OBREAL Global

INTER-REGIONAL TRAINING COURSES IN THE FRAMEWORK OF THE 2ND INTER-REGIONAL DIALOGUE ON EDUCATION AND DEVELOPMENT

UNLOCKING THE POTENTIAL OF BIOTECHNOLOGY IN FOOD PRODUCTION

MODULE 4

BIOTECHNOLOGY FOR LIVESTOCK AND FISHERIES

May 23, 2024

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OBJECTIVE OF MODULE 4 BIOTECHNOLOGY FOR LIVESTOCK AND FISHERIES

- Gaining understanding about the fundamental concepts and principles of biotechnology as applied to livestock and fisheries.
- Assessing the various biotechnological applications in livestock and fisheries management.
- Understanding the ethical, legal and societal implications of biotechnological interventions in livestock and fisheries management.
- Analyze the effective communication with diverse stakeholders.

The main challenges for the animal production

- **Satisfy the demands in animal products of our present and future human populations**
- **Ensure a worthy standard of living for our livestock farmers** generating wealth and reducing poverty in our rural communities.

For this to be achieved our main avenues are :

  - **The Increase Productivity and competitiveness and ensure sustainability**

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The key is the productivity

No one system with low productivity is sustainable through time , nor can achieve competitiveness

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PRODUCTIVY :

More quantity and better quality of product obtained per unit of resource (soil, water, energy) applied.

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The one that uses and transforms the resources is the living organism (plant,animal)

The efficiency of the living organism (of the animal for the case of livestock and fish) in the transformation of the resources into products **depends of the design of that animal** (**their genes, the genetic composition**)

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- **The genes provide the operational plan of the animal, are responsible for its structure and function and their metabolic efficiency**,
- **The genes interacting with the environment determine**
  - **The type of product and the quantity of animal product** able to be produced by the animal from each unit of resource applied **(production and productivity)**
  - **The structure and composition of the products** produced by the animal determining the **quality of the animal product.**
  - **The capacity of the animals to resist sickness** (either caused by infections of virus, bacteria and fungus or by lack of nutrients, stress, etc.)
  - **The capacity to tolerate/adapt to adverse environmental conditions** (low or high temperatures and humidity, strong winds, water scarcity, type of soil and forages, etc).

UNDERSTANDING the animal machinery based on **deeper knowledge of the genes and their functions** has open a total new era:

The era of **biotechnological applications in animal improvement**

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BIOTECHNOLOGY is referred to technologies based on the understanding of the biology of the living organisms and its manipulation to develop products, methods and organisms .

MODERN BIOTECHNOLOGY more specifically , is based on molecular biology, began with the discovery of the genetic code and is considered our more potent tool in the XXI century to achieve animal genetic improvement.

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Biotechnology in animal (livestock and fish) genetic improvement

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- Animal genetic improvement has been practiced for millennia from domestication of animals 8,000 years ago and subsequent **selective breeding**

Source of picture: **MARVIN A. VILLANUEVA, DVM, PhD**
Genetically Modified (GM) Animals: Developments in Research and Policy Framework
Research and Development Division, DA- Philippine Carabao Centre

- **Genetic improvement in animals has been done traditionally by SELECTIVE BREEDING**

- **Selective breeding** is the preferential use as parents of the next generation to the individuals with the highest probabilities of having the desirable genes for the productive characteristics of interest. Implies:
  - FINDING the animals in the population that have the good genes
  - INTENSIVELY DISSEMINATE in the population their good genes

Before disseminating the genes in the population through intensive reproduction we need to find the animals with the desirable genes to be used as parents

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- **The genetic progress in productivity and quality of product achieved with selective breeding per unit of time depends on**:
  - **How high above the population mean is the performance of the selected individuals** (selection intensity depends on how many individuals can be generated from the selected parents; more offspring generated per parent, less parents needed, select only the top)
  - **How close are the ESTIMATED genetic values** to the **TRUE genetic values** of the selected parents (selection accuracy based on evaluation of phenotypic performance of the individual and its relatives; more relatives, more accuracy)
  - **How early the selected individuals can be used as parents** (generation interval)
  - **How variable is the genetic composition of the animals of the population** (If all are the same, there is no selection).

Factors determining the Genetic progress (△G)

$$\triangle G = \frac{\text{Selection Intensity x Selection accuracy X Genetic variability}}{\text{Generation Interval}}$$

Each of these factors is affected by the reproductive technology used and by the method used to find the animals with the higher probabilities of having the desired genetic composition

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- The first and main contributions of **modern biotechnology** in animal genetic improvement are the **PARENTAGE CERTIFICATION** (increasing accuracy in the genealogic records) **and the GENOMIC SELECTION** which predicts the genetic value of the animals with **HIGHER ACCURACY than the conventional selection**.
- **GENOMIC SELECTION** can be done very early in life consequently **reducing dramatically the generation interval INCREASING THE GENETIC PROGRESS PER UNIT OF TIME**
- **GENOMIC SELECTION** is a form of Marker Assisted Selection (MAS) that predicts the genetic values by associating their traits with their high-density **genetic marker scores**
- **GENOMIC SELECTION** is practiced in Peru in dairy and beef cattle but using genetic markers developed mainly in the USA. There are efforts today in Peru to **develop genetic markers for fiber characteristics in alpacas**. We are in the process of generating a population of reference with phenotypic evaluation and DNA determination to develop genetic markers .

Molecular genetics of alpacas in Peru

- Silvestre R., Rivas E., Veli E., Aquino, Y. and Vivanco W. 2008. Análisis genético molecular en alpacas de la raza Huacaya (Molecular genetic analyses in Alpacas of the Huacaya breed) **13Avo Congreso Latinoamericano de Genética. Resúmenes. Lima 2008.**

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More M., Gutierrez G., Rothschild M., Bertolini F., de Leon F.A.P. **Evaluation of SNP Genotyping in Alpacas Using the Bovine HD Genotyping Beadchip**. *Front Genet.* 2019;10:361. doi: 10.3389/fgene.2019.00361.

Mendoza M.N., Raudsepp T., Alshanbari F., Gutierrez G., de Leon F.A.P. **Chromosomal Localization of Candidate Genes for Fiber Growth and Color in Alpaca (*Vicugna pacos*)** *Front Genet.* 2019;10:583. doi: 10.3389/fgene.2019.00583.

Barreta J., Gutierrez-Gil B., Iniguez V., Saavedra V., Chiri R., Latorre E., Arranz J.J**. Analysis of mitochondrial DNA in Bolivian llama, alpaca and vicuna populations: A contribution to the phylogeny of the South American camelids.** *Anim. Genet.* 2013;44:158–168. doi: 10.1111/j.1365-2052.2012.02376.

- In addition to **parentage certification** and the increment of the **rate of genetic gain** in the animal populations , **MODERN BIOTECHNOLOGY** is used to
  - **Adapt the animals to the climate changes** and **confer characteristics necessary for the successful animal production in different ecological environments**
  - **Solve phisiological limitations , gene terapy**
  - **Confer resistance and even inmunity to different sickness**
  - **Produce special proteins using animals as bioreactors**
  - **Use of animals to produce organs for humans**

GMO in fish

- The use of GMO technologies in fish has been deemed useful for obtaining an increase in
- production,
- size,
- disease resistance,
- optimal food consumption,
- and pharmaceutical development.

Common species selected for research into the application of transgenic manipulations include salmon, tilapia, channel catfish, and medaka.

Forms of genetic modifications in animals

- **Increase gene copies** of the same specie to increase their expression.
- **Deletion of genes** to eliminate them from the genome of the animal
- **Transfer genes** :
  - **TRANSGENESIS**, transfer from one specie to another to incorporate characters that do not exist in the specie
  - **CISGENESIS** transfer from same specie sexually compatiple (crossable) organism.

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Transgenic Construction in fish

Transgenic fish is mainly created by injecting bacterial artificial chromosomes (*BACs*) or plasmids containing the desired DNA into single-cell embryos. Including sequences with recognition sites for DNA-modifying enzymes allows the DNA to insert randomly into the genome. Most commonly, the Tol2 *transposon* is used in zebrafish .

Transgenic Salmon (growth hormone transgene) in comparison with a non transgenic Salmon at the same age.
IS NOW IN COMMERCIAL USE, approved by FDA (UCM 3331º2.pdf)

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Use of **"sequencial gene Targeting"** to **eliminate** the genes that regulate the production of PRIONS consequently are inmune to the "crazy cow disease" (Bovine spongioform encephalopatie)

PRNP-/- Cloned calves
immune to Bovine spongiform encephalopathy

J.A. Richt et al. 2007

USDA Ames IOWA

Nature Genetics (2004), Nature Biotechnology (2007)

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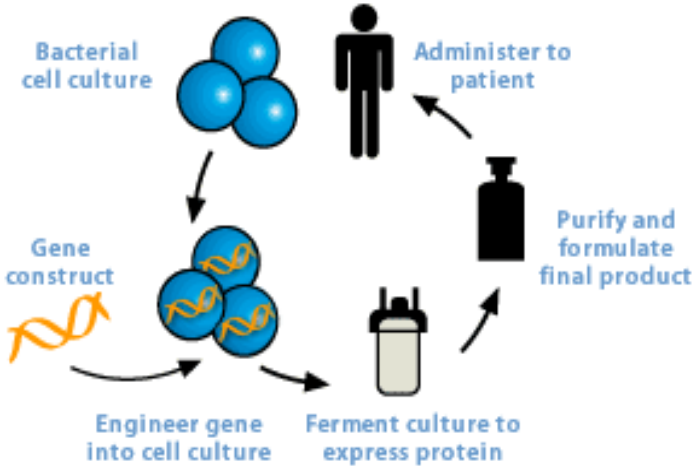
Transgenesis used for production of specific proteins .



The mammary gland acts as BIOREACTOR to produce large quantities of specific proteins (i.e. human myelin basic protein)

Final product

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Production of terapeutic milk. AgResearch New Zealand

- Production of human myelin basic protein (rhMBP) for the treatment of muliple sclerosis in humans.
- Journal of Chromatography B. Volume 877, Issues 16–17, 1 June 2009, Pages 1667-1677

On-line casein micelle disruption for downstream purification of recombinant human myelin basic protein produced in the milk of transgenic cows

A. Al-Ghobashy , Martin A.K. Williams , Brigid Brophy , Götz Laible , David

R.K. Harding

Rosita a twice TRANSGENIC cow developed in Argentia is producing milk containing proteins similar to proteins in human milk (lisozime and lactoferine).

INTA Balcarce, Buenos Aires and Universidad Nacional de San Martín (UNSAM)
https://dossierweb.com.ar

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Nexia. Canada: TRANSGENIC Goats producing SPIDER WEB proteines in their milk

Canadian scientists through genetic engineering have produced the proteins of spider-silk from goats' milk, and the proteins have already yielded fibres strong enough for surgical use. Some spider silk is as strong as Kevlar used for ballistic protection; being as strong as steel it could substitute for Kevlar.

Transgenic goats producing spider silk protein in their milk; behaviour, protein purification and obstacles Justin Jones*, Heather Rothfuss, Holly Steinkraus & Randy Lewis Department of Molecular Biology, University of Wyoming, USA. Transgenic Res (2010) 19:131–153 DOI 10.1007/s11248-009-9326-4

TRANSGENIC cows produce 3 times more caseine in their milk

AgResearch
New Zealand

Increased gene dosage for β- and κ-casein in transgenic cattle improves milk composition through complex effects
Götz Laible,[a,1] Grant Smolenski,[1] Thomas Wheeler,[1] and Brigid Brophy[1]

Sci Rep. 2016; 6: 37607.
Published online 2016 Nov 23. doi: 10.1038/srep37607

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Milk of transgenic cows with high level of casein (3 times higher) VS milk of non transgenic control cows.

control TG control TG

Whole milk Skim milk

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Transgenic animals generated in Ibero-America

Gluca: **Transgenic Goat** generated by Unifor Brasil in 2005 , has a human protein in her milk which is used for treatment of the Gaucher Disease

Mol Biotechnol. 2016 Jan;58(1):47-55. doi: 10.1007/s12033-015-9902-1.. Kaio Cesar Simiano Tavares et al.

Gaucher disease is a rare, inherited metabolic disorder due to deficiency of the enzyme glucocerebrosidase

Rosita, Argentina , Human milk proteins. 2011

Fluorescent sheep with gene from medusa Uruguay. Menchaca. 2013 https://trama.uy/proyecto/10/ovejas-fluorescentes-fluo-sheep/

GENE EDITING THE ULTIMATE TECHNOLOGY CRISPR/Cas9

GENETIC EDITING: CRISPR/Cas9

- The technology **CRISPER/Cas9** is a molecular tool utilized to EDIT ,REPAIR/CORRECT the genome of any cell. Is like having a molecular scissors that are capable of cutting any molecule of DNA **in a precise and controled form.**
- This capability of cutting the DNA is what allows us to **modify the sequence** eliminating and or inserting new DNA.
- CRISPR is the abreviation of *Clustered Regularly Interspaced Short Palindromic Repeats*.
- CAS9 is the name of a series of proteins, **mainly nucleases** associated to CRISPR

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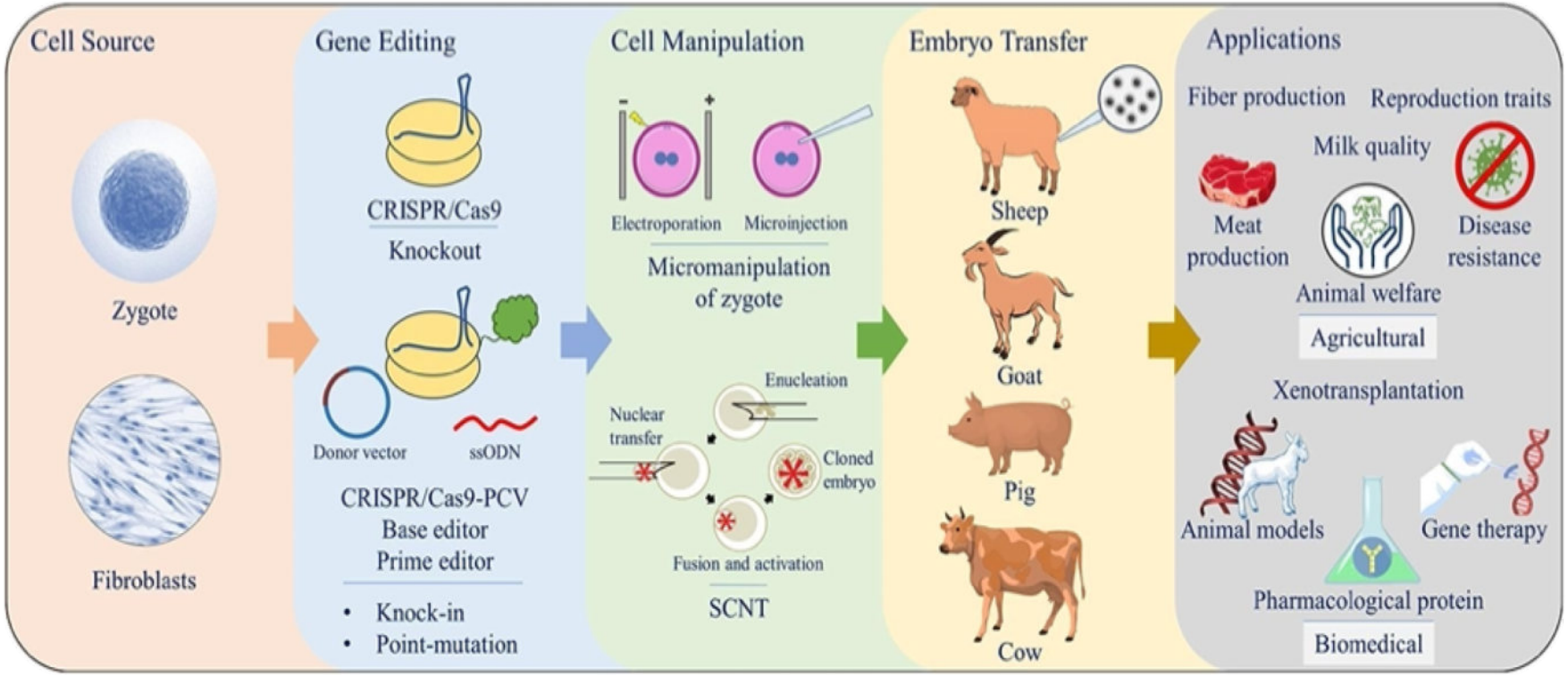
CRISPR offers a major advance over previous gene-editing tools.

- It is very precise, predictable, free of transgene
- Newer forms of CRISPR allow scientists to do more to a genome:
  - Some forms allow us to change the base letters of DNA.
  - Other forms let us insert entirely new genes
- It is relatively cheap, quick, and easy to use

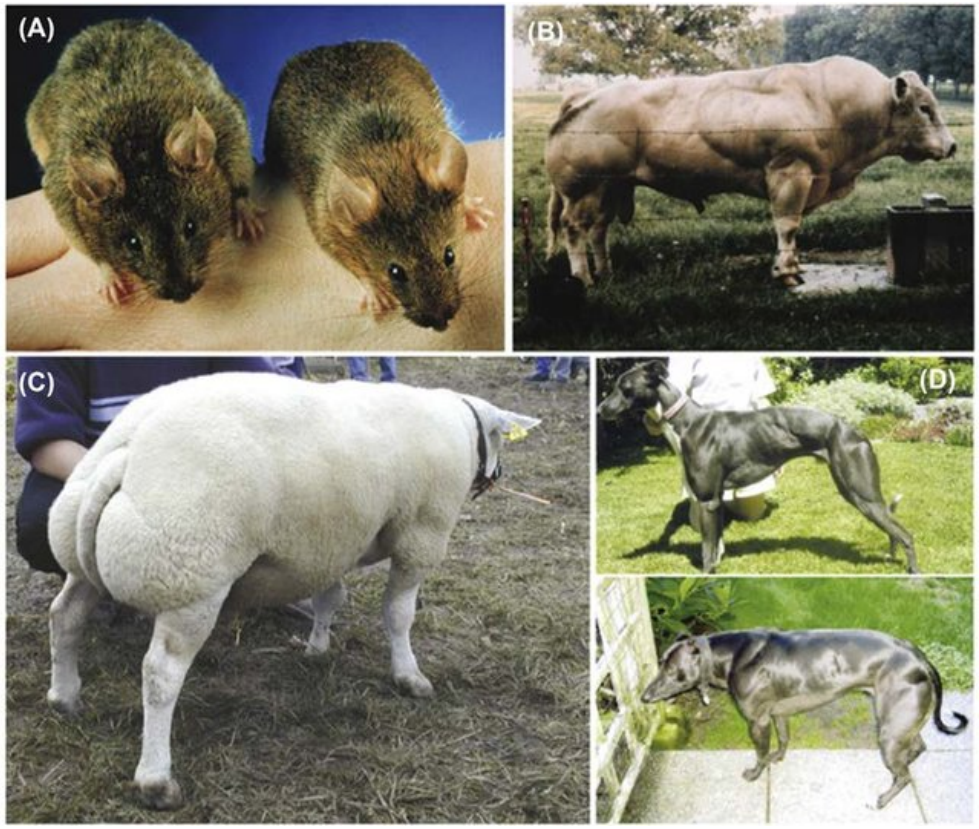
Cell mediated Genomic Editing CRISPER/Cas9



Perisse *et al.*, Front Genet 2021 Jan 8:11:614688.
doi: 10.3389/fgene.2020.614688. eCollection 2020.
Improvements in Gene Editing Technology Boost Its Applications in Livestock
DOI: 10.3389/fgene.2020.614688

Source of graphic: **MARVIN A. VILLANUEVA, DVM, PhD**
Genetically Modified (GM) Animals: Developments in Research and Policy Framework
Research and Development Division, DA- Philippine Carabao Centre

Some applications of CRISPR in livestock and fisheries



Source: Lee SJ. Sprinting without myostatin: a genetic determinant of athletic prowess. Trends Genet. 2007;23(10):475-477.

One popular target is a gene called myostatin, which codes for a protein that controls muscle growth. **Interfering with this gene can lead to muscle overgrowth .**

Scientists have already experimented with using CRISPR to generate **super-muscled** cattle, pigs, sheep, rabbits, and goats.

University of La MOLINA. Lima Peru has initiated a program for developing **double muscle in guinea pigs** and there are some efforts in Uruguay for a double muscle lamb.

Cosmo , bull produced by gene editing CRISPER making gene knock-IN of the

gene SRY responsible of initiating the development of masculine phenotype.

The offspring of Cosmo inherit this gene SRY and will be phenotypically MALES independently of having Y chromosome or not

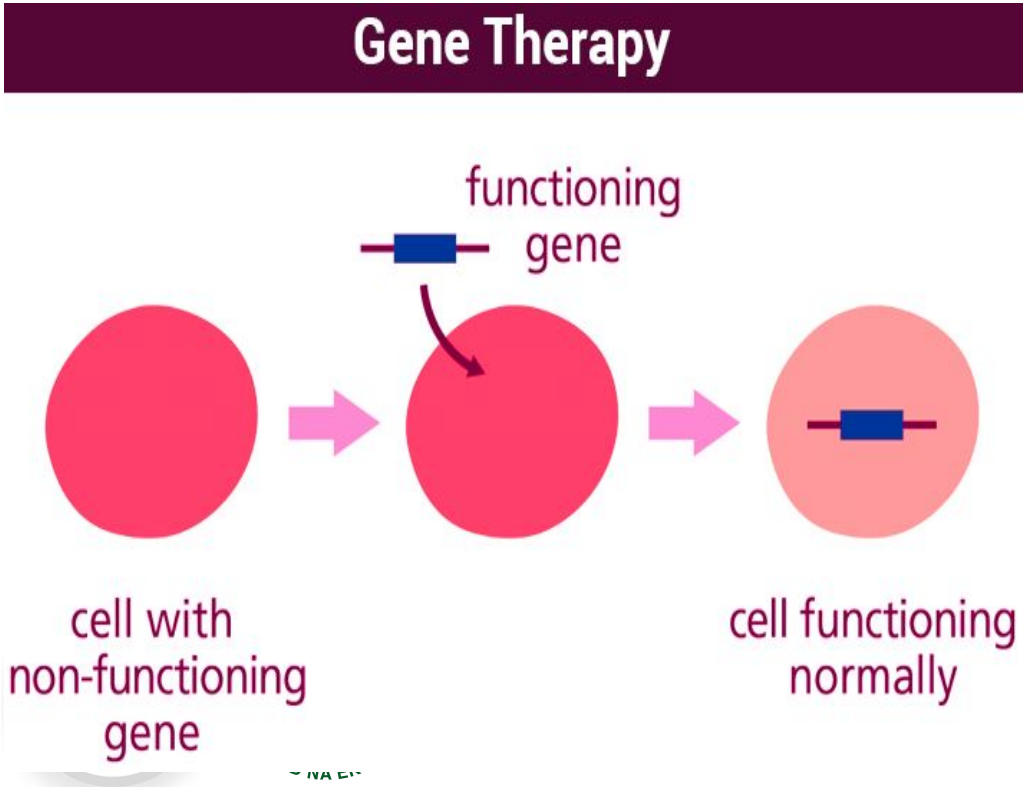
The presence of the SRY gene blocks the genetic expression of the female sex hence all individuals will be phenotypically male.

Dr. Alison Van Eenennaam University of Davis California with 2 bulls born genetically females,

Van Eenennaam A. L., (2020). How to make a CRISPR cow. *Research OUTREACH* 119. Available at: https://researchoutreach.org/articles/how-to-make-a-crispr-cow/ (Accessed YYYY/MM/DD)

CRISPR in FISH

- Dunham, Baofeng Su et al. at Auburn University CRISPR to insert the **alligator gene** for **cathelicidin**.
- **The resulting fish do seem to be more resistant to infections**., the survival rate of the **cathelicidin** transgenic fish was between **two- and five-fold** higher than controls.



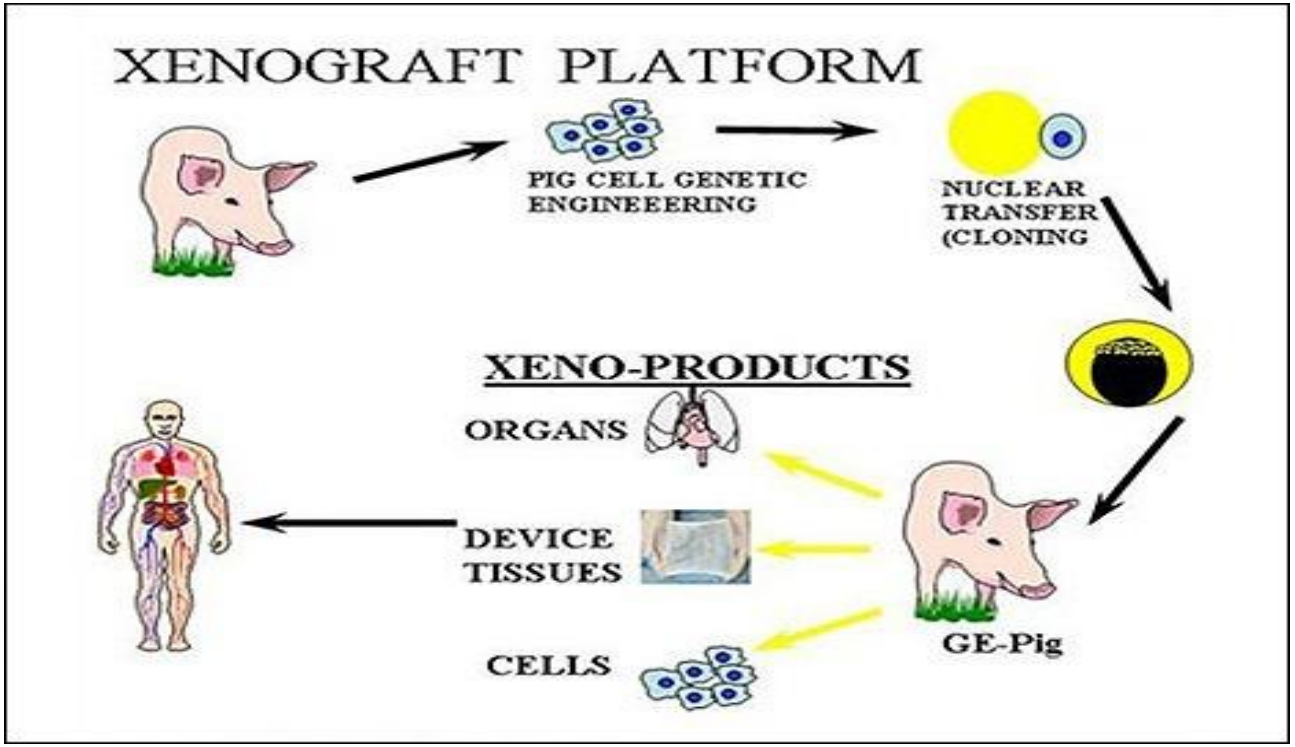
Source of graphic: **MARVIN A. VILLANUEVA, DVM, PhD**
Genetically Modified (GM) Animals: Developments in Research and Policy Framework
Research and Development Division, DA- Philippine Carabao Centre

Front Pharmacol. 2022; 13: 1015926.
Published online 2022 Oct 11. doi: 10.3389/fphar.2022.1015926
PMCID: PMC9592762
PMID: 36304167

Gene therapy for cystic fibrosis: Challenges and prospects

Hongshu Sui, [1,*,†] Xinghua Xu, [1,†] Yanping Su, [1] Zhaoqing Gong, [1] Minhua Yao, [1] Xiaocui Liu, [1] Ting Zhang, [1] Ziyao Jiang, [1] Tianhao Bai, [2] Junzuo Wang, [3] Jingjun Zhang, [4] Changlong Xu, [5,6,*] and Mingjiu Luo [2,*]

Xenotransplantation



Source of graphic: **MARVIN A. VILLANUEVA, DVM, PhD Genetically Modified (GM) Animals: Developments in Research and Policy Framework** Research and Development Division, DA-Philippine Carabao Centre

Efficacy of porcine skin Xenotransplants indistinguishable from Allograft in first-in-human Clinical evaluation. Mar 2022 Journal of burn care&research. DOI:10.1093/jbcr/irac012.093. Paul Holzer et at.

Animal genetic improvement or animal genetic modifications in general should be understood as the art of combining **genetics** and **reproduction**

The **reproductive techniques** are required for both: **The generation** of the animals with the desired genetic compositions as well as for the **dissemination** of their genes in the population.

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- **REPRODUCTIVE TECHNOLOGIES IN LIVESTOCK**

  - Controled and directed **natural mating**
  - Semen collection , preservation and **artificial insemination**.
  - Control and **synchronization of ovarian cycles**
  - Inmunization for **increment of ovulatory rate**
  - **Superovulatión,** IN VIVO embryo production and transfer.
  - **Ovum pick up, and IN VITRO maturation and fertilization with in vitro capacitated sperm**
  - **IN VITRO embryo cultutre**
  - Preservation of Oocytes and embryos
  - Cell culture (reproduction)
  - Embryo sexing
  - Sperm sexing
  - **Embryo micromanipulation: biopsy, bisection (true cloning), enucleation and cloning by nuclear transfer, ICSI, embryo micro injection**
  - *Use of primordial and STEM cells for reproduction.*

Reproductive techniques to generate and reproduce genetically modified fish

Goro Yoshizaki, in Encyclopedia of Reproduction (Second Edition), 2018

- **Microinjection of genetically modified genes into fertilized eggs**
- **Germ cell transplantation via transfected germ cells.**
  - **Germ cell transplantation methods can be divided into the following:**
  - transplantation of migrating primordial germ cells into blastula embryos,
  - transplantation of post-migratory primordial germ cells into the body cavity of hatchlings,
  - transplantation of germ-line stem cells into the body cavity of hatchlings, and
  - transplantation of spermatogonial stem cells into adult gonads.

Building capacities for advanced reproductive technologies is crucial for the application of modern genetic biotechnologies in livestock and fisheries

In Peru we have installed advanced reprodutive technologies laboratories both in private organizations (LACTEA SA ; MICHELL and CIA) as well as in different centres of INIA (National Agrarian Innovation Institute)

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Eighteen offspring from one cow in 60 days by in vitro embryo production at LACTEA SA, Peru..

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Cattle: Collections by OPU and IVF in Ibero America.

Countrty	Sessions of OPU-IVF with conventional semen	Sessions of OPU-IVF with SEXED semen	Total OPU-IVF Sessions
BRASIL	17,280	33,700	50,980
PERU	5,473	1,081	6,554
ARGENTINA	1,618	303	1,921
PANAMA	396	265	661
REPUBLICA DOMINICANA	281	188	469

Estadísticas oficales de la IETS, 2016

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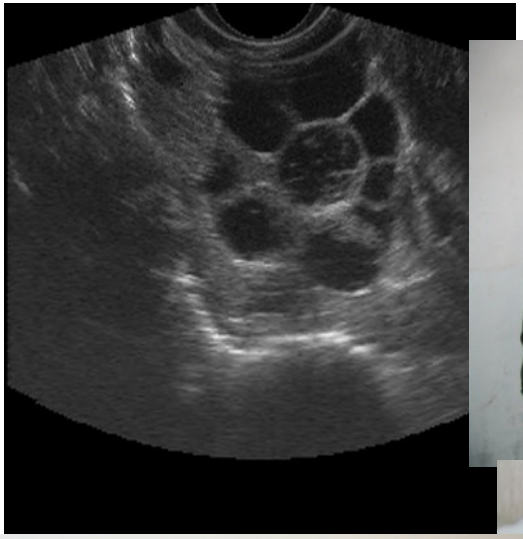
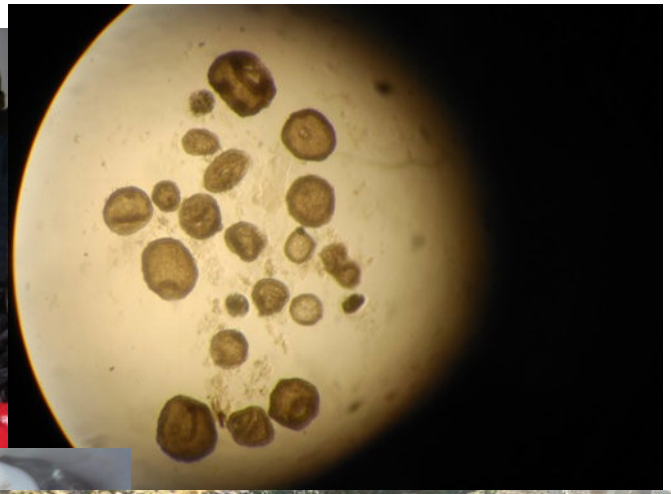
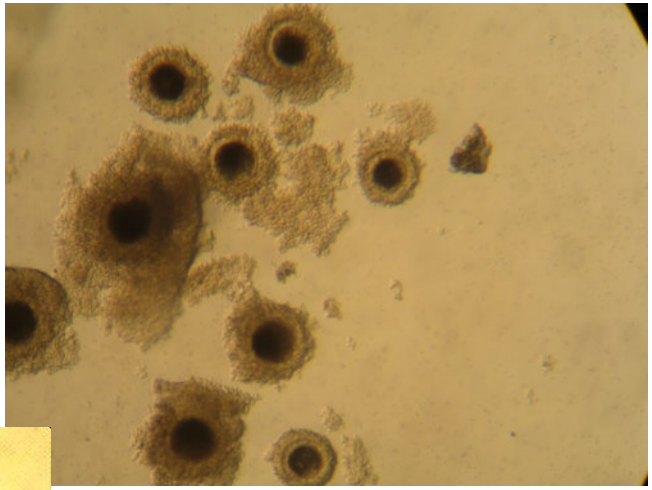
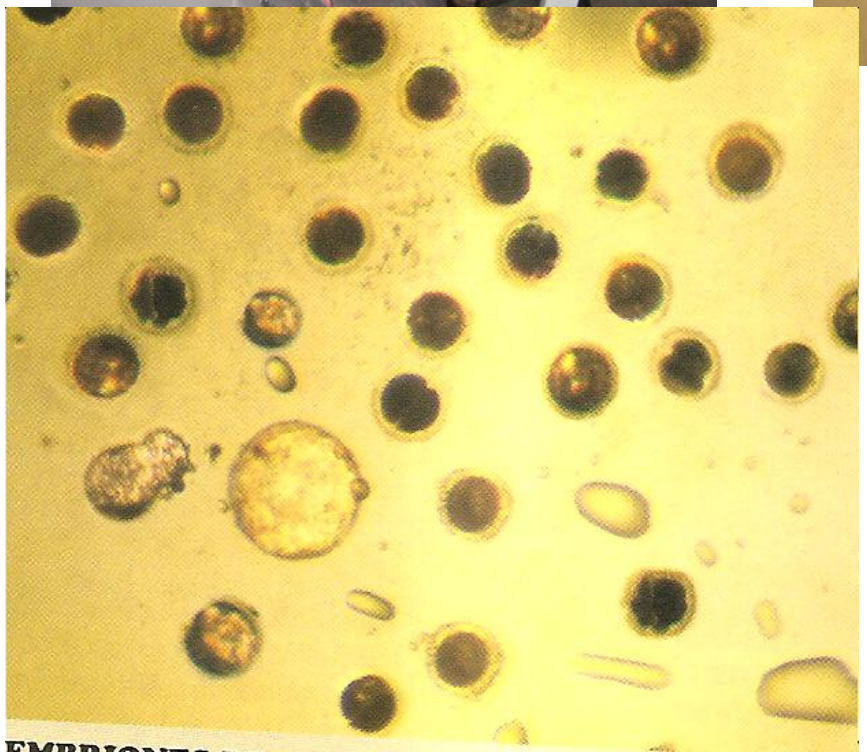
Development of artificial insemination in alpacas in Peru

Development of IN VIVO embryo production and transfer in alpacas. Peru









G. GAMARRA, E.HUAMAN, S LEON, M CARPIO, E. ALVARADO, M. ASPARRIN and H. W. VIVANCO. 2009. First In Vitro Embryo production in Alpacas (Lama Pacos). Reproduction, Fertility and Development. January 2009.Abst. 157.

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Cloning for generation of transgenic animals

Promoter or driver

Gene

Gene construct

Somatic cells

Donor cell

Oocyte enucleation

Cell injection

Cell electro-fusion

Embryo activation

In vitro culture

Day 7 blastocyst

Embryo transfer

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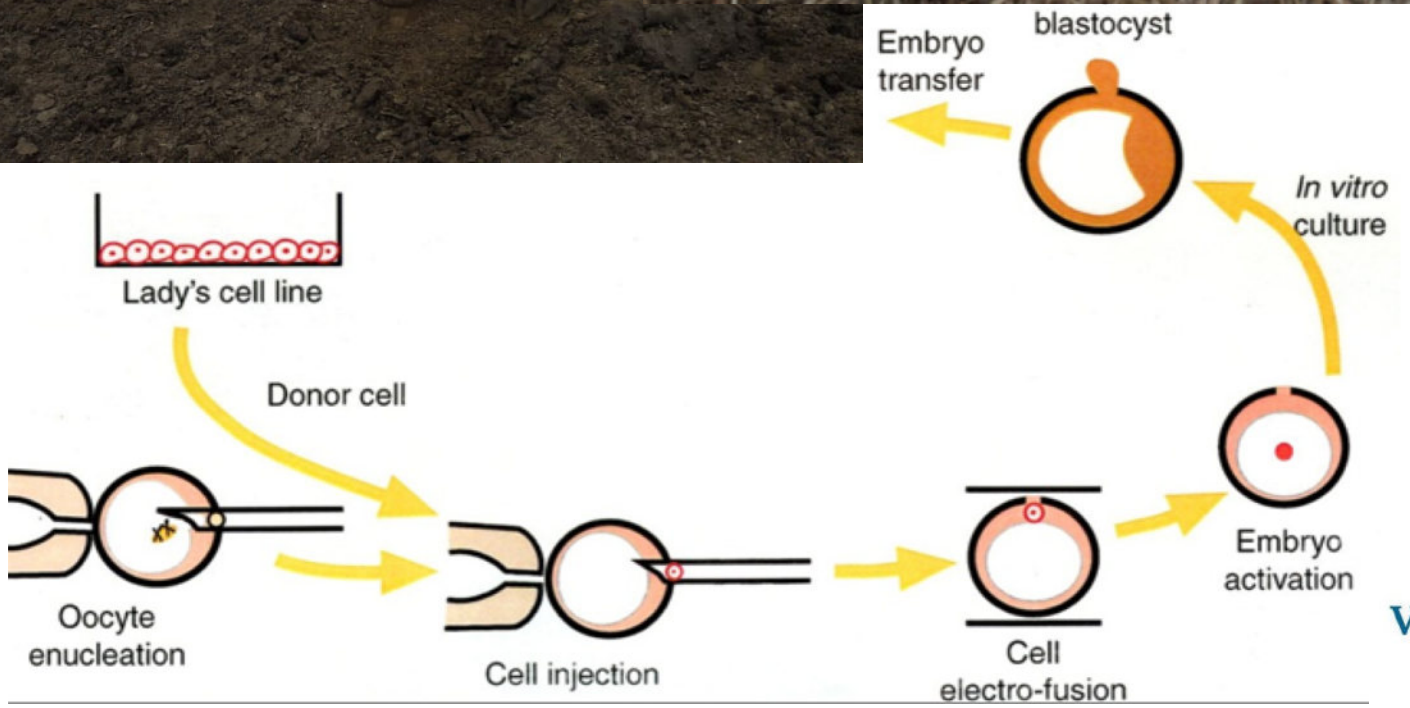
Cloning for dissemination of the genes (LACTEA Peru)



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Cloning of adult cows by nuclear transfer of ovarian mural granulosa cells

Irina Lagutina, Daniel Ponce, Roberto Diaz, Flor Castañeda, Iván Mesía, Cesare Galli and William Vivanco

Spermova 2019; 9(1): 19 - 27 - *Original paper* DOI. 10.18548/aspe/0007.03

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A cloned herd developed in AgResearch New Zealand with copies of a high breeding value cow

40 calves were produced by SCNT

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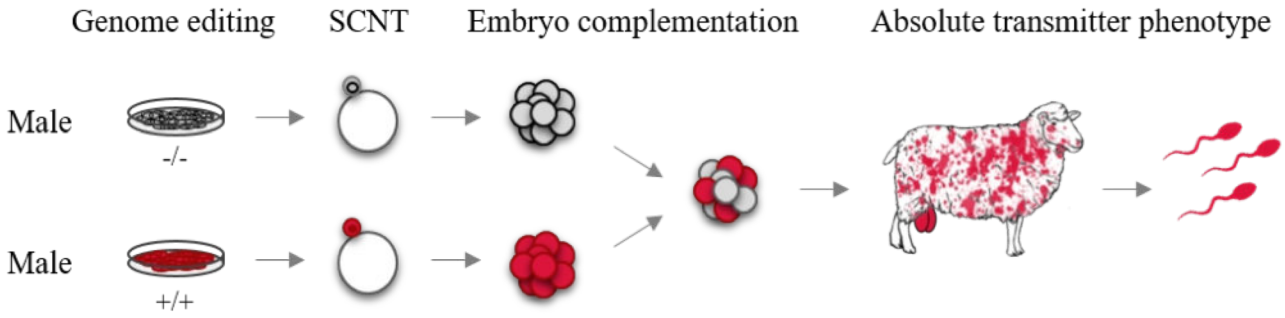
Chimaeras produced with edited cells : production of absolute transmitters



Generating absolute transmitter rams by genome editing and embryo complementation

Chimeras complementation and controling male germline

Bjorn Oback and David A. Cossey.

May 2023. Trends in Biotechnology. DOI: 10.1016/J. tibtech.2023.03 020

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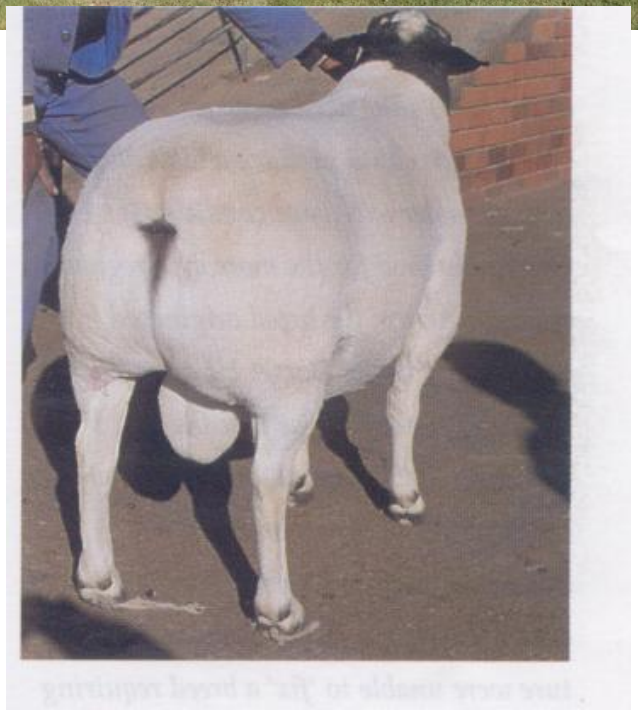

Utilization of the strategy of ABSOLUTE TRANSMITTERS Could be used to solve the limited Spermatogesis in SA camelids

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Ethical implications of biotechnological interventions in livestock and fisheries

An accepted ethic of animal use in science, considers

- The **Reduction** of animal numbers in the experiments as much as possible
- The **Refinement** of the practices and husbandry to minimize pain and distress,
- **Replacement** of animals with non-animal alternatives wherever possible)

The aim is to minimize any **pain and distress** experienced by the animals used, and as such, they are considered the principles of humane experimental technique.

- Can Vet J. 2011 May; 52(5): 544–550.

PMCID: PMC3078015

PMID: 22043080

Genetic engineering of animals: Ethical issues, including welfare concerns

Elisabeth H. Ormandy, Julie Dale, and Gilly Griffin

However, despite the steps taken to minimize pain and distress, there is evidence of public concerns that go beyond the concerns in animal welfare regarding the creation and use of genetically engineered animals.

People express ethical concern over GM animals, including unanticipated results and their sense of the need of GM animals versus animals generated by selective breeding practices.

A sociologic study by P. Macnagthen suggest that GM animals are going to be an issue of public controversy for very long time due to **the ways in which they symbolize and give voice to underlying tensions between 'moral 'and 'instrumental approaches to animals**

Animals in their nature: A case study on public attitudes to animals, genetic modification and 'nature'. P Macnaghten. Sociology, 2004 journals.sagepub.com

The need of defined policies, regulation and education

- As clearly stated by MA Villanueva (Philippine Carabao Centre) In order to successfully develop genetic engineering and its applications in livestock and fisheries for the benefit of the society, is necessary to:
- Implement **CLEAR, PREDICTABLE , SCIENCE BASED and RISK PROPORTIONATE** regulations
- Establish **ADAPTATIVE AND RESPONSIVE POLICIES** that can adapt to rapid advancements and emerging technologies
- Foster **INTERNATIONAL COOPERATION AND ARMONIZATION OF REGULATORY STANDARS** to streamline global biotech development and facilitate cross-border research and trade
- **ALLOCATE SUFFICIENT FUNDING** for biotech, research and innovation
- **FOSTER EDUCATION AND PUBLIC AWARNEESS**

Many thanks for your kind attention

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