A fronteira tecnológica da reprodução animal:

Como a edição gênica (CRISPR) pode colaborar com a produção animal

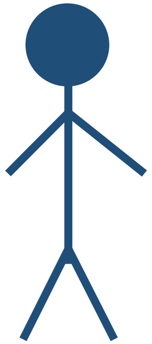
Alejo Menchaca

Fundación IRAUy, Instituto de Reproducción Animal Uruguay.
Instituto Nacional de Investigación Agropecuaria, INIA, Uruguay.

INIA
Instituto Nacional de Investigación Agropecuaria
URUGUAY

Fundación IRAUy
Instituto Reproducción Animal Uruguay

Uruguay



per capita → ~ 6 hectáreas

~ 4 vacas

~ 2 ovejas

- Productividad
- Salud animal
- Bienestar animal
- Impacto ambiental
  Cambio climático?
- Trazabilidad total
- Inocuidad

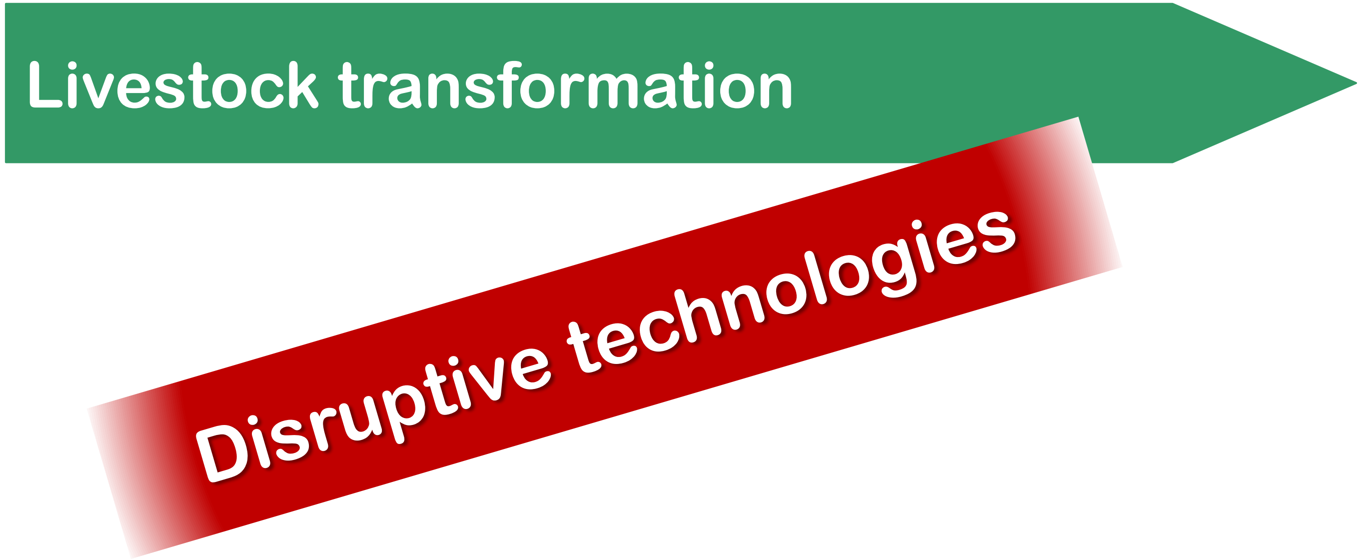
sustainability

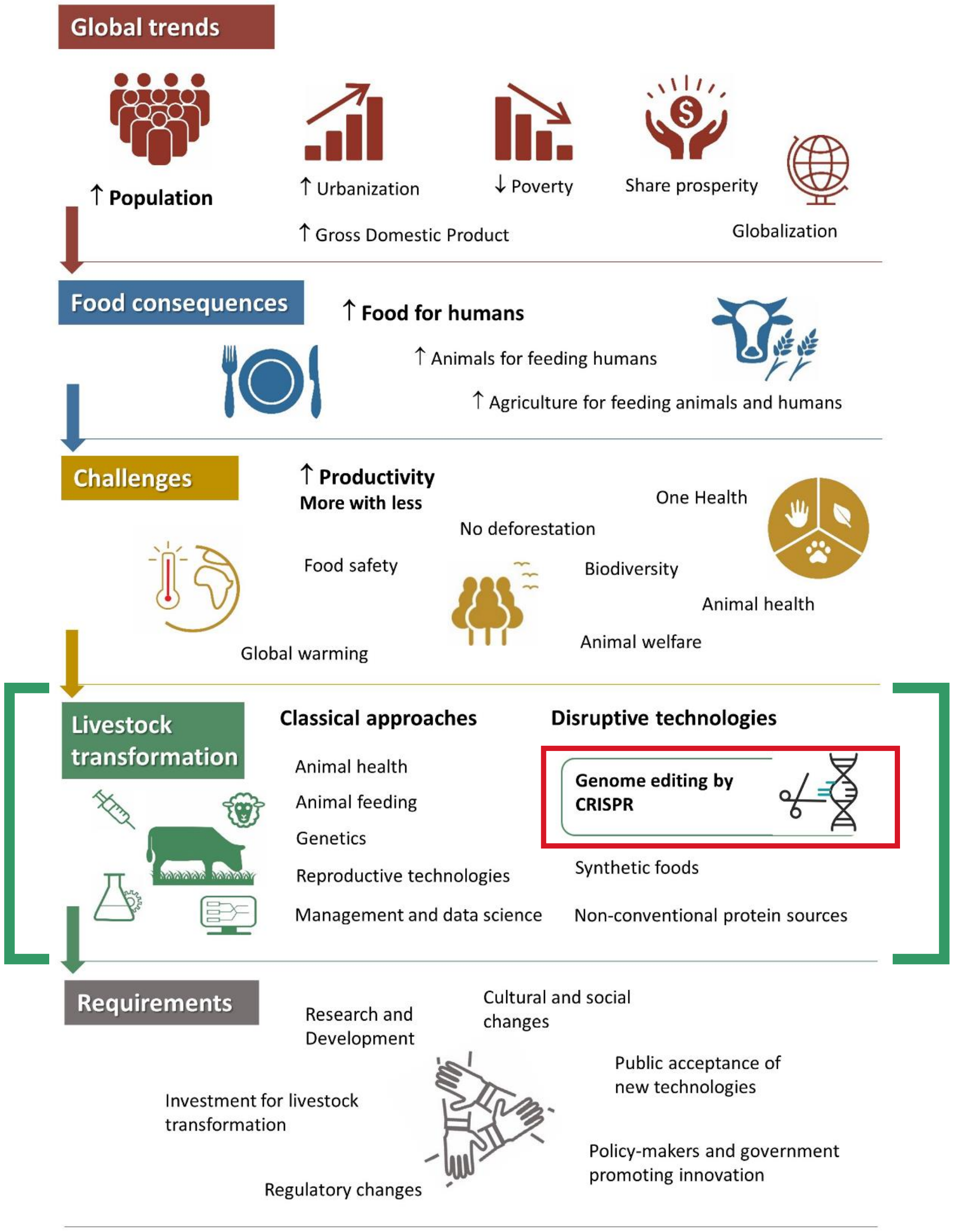
Review

Sustainable Food Production: The Contribution of Genome Editing in Livestock

Alejo Menchaca [1,2]

[1] Fundacion IRAUy, Instituto de Reproducción Animal Uruguay, 12200 Montevideo, Uruguay; menchaca.alejo@gmail.com
[2] Institut Pasteur Montevideo, Unidad Mixta Pasteur INIA, 11400 Montevideo, Uruguay





CRISPR in livestock

- The CRISPR/Cas system.
- How to produce edited animals.
- CRISPR for livestock improvement.

Que?
Como se faz?
Para que?

Genética clásica

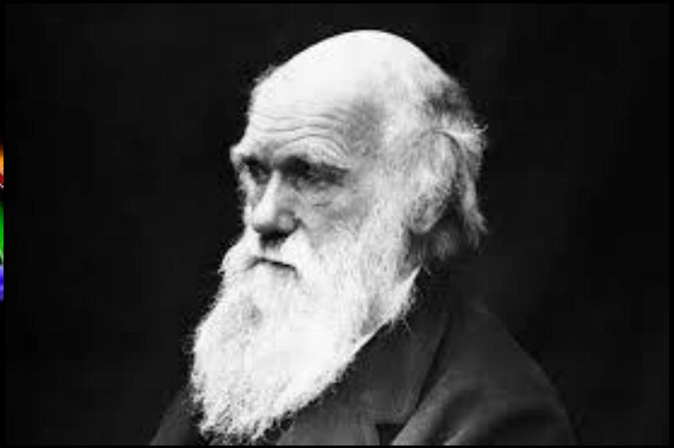
Fenotipo

↓

Genotipo

- Selección natural “Darwinismo”
- Cruzamiento y selección “Mendelismo”
- Selección genómica

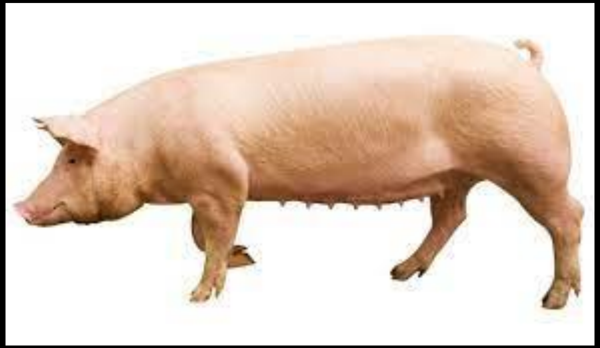
Genética clásica



Darwin
El origen de las especies
1859

Mendel
Leyes de la herencia
1866

Watson & Crick
La doble hélice del ADN
1953

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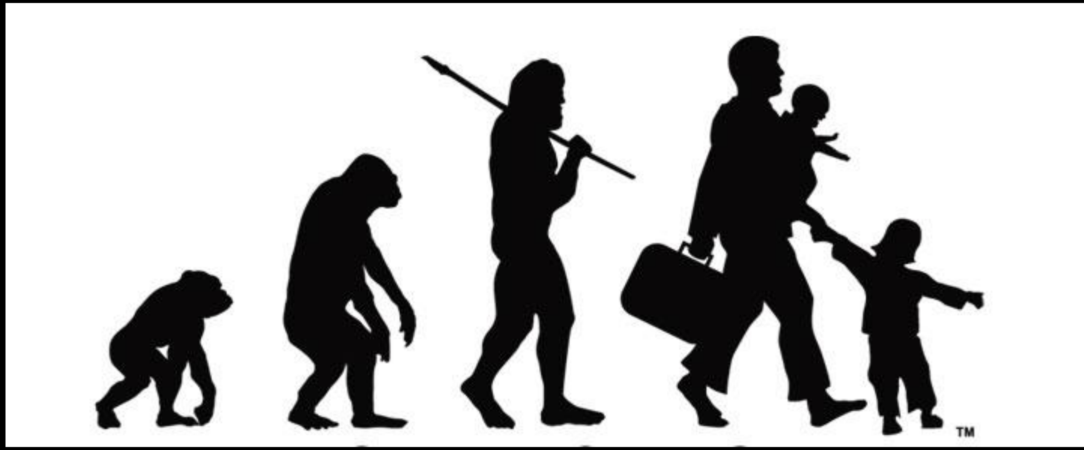
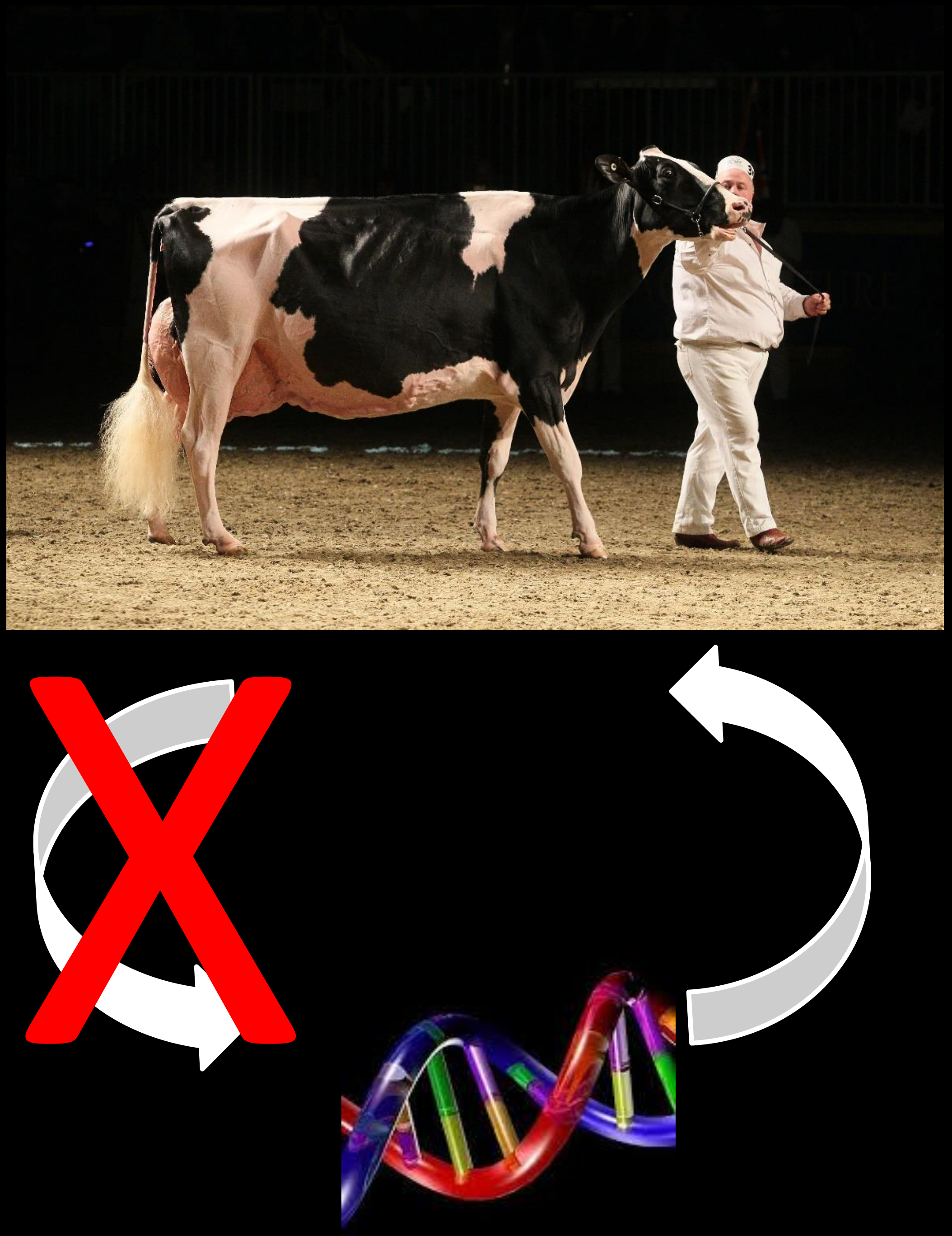

TM



Genética inversa

Desde la biología molecular a la biotecnología

ADN

ARN

Proteína

Función

Con la Edición Génica **(GE)** podemos alterar el **ADN en la célula** y cambiar el **fenotipo en el animal.**

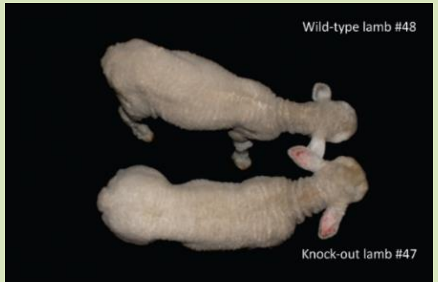
Genética inversa

Genotipo

Agregar una función
Transgénesis

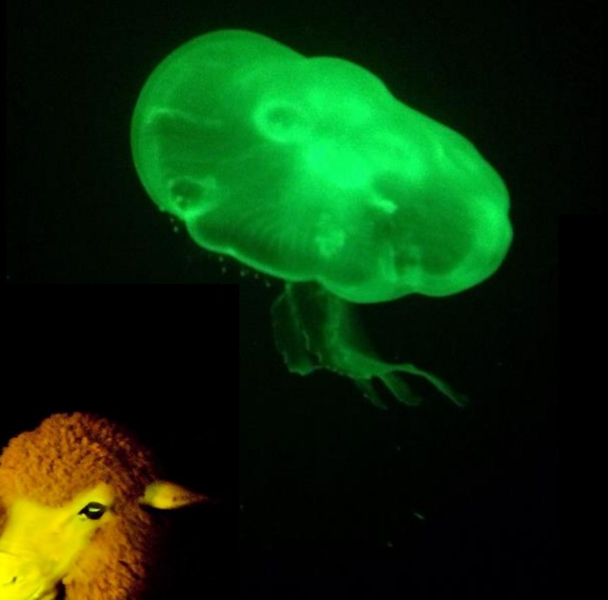
Suprimir, corregir, agregar
Edición Génica

Fenotipo



Transgénesis

Wild type Transgenic lambs



ORIGINAL PAPER

Transgenic Res (2015) 24:31–41

Embryo development, fetal growth and postnatal phenotype of eGFP lambs generated by lentiviral transgenesis

M. Crispo · M. Vilariño · P. C. dos Santos-Neto · R. Núñez-Olivera · F. Cuadro · N. Barrera · A. P. Mulet · T. H. Nguyen · I. Anegón · A. Menchaca

Genética clásica

Fenotipo

↓

Selección natural
"Darwinismo"

Cruzamiento y selección
"Mendelismo"

Selección genómica

Genotipo

Genética inversa

Genotipo

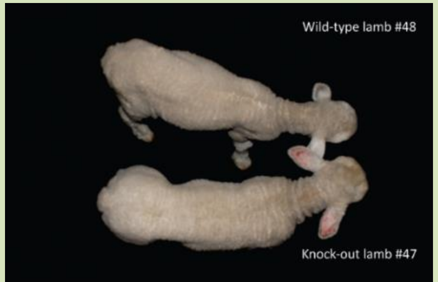
↙ ↘

Agregar una función
Transgénesis

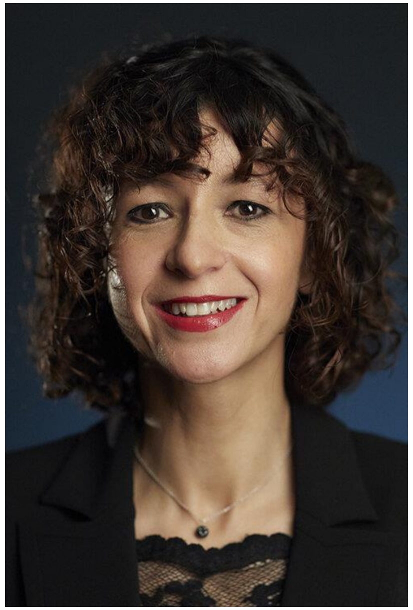
Suprimir, corregir, agregar
Edición Génica

↘ ↙

Fenotipo



The Nobel Prize in Chemistry 2020



© Nobel Prize Outreach. Photo: Bernhard Ludewig

Emmanuelle Charpentier

Prize share: 1/2

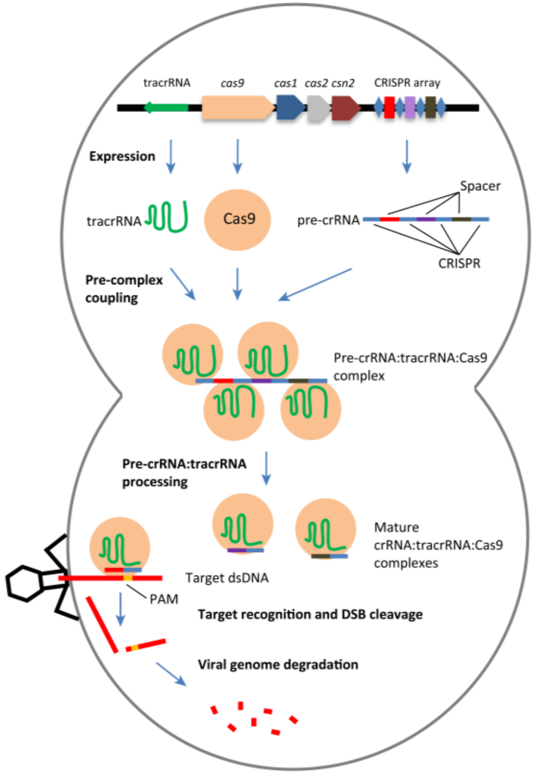
© Nobel Prize Outreach. Photo: Brittany Hosea-Small

Jennifer A. Doudna

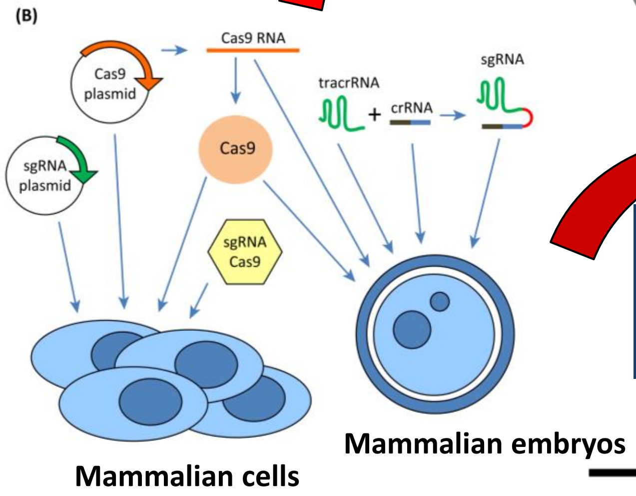
Prize share: 1/2

The Nobel Prize in Chemistry 2020 was awarded jointly to Emmanuelle Charpentier and Jennifer A. Doudna "for the development of a method for genome editing."

CRISPR/Cas: from prokaryotes…



…to cells



…to animals

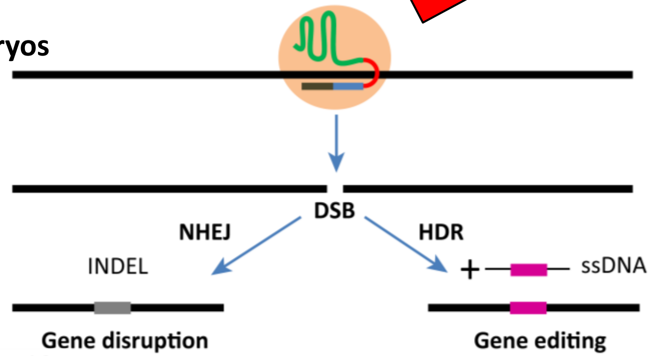


Table 1. Milestones, Discoveries, and Achievements in the History of CRISPR-Cas Technology (1987–2013)

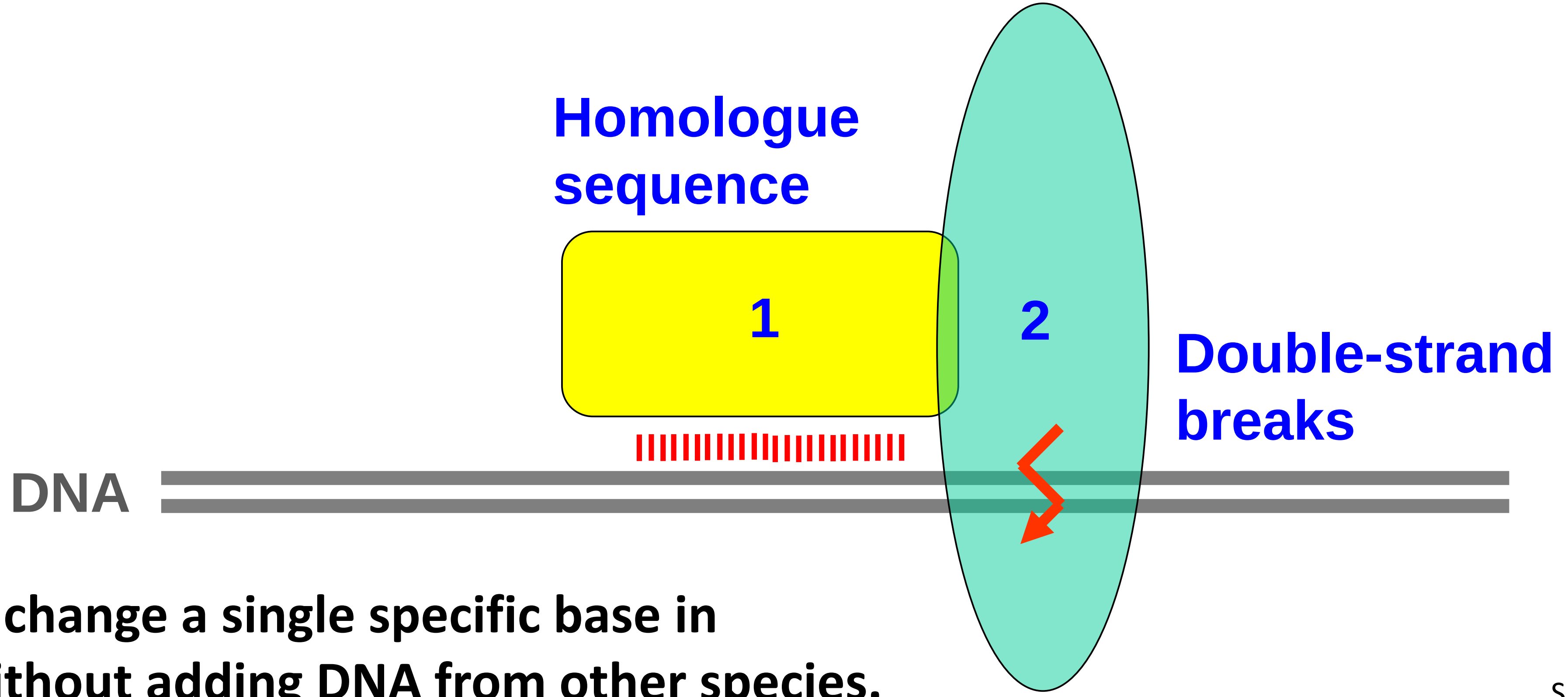
Year	Milestone	Refs
1987	First report of CRISPR arrays in Gram-negative bacteria	[14]
1991	First report of CRISPR arrays in Gram-positive bacteria	[16]
1993	First report of CRISPR arrays in archaea	[20]
1995	First insight on CRISPR functionality	[21]
2000	Large number of regularly spaced repeats are found in bacteria and archaea, suggesting a relevant function	[22]
2002	Regularly spaced repeats of bacteria and archaea are termed with the acronym CRISPR	[23]
2002	First identification of CRISPR-associated (*cas*) genes	[23]
2005	First identification of CRISPR spacers as homologous to sequences in bacteriophages and plasmids	[24–26]
2005	First suggestion that CRISPR-Cas systems would represent a bacterial defense mechanism	[24]
2007	First experimental demonstration that CRISPR-Cas systems are involved in acquired immunity against bacteriophages	[31]
2008	First experimental demonstration that CRISPR-Cas systems interfere with plasmid horizontal transfer, by targeting DNA	[34]
2008	First description of the role of CRISPR small RNAs (crRNA) as the guides for CRISPR interference	[33]
2008	It is anticipated that conserved sequences next to protospacers are important for CRISPR-mediated phage resistance	[37]
2010	First description of the CRISPR-Cas interference mechanism through Cas proteins cutting target DNA at precise sites	[38]
2011	Identification of trans-activating crRNAs (tracrRNAs)	[41]
2011	First successful transfer of a CRISPR-Cas system between two evolutionary distant organisms: from *Streptococcus thermophilus* to *Escherichia coli*	[80]
2012	First reports documenting functional CRISPR-Cas systems reconstructed *in vitro* and suggesting their potential application as RNA-programmable genome editing tools	[42,48]
2013	First reports demonstrating the use of CRISPR-Cas tools for efficient genome editing in mammalian cells	[52,53]
2013	First reports showing efficient genome engineering at multiple loci in mice, through the use of CRISPR-Cas tools	[59–61]

CellPress
Mojica & Montoliu
Trends in Microbiology, October 2016, Vol. 24, No. 10

The CRISPR tool: 2 elements

1 - gRNA

2 – CAS9

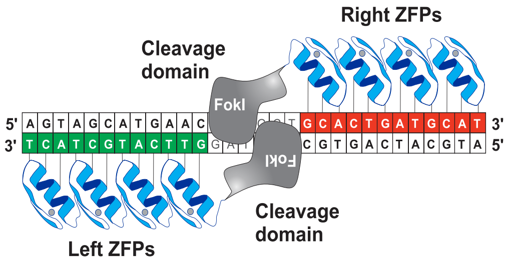


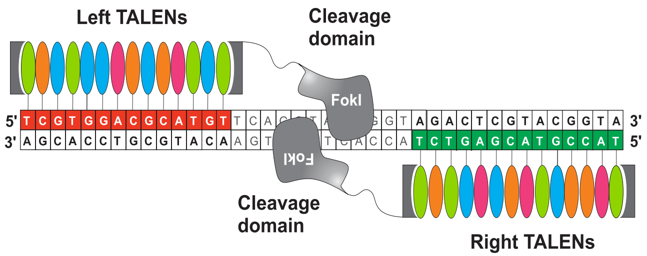
It is possible to change a single specific base in the genome, without adding DNA from other species.

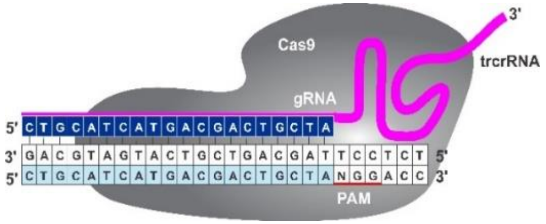
Slide: Lluis Montoliu

Genome editing

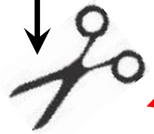
A. Menchaca et al. / Theriogenology 86 (2016) 160–169







Nucleases | **ZFNs** | **TALENs** | **CRISPR/Cas**



Target genomic sequence

Double DNA strand breaks

DNA-repair mechanisms

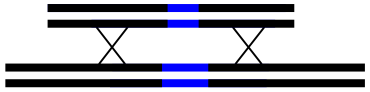
Non-Homologous End Joining (NHEJ)

Gene disruption in ~70 % out of frame deletions = STOP codon
Targeted Knockout

Homology-directed repair (single-strand annealing) ssODNs

Precise repair (user–defined)
Targeted integration nucleotides

Homology-directed repair (homologous recombination) several kbDNA donors



Precise repair (user–defined)
Targeted integration expression cassette

DNA analyses

PCR+sequence
T7 endonuclease I
look for frame shift mutations

PCR+sequence
look for specific mutations

PCR+sequence
Southern blot
look for specific mutations

CRISPR in livestock

- The CRISPR/Cas system.
- How to produce edited animals.
- CRISPR for livestock improvement.

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Genética clásica

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"Darwinismo"

Cruzamiento
"Mendelismo"

Selección

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ción
esis

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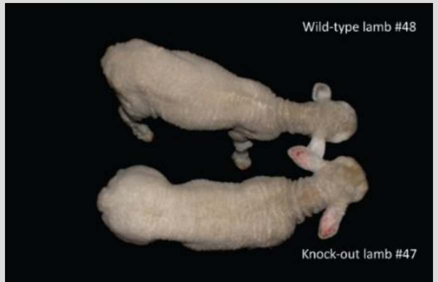


Advanced Reproductive Technologies (ARTs)

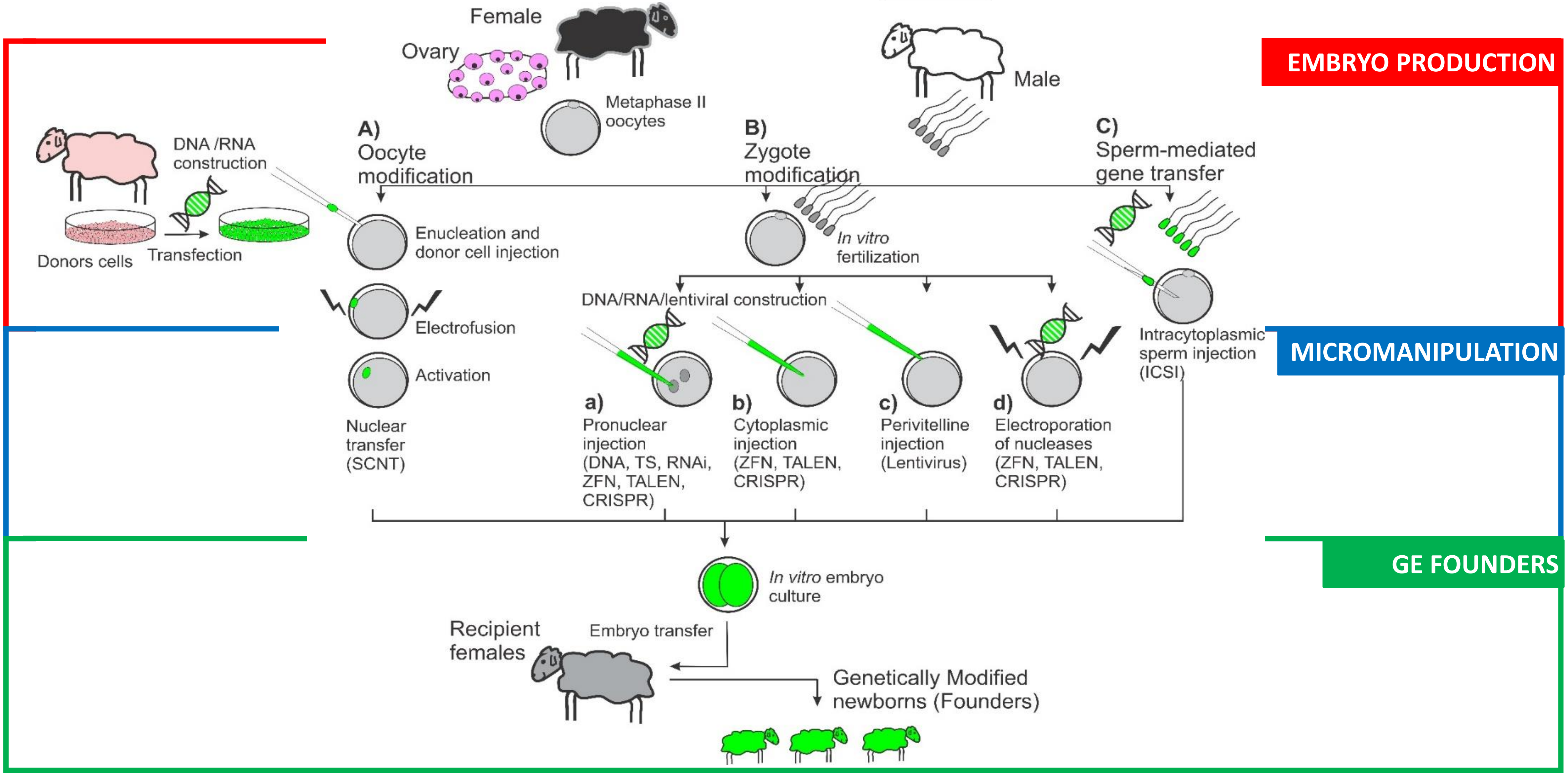
Não há como trabalhar com CRISPR se não tivermos sucesso absoluto nas técnicas de reprodução assistida.

Genetic engineering (GE) basic pipeline

Review article

New insights and current tools for genetically engineered (GE) sheep and goats

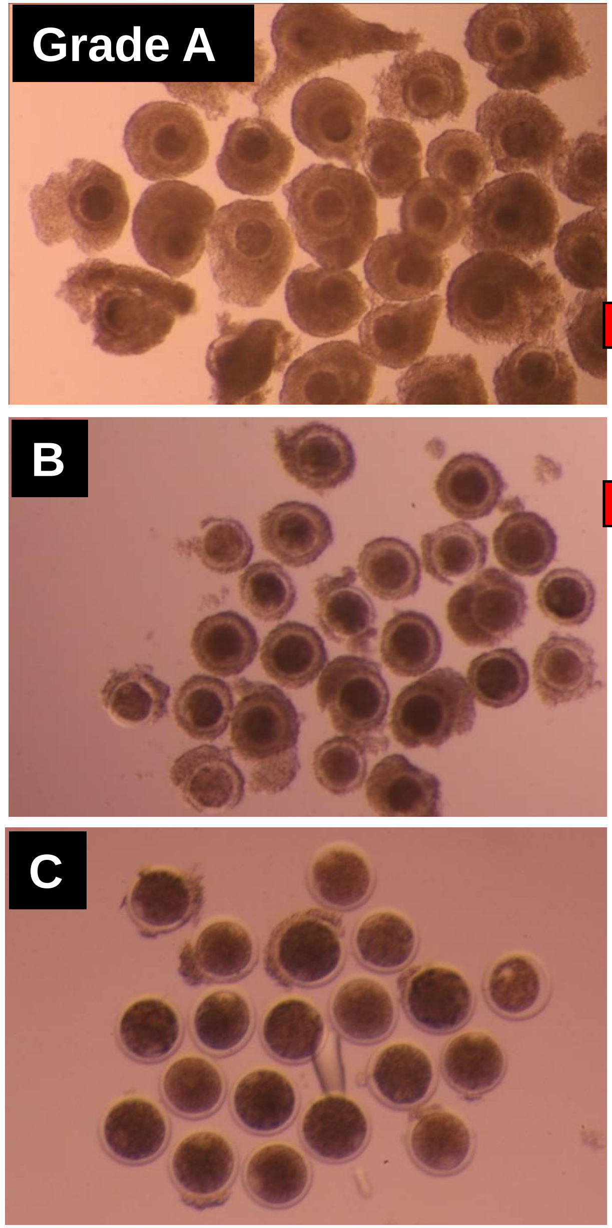
A. Menchaca [a,*], I. Anegon [b], C.B.A. Whitelaw [c], H. Baldassarre [d], M. Crispo [e,*]

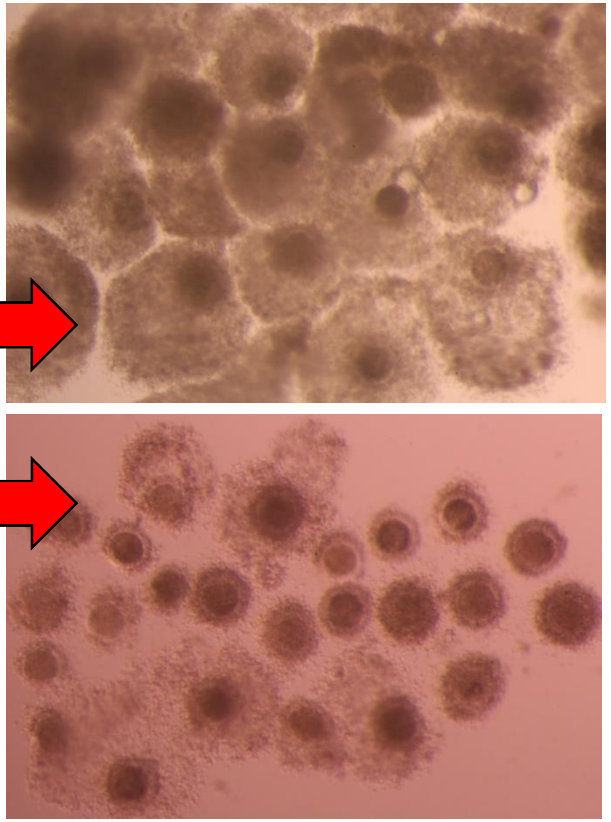


Oocytes from live animals:
US-guided folicular aspiration (cattle)
Laparoscopic Ovum Pick Up (sheep and goats)

Follicular aspiration: Laparoscopic ovum pick-up (LOPU)

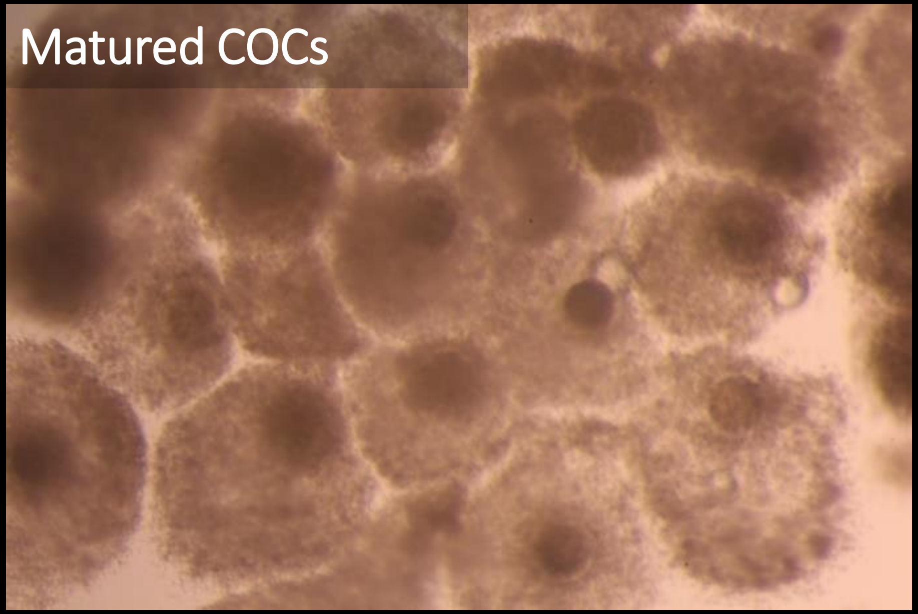
In vitro MATURATION

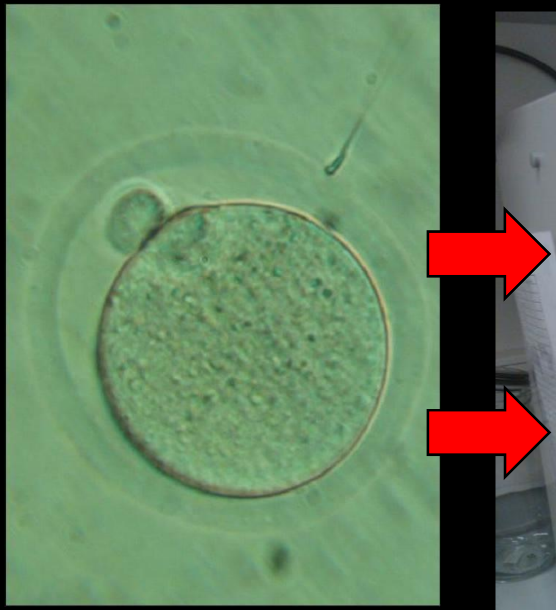


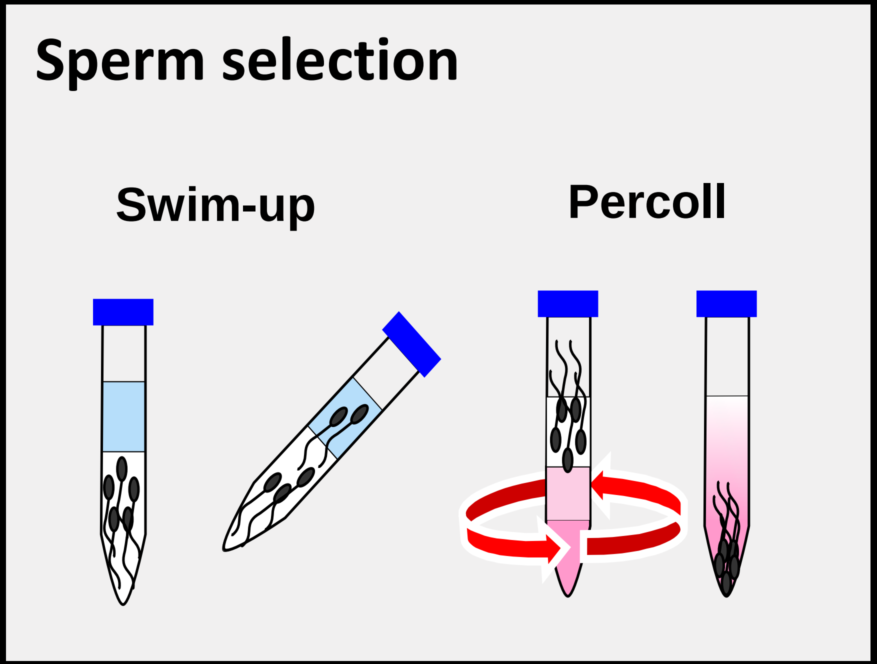


- Nuclear, cytoplasmic and CC maturation.
- During 22h, 39°C, 5% CO_2.
- TCM 199, FSH, LH, estrus sheep serum, ATB, cysteamine.
- pH 7.2 – 7.4; 280 mOsm/kg.
- **~90% success (maturation rate).**

In vitro FERTILIZATION

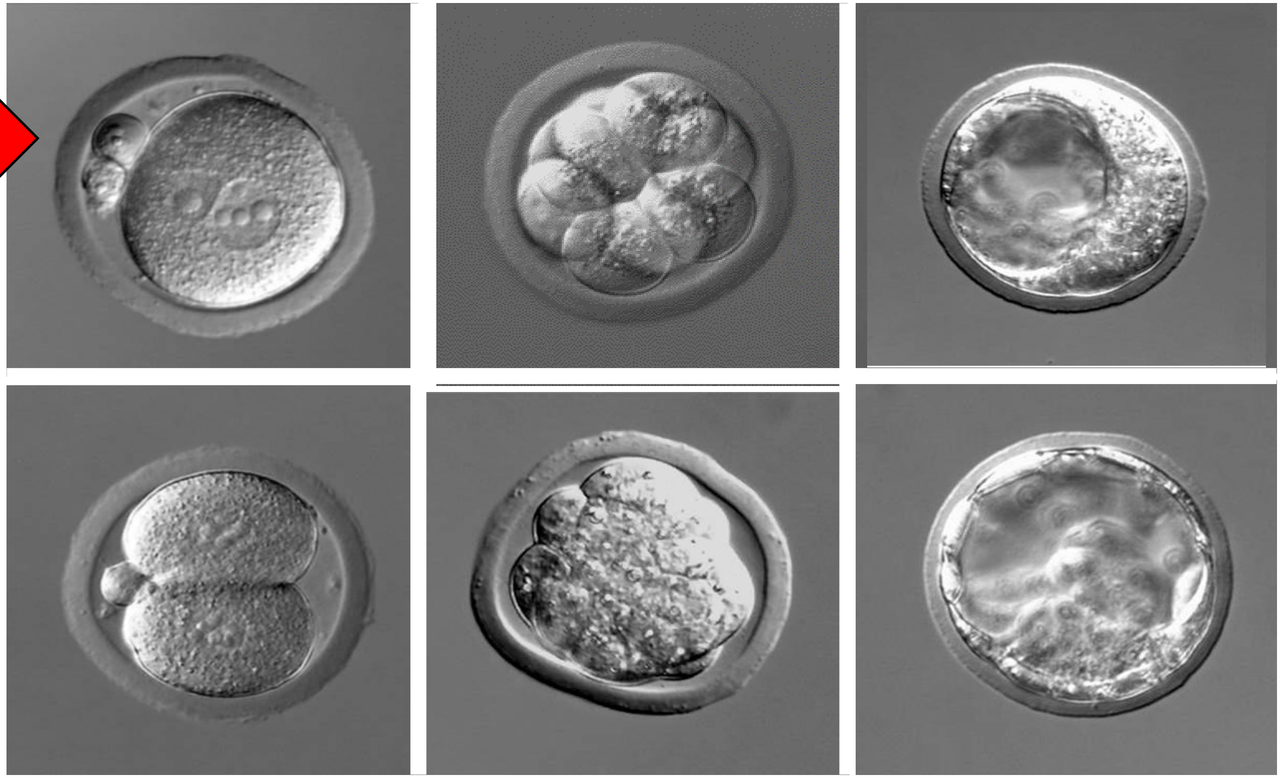






- 18-22h in co-incubation.
- SOF + heparin + hypotaurine + estrus sheep serum + ATB.
- 1x10^6 spz in 100 µl drops with 20-30 COCs.
- **80-90% cleavage rate.**

In vitro CULTURE



Changing IVC medium improves blastocyst yield

n= 712 COCs	Cleavage rate	Blastocysts/ oocytes	Blastocysts/ cleaved
No change	**82.8%[a]** (192/232)	**33.6%[ab]** (78/232)	**40.6%[a]** (78/192)
Day 3	**80.2%[a]** (195/243)	**42.0%[b]** (102/243)	**52.3%[b]** (102/195)
Day 2 & Day 4	**83.1%[a]** (197/237)	**30.8%[a]** (73/237)	**37.1%[a]** (73/197)

a vs. b, $P<0.05$.

Vilariño et al., ICAR 2012.

- 6 days (sheep) and 7 days (goats) in culture.
- SOFaa + BSA.
- 5% O2, 5% CO2, 90% N2; pH 7.2-7.4; 280 mOsm/kg.
- **30-40% development rate** (blastocysts/COCs).

Embryo survival after zygote CRISPR/Cas microinjection in large scale programs (n= 8,520; in four GE programs).

Embryo development of CRISPR/Cas microinjected sheep zygotes subjected to *in vitro* culture.

	n	Cleavage rate	Blastocyst rate	Blastocyst/cleaved
Microinjected zygotes	7,819	69.1 % (5,400/7,819)	20.0 % (1,560/7,819)	28.9 % (1,560/5,400)
Control zygotes*	701	84.0 % (589/701)	44.9 % (315/701)	53.4 % (315/589)
P-value		<0.01	<0.01	<0.01

*Each session includes about 10% of non-microinjected zygotes serving as control group.

dos Santos-Neto et al., in preparation.

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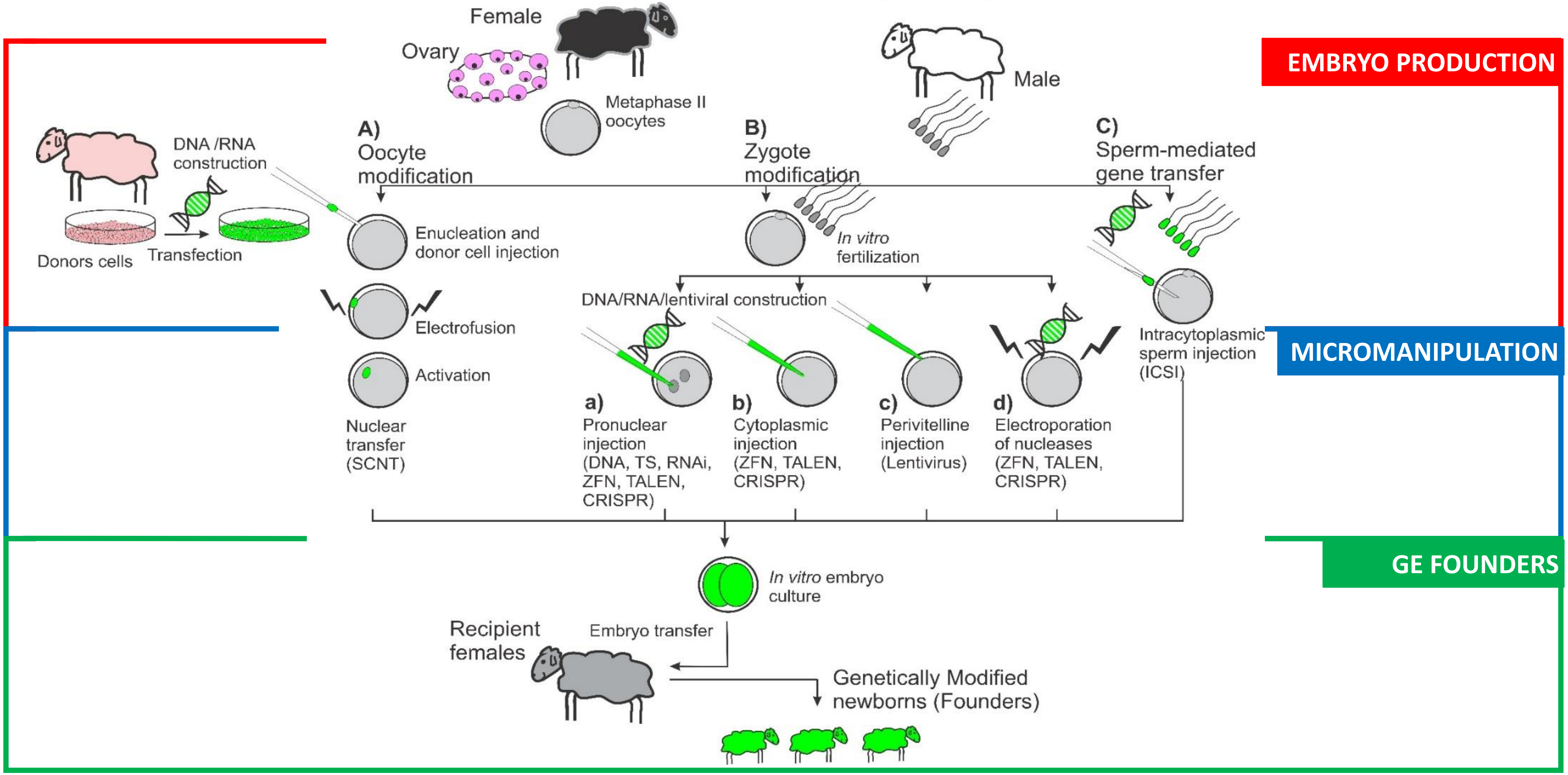
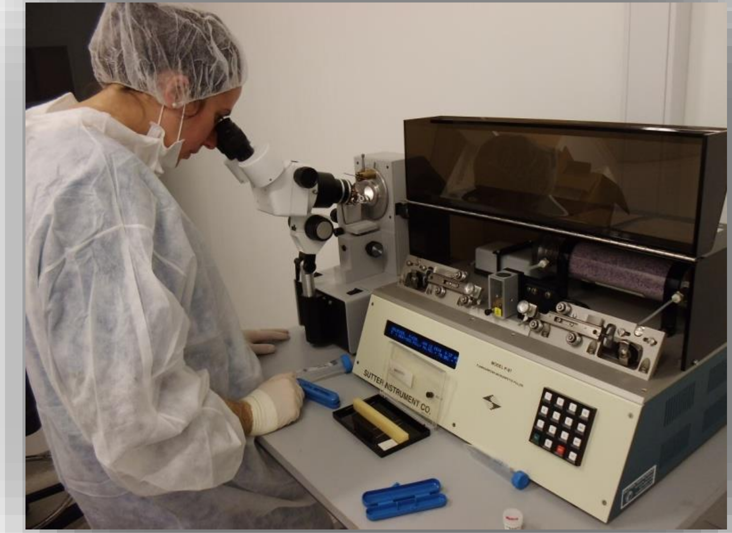


Laboratory of micromanipulation



Institut Pasteur de Montevideo

Fundación IRAUy Instituto Reproducción Animal Uruguay

Embryo micromanipulation

Cytoplasmic injection of CRISPR/Cas

Institut Pasteur de Montevideo

Fundación IRAUy

Instituto Reproducción Animal Uruguay

Efficiency of CRISPR/Cas system in KO lambs produced by NHEJ mechanism.

RESEARCH ARTICLE

Efficient Generation of Myostatin Knock-Out Sheep Using CRISPR/Cas9 Technology and Microinjection into Zygotes

M. Crispo[1]*, A. P. Mulet[1], L. Tesson[3], N. Barrera[2], F. Cuadro[2], P. C. dos Santos-Neto[2], T. H. Nguyen[3], A. Crénéguy[3], L. Brusselle[3], I. Anegón[3]*, A. Menchaca[2]*

PLOS ONE

August 25, 2015

CRISPR/Cas9 microinjection into zygotes and embryo development.

	No. zygotes	Cleavage rate on Day 2	Morulae and Blastocysts on Day 6	No. of embryos on Day 6/ cleaved	Mutant embryos at blastocyst stage
CRISPR injection	216	63,9% (138/216)[a]	25,0% (54/216)[a]	39,1% (54/138)[a]	50.0% (10/20)
Buffer injection	183	60,7% (111/183)[a]	20,2% (37/183)[a]	33,3% (37/111)[a]	—
Non-injected	173	86,1% (149/173)[b]	35,8% (62/173)[b]	41,6% (62/149)[a]	—

For different superscripts, P<0.05.

CRISPR/Cas9 system is a highly efficient method to produce mutant lambs.

	Embryos on Day 30	Pregnant ewes	Fetal loss	Mutant/ born lambs	Biallelic/ mutant lambs	Homozygous/ mutant lambs
CRISPR/Cas9 efficiency	41.5% (22/53)	65.5% (19/29)	0.0% (0/ 22)	45.5% (10/22)	80.0% (8/10)	50.0% (5/10)

Efficiency of CRISPR/Cas system induced to Homology Directed Repair (HDR) mechanism.

Otoferlin gene editing in sheep via CRISPR-assisted ssODN-mediated Homology Directed Repair

A. Menchaca[1*], P. C. dos Santos-Neto[1], M. Souza-Neves[1], F. Cuadro[1], A. P. Mulet[2], L. Tesson[3,4], V. Chenouard[3,4], A. Guiffès[3,4], J. M. Heslan[3,5], M. Gantier[3,5], I. Anegón[3,4,5*] & M. Crispo[2*]

SCIENTIFIC REPORTS
natureresearch

Menchaca et al., 2020.

	Overall results	Cas9 mRNA *vs.* Cas9 RNP				
		mRNA 50 ng/µl	RNP 100 ng/µl	RNP 250 ng/µl	RNP 500 ng/µl	*P* value
Number of recipient females	247	103	48	49	47	—
Number of transferred embryos	1,316	629	218	236	233	—
Pregnant/transferred recipients	25.1% (62/247)	16.5%[a] (17/103)	45.8%[b] (22/48)	24.5%[a] (12/49)	23.4%[a](11/47)	<0.05
Embryos alive at 30 d of gestation	78	19	33	14	12	—
Fetal losses (from 30 d of gestation to birth)	6.4% (5/78)	5.3% (1/19)	6.1% (2/33)	7.1% (1/14)	8.3% (1/12)	NS
Lambs born	73	18	31	13	11	—
Lamb survival rate*	89.0% (65/73)	77.8% (14/18)	93.5% (29/31)	100% (13/13)	81.8% (9/11)	NS
Mutants/lambs born	17.8% (13/73)	27.8%[a] (5/18)	[illegible].5%[b] (2/31)	7.7%[b] (1/13)	45.5%[a] (5/11)	<0.05
KI/mutant lambs	61.5% (8/13)	60.0% (3/5)	50.0% (1/2)	100% (1/1)	60.0% (3**/5)	NS
KI/total lambs	11.0% (8/73)	16.7% (3/18)	3.2% (1/31)	7.7% (1/13)	2.7% (3**/11)	NS

CRISPR in Biomedicine: Deafness lambs

SCIENTIFIC REPORTS
natureresearch

OPEN

Otoferlin gene editing in sheep via CRISPR-assisted ssODN-mediated Homology Directed Repair

A. Menchaca[1*], P. C. dos Santos-Neto[1], M. Souza-Neves[1], F. Cuadro[1], A. P. Mulet[2], L. Tesson[3,4], V. Chenouard[3,4], A. Guiffès[3,4], J. M. Heslan[3,5], M. Gantier[3,5], I. Anegón[3,4,5*] & M. Crispo[2*]

HARVARD MEDICAL SCHOOL

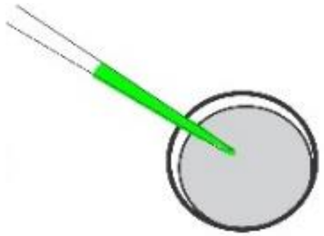
Inserm France

Institut Pasteur de Montevideo

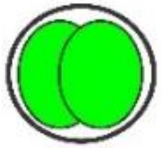
Fundación IRAUy Instituto Reproducción Animal Uruguay

Beyond CRISPR and embryo manipulation, let’s go to the field.

CRISPR/Cas design and *in vitro* analysis (in cells and embryos), and then to the Program.



In vitro embryo production



In vitro embryo culture

Embryo transfer

Genome edited newborns

Recipient preparation for embryo transfer, pregnancy and parturition

Embryo transfer: CRYOPRESERVATION?

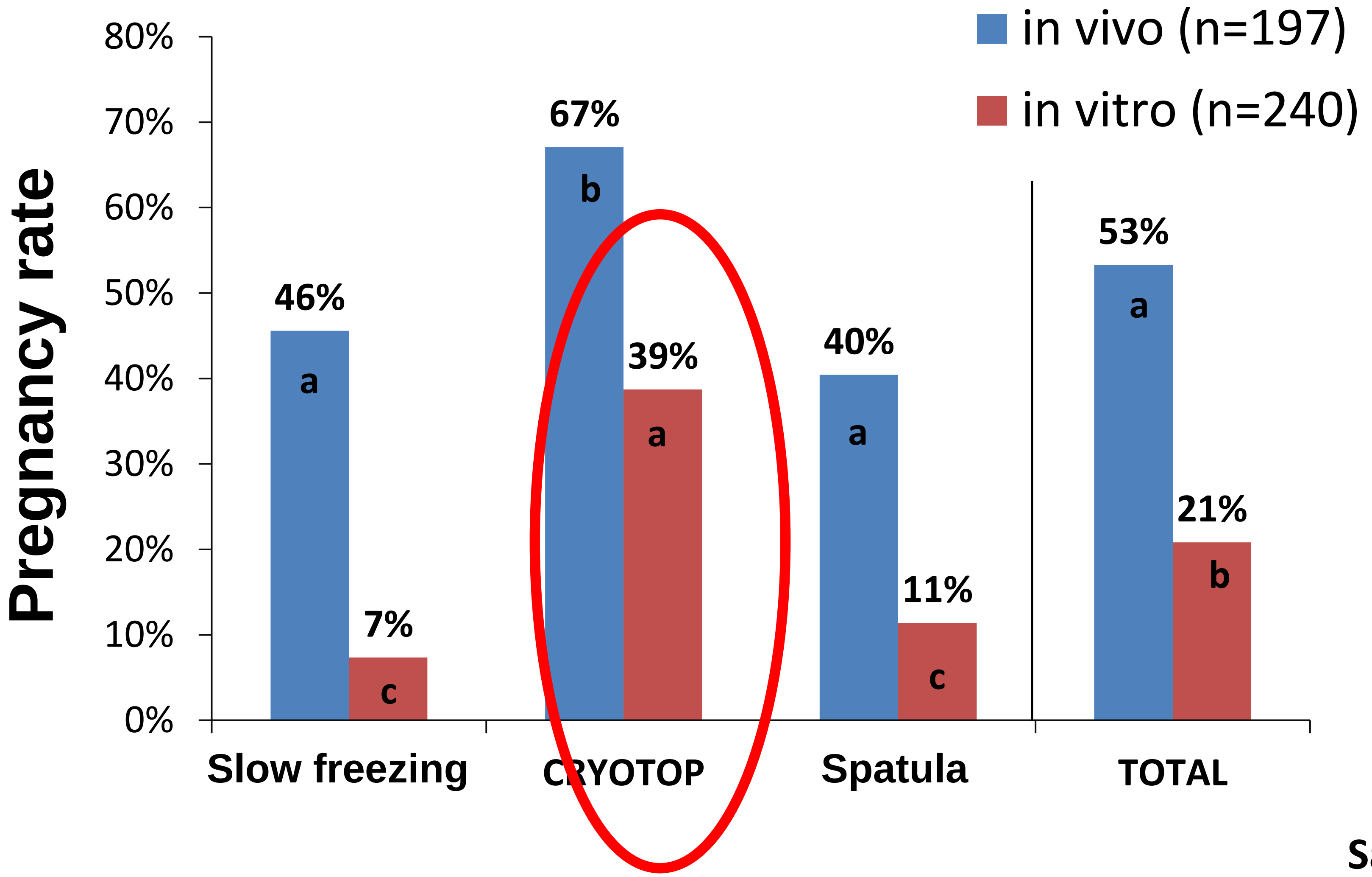
Can any embryo survive?

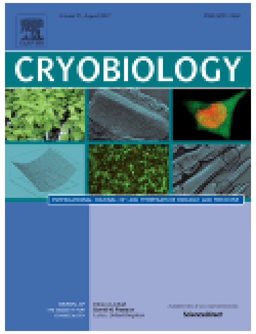
in vitro produced +

Microinjected zygotes

Pregnancy rate
in vivo vs. *in vitro* embryos

n= 437 embryos

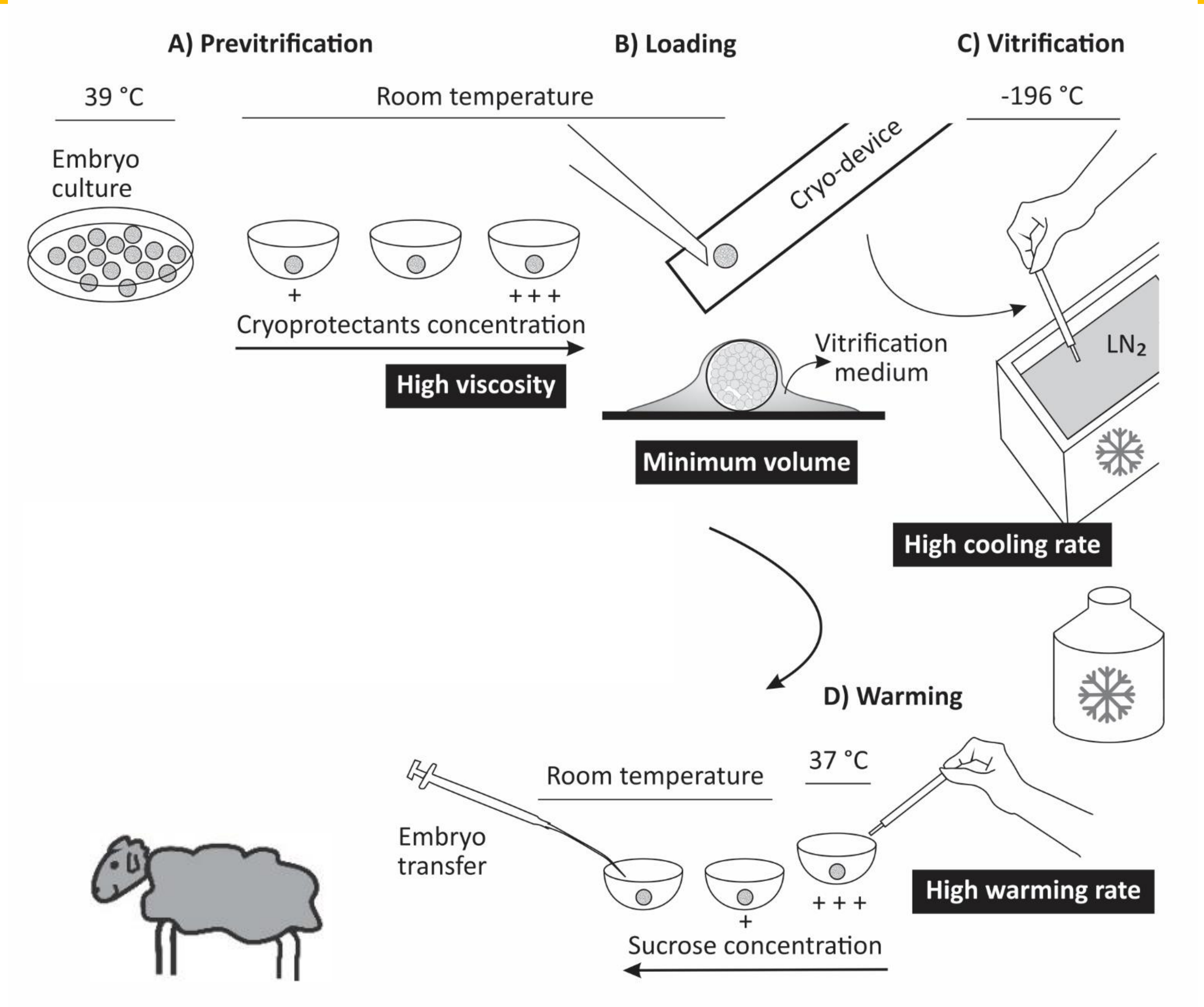




Santos-Neto et al., Cryobiology (2015).
Santos-Neto et al., Cryobiology (2017).

For different letters, P<0.05.

VITRIFICATION protocol for minimum volume methods



$$\text{Probability of vitrification} = \frac{\text{Cooling/warming rate x Viscosity}}{\text{Volume}}$$

Arav, 2014.

Santos-Neto et al., 2015; Cryobiology.
Santos-Neto et al., 2017; Cryobiology.
Meikle et al., 2018; Cryobiology.
Barrera et al., 2018; Plos One.
Menchaca et al., 2018; Anim Reprod.

Vitrification by the minimum volume Cryotop method in CRISPR/Cas embryos

Pregnancy outcomes obtained with vitrified blastocysts produced by CRISPR/Cas microinjected zygotes.

	No. of recipients	No. of transferred embryos	Pregnant/transferred recipients	Embryo survival/transferred embryos	Birth/pregnant embryos
Vitrified embryos	159	474	30.8% (49/159)	14.8% (70/474)[a]	85.7% (60/70)[a]
Fresh embryos	25	75	48.0% (12/25)	21.3% (16/75)[a]	75.0% (12/16)[a]
P-value			0.09	0.15	0.30

*Each embryo transfer (ET) session includes 10-20% fresh embryos serving as control group. Data from 5 ET sessions (replicates).

dos Santos-Neto et al., in preparation.

Can we transfer 2-day embryos into the uterine horn?

Can we transfer 2-day embryos into the uterine horn?

Sheep & goats

Why?

- To avoid oviductal ET.
- To avoid *in vitro* culture.

Sheep, goats, cattle

Why not?

Embryo transfer in sheep (CRISPR microinjected zygotes)

INTO THE OVIDUCT
2-day embryos

Embryo transfer in sheep (CRISPR microinjected zygotes)

INTO THE OVIDUCT
2-day embryos

UTERINE HORN
6-day blastocysts

- Faster
- Easier
- Less invasive
- Pregnancy rate?

What happen if we transfer 2-day embryos into the uterine horn?

Embryo transfer of 2-day embryos into the uterine horn (n= 538 embryos).

Effect of the site of embryo transfer (*i.e.*, **Oviduct *vs.* Uterine horn**) on Day 2 after in vitro fertilization. Ovine zygotes were subjected to **CRISPR/Cas microinjection.**

Site of embryo transfer	No. of recipients	No. of transferred embryos	Pregnant/transferred recipients	Embryo survival/ transferred embryos (Pregnancy 30 d after ET)	Birth/pregnant embryos
Oviduct	50	262	24.0% (12/50)	6.9% (18/262)	72.2% (13/18)
Uterine horn	52	276	25.0% (13/52)	6.2% (17/276)	100.0% (17/17)

P= NS

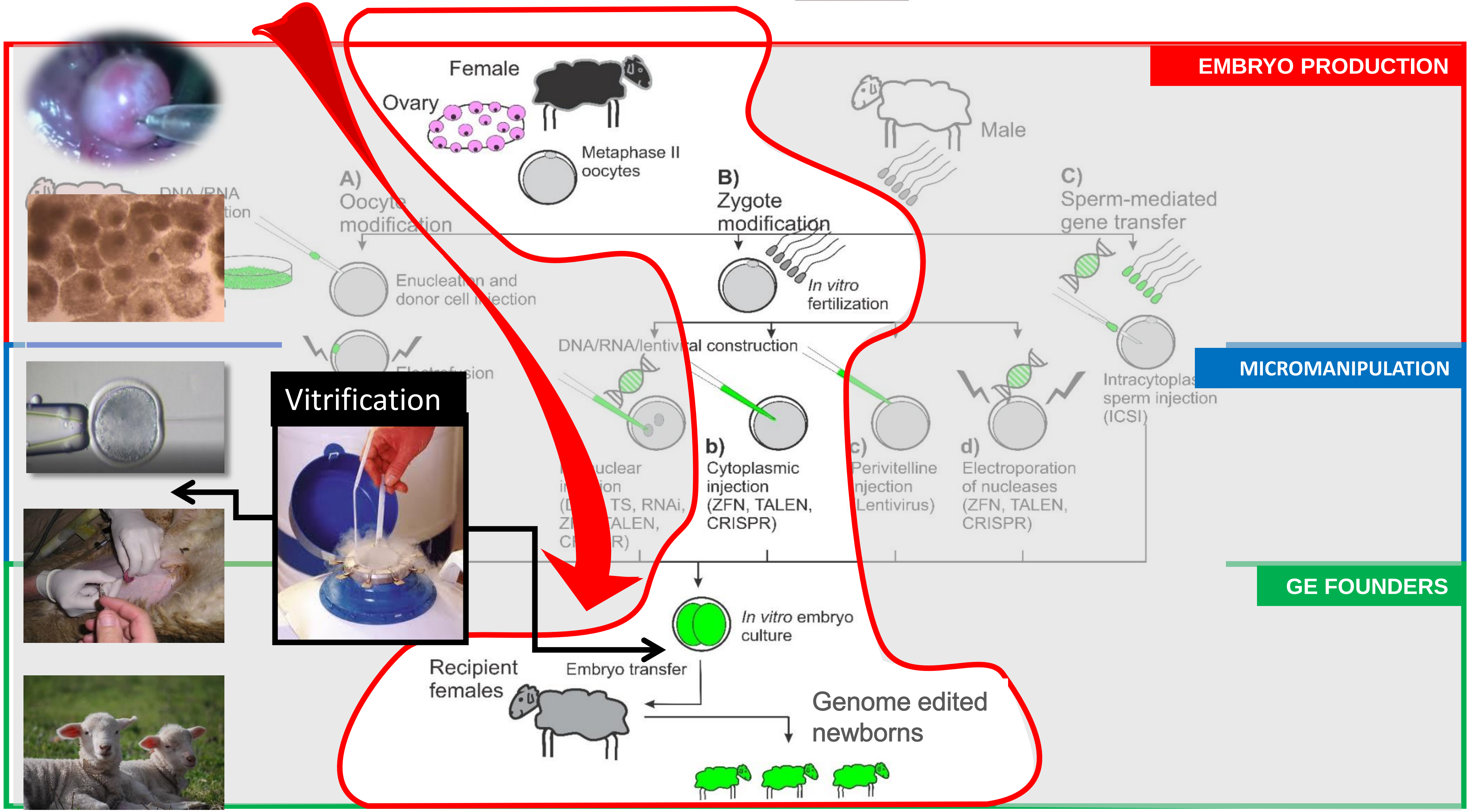
dos Santos-Neto et al., in preparation.

Genetic engineering (GE) basic pipeline

Review article

New insights and current tools for genetically engineered (GE) sheep and goats

A. Menchaca [a,*], I. Anegon [b], C.B.A. Whitelaw [c], H. Baldassarre [d], M. Crispo [e,*]

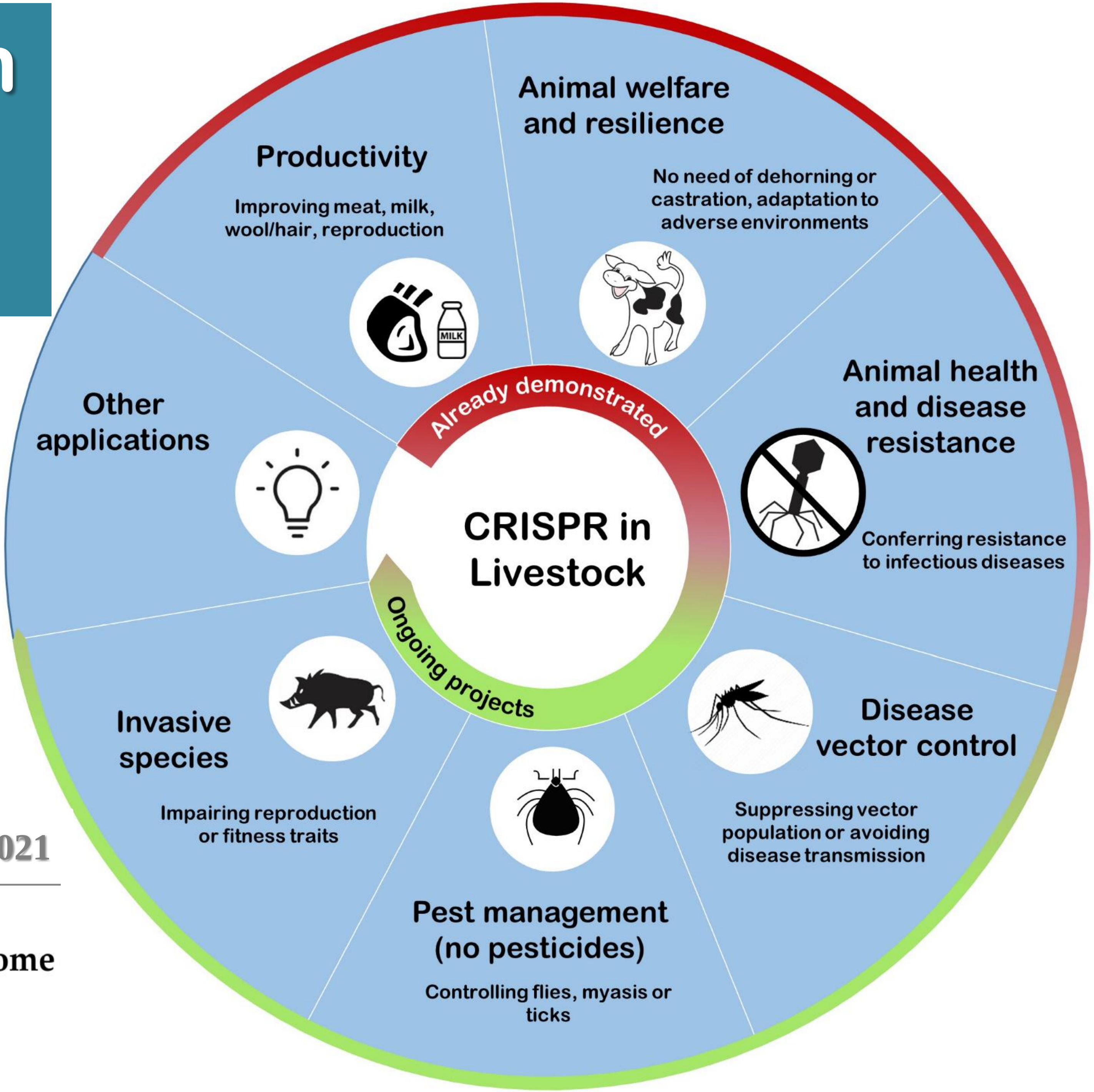


CRISPR in livestock

- The CRISPR/Cas system.
- How to produce edited animals.
- CRISPR for livestock improvement.

Que?
Como se faz?
Para que?

Potential contribution of Genome editing in Livestock



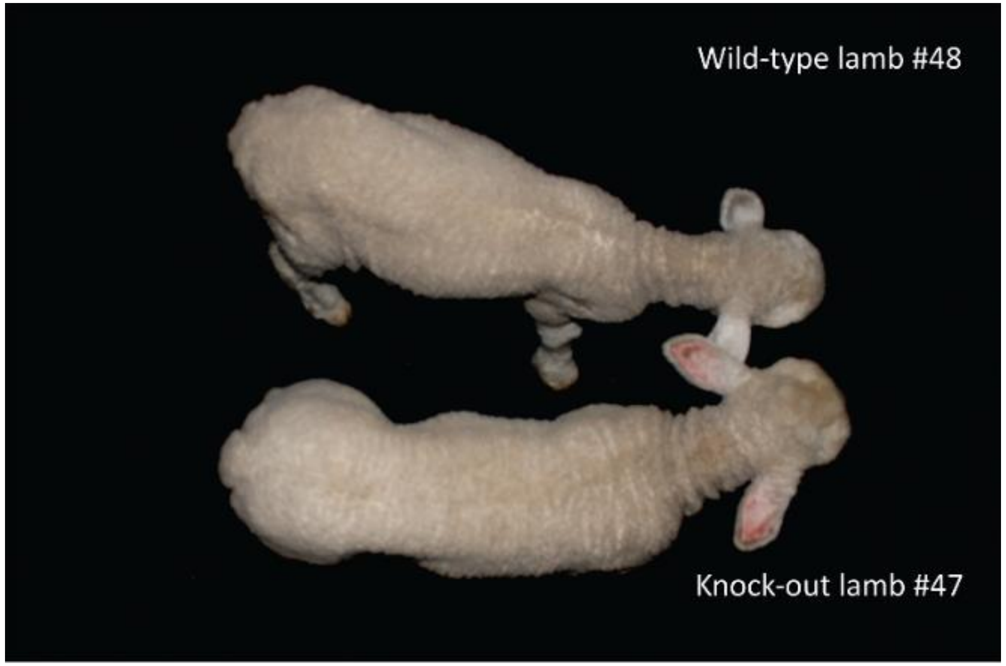
sustainability 2021

Review

Sustainable Food Production: The Contribution of Genome Editing in Livestock

Alejo Menchaca [1,2]

Double purpose animals



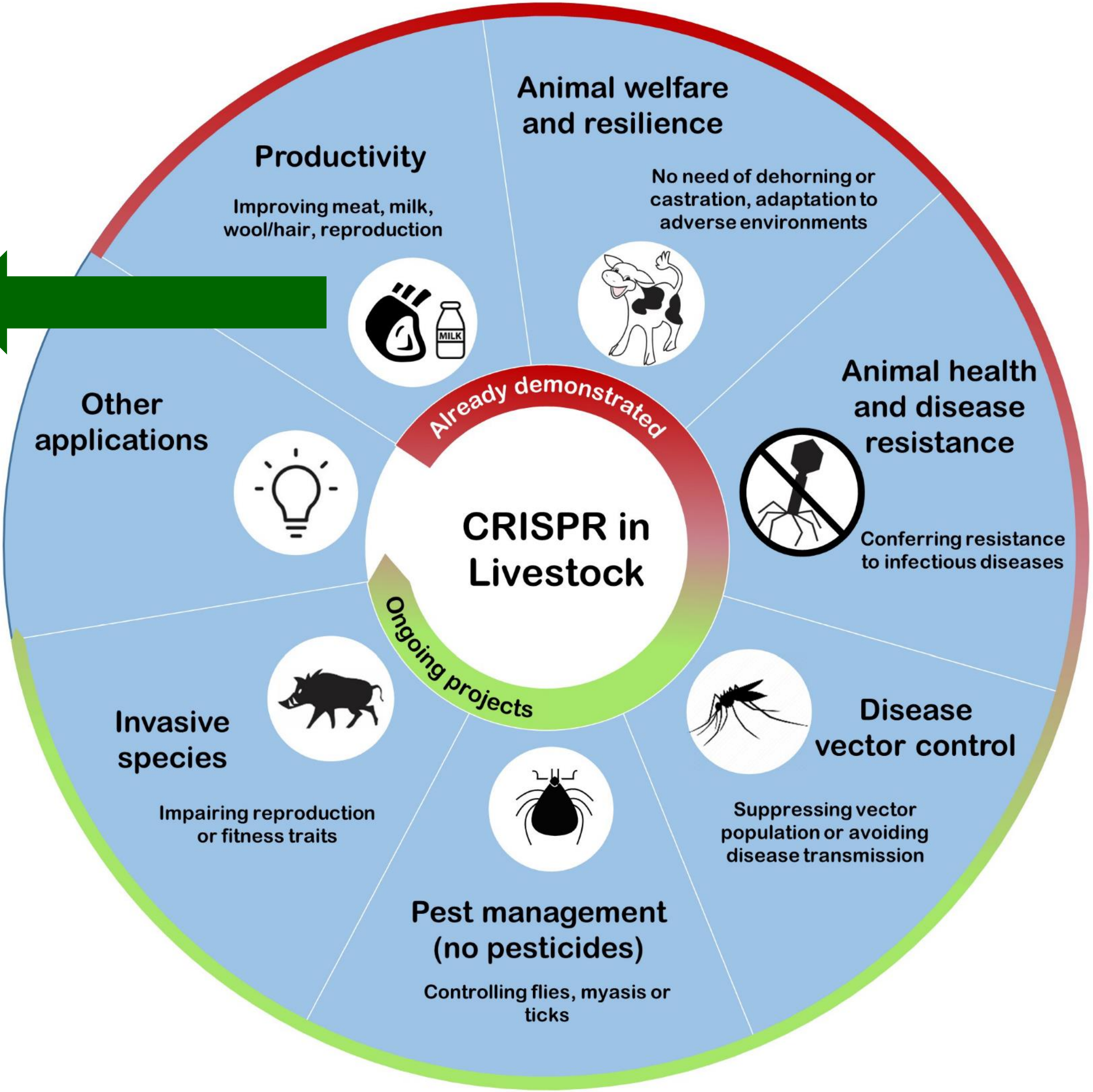
PLOS ONE

August 25, 2015

RESEARCH ARTICLE

Efficient Generation of Myostatin Knock-Out Sheep Using CRISPR/Cas9 Technology and Microinjection into Zygotes

M. Crispo[1]*, A. P. Mulet[1], L. Tesson[3], N. Barrera[2], F. Cuadro[2], P. C. dos Santos-Neto[2], T. H. Nguyen[3], A. Crénéguy[3], L. Brusselle[3], I. Anegón[3]*, A. Menchaca[2]*



Superfine wool

e.g., Australian Merino sheep

vs.

Meat breeds

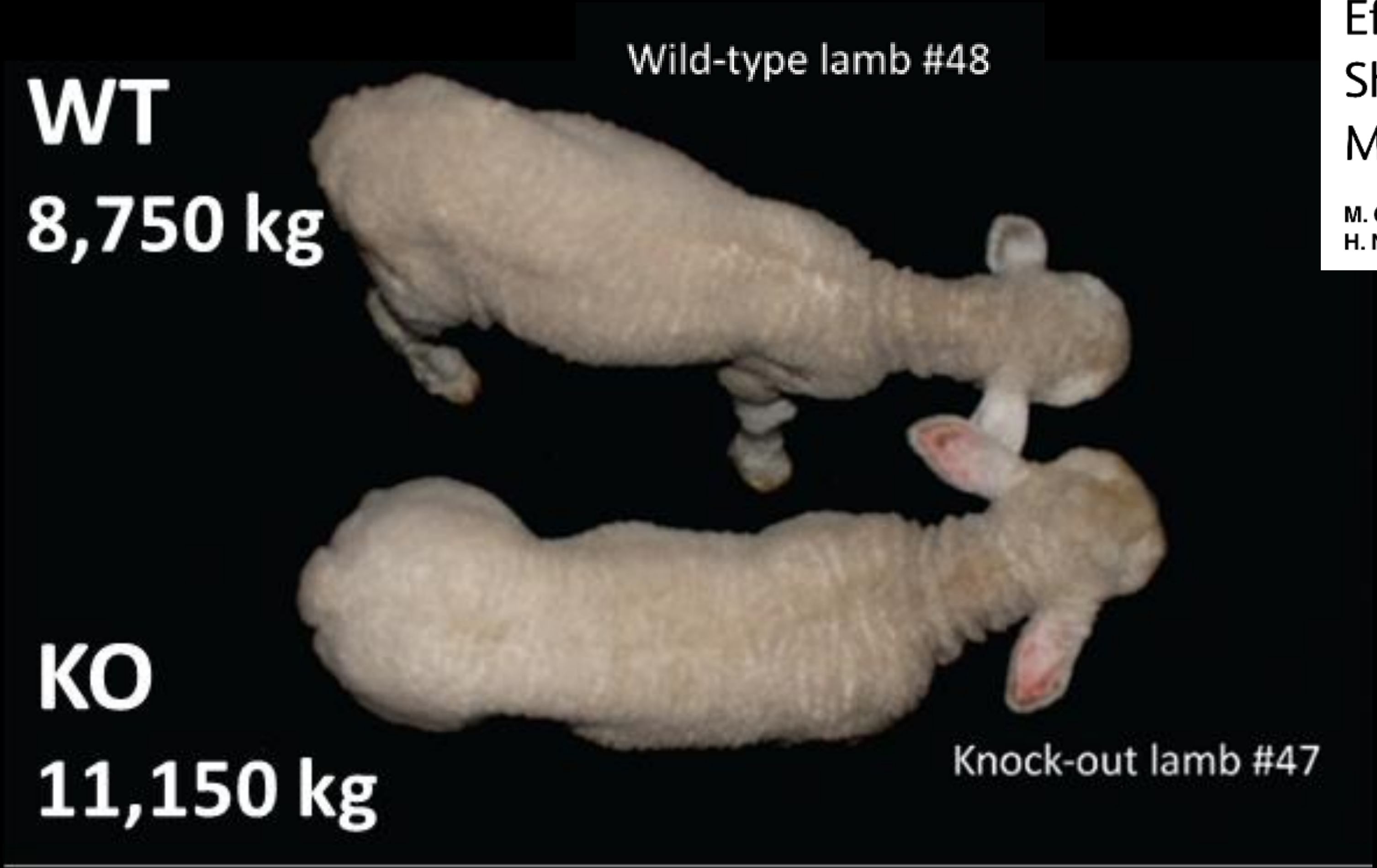
e.g., Texel sheep

PLOS | ONE

RESEARCH ARTICLE

Efficient Generation of Myostatin Knock-Out Sheep Using CRISPR/Cas9 Technology and Microinjection into Zygotes

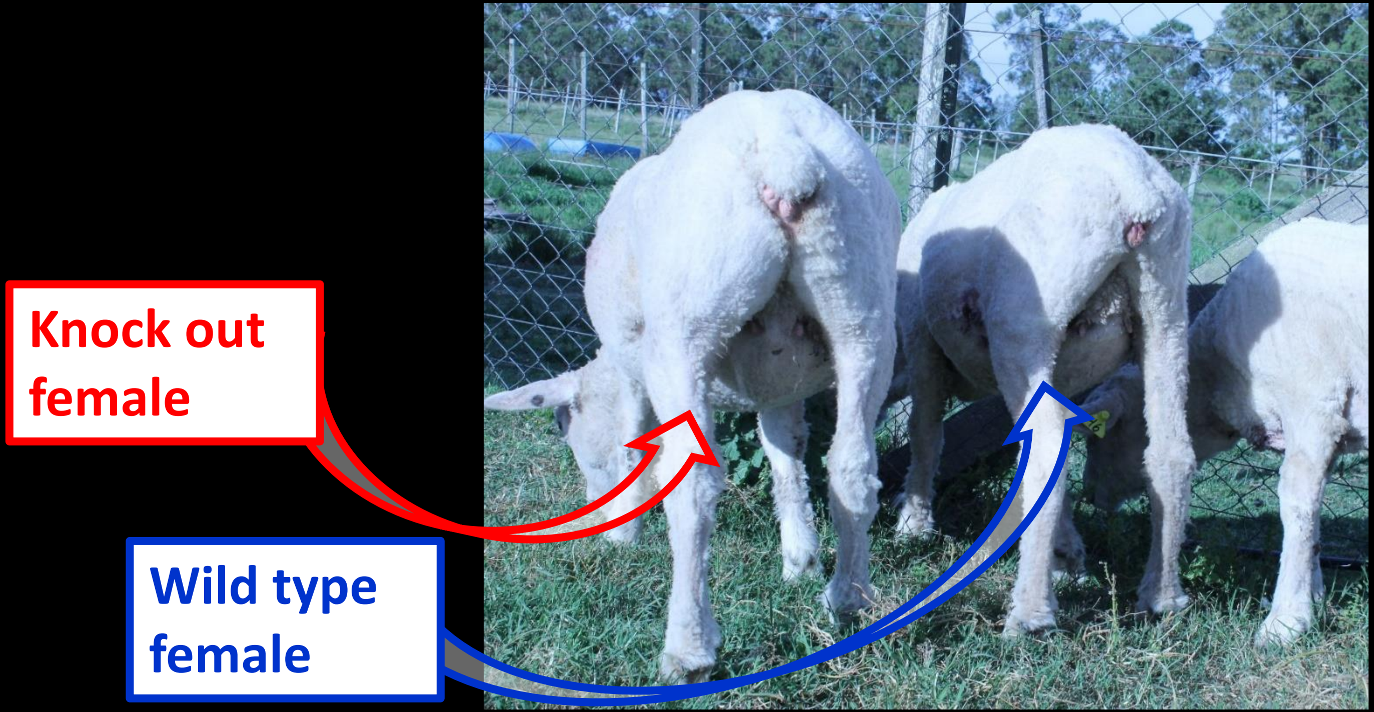
M. Crispo[1]*, A. P. Mulet[1], L. Tesson[3], N. Barrera[2], F. Cuadro[2], P. C. dos Santos-Neto[2], T. H. Nguyen[3], A. Crénéguy[3], L. Brusselle[3], I. Anegón[3]*, A. Menchaca[2]*



Corderos de 30 días comparando un animal Wild Type (WT) con uno producido mediante la técnica CRISPR (KO).

Wool production

Menchaca et al., 2020.



Similar yearling wool production (10 months old lambs).

	Yearling fleece weight	Fiber diameter			Clean fleece yielding	Fiber length	Colour	
		Mean diameter	CV	>30 microns fibers			Y º of brightness	Y-Z yellowness
WT (n=9)	2,4 kg	19,8 µm	19,7%	1,3%	71,9%	9,1 cm	70,7	-0,5
KO (n=4)	1,8 kg	18,0 µm	20,4%	0,8%	74,8%	8,5 cm	69,8	-0,1

P:NS

Superfine Merino wool

Meat breeds

Classical genetics

~10,000 years

CRISPR

few months

More wool

More meat

PlosOne, 2015.

Genome editing beyond research

Producing Healthier, Happier Animals and More Sustainable Farming

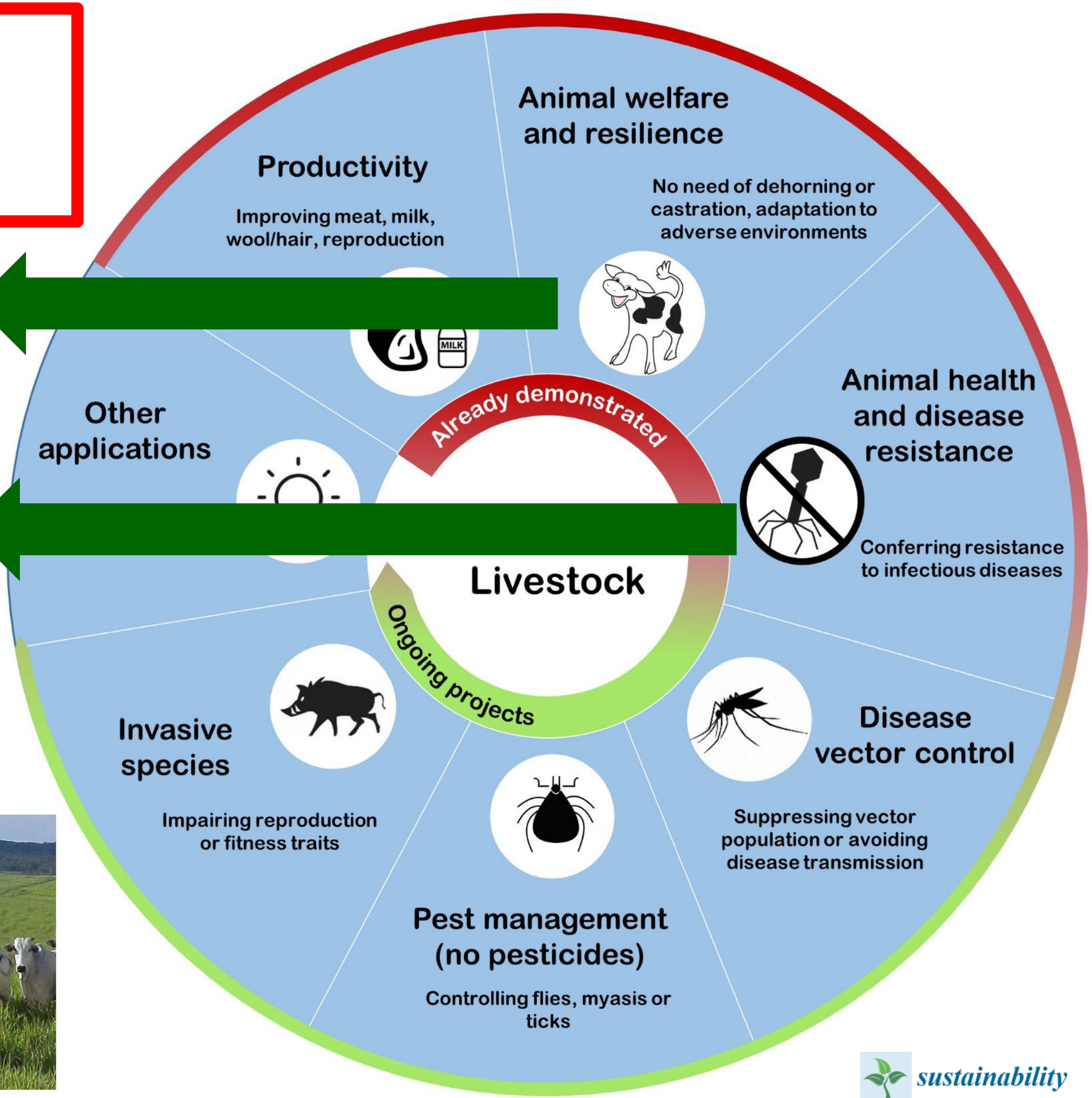
acceligen™

Learn More

recombinetics™

For Beef & Dairy Cattle

- Bovine tuberculosis (BTB) resistance
- Foot-and-mouth disease virus (FMDV) resistance
- Pest resistance
- Improved heat tolerance
- Dehorning procedure prevention
- Greater genetic diversity



https://recombinetics.com/acceligen/

sustainability

nature biotechnology 2016

Correspondence | Published: 06 May 2016

Production of hornless dairy cattle from genome-edited cell lines

Daniel F Carlson, Cheryl A Lancto, Bin Zang, Eui-Soo Kim, Mark Walton, David Oldeschulte, Christopher Seabury, Tad S Sonstegard & Scott C Fahrenkrug

Nature Biotechnology **34**, 479–481 (2016) | Download Citation

https://www.recombinetics.com/2018/05/29/recombinetics-and-semex-form-alliance-to-improve-animal-we

eia-1298_ifu--pmsg_2... Product Search | OriGe... Google Calendar Translater Scopus Publons Scimago Google e-BROU BBVA Scotiabank SNI Ley 18.611 iCloud

recombinetics Precise Gene Editing Our Team Our Story Newsroom

Recombinetics and Semex Form Alliance to Improve Animal Well-being

May 29, 2018

Precision Breeding Partnership to Eliminate the Need to Dehorn Cattle

recombinetics | SEMEX® Genetics for Life®

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.

SEMEX®
Genetics for Life®

FDA NEWS RELEASE

FDA Makes Low-Risk Determination for Marketing of Products from Genome-Edited Beef Cattle After Safety Review

Decision Regarding Slick-Haired Cattle is Agency's First Enforcement Discretion Decision for an Intentional Genomic Alteration in an Animal for Food Use

Share | Tweet | Linkedin | Email | Print

FDA APPROVED

For Immediate Release: March 07, 2022

Summary

Acceligen submitted genomic data and other information to FDA to demonstrate that the IGA contained in PRLR-SLICK cattle is the equivalent to naturally occurring mutations that occur in conventionally raised cattle with a history of safe use as a source of human food. These mutations result in the same short, slick haircoat seen in cattle with the IGA, and people have safely eaten food products derived from cattle with the slick haircoat for years.

https://www.fda.gov/media/155706/download

Tropical slick-haired cattle breeds.
Criollo composite cattle breeds **(A)** Senepol, **(B)** Carora, and **(C)** Romosinuano, exhibit the slick hair coat which has been associated with thermo-tolerance in tropical and sub-tropical climates.The slick phenotype is characterized as a fine, sleek hair coat with fewer hair follicles, shorter hair length, and larger sweat glands.
Huson et al., 2014; Frontiers in Genetics.

Annual losses:
>600 million USD in USA
>1,500 millon € in Europe

nature biotechnology

Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus

Kristin M Whitworth[1], Raymond R R Rowland[2], Catherine L Ewen[2], Benjamin R Trible[2], Maureen A Kerrigan[2], Ada G Cino-Ozuna[2], Melissa S Samuel[1], Jonathan E Lightner[3], David G McLaren[3], Alan J Mileham[3], Kevin D Wells[1] & Randall S Prather[1]

[1]Division of Animal Science, University of Missouri, Columbia, Missouri, USA. [2]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, Kansas, USA. [3]Genus plc, DeForest, Wisconsin, USA.

email: pratherr@missouri.edu

VOLUME 34 NUMBER 1 JANUARY 2016 NATURE BIOTECHNOLOGY

PRRSV resistant pigs

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology®

Pigs Lacking the Scavenger Receptor Cys of CD163 Are Resistant to Porcine Reproductive and Respiratory Syndrome Virus 1 Infection

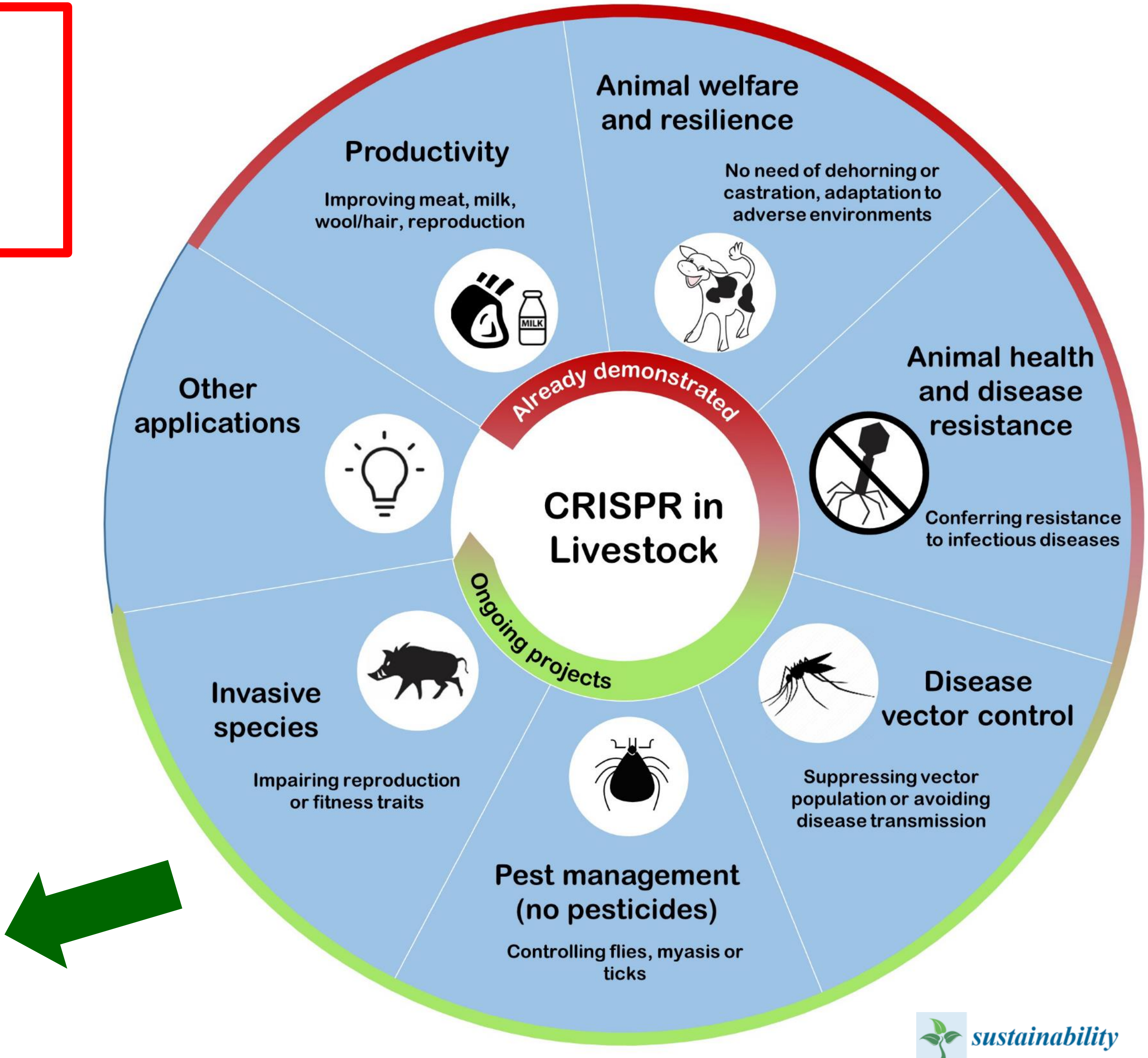
Christine Burkard,[a] Tanja Opriessnig,[a,b] Alan J. Mileham,[c] Tomasz Stadejek,[d] Tahar Ait-Ali,[a] Simon G. Lillico,[a] C. Bruce A. Whitelaw,[a] Alan L. Archibald[a]

[a]The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, United Kingdom

[b]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

[c]Genus plc, DeForest, Wisconsin, USA

CRISPR-based Gene drive

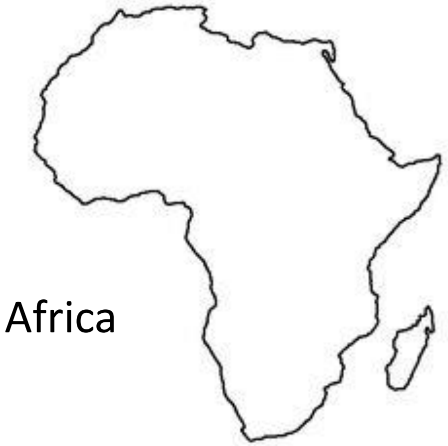


Pest management in livestock/agriculture

CRISPR para controlar plagas: Gene drive

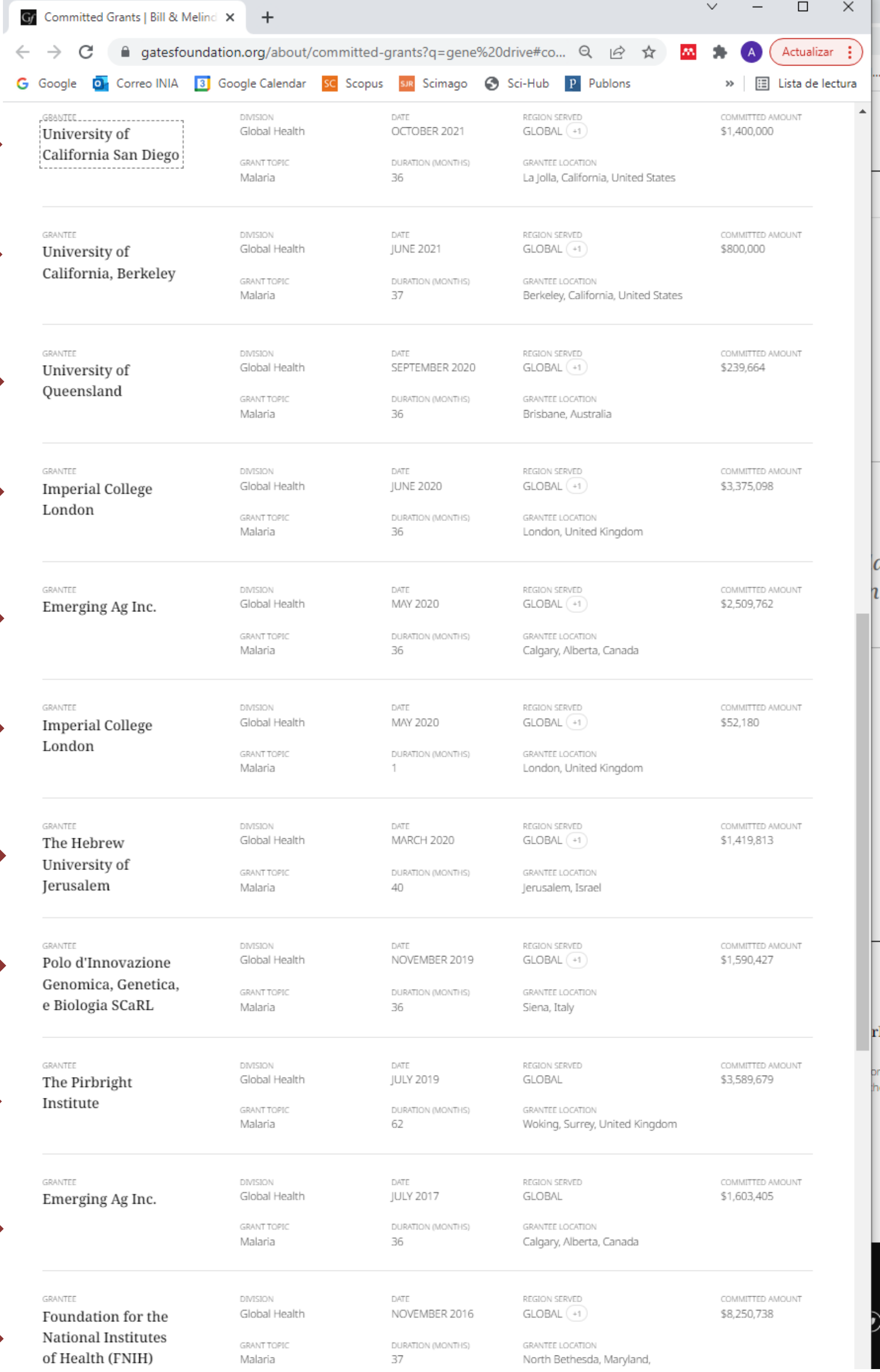
CRIPSR/Cas-based Gene drive

Malaria



Global efforts:
B&M Gates Foundation supports 11 projects in Gene drive

BILL & MELINDA GATES foundation



Grantee	Division	Date	Region served	Committed amount	Grant topic	Duration (months)	Grantee location
University of California San Diego	Global Health	OCTOBER 2021	GLOBAL +1	$1,400,000	Malaria	36	La Jolla, California, United States
University of California, Berkeley	Global Health	JUNE 2021	GLOBAL +1	$800,000	Malaria	37	Berkeley, California, United States
University of Queensland	Global Health	SEPTEMBER 2020	GLOBAL +1	$239,664	Malaria	36	Brisbane, Australia
Imperial College London	Global Health	JUNE 2020	GLOBAL +1	$3,375,098	Malaria	36	London, United Kingdom
Emerging Ag Inc.	Global Health	MAY 2020	GLOBAL +1	$2,509,762	Malaria	36	Calgary, Alberta, Canada
Imperial College London	Global Health	MAY 2020	GLOBAL +1	$52,180	Malaria	1	London, United Kingdom
The Hebrew University of Jerusalem	Global Health	MARCH 2020	GLOBAL +1	$1,419,813	Malaria	40	Jerusalem, Israel
Polo d'Innovazione Genomica, Genetica, e Biologia SCaRL	Global Health	NOVEMBER 2019	GLOBAL +1	$1,590,427	Malaria	36	Siena, Italy
The Pirbright Institute	Global Health	JULY 2019	GLOBAL	$3,589,679	Malaria	62	Woking, Surrey, United Kingdom
Emerging Ag Inc.	Global Health	JULY 2017	GLOBAL	$1,603,405	Malaria	36	Calgary, Alberta, Canada
Foundation for the National Institutes of Health (FNIH)	Global Health	NOVEMBER 2016	GLOBAL +1	$8,250,738	Malaria	37	North Bethesda, Maryland,

Mosca de la bichera (Screwworm)

Cochliomyia hominivorax

Big problem in South America

https://upload.wikimedia.org/wikipedia/commons/7/7d/Cochliomyia_hominivorax_%28Coquerel%2C_1858%29.jpg

Mosca de la bichera (Screwworm)

Cochliomyia hominivorax

Sustainability?

Pesticides: 6 Million tons/yr

Bernhardt et al., 2017

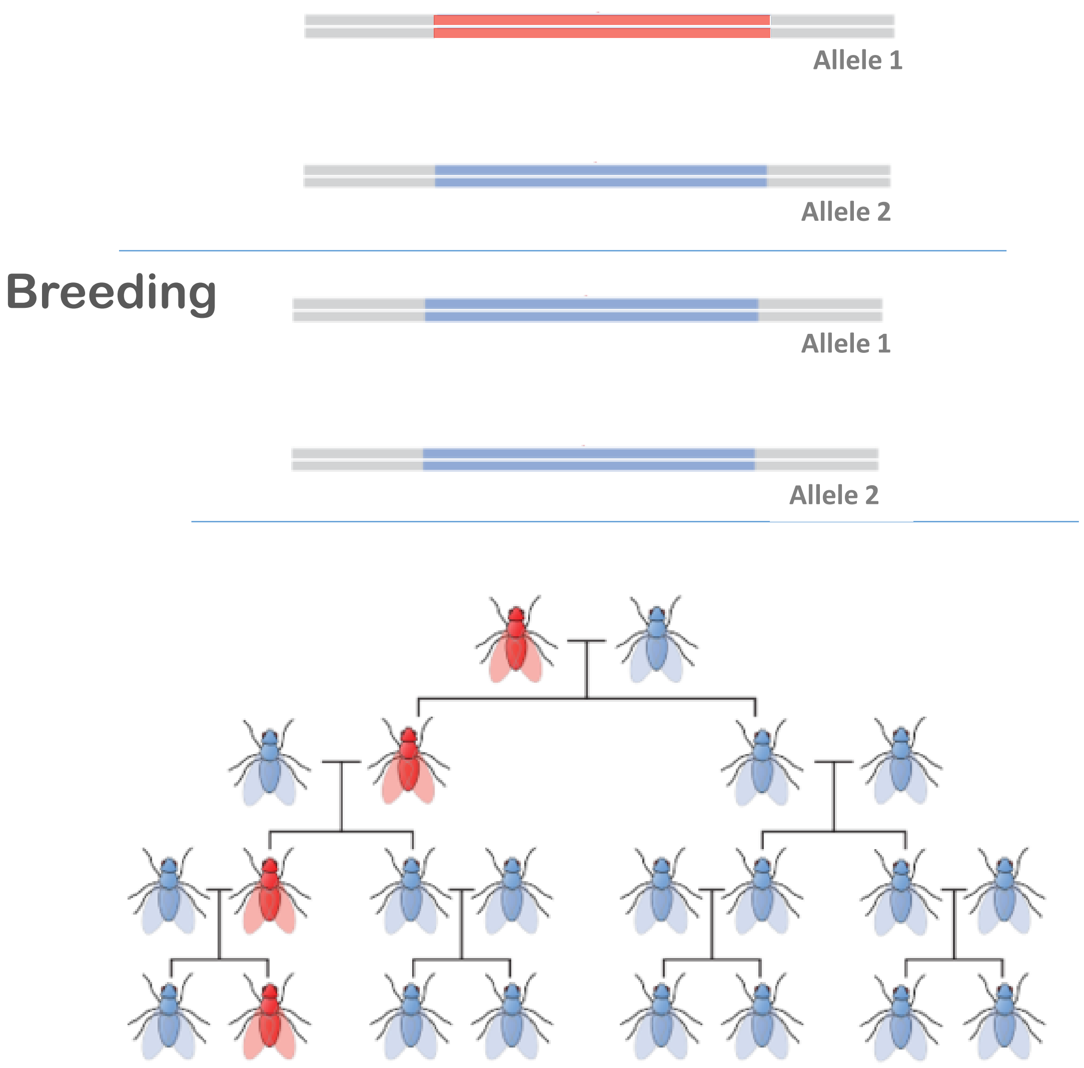
$$$

Losses in South America US$ 3.5 bill/year

Also in humans

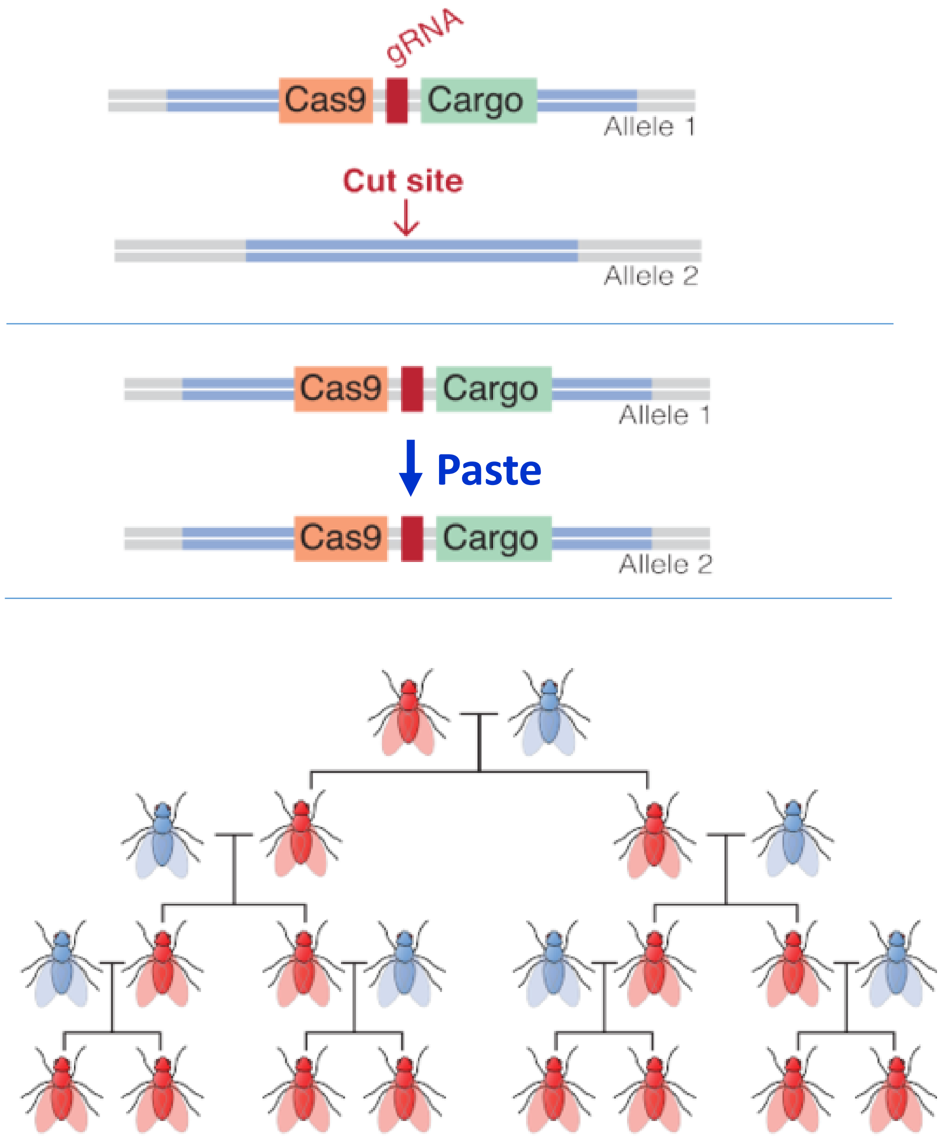
Animal welfare?

Natural breeding



The genetic trait is lost in the wild population

Gene drive by CRISPR

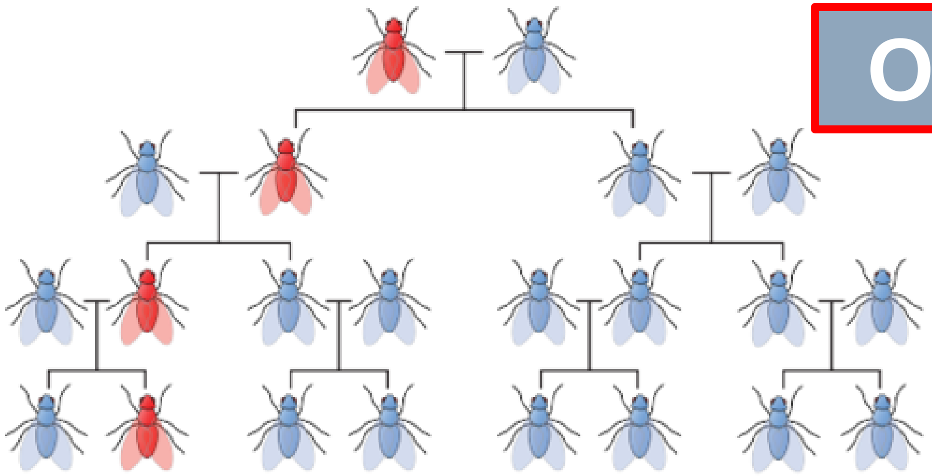


The trait is always inherited in all the progeny

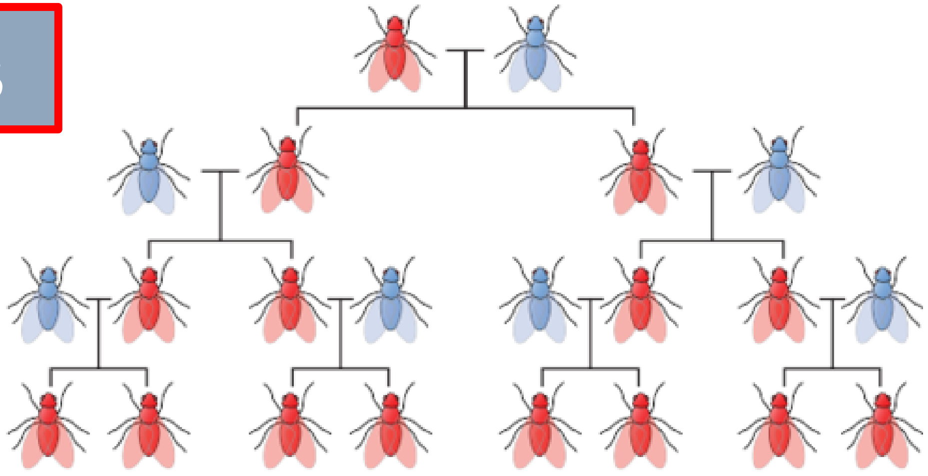
Mosca de la bichera (Screwworm)

Cochliomyia hominivorax

Only males

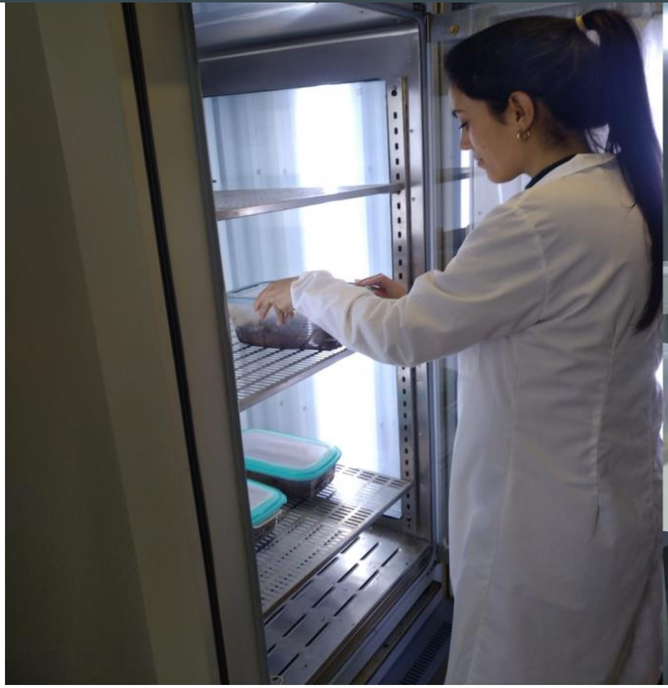


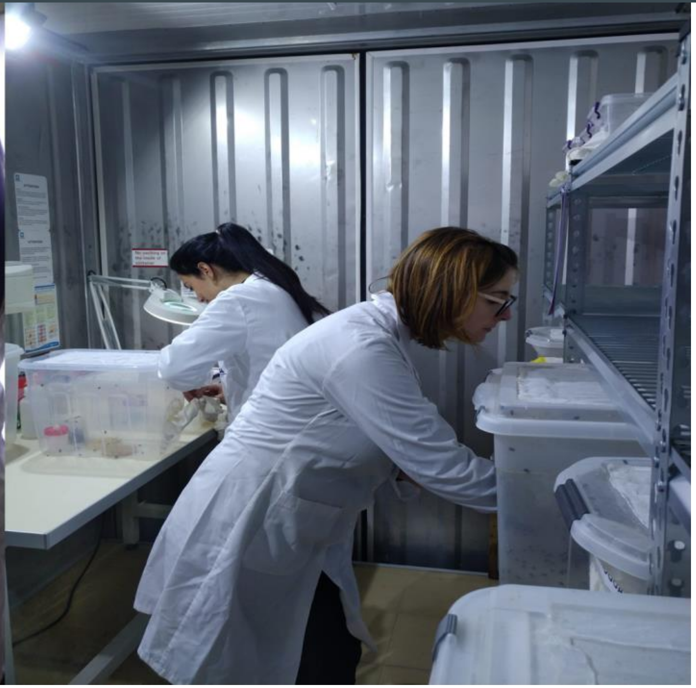
The genetic trait is lost in the wild population

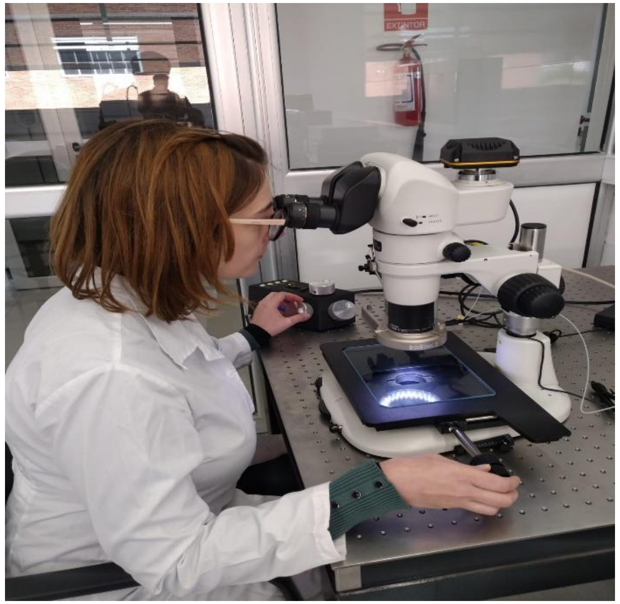


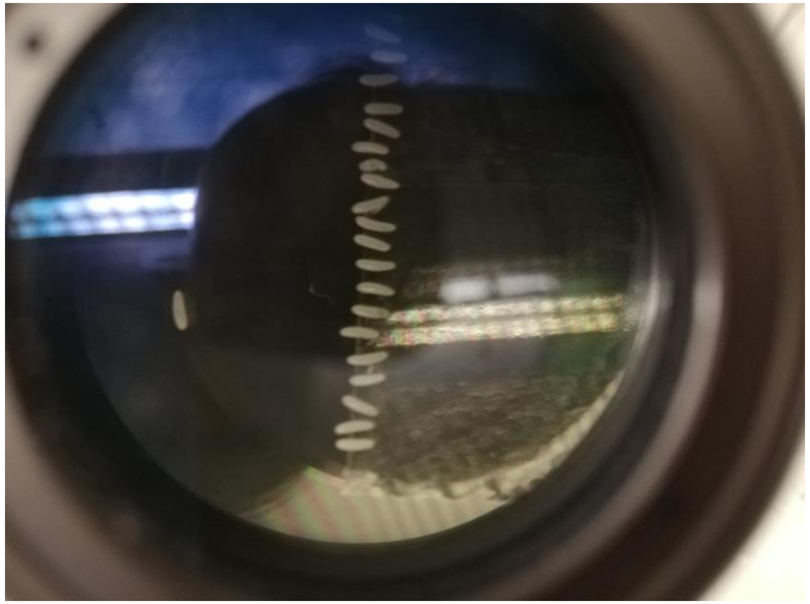
The trait is always inherited in all the progeny

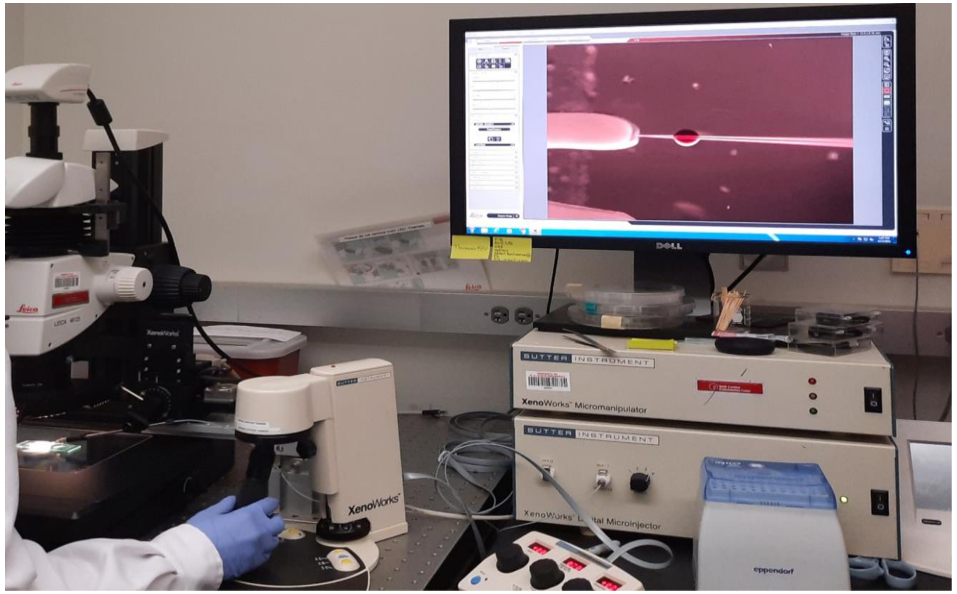
CRISPR/Cas system, fly rearing, and embryo microinjection:



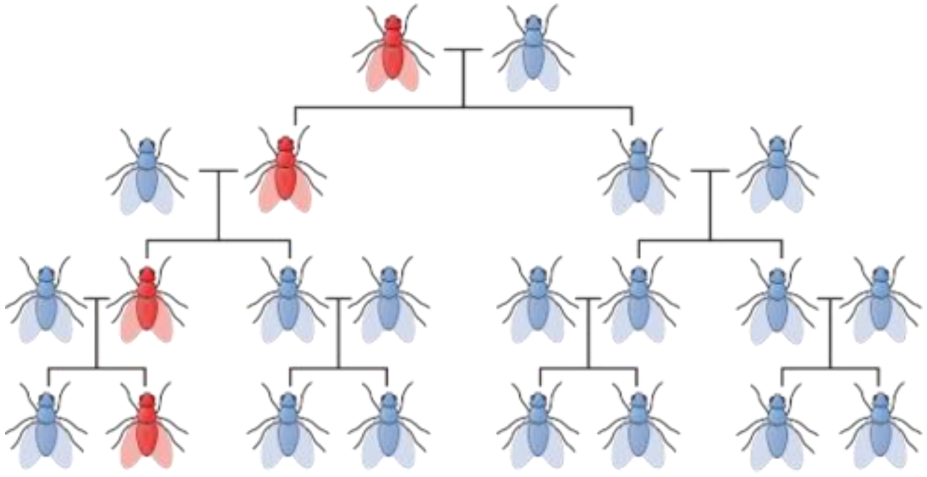




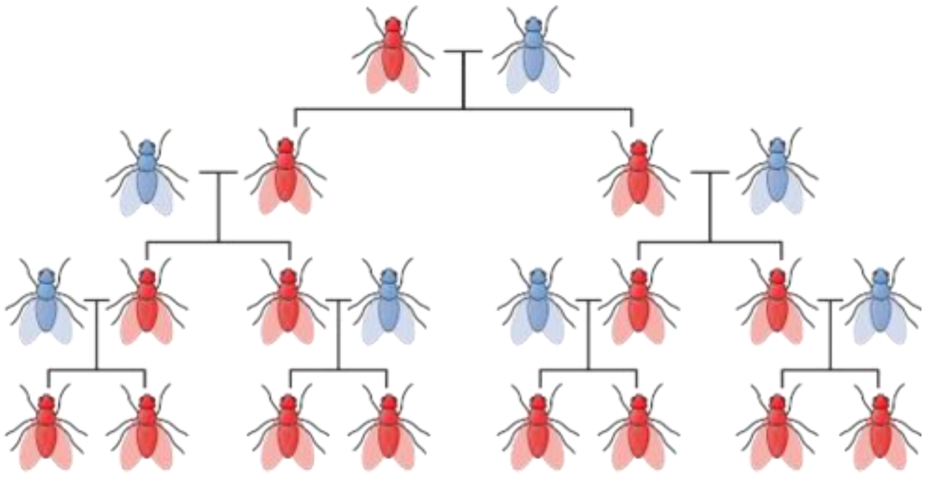




Garrapata?



El gen de interés se pierde en la población

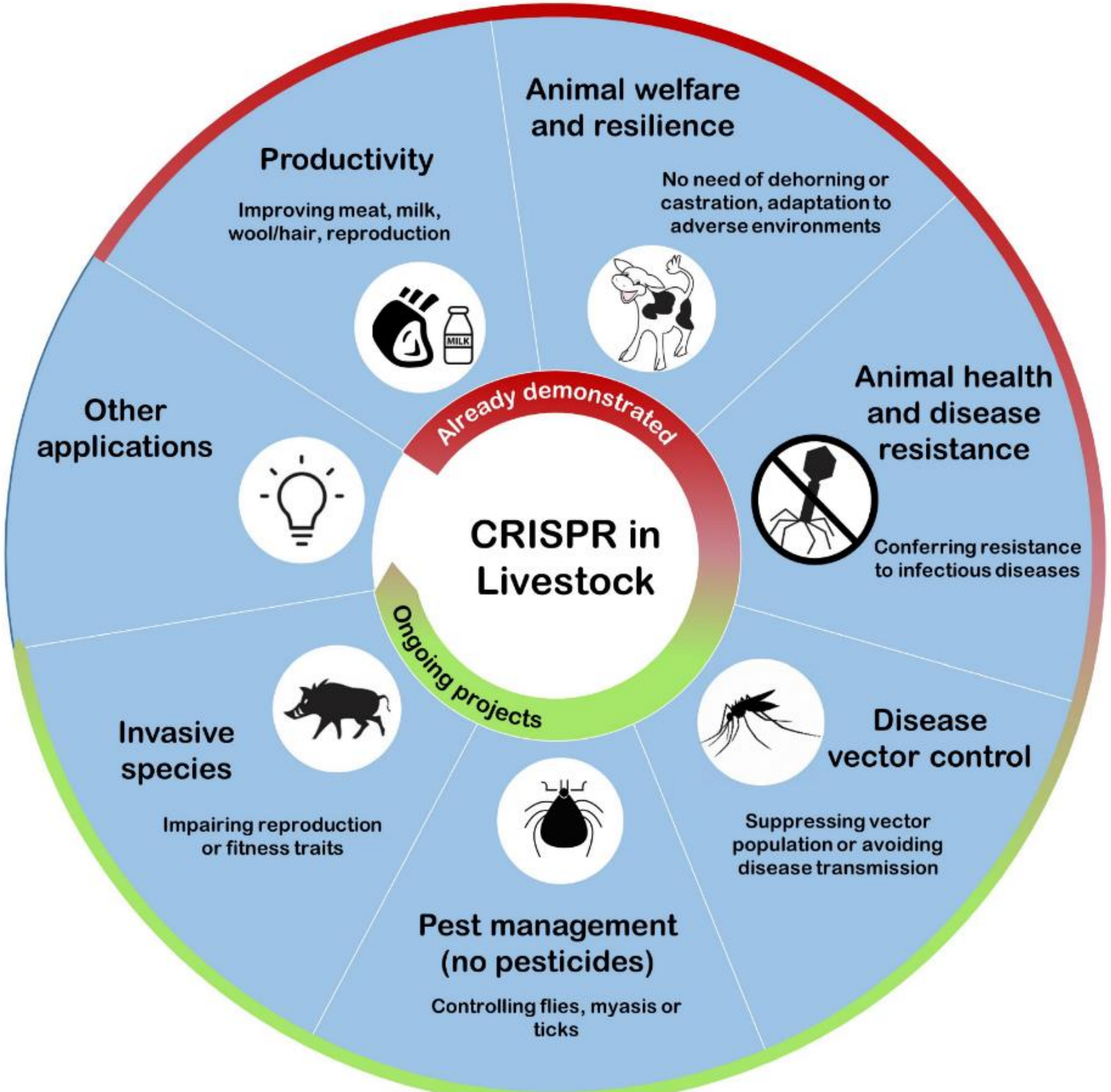


El gen de interés siempre se hereda

CRISPR in livestock

Preguntas y nuevas ideas??

Que?
Como?
Para que?



INIA
URUGUAY

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Anderson Saravia

Alejo Menchaca

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UNIDAD MIXTA PASTEUR + INIA

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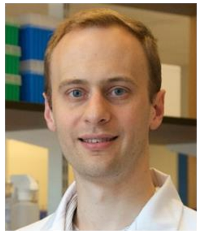
Institut Pasteur
de Montevideo

NC STATE
UNIVERSITY

Max Scott's Lab



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Institute of
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Ignacio Anegón's Lab

Inserm
Nantes, France

Obrigado.