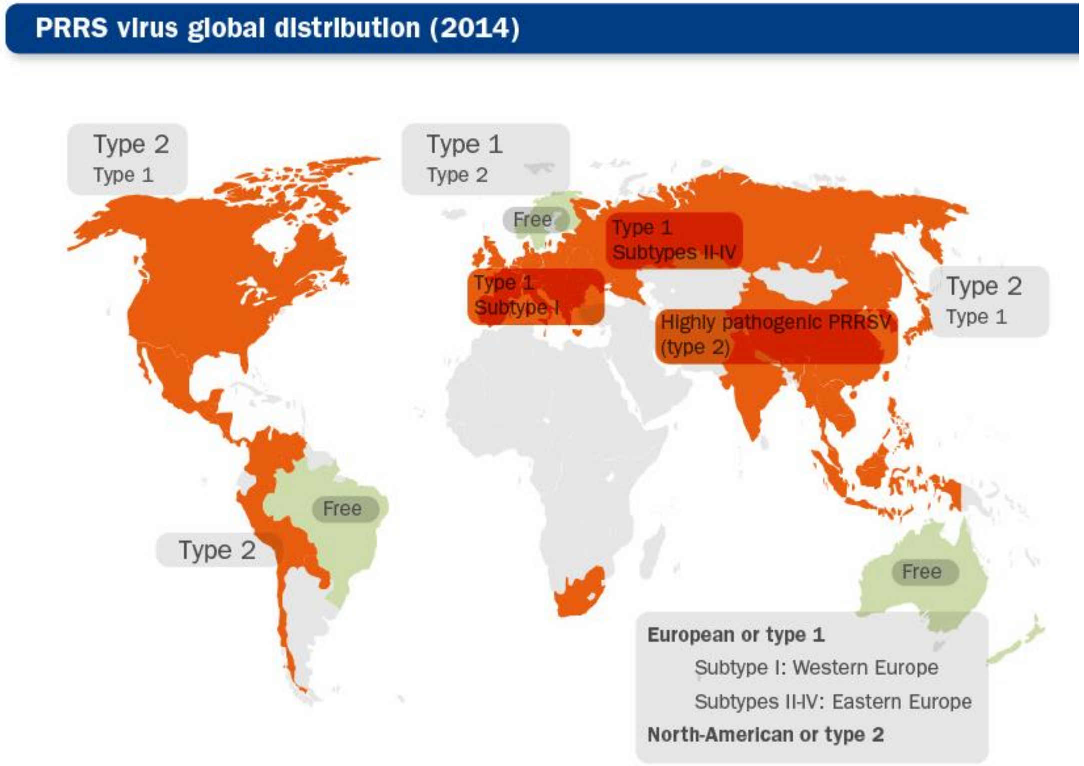
Genus breeds first pigs resistant to major infection

The genetically-enhanced porkers are a "potential game-changer" for the industry

27 | 0 | 14 | 41 | Email

Genus helps farmers breed high quality livestock by supplying them with semen from genetically superior animals Photo: EPA



Whitworth et al. 2016. **Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus (PRRSV).** Nature Biotechnology 34:20-22.
University of Missouri, USA

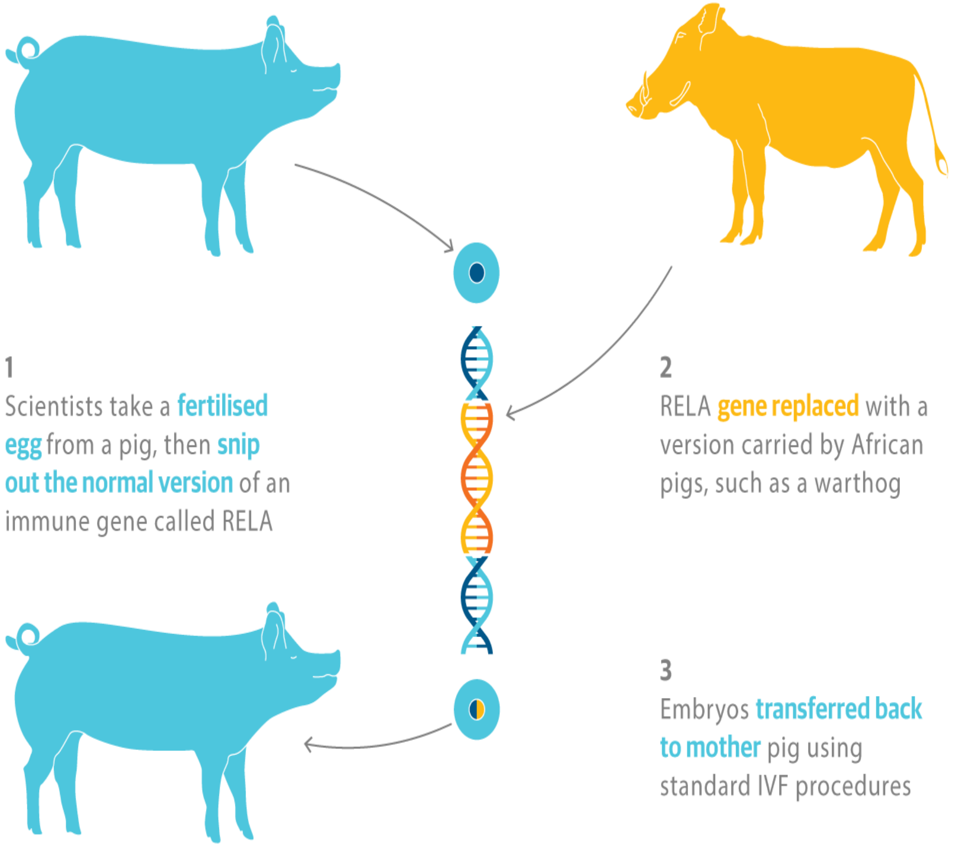
Gene editing to produce African Swine Fever resistant pigs

Pigs resting in a pen at a pig farm in Yiyang county, in China's central Henan province. On Aug.10, 2018. (GREG BAKER/AFP/Getty Images)

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

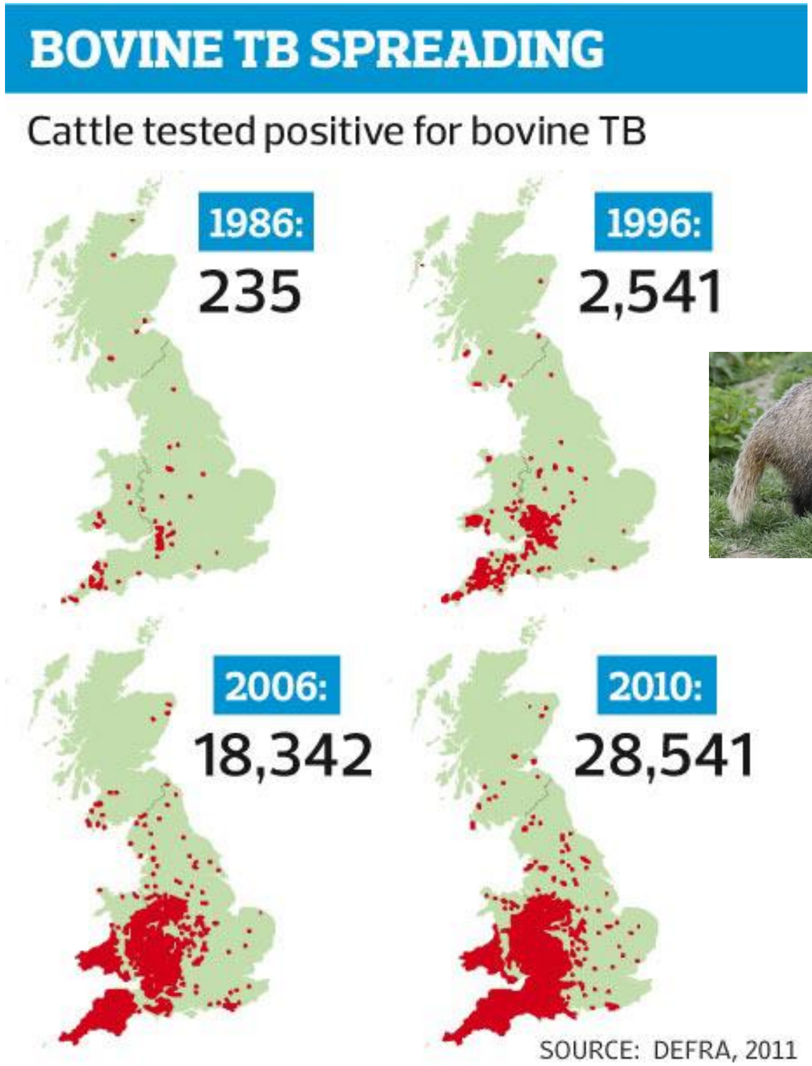
BY FRANK FANG, EPOCH TIMES

Updated: August 27, 2018



Lillico et al. 2016. **Mammalian interspecies substitution of immune modulatory alleles by genome editing**. Sci Rep 6:21645.
Roslin Institute, Scotland

Gene editing to produce Tuberculosis resistant cattle



SCIENCE TICKER GENETICS, ANIMALS, AGRICULTURE

CRISPR used in cows to help fight tuberculosis

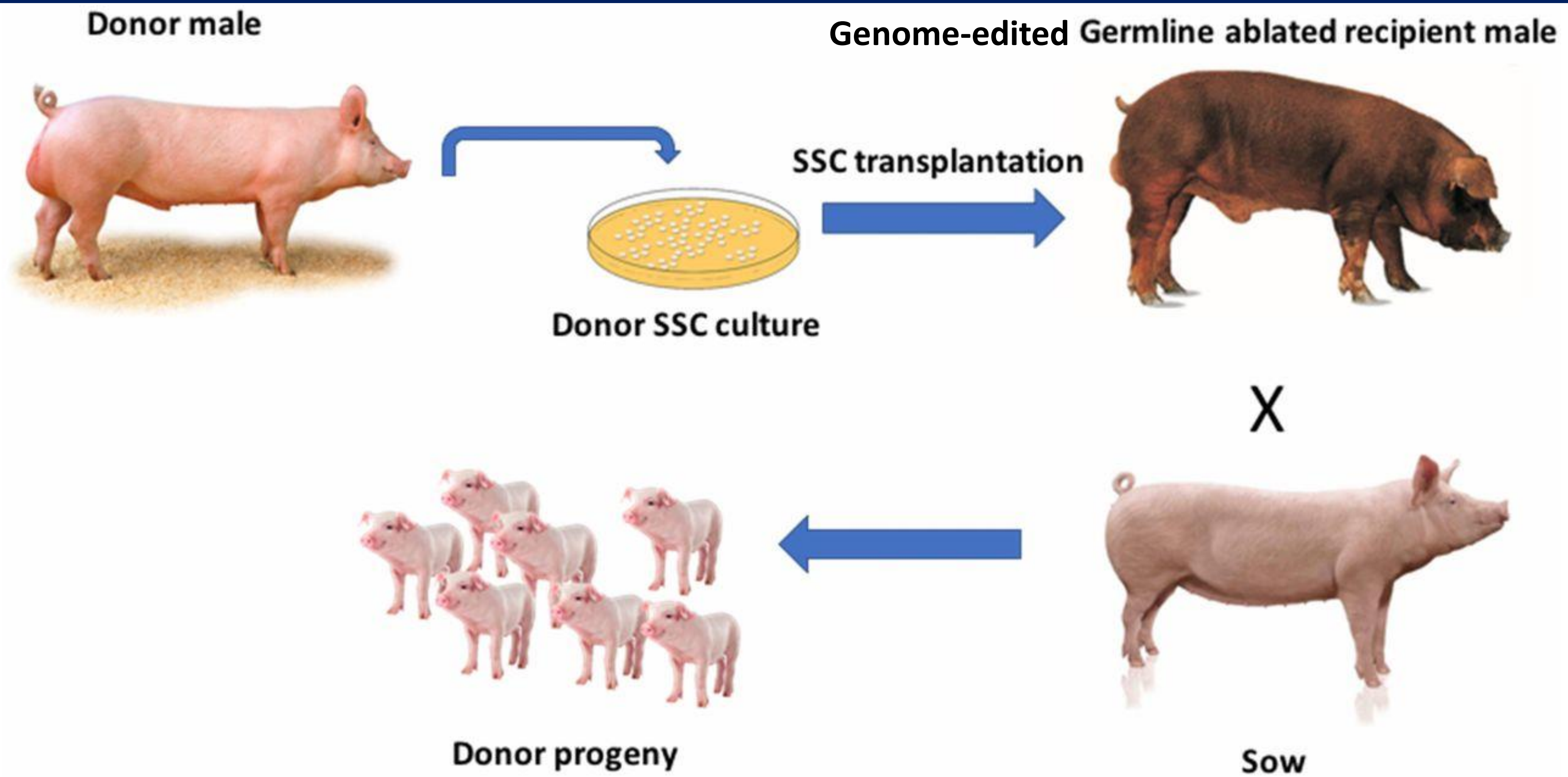
BY HELEN THOMPSON 1:00PM, FEBRUARY 3, 2017

The CRISPR/Cas 9 system could give dairy cows a protein that helps fight off bovine tuberculosis.

Gao et al. 2017. **Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects**. Genome Biol. Feb 1;18(1):13.
Northwest A&F University, Yangling, China

Genetic improvement (permanent, cumulative) as a solution to animal disease rather than antibiotics/chemicals

What if we could replace the testicles of average animals with the germ cells of the best animals in the breed?
Surrogate sire technology



Gottardo P, Gorjanc G, Battagin M, Gaynor RC, Jenko J, Ros-Freixedes R, Bruce A Whitelaw C, Mileham AJ, Herring WO, Hickey JM. 2019.
A Strategy To Exploit Surrogate Sire Technology in Livestock Breeding Programs. G3 9(1):203-215. doi: 10.1534/g3.118.200890. PubMed PMID: 30563834

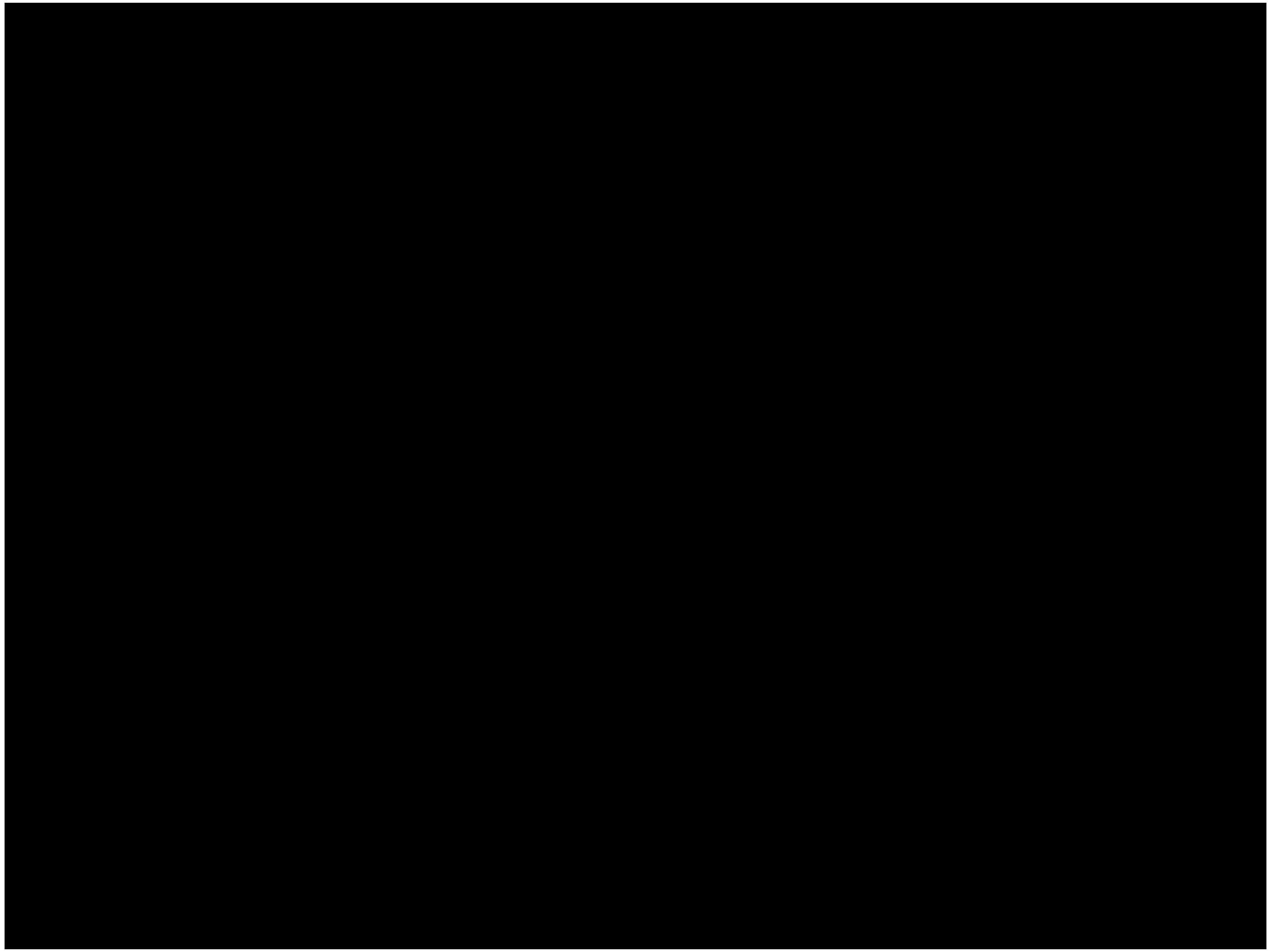
Gene Edited Polled Calves

Naturally-occurring bovine allele at polled locus

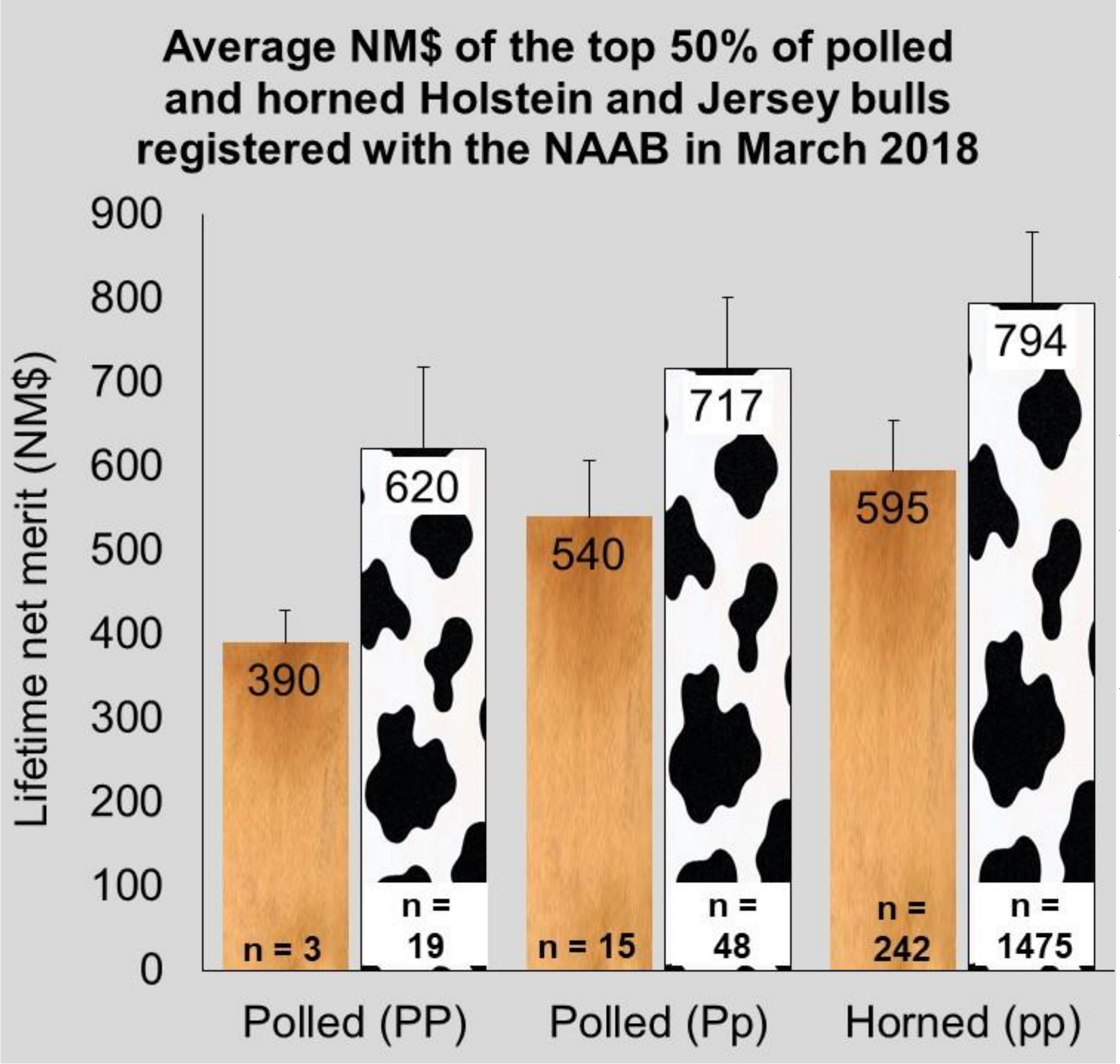
Carlson DF, Lancto CA, Zang B, Kim E-S, Walton M, et al. 2016. Production of hornless dairy cattle from genome-edited cell lines. Nat Biotech 34: 479-81

Precision breeding offers a new alternative to dehorning

YouTube: https://youtu.be/-Qks_LMmodw



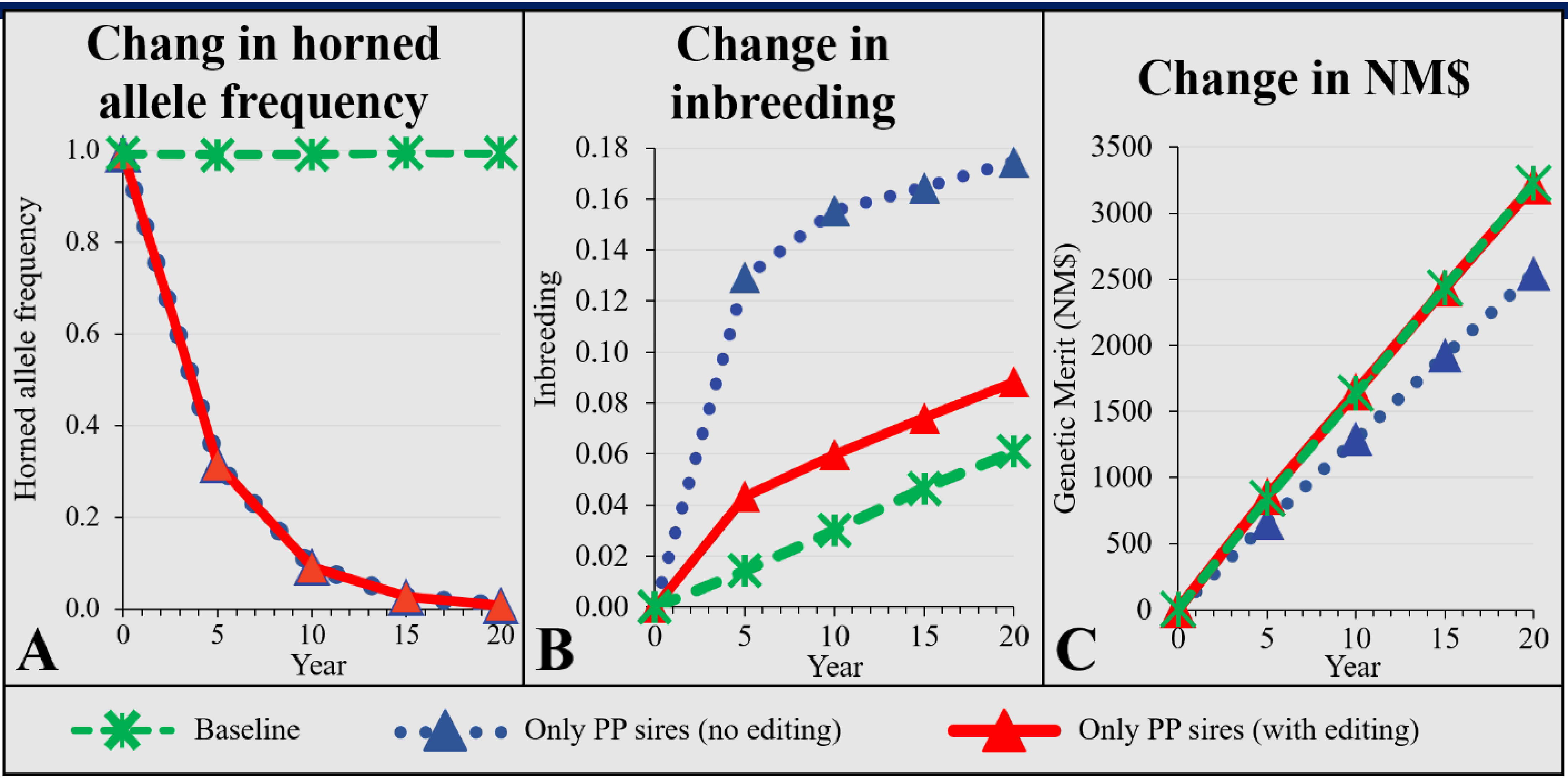
Current polled dairy sires have inferior genetic merit



- ❖Daughters of polled Holstein sires will earn less over their lifetimes
- ❖**Polled allele frequency is 0.0071**
- ❖Adding polled to selection indices is not effective
- ❖If used exclusively polled sires would increase inbreeding & decrease genetic gain

Mueller, M, J.B. Cole, T.S. Sonstegard, A.L. Van Eenennaam 2019. Comparison of gene editing versus conventional breeding to introgress the *POLLED* allele into the US dairy cattle population. Journal of Dairy Science. *In press.* https://doi.org/10.3168/jds.2018-15892

Simulation of introgression of the POLLED allele via conventional breeding versus gene editing



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Gene Edited Polled Calves

Naturally-occurring bovine allele at polled gene

10 base pairs (p)

212 base pairs (P)

POLLED GENE

Even a female cow has to get “made up” for a glamor shot!

Princess gets her 15 minutes of fame

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https://www.wired.com/story/crispr-gene-editing-humane-livestock 3/19/2019

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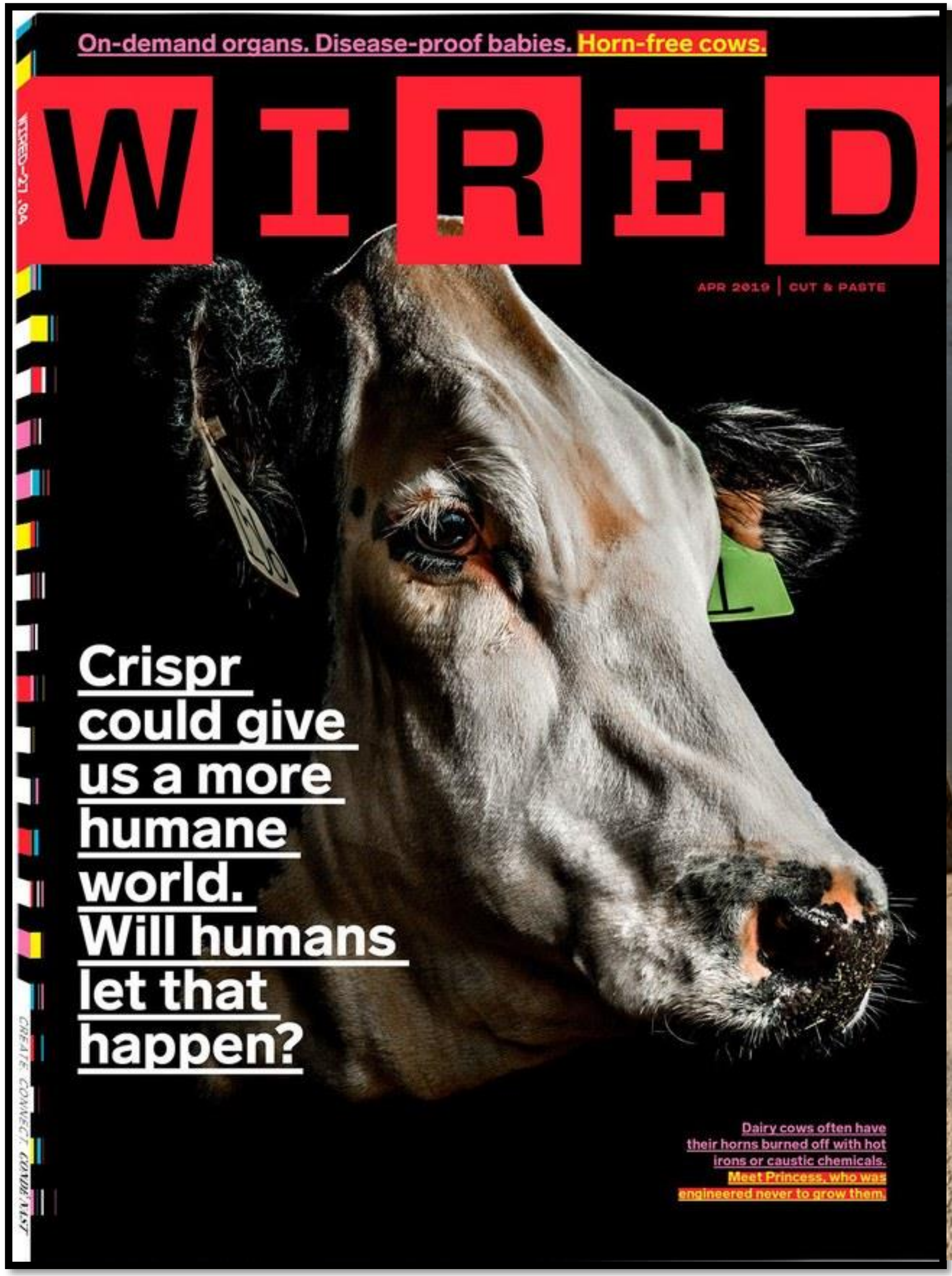
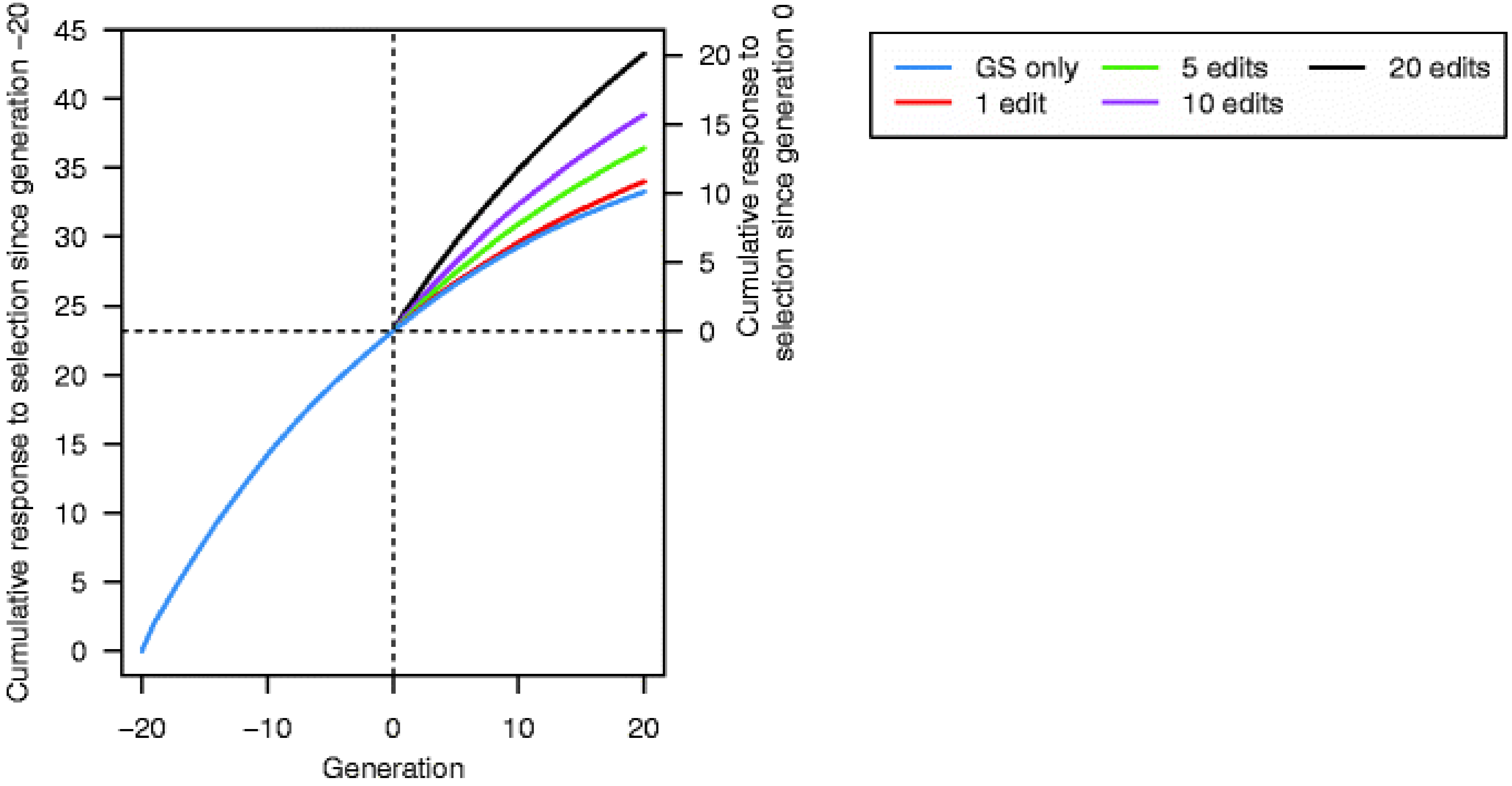


https://www.wired.com/story/crispr-gene-editing-humane-livestock 3/19/2019

Accelerated rate of gain when promoting 1-20 genome edits in genomic selection



Jenko, J. et a. 2015. Potential of promotion of alleles by genome editing to improve quantitative traits in livestock breeding programs. Genetics Selection Evolution 47: 1-14.

Editing as a Cherry on Top of the Breeding Sundae

It will be able to introduce useful alleles without linkage drag, and potentially bring in useful novel genetic variation from other species

Genome Editing

In vitro embryo fertilization (IVF)

Genomic Selection

Embryo Transfer

Artificial insemination

Progeny testing

Performance recording

Development of breeding goals

Association of like minded breeders

March 28th, 2018 USDA statement

No additional regulatory requirements if plants could otherwise have been developed through traditional breeding

USDA U.S. DEPARTMENT OF AGRICULTURE

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Secretary Perdue Issues USDA Statement on Plant Breeding Innovation

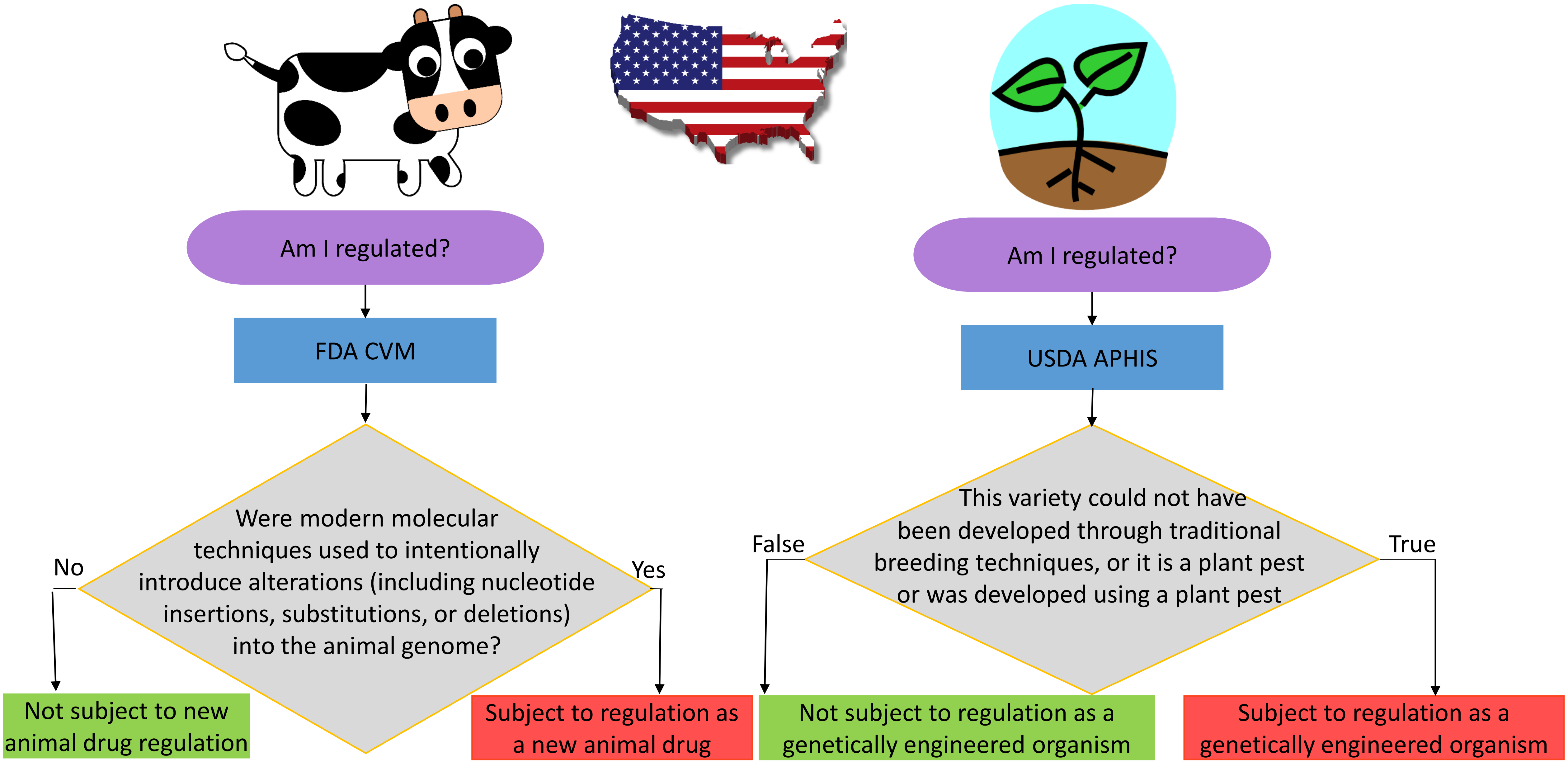
(Washington, D.C., March 28, 2018) – U.S. Secretary of Agriculture Sonny Perdue today issued a statement providing clarification on the U.S. Department of Agriculture's (USDA) oversight of plants produced through innovative new breeding techniques which include techniques called genome editing.

Press Release
Release No. 0070.18

Contact: USDA Press
Email: press@oc.usda.gov

Under its biotechnology regulations, USDA does not regulate or have any plans to regulate plants that could otherwise have been developed through traditional breeding techniques as long as they are not plant pests or developed using plant pests. This includes a set of new techniques that are increasingly being used by plant breeders to produce new plant varieties that are indistinguishable from those developed through traditional breeding methods. The newest of these methods, such as genome editing, expand traditional plant breeding tools because they can introduce new plant traits more quickly and precisely, potentially saving years or even decades in bringing needed new varieties to farmers.

January 18th, 2017 FDA draft guidance 187 considers all gene edited animals whose genomes have been "altered intentionally" to be drugs



Van Eenennaam, et al. 2019. Proposed U.S. regulation of gene-edited food animals is not fit for purpose. npj Science of Food. *In press.*

Van Eenennaam 2/15/2019

October 31, 2018

Brazil has ruled an intraspecies allele substitution not a GMO

https://sei.mctic.gov.br/sei/controlador_externo.php?acao=documento_conferir&codigo_verificador=3509624&codigo_crc=FFECC97F&hash_download=337f22c112c3227a38c8307d39e7d1c4764...

MINISTRY OF SCIENCE, TECHNOLOGY, INNOVATIONS AND COMMUNICATIONS
TECHNICAL OPINION No. 6125/2018

Process nº: 01250.045811 / 2018-98
Applicant: AgroPartners Consulting
CNPJ: 24.742.277 / 0001-58
Address : Teresina Street, 57, Itu-SP. CEP 13301-490.
Subject: Consultation on the application of Normative Resolution 16 in animal products developed with innovative precision improvement techniques - TIMP
Extract No. 6193/2018, published in the DOU on October 4, 2018.
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.

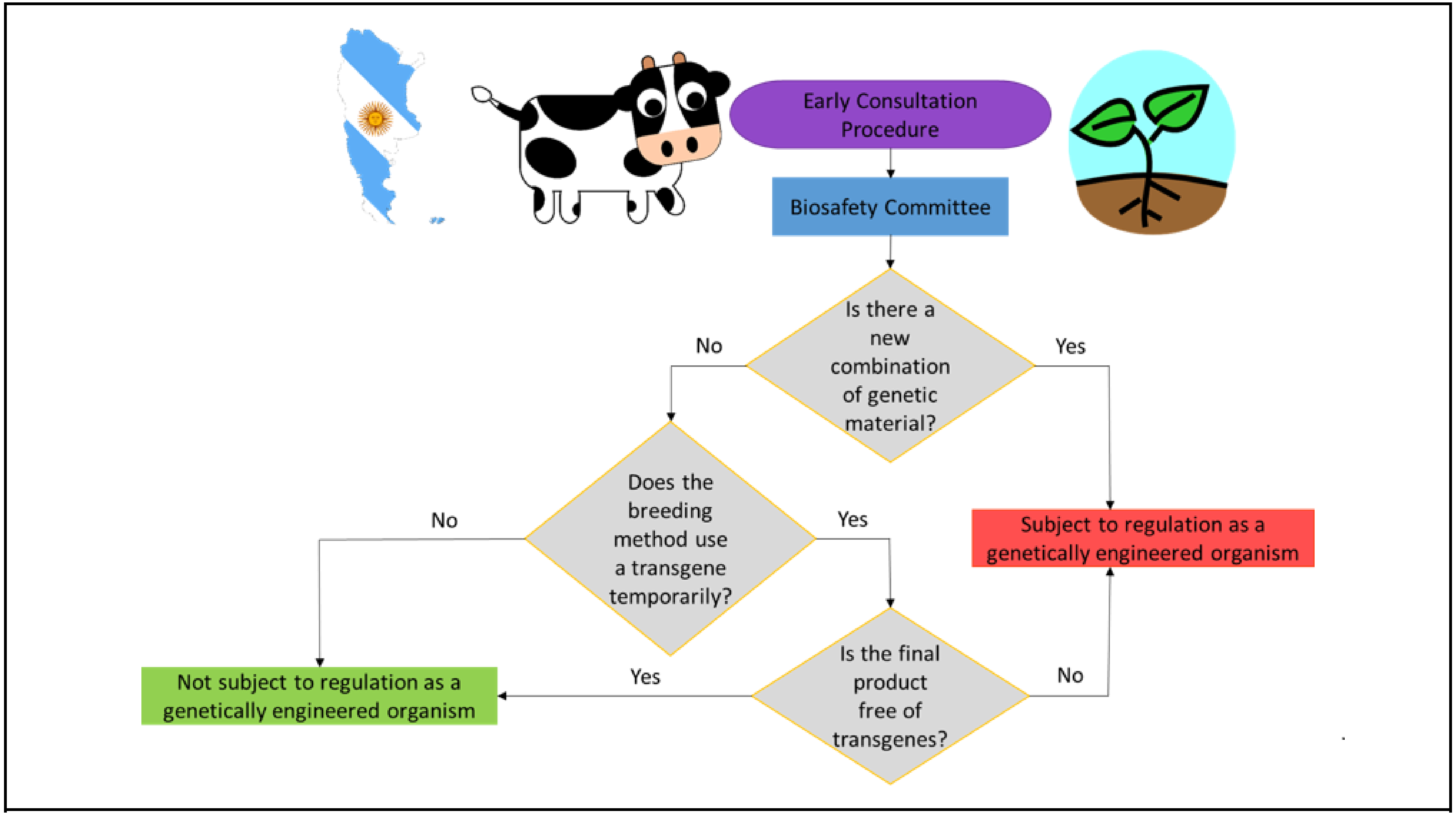
TECHNICAL BACKGROUND

The company Agro Partners 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 (Pc) owl allele (which naturally determines the characteristic absence of horns in cattle).

Regulation of New Breeding Techniques (NBTs) 2015
Argentina



Whelan AI, Lema MA. Regulatory framework for gene editing and other new breeding techniques (NBTs) in Argentina. GM Crops Food 2015;6:253–265.

Van Eenennaam Alison L. 2018. The Importance of a Novel Product Risk-Based Trigger for Gene-Editing Regulation in Food Animal Species. The CRISPR Journal. 1. https://doi.org/10.1089/crispr.2017.0023

May 29, 2018
Canada has novel product-based regulations

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Eliminating dehorning in dairy cattle

By **Other News** - June 1, 2018 504 0

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The world's first precision-bred naturally hornless cow. Recombinetics and Semex have formed an alliance to improve animal health and well-being. In the gene editing process the cell's natural repair function was used to replace the horned gene with a naturally occuring polled gene. (Submitted photo)

SAINT PAUL, Minn. — Recombinetics has formed an alliance with Semex, a Canadian-based, farmer-owned cattle genetics organization, to implement a precision breeding program that improves animal health and well-being through hornless dairy cattle genetics.

recombinetics SEMEX

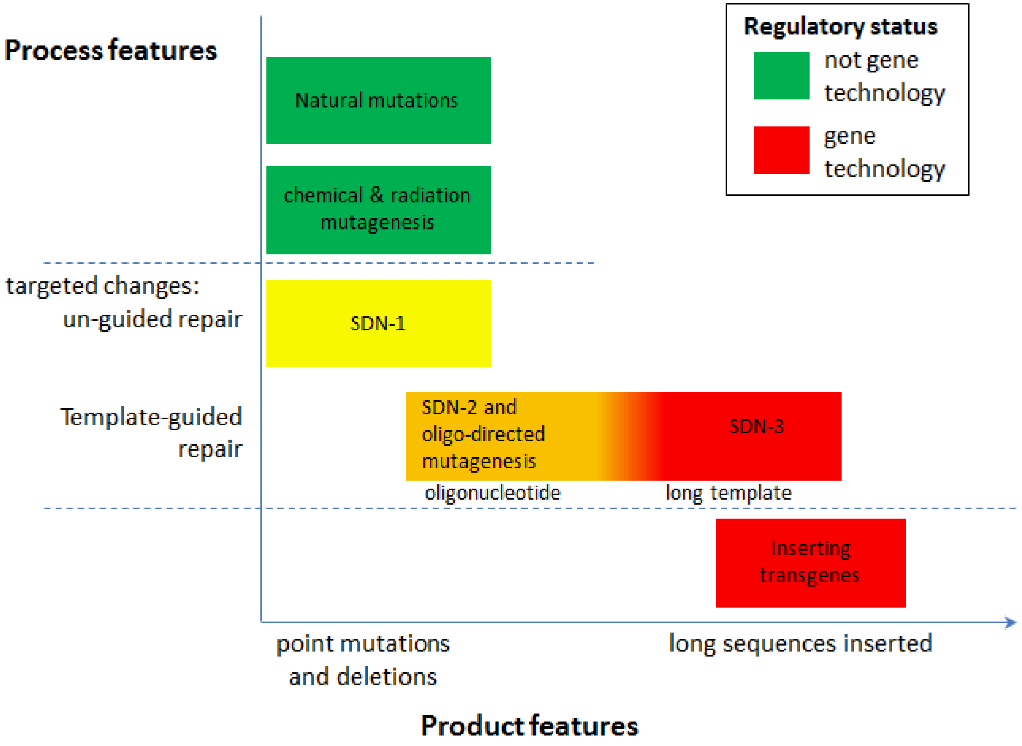
Recombinetics formed an alliance with Semex, a Canadian-based, farmer-owned cattle genetics organization to implement a precision breeding program to introduce hornless into elite dairy cattle genetics using genome editing

February, 2018
Australian OGTR


10 base pairs (p)

212 base pairs (P)

POLLED GENE



Is 202 base pairs long?

Van Eenennaam 2/15/2019

July 25, 2018

European High Court rules all genome edits are "GMOs"

Court of Justice of the European Union
PRESS RELEASE No 111/18
Luxembourg, 25 July 2018

CVRIA

Press and Information

Confédération paysanne

Organisms obtained by mutagenesis a
obligations laid d

However, organisms obtained by mutagenes
a number of applications and have a long safe
understanding that the Member States are fr
obligations laid down by t

Unlike transgenesis, mutagenesis is a set of techniques … alter the genome of a living species without the insertion of foreign DNA. Mutagen… ques have made it possible to develop seed varieties which are resista… elective herbicides.

… interests of small-scale … efore the Conseil d'État … ich exempts organisms … on genetically modified … be authorised following … e environment and also …

… enesis techniques have … conventional or random … ntly, technical progress … it possible to target the … Confédération paysanne … seed varieties carries a … lth, in the same way as …

It … seil d'État to determine, … whether they are subject to the obligations laid down by the GMO Directive.

"**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 polled Holsteins be subject to additional regulations in this country?

Country	Additional Regulations?	Basis of trigger/regulation?
Argentina	No	Novel DNA sequence/transgene
Australia	Yes	Use of “long” template
Brazil	No	Novel DNA sequence/transgene
Canada	No	Trait novelty (i.e. novel product risk)
European Union	Yes	Is a GMO if used a mutagenesis technique not in existence before 2001
Japan	No	No exogenous genes
New Zealand	Yes	Using of in vitro technique that modifies the genes/genetic material
United States	Yes	New Animal Drug

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.

Can’t Stop the Feeding

YouTube: https://youtu.be/C0MBl0BANHg

Thanks for inviting me!

UC DAVIS ANIMAL SCIENCE

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USDA United States Department of Agriculture National Institute of Food and Agriculture