



APYS Biotechnology

LIVESTOCK GENE EDITING FOR SERVICE

*Perspectives of a University Spin-Off in the Animal
Biotechnology Space*

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ADVANCING SCIENTIFIC DISCOVERY TO SOCIETAL IMPACT

Basic
Discovery

Biological
Proof-of-Concept

Commercial
Proof-of-Concept

Livestock
Producer

Food
Supply

WASHINGTON STATE
 UNIVERSITY



APYS Biotechnology





Impact

**Governmental
Regulation**

**Consumer Perception
& Acceptance**

Commercialization

**Societal Issue & End
User Need/Interest**

Basic Discovery

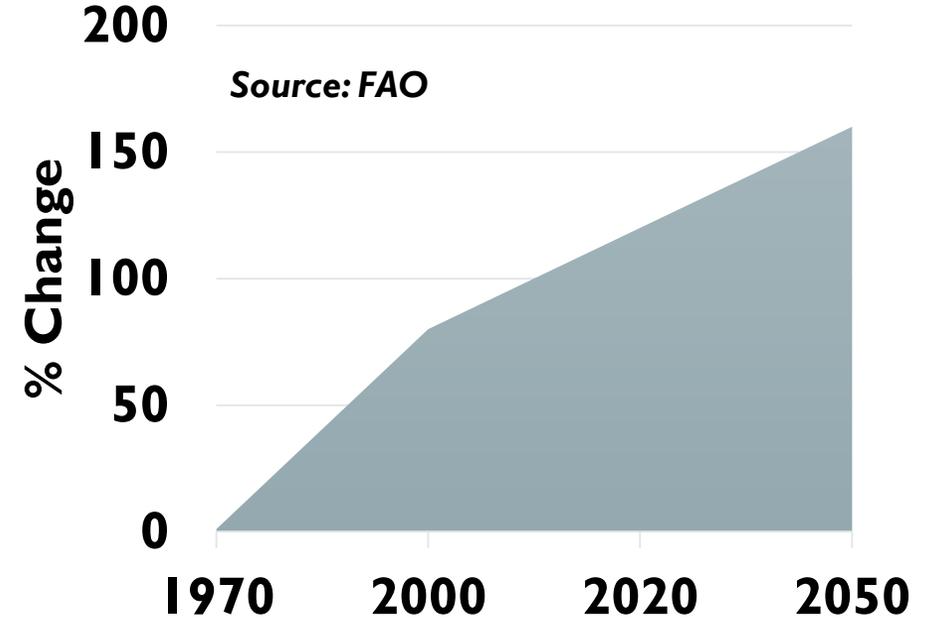


GLOBAL PROBLEM

↓ Agricultural Land

↑ Demand for Food

Demand for Animal Protein

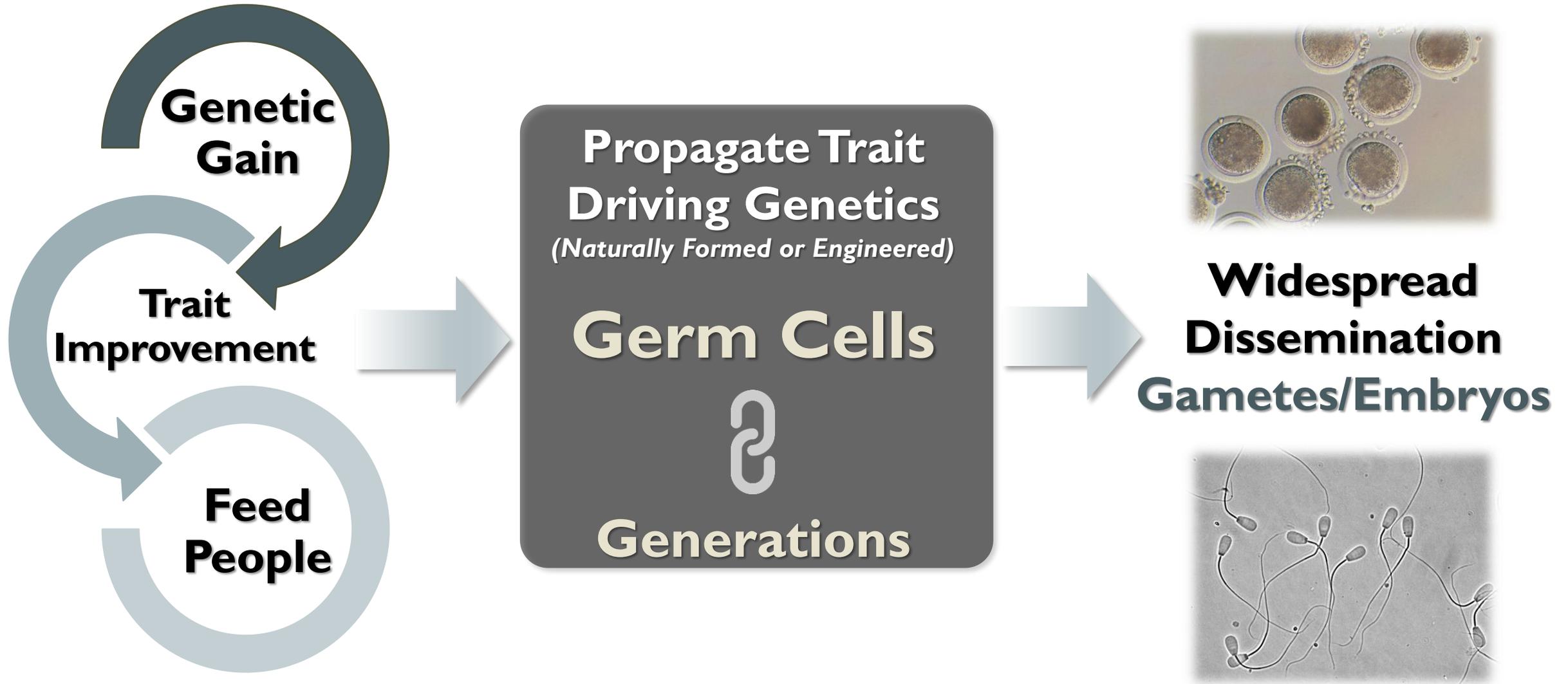


Solution



Enhance the Efficiency of Livestock Production

OUR GUIDING PRINCIPLE



OVERARCHING GOAL

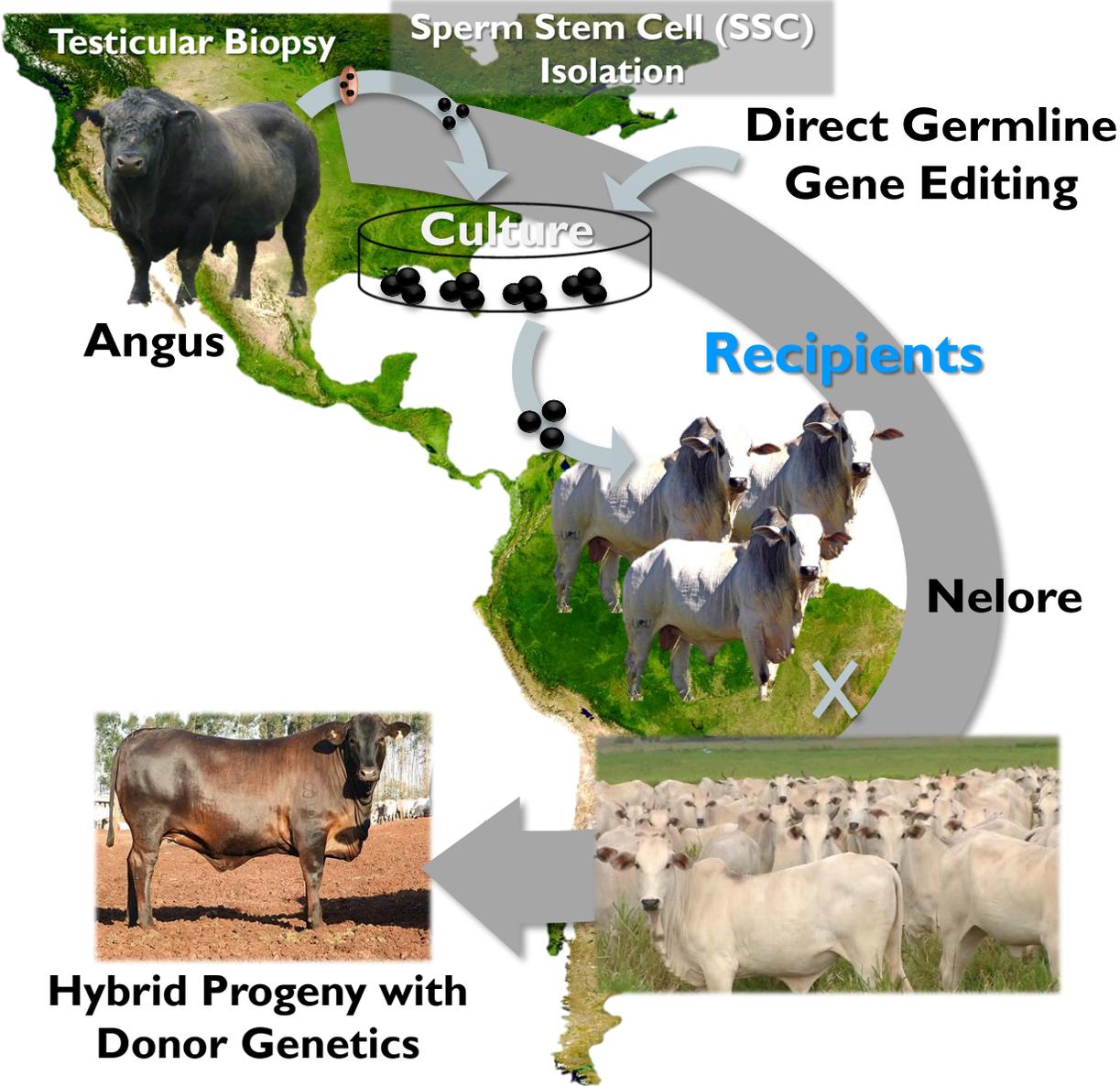
**Devise Technologies that Enable Access to Sperm Containing Trait
Driving Genetics and Dissemination via Natural Mating**

NextGen Sires

(Surrogate Sires, Giassetti et al., 2019, Ann Rev Anim Biosci)



FOUNDATIONAL CONCEPT GERMLINE TRANSPLANTATION IN LIVESTOCK



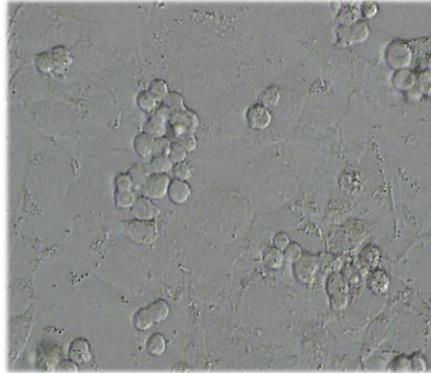
Key Aspects

In Vitro Expansion of Donor Stem Cells

Recipient Germline Ablation & Transplantation

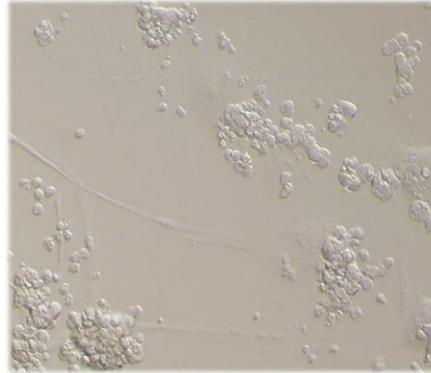
LIVESTOCK SPERMATOGENIAL CULTURE

Mouse



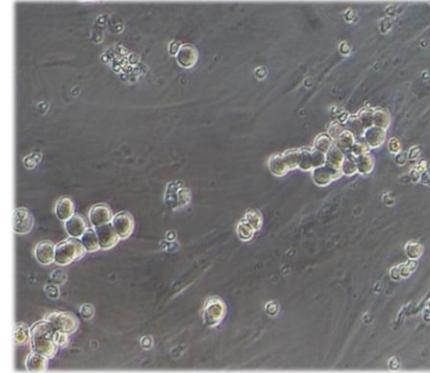
Oatley et al., 2006, PNAS

Pig



Unpublished

Cattle



Oatley et al., 2016, Biol. Reprod.

Sheep



Unpublished

POC Pipeline

Germ Cell Biomarker



Spermatogonial Biomarker



Exponential Expansion



Sperm Generation via Transplantation

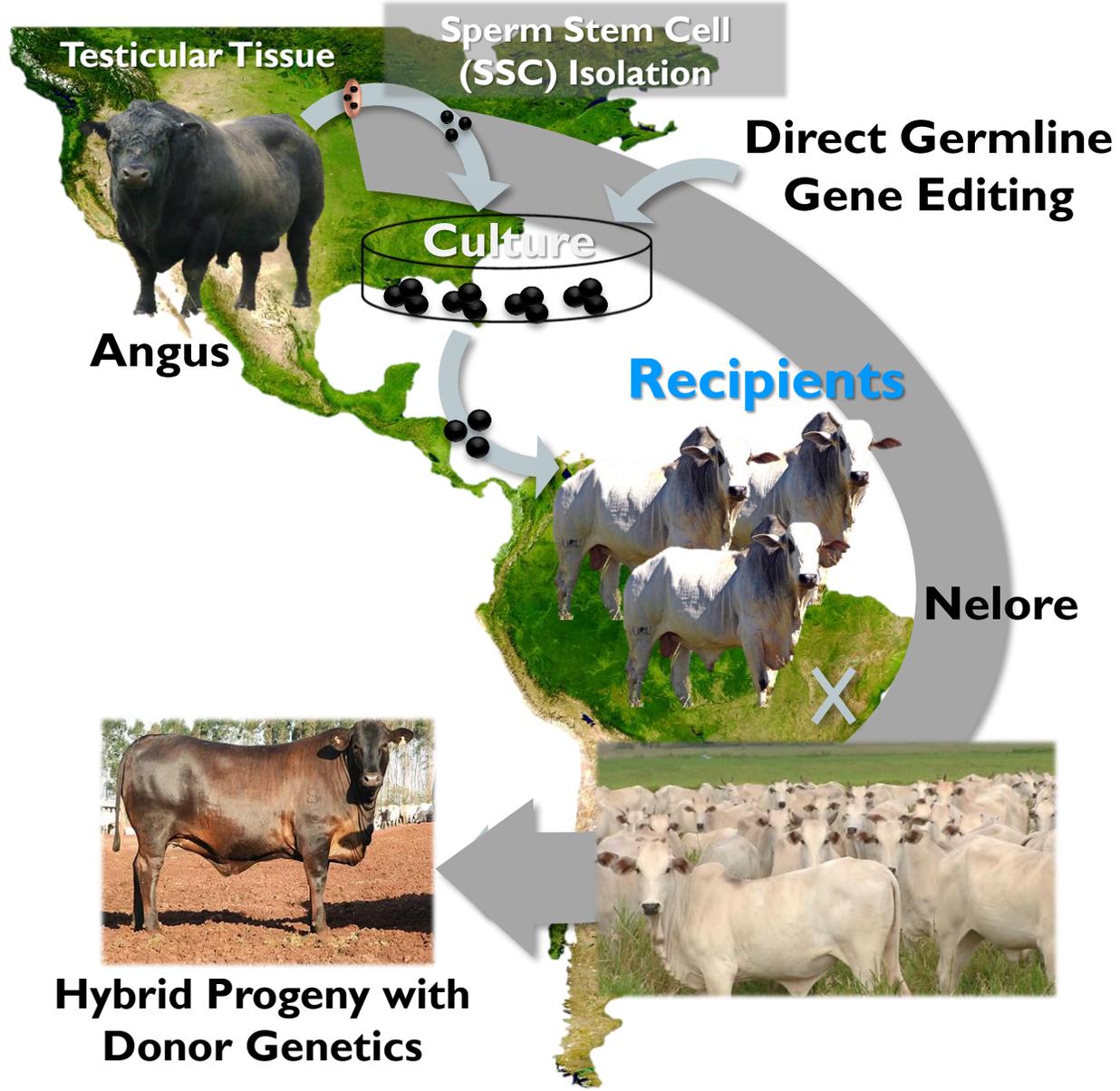


In Development

In Development

FOUNDATIONAL CONCEPT

GERMLINE TRANSPLANTATION IN LIVESTOCK



Key Aspects

In Vitro Expansion of Donor Stem Cells

Recipient Germline Ablation & Transplantation

EDITING NANOS2

Nanos



Nanos KO



**Male & Female
Sterile**



Nanos 1/2/3



Nanos2 KO
ES Cell Gene Targeting



**Males – Sterile
Females – Fertile**



Nanos 1/2/3

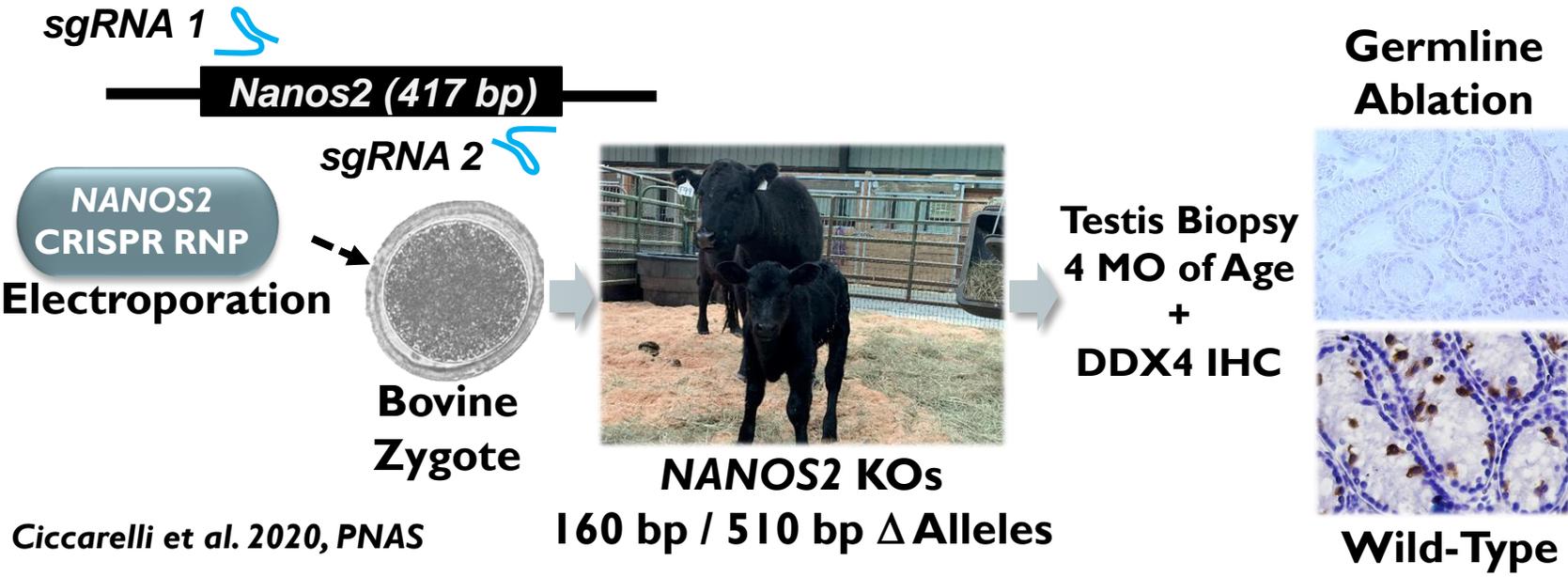


Nanos2 KO
CRISPR/Cas9 Editing

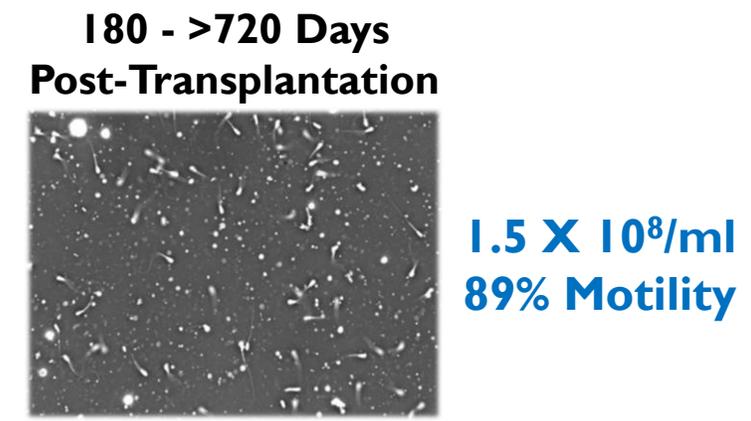


**Males – Sterile
Females – Fertile**

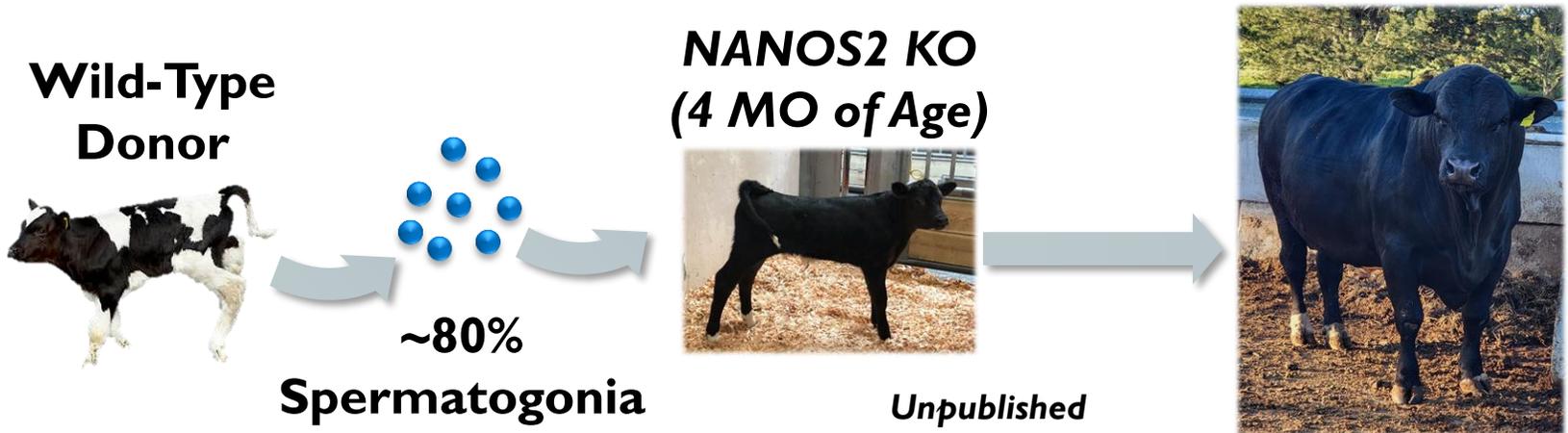
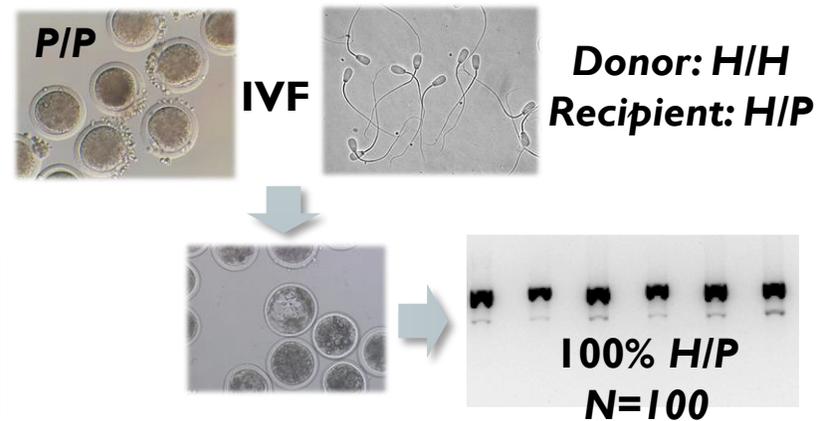
SSC TRANSPLANTATION NANOS2 KO MALE CATTLE



Donor-Derived Spermatogenesis



Sperm Genotyping Horned/Polled Allele



NATURAL MATING NANOS2 KO SURROGATE BULLS



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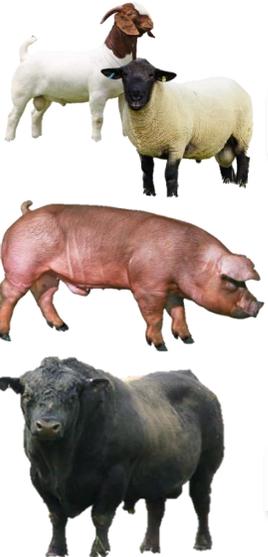
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➔ **APYS Biotechnology**



Custom Genome
Editing

NextGen Sires

Germline Edited
Traits

LIVESTOCK PRODUCER INTERESTS

Welfare

Disease Resistance

Growth

Reproduction

NextGen Sires

Beef Cattle, Sheep, Goat

- Amplify Breeding of Desirable/Elite Sires
- Increased Access to New Breeds
- Preservation of Legacy Breeds

Germline Edited Traits

Beef Cattle

- Dehorning
- BRD & ECF Resistance
- Increased FCR
- Polyovulation

Sheep

- Increased FCR
- Scabby Mouth Resistance
- Tail Docking

NAVIGATING THE US FEDERAL REGULATORY LANDSCAPE

Who is responsible for gaining federal approval?

Developer?

- University
- Startup
- Sublicensee

End User?

- Livestock Producer

NANOS2 Edited Animal

Welfare Concerns?

- NANOS2 is expressed in testicular germ cells only

Altered Composition of Edible Product?

- Germline restricted expression
- Germ cells die

Environmental Impact?

- KO is male sterile

Offspring of NextGen Sire

Non-Edited Donor SSCs

- Won't possess a genome edit
- Regulated or not?

Trait Edited Donor SSCs

- Genome alterations that could be attained with selective breeding
- Regulated or not?

GENETIC ENGINEERING – WHAT ARE WE FEARING?

- **DNA changes from CRISPR-Cas generated INDEL mutations or polymorphisms**
 - Are these different than changes from endogenous DSB repair, replication errors, or meiotic recombination?
- **Concerns of changing DNA sequence with gene editing?**
 - *Generation of a novel protein*
 - Also possible through random mutation during DNA replication and meiosis
 - *Negative impact on welfare of the animal*
 - Self-regulating – developers/producers unlikely to advance genome edits that reduce efficiency or health of an animal
 - *Negative environmental impact*
 - Also possible through random mutagenesis & selective breeding
 - *Altered composition of the food product*
 - Multifaceted – all animals, including non-edited, impacted by diet and environment
- **GMO trigger (in my opinion)**
 - Incorporation of foreign DNA into the genome that could not occur without human intervention
 - Animals with genome edits that are changes in DNA that could arise in nature should not be considered a GMO

Thank You