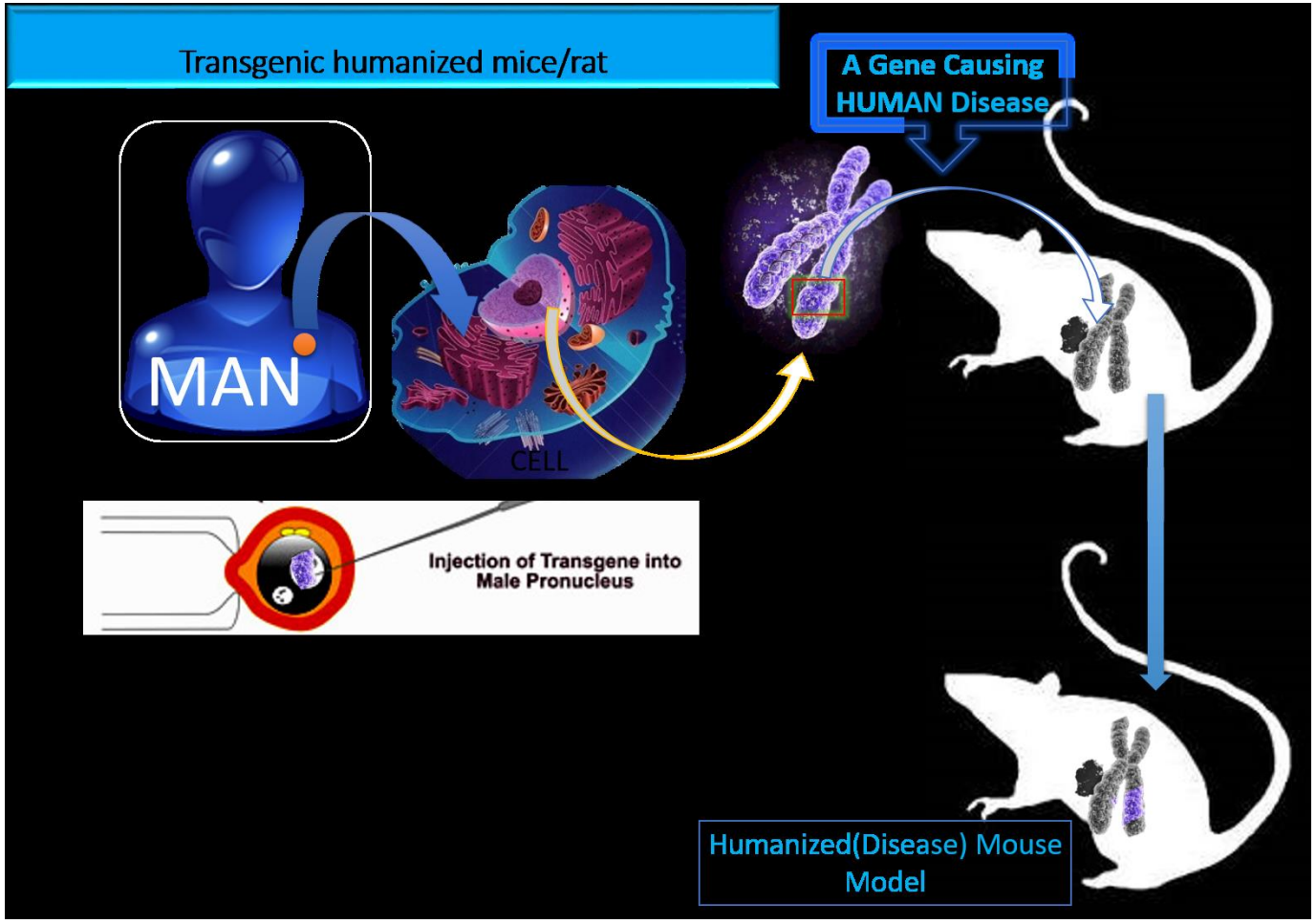
Centers and studies of transgenic animals in India

Subeer S. Majumdar

National institute of Immunology, National Institute of Animal Biotechnology & Gujarat Biotechnology University, Gandhinagar



Summary: Gene editing / expression in animals in India

There are institutions who undertook studies of gene overexpression generating transgenic mice, embryonic manipulation

National Inst of Immunology, New Delhi
Indian Inst of Science, Bangalore
CCMB, Hyderabad
IISER, Pune
Instem, Bangalore

NII , Delhi developed testicular transgenesis, avoiding embryo use.

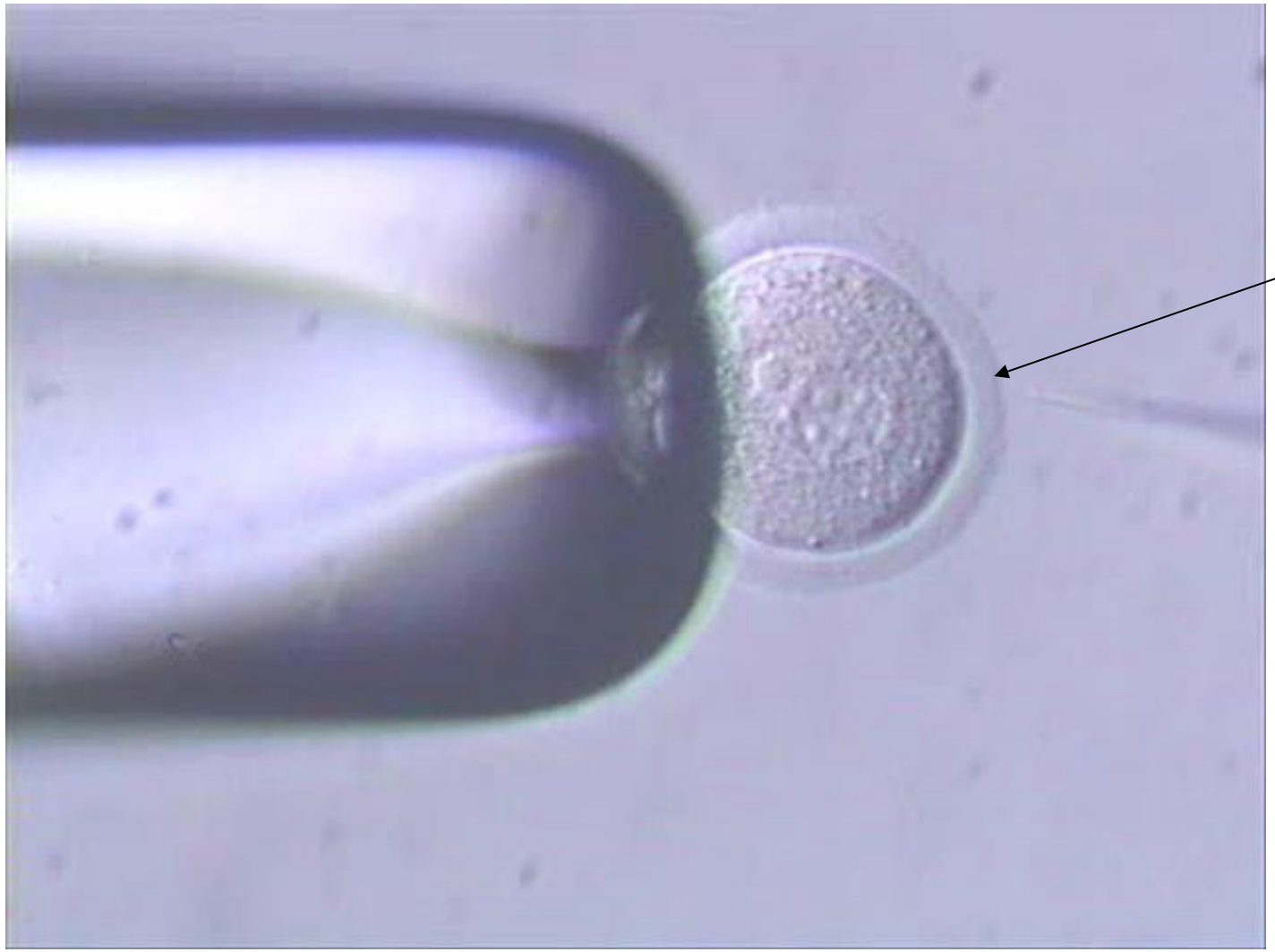
Recently: Genome editing using CrispR Cas9 is being practiced at 1)CCMB, 2)IISER, Pune and 3)Instem, Bangalore, mostly in mice

Large animal genome editing is only for overexpression of therapeutic proteins in the milk in goat, attempts also undertaken for this in Buffalo

Many institutes use genome edited mouse procured from Jackson labs etc. for their specific studies.

2 studies are taken up for knocking down myoststin gene for enhanced meat production in goats (no major success story yet)

Developed New procedures of transgenesis at National Institute of Immunology transgenic facility, New Delhi, India

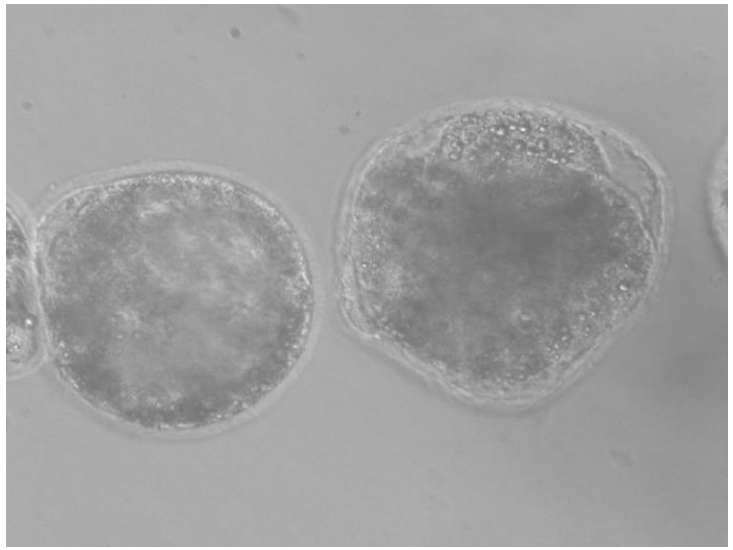


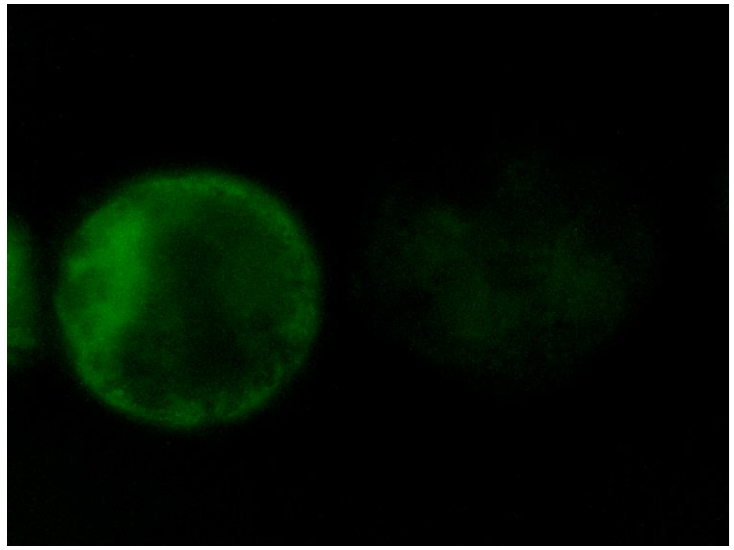
Traditional Procedure

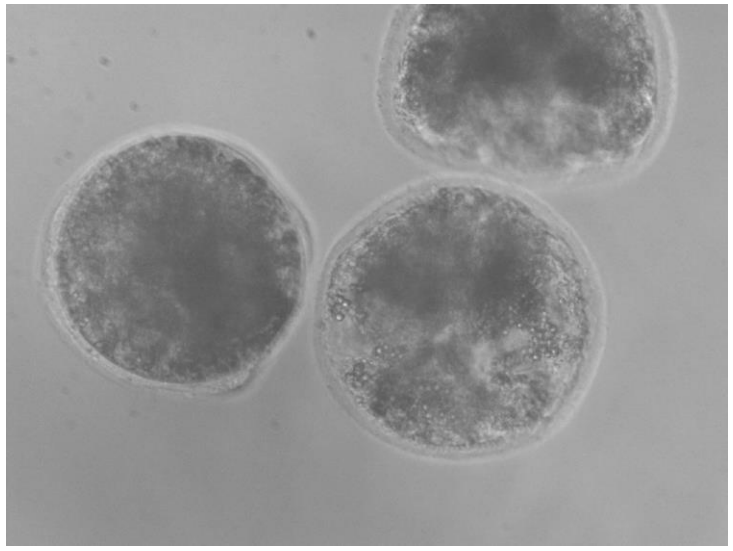
use male Pronuclear Gene Insertion to make transgenic animals

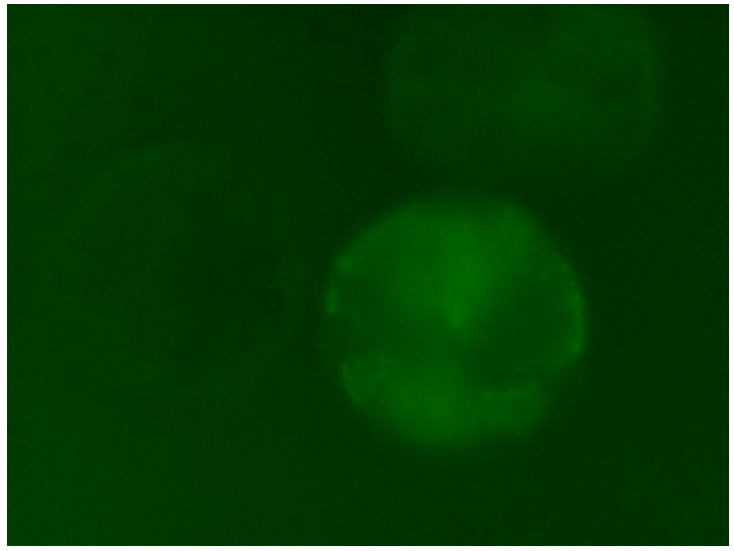
Need 1000s of eggs at the cost of 100s of females

National Inst of Immunology : Buffalo embryo transgenesis via cloning









EGFP EXPRESSING TRANSGENIC CLONED BUFFALO BLASTOCYSTS

Embryogenesis

Requirement

Existing technique	Females 100-200 (sacrifice!)	Eggs- 1000s	Founder 1

Equipments → inverted microscope, micromanipulator, microinjector, pipette puller, CO_2 incubator + Dedicated laboratory space + Dedicated well trained Personnel (Hard to get)

Alternate Method

Testicular Stem Cells

Requirement

We developed	Male –1 (alive!)	Eggs- Nil	Founders 100s

Added advantage: **All scientists with barely minimal infrastructure can produce transgenic mice of their interest.**

No special facility is needed.

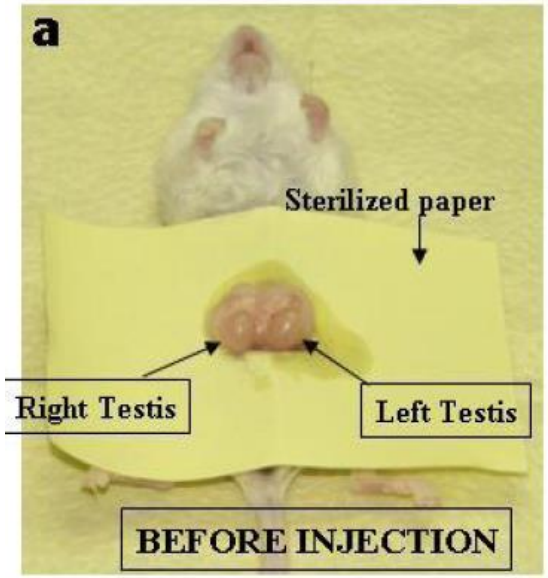
. Male germ cell mediated

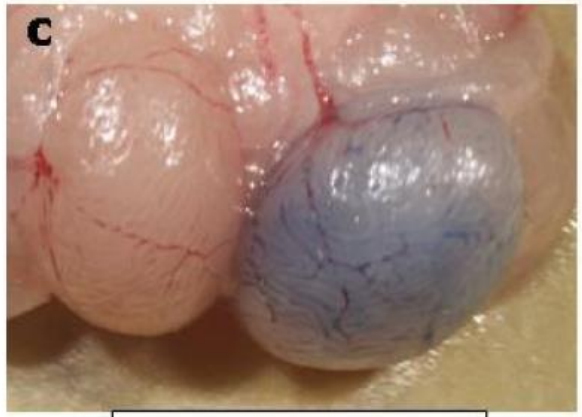
. Quick/Rapid

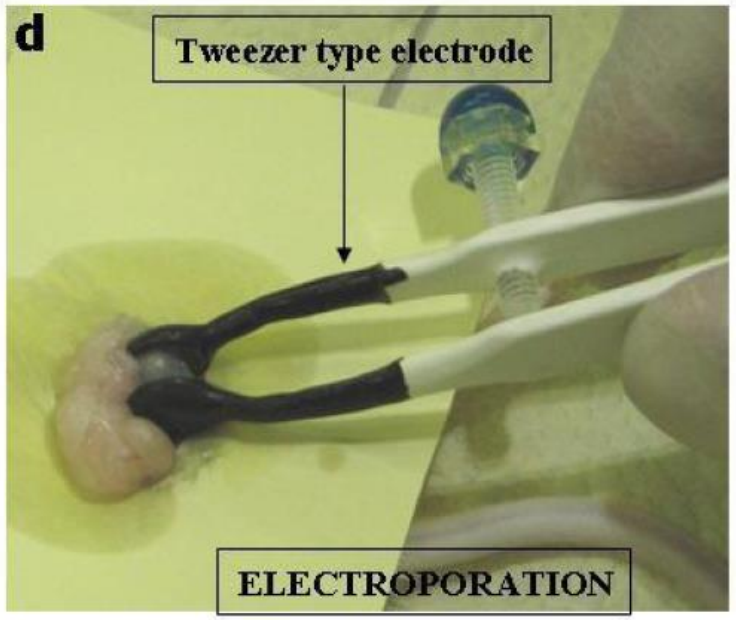
. No superovulation or oocyte

. Natural mating

. Can select progeny with desired level of gene expression



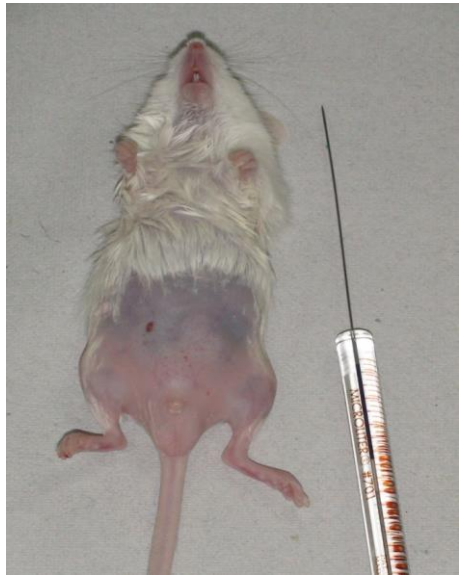


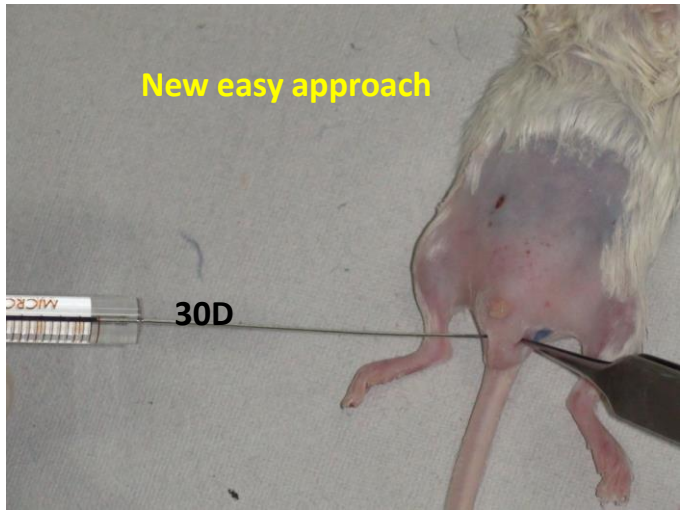


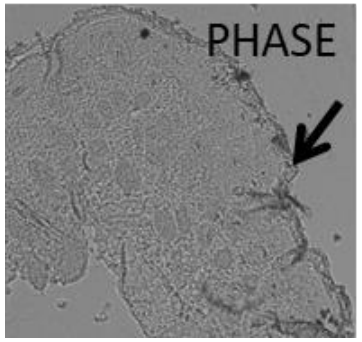
8 squre Pulses of 40V for 0.05 sec (inter Pulse Interval of 1sec)

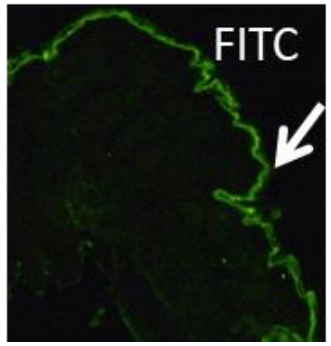
Procedure of testicular electroporation

Suveera Dhup and **Subeer S. Majumdar.** Transgenesis via permanent integration of genes in repopulating spermatogonial cells in vivo. ***Nature Methods*, 2008 5(7):601-603**



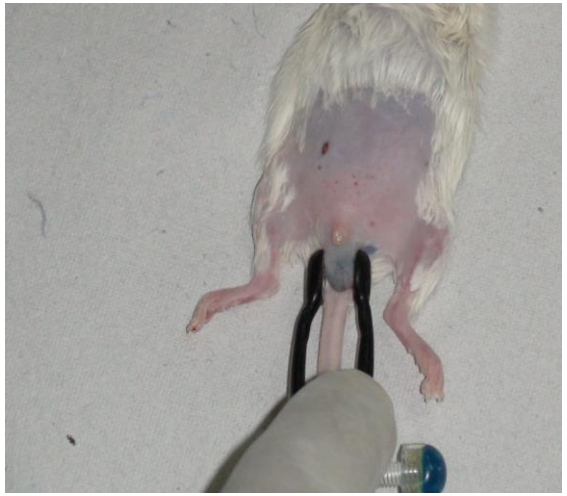


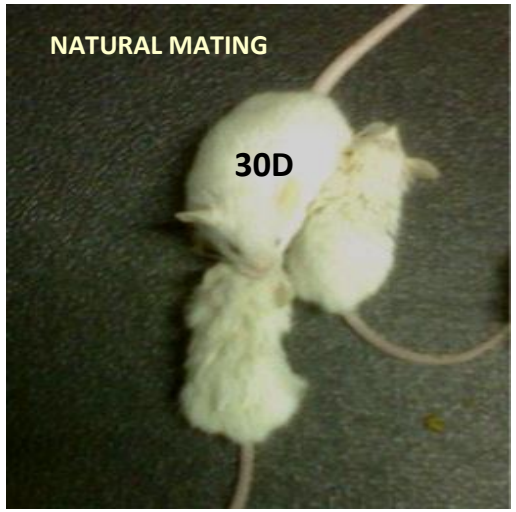




Keratin promoter driven GFP expression on upper layer of the skin of transgenic animal using testicular transgenesis

Arrow: Keratin layer





Abul Usmani, Nirmalya Ganguli, Hironmoy Sarkar, Suveera Dhup, Suryaprakash R. Batta, Manoj Vimal, Nilanjana Ganguli, Sayon Basu, P. Nagarajan and **Subeer S. Majumdar.** A non-surgical approach for male germ cell mediated gene transmission through transgenesis. **Scientific Reports, 2013, 3, Article number: 3430 doi: 10.1038/srep03430.**

Dickkopf-related protein 3 is a protein that in humans is encoded by the *DKK3* gene.

This gene encodes a protein that is a member of the dickkopf family. The secreted protein contains two cysteine rich regions and is involved in embryonic development through its interactions with the Wnt signaling pathway.

The expression of this gene is decreased in a variety of cancer cell lines and it may function as a tumor suppressor gene.

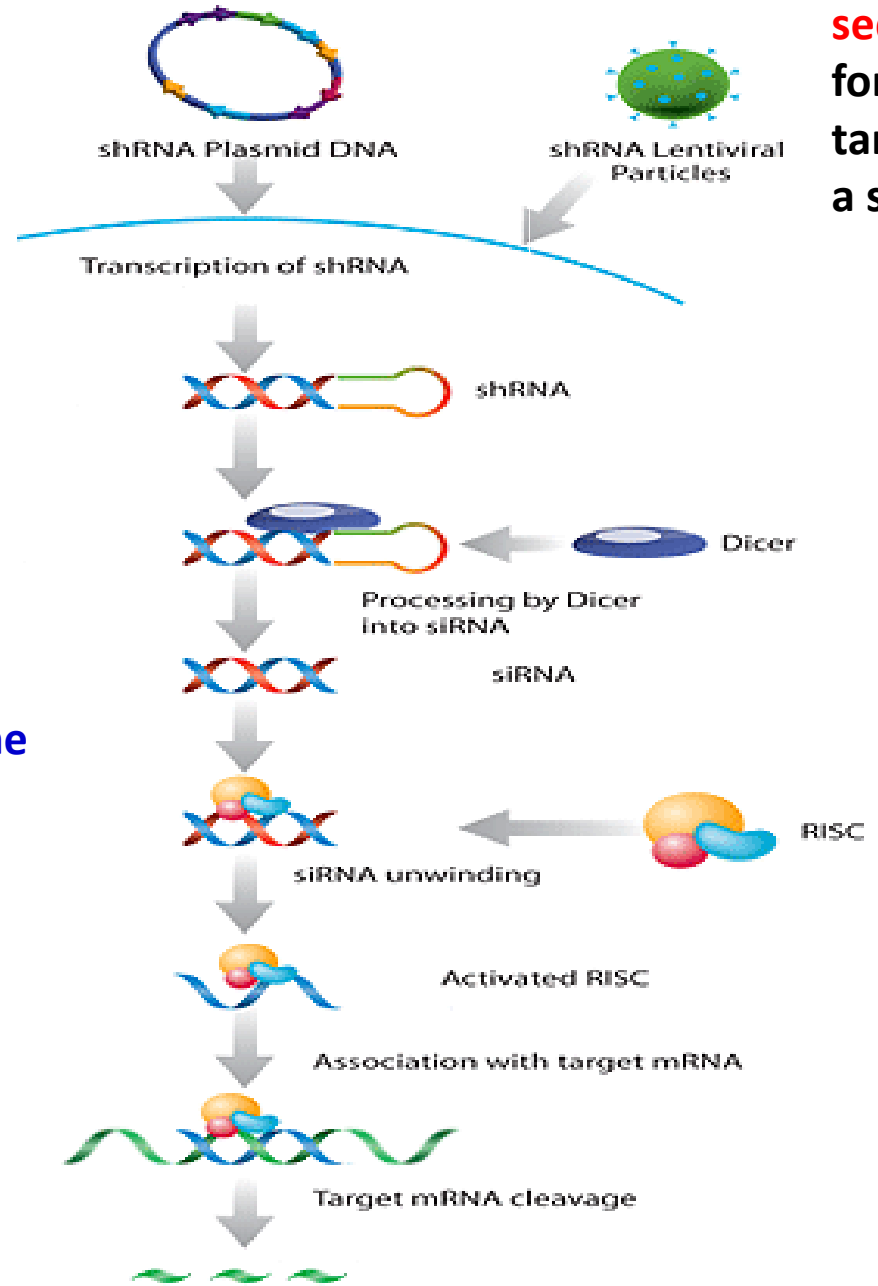
We compared fertile and infertile testicular gene expression and selected DKK3

DKK3 is over expressed in the testis in adulthood

We chose to knock down *DKK3* "in-vivo" via

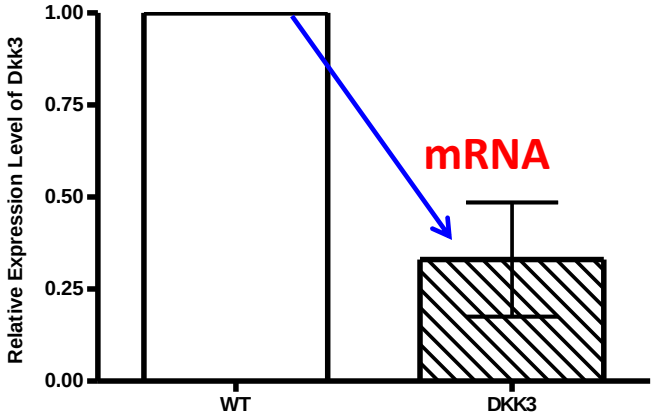
transgenesis by making shRNA knock down model

to see whether we can induce infertility in such mouse. .

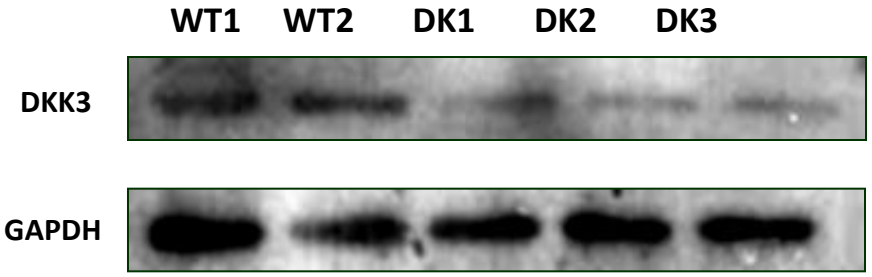


Can we do so with DNA sequences coding for shRNA targeting a specific gene?

Immunohistochemical localization and Western blot of DKK3 in the testis of 10 weeks old WT and shRNA knock down mice



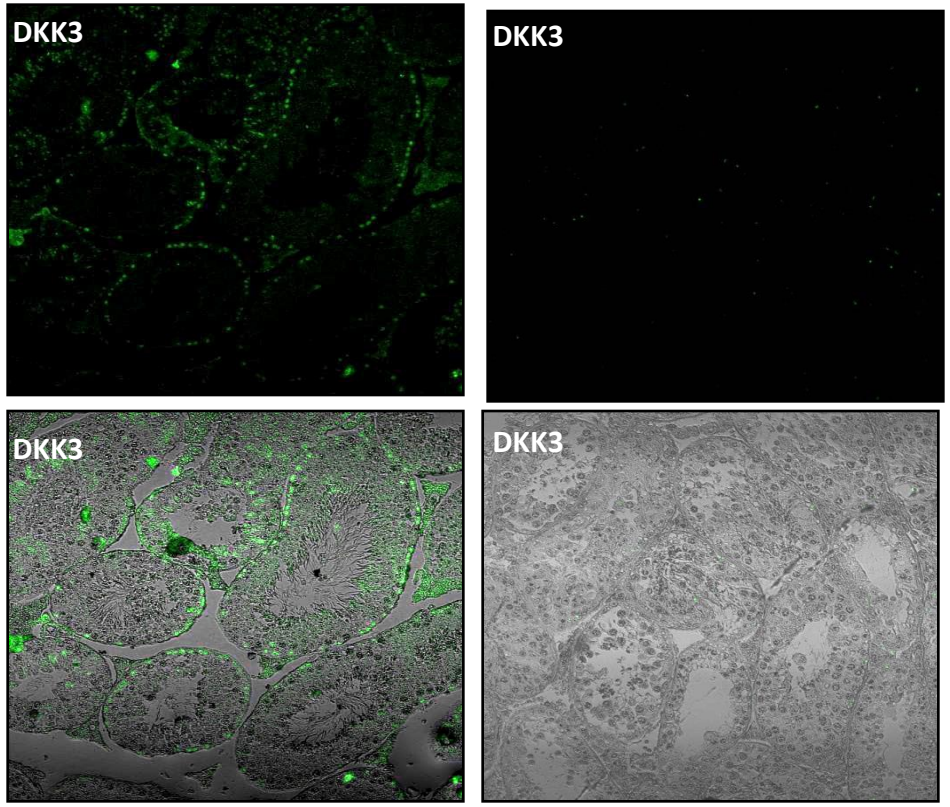
Western blot



IHC

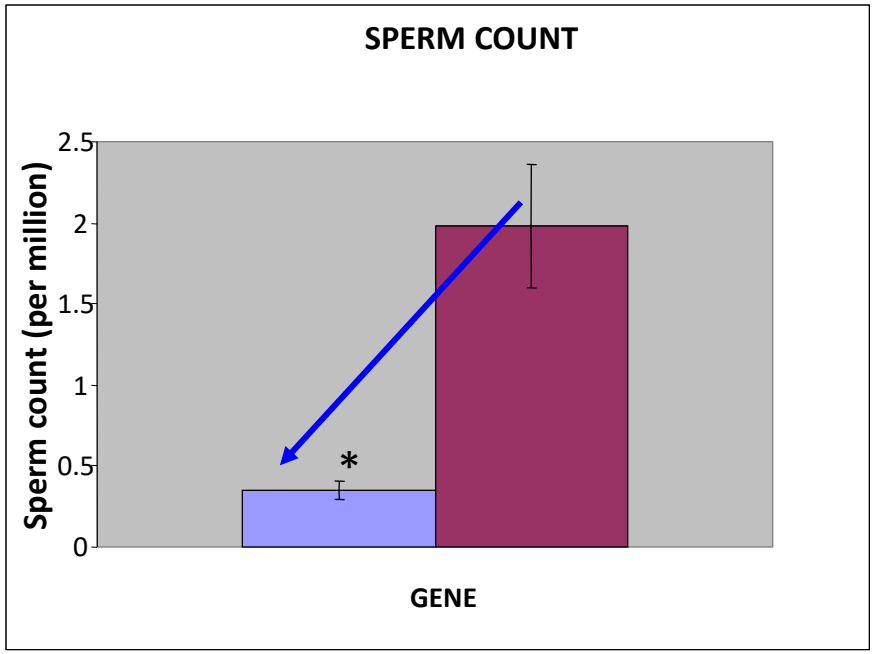
WT mice

Knock down mice



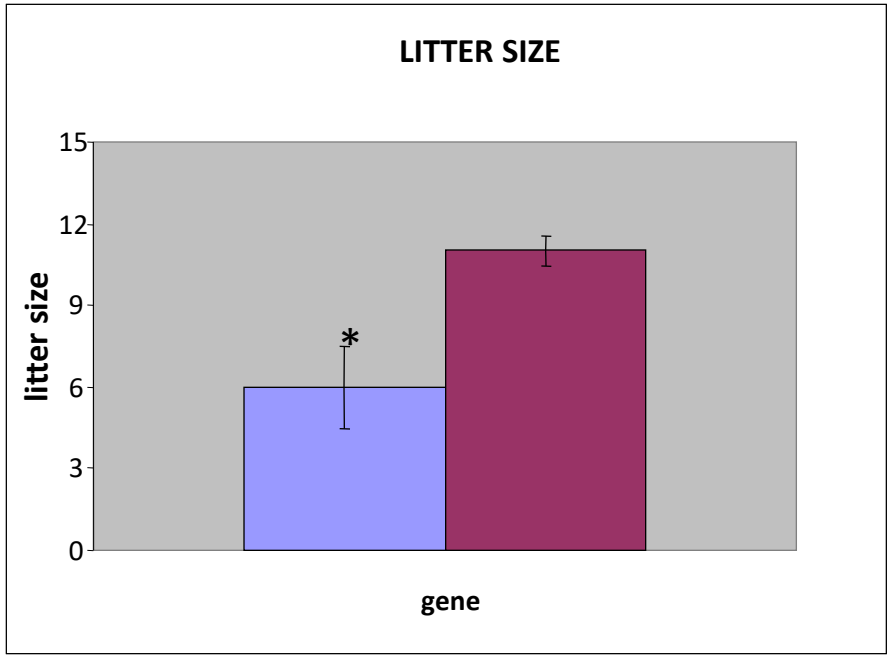
These mice were infertile

SPERM COUNT



Mean sperm count (million/ml) from epididymis of *DKK3* knock down mice and wild type mice at 10 weeks of age (n=15).

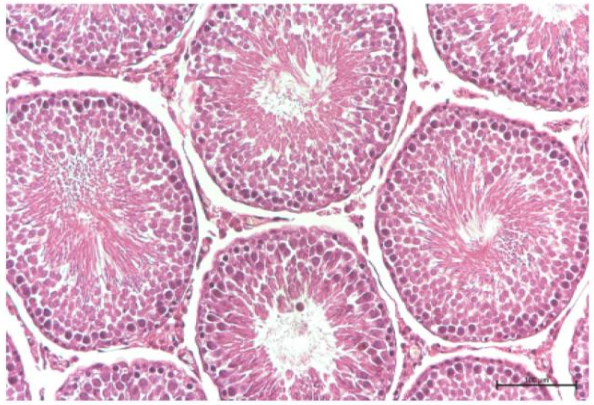
LITTER SIZE

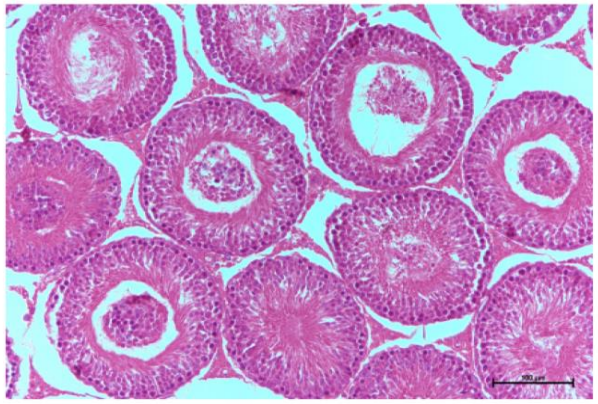


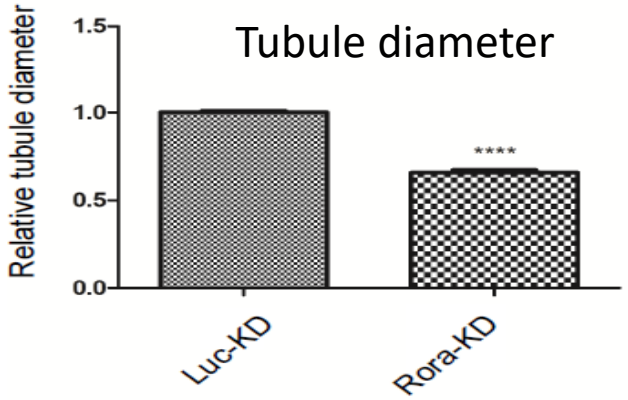
Litter size of *DKK3* knock down mice compared to that generated from wild type mice (n=4).

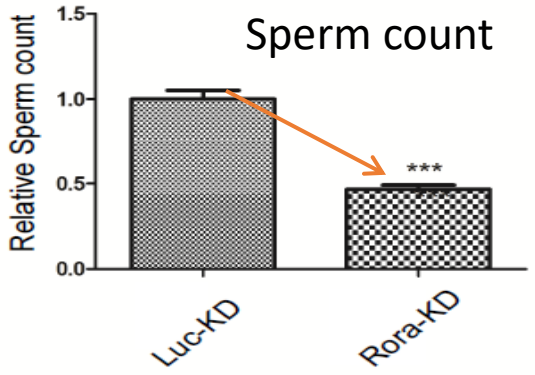
Histological analysis of testis of the Rora-KD animals

Knock down of transcription factor Rora in the testis









Using transgenic mice, we discovered role of some (about 10) proteins, transcription factors and micro RNAS in hormone independent male infertility using rat and mice models. National Inst of Immunology and National inst of Animal Biotechnology , India

Generation of K8 Mutant Mice Model

Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai , India

Vector for Skin Specific over expression of K8 Mutant

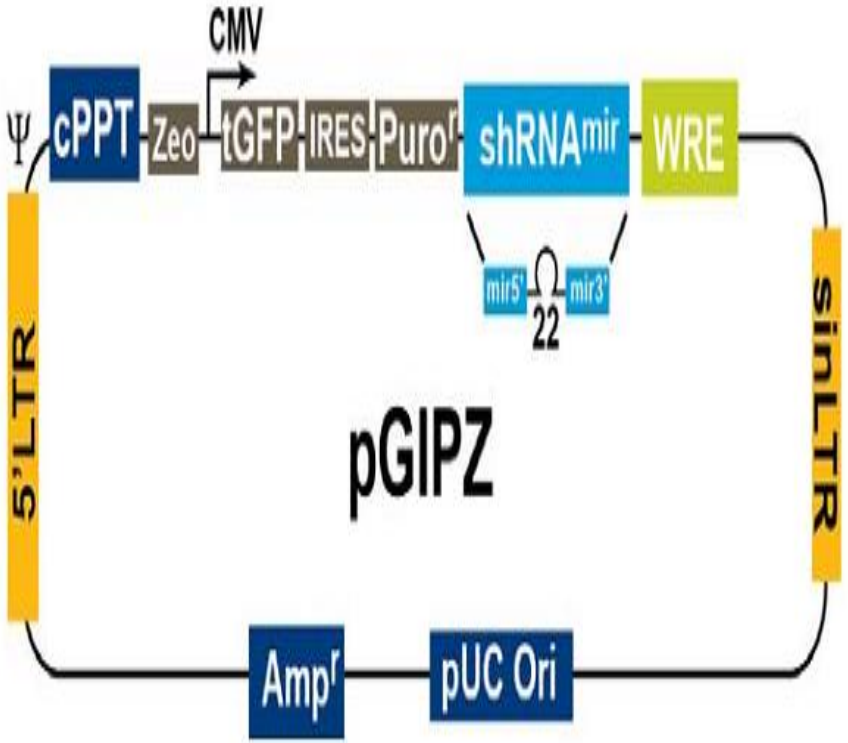
We generated transgenic mice for them

The K8 Mutant mice showed less tumorigenesis as compared to the Wt control.

Generation of a tissue-specific transgenic model for K8 phosphomutants: A tool to investigate the role of K8 phosphorylation during skin carcinogenesis in vivo. Richa Tiwari, Nirmalya Ganguli, Hunain Alam, Indrajit Sahu, Chella Krishna Vadivel, Shruti Sinha, Shweta Patel, Sayli Nitin Jamghare, Sanjay Bane, Rahul Thorat, **Subeer S. Manjumdar, Milind M. Vaidya.** Cell Biology International, 2021; doi.org/10.1002/cbin.11611

Generation of HOXA10 Knock Down Mice Model

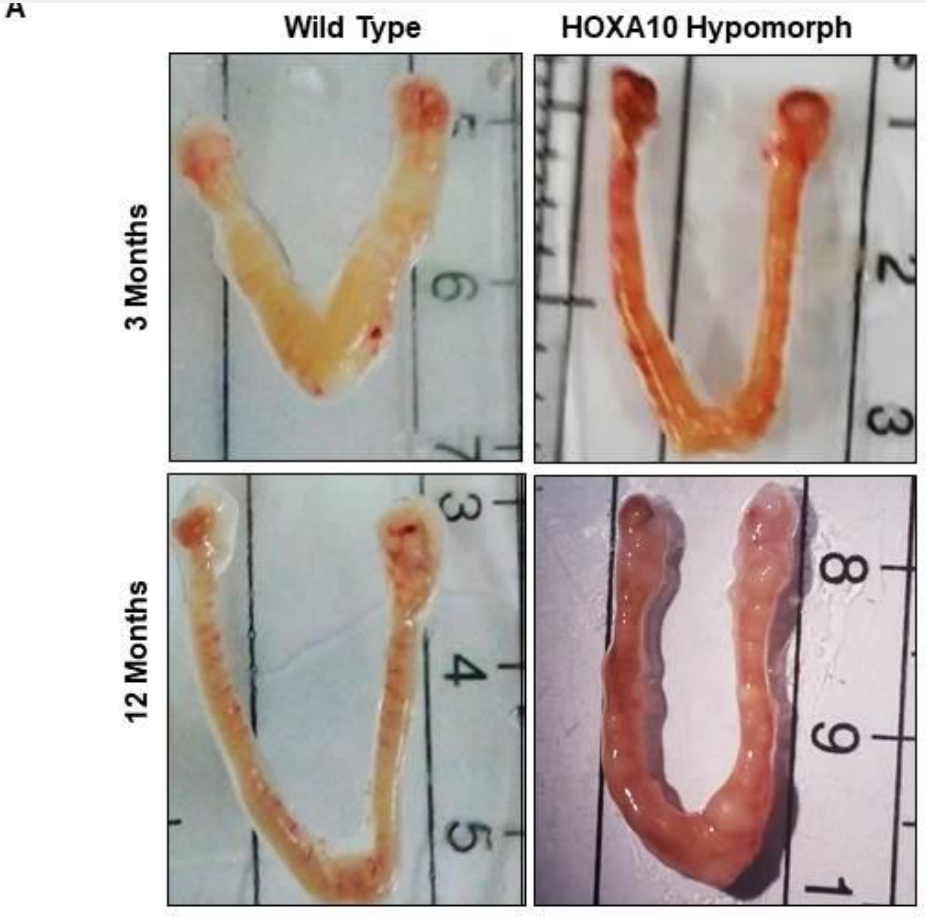
We generated transgenic mice for them



Vector for shRNA mediated knock down of HoxA10.

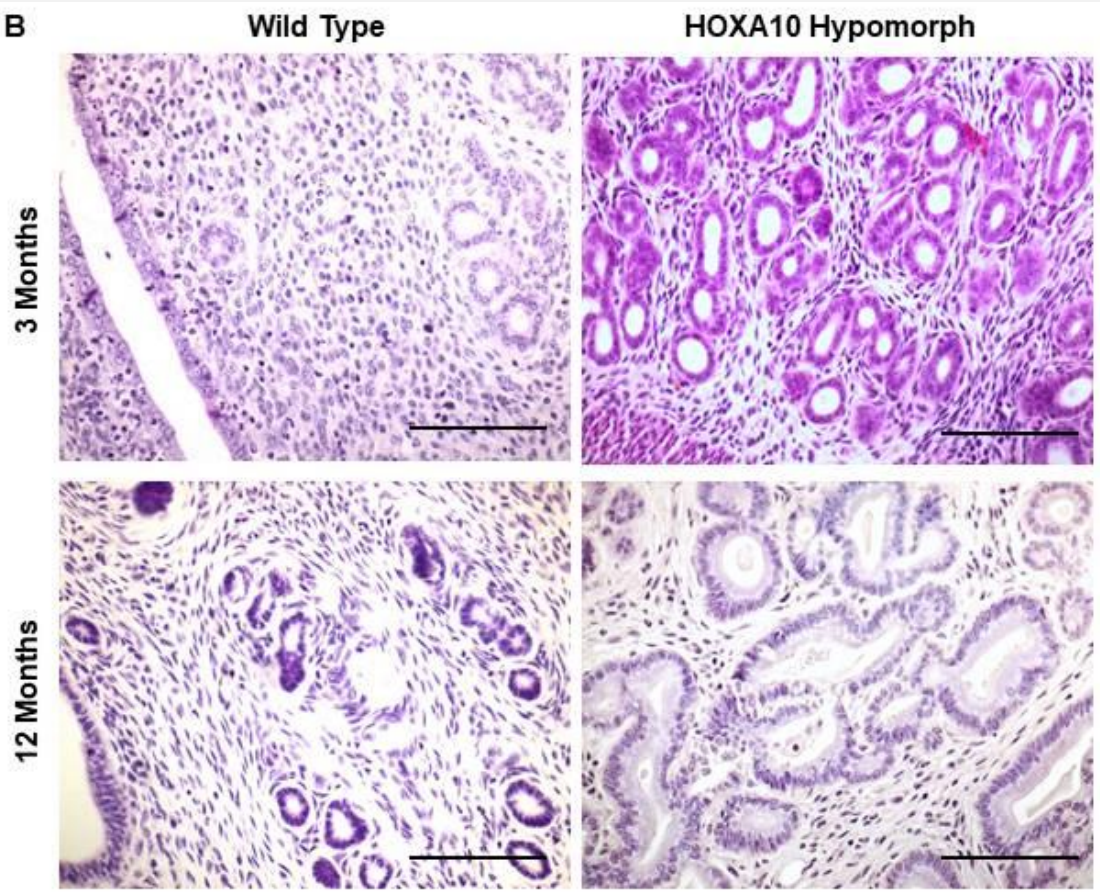
Loss of HOXA10 causes endometrial hyperplasia progressing to endometrial cancer. Anuradha Mishra, Nirmalya Ganguli, **Subeer S Majumdar, and Deepak N Modi**. *Journal of Molecular Endocrinology.* 2022. doi.org/10.1530/JME-22-0051

Generation of HOXA10 Knock Down Mice Model



Representative images of uteri from young (3 months) and aged (15 months) WT and HOXA10 hypomorphs

Gross morphology of the uterine horns was similar in the young hypomorphs and WT controls. However, in the aged hypomorphs, the uteri appeared thicker and often had nodule-like structures



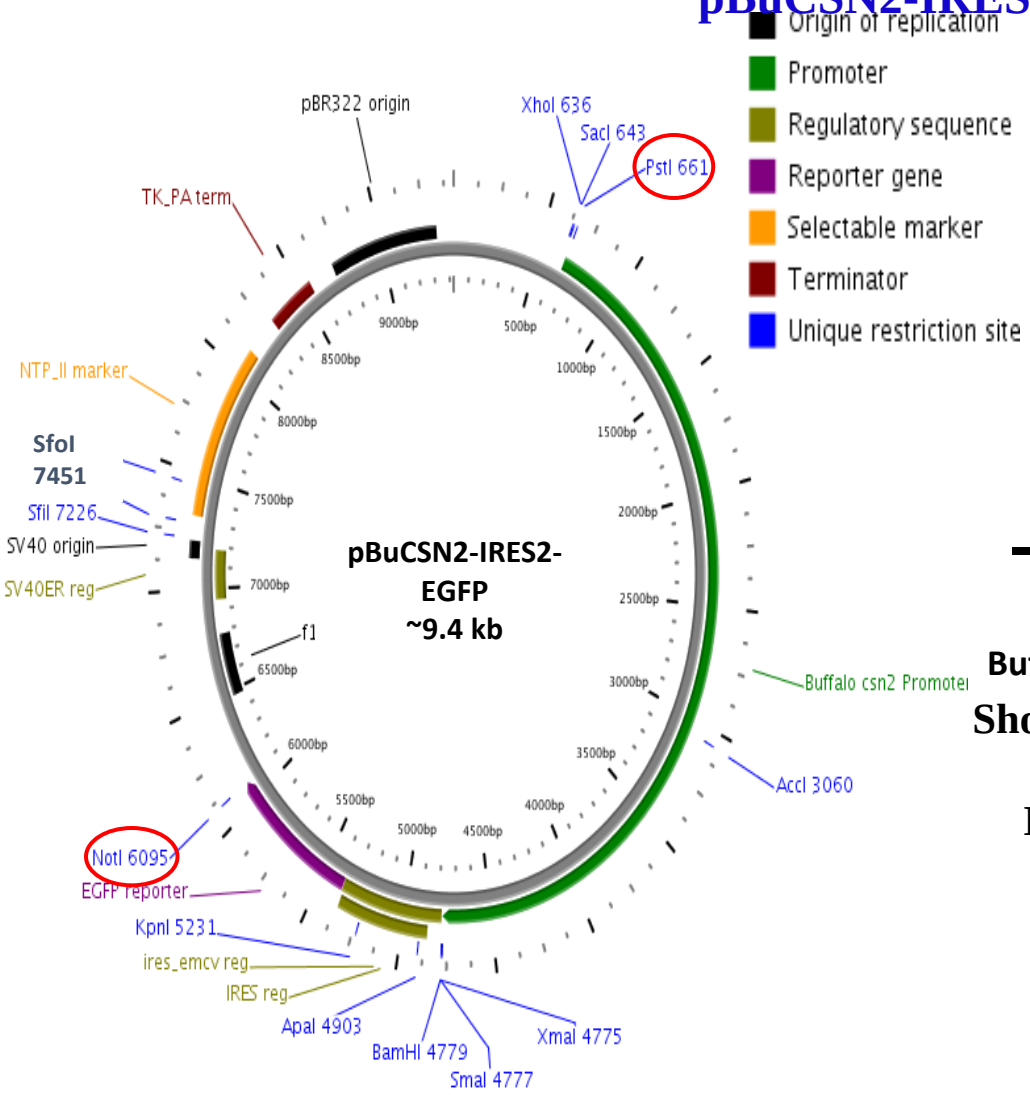
Histologically, as compared to the age-matched WT females (*n* = 10), the endometrium of young hypomorphs (*n* = 10) had a large number of glands. There was a wide variation in gland size, many had cystic dilatation or irregular luminal contours in older animals.

National Inst of research in reproductive and child health, Mumbai, INdia

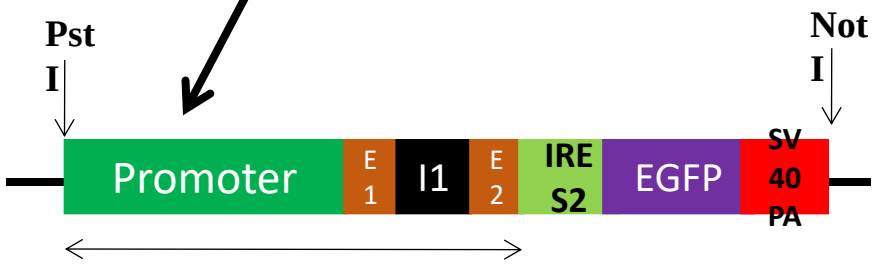
ISOLATION of BUFFALO βCASEIN PROMOTER

Mammary Luminal Epithelial Cell Specific β-Casein Gene Promoter Of Indian River Buffalo (*Bubalus bubalis*)

pBuCSN2-IRES2-EGFP.



Mammary gland specific promoter (buffalo β-Casein)

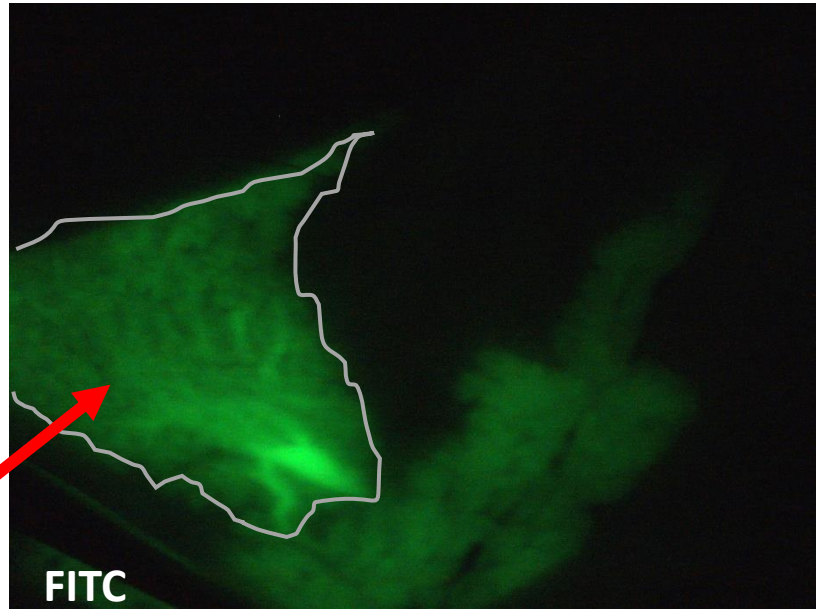


Buffalo Betacasein(csn2) genomic Region

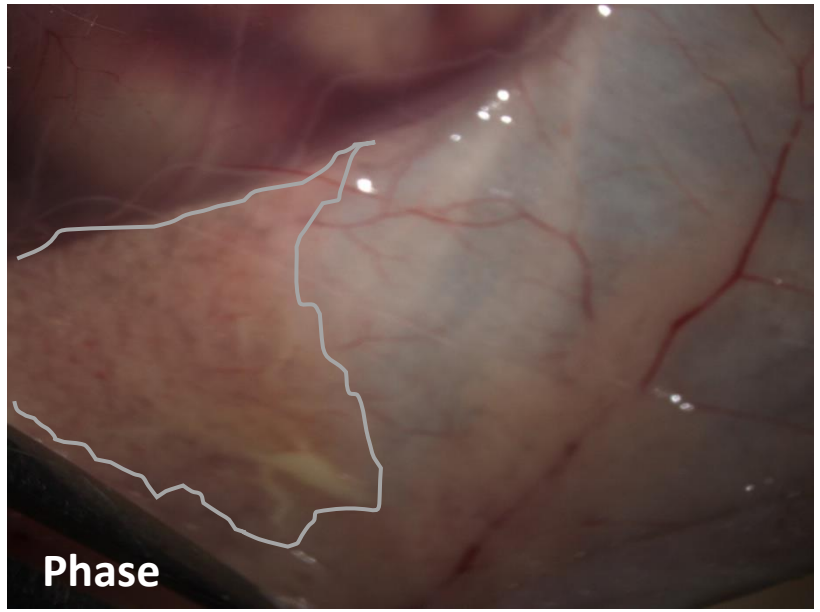
Showing Various Elements of Functional Cassette of pBuCSN2-IRES2-EGFP.
E1 and E2 refer to Exon1 and 2 respectively. I1 refer to Intron1.

Generated transgenic mice using construct having beta casein promoter driven GFP

Udder gland specific gene expression in transgenic mice



Now GFP and later, human gama interferon



Western blotting of Beta Casien EGFP transgenic mice for detection of EGFP

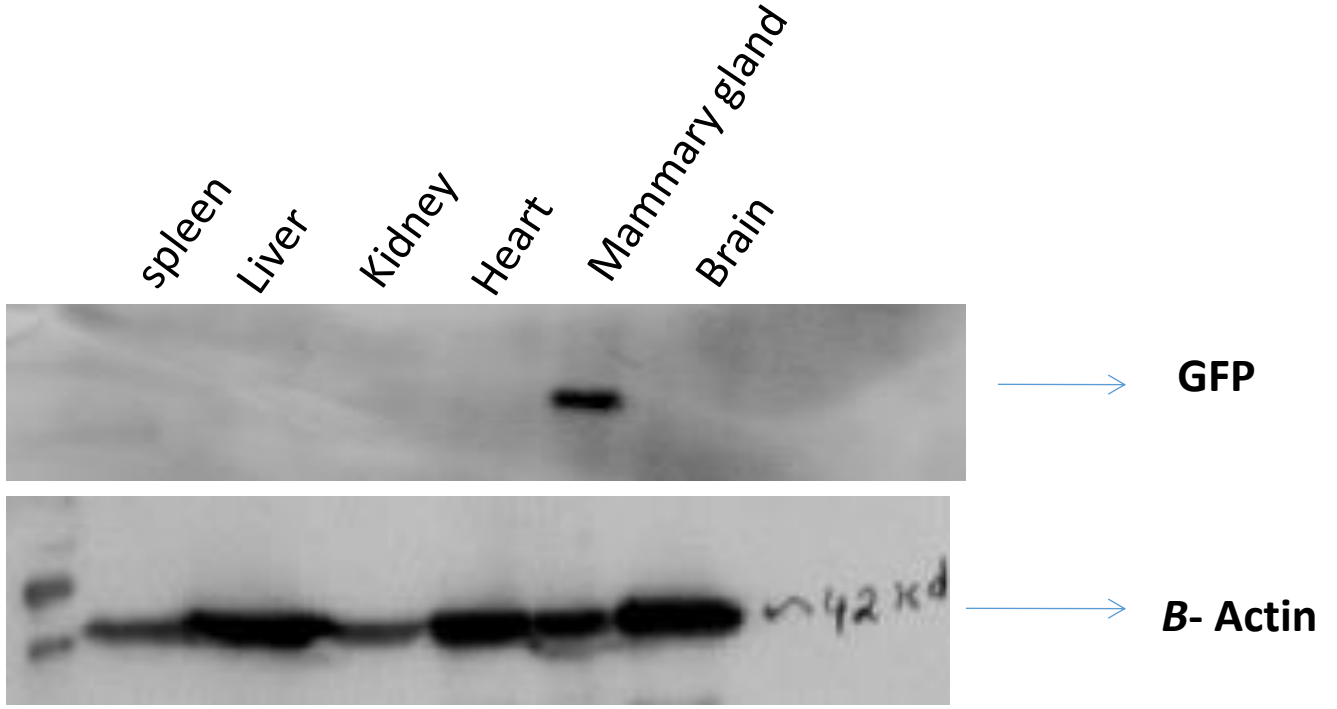
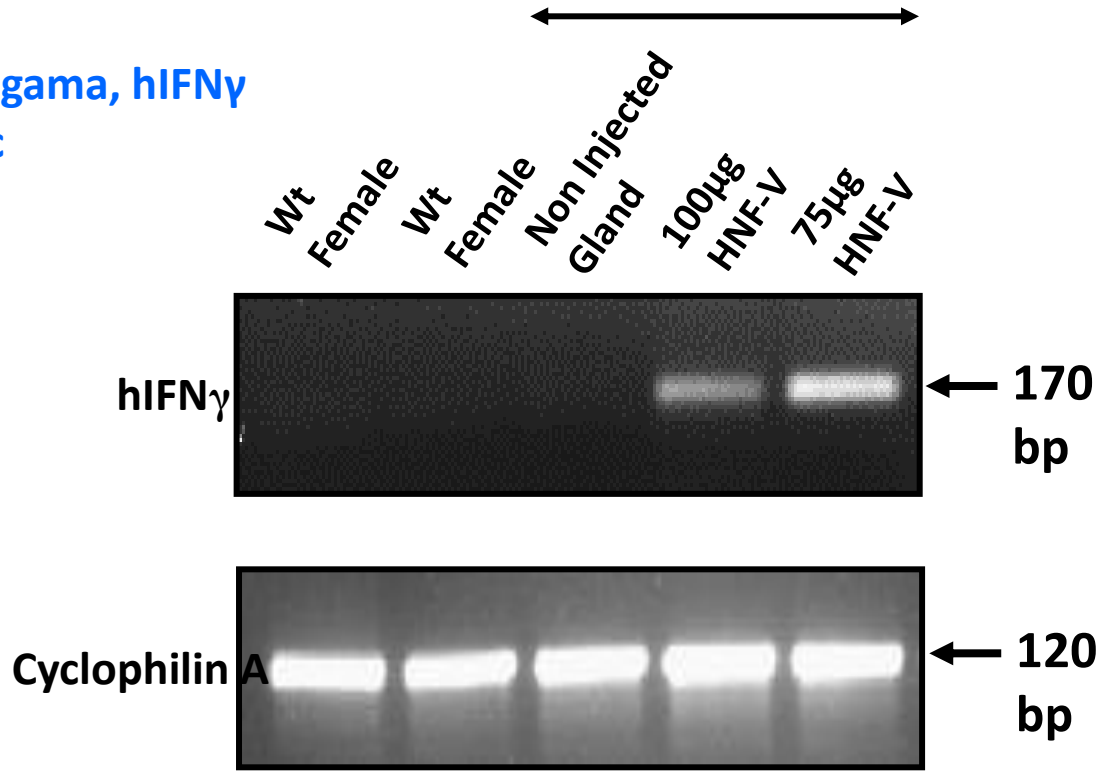


Figure showing the EGFP expression specifically in the mammary gland of the transgenic mice. There is no EGFP expression in the other tissue type of the same animal.

RT PCR ANALYSIS of hIFNγ TRANSCRIPT

MY GROUP

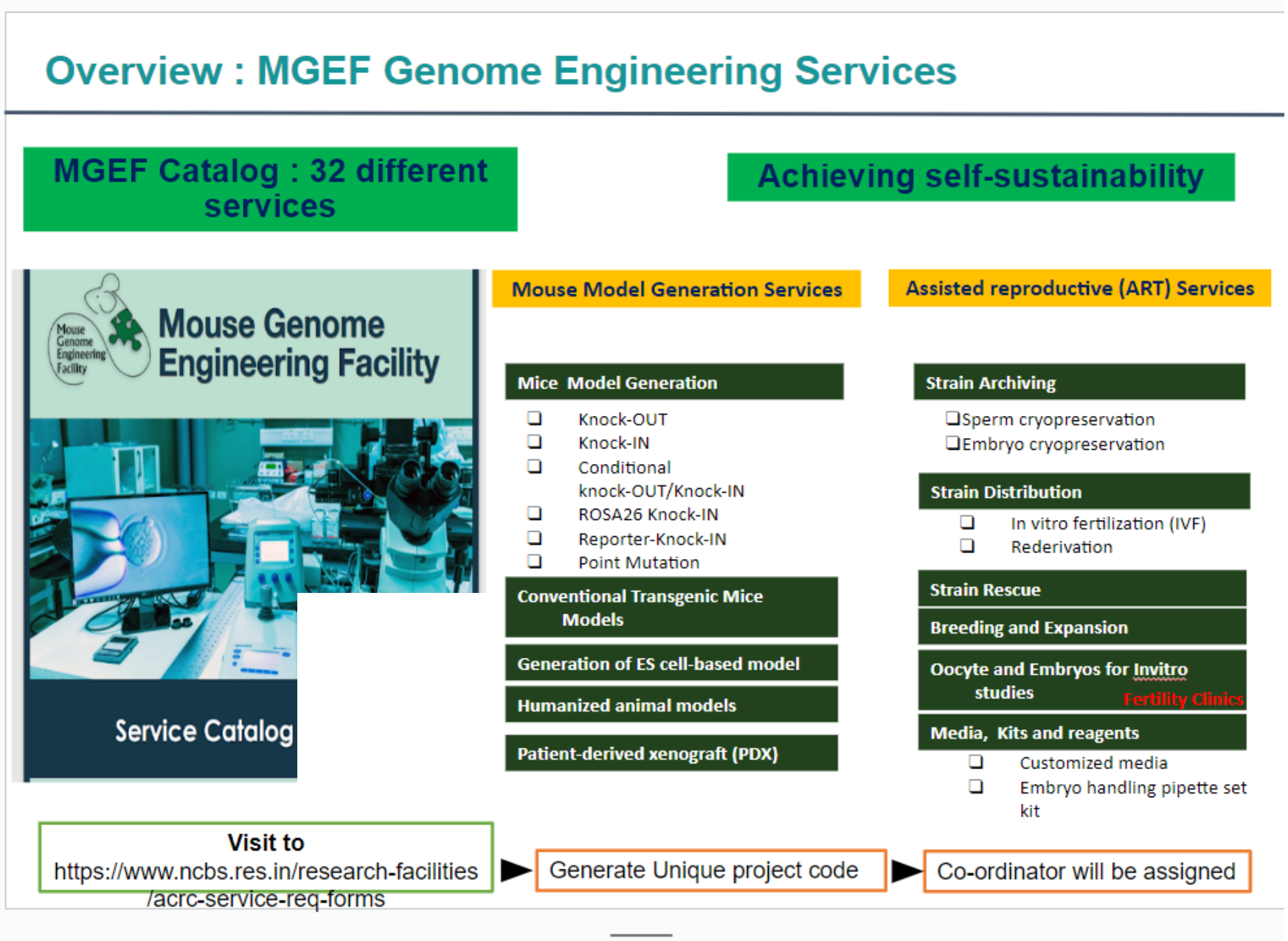
Expression of human interferon gama, hIFNγ
In mammary gland of transgenic
animals



Generated hIFNγ, bone morphogenic protein and bovine FSH in the milk of mice

Initiated major work in Goats

inStem, Bangalore, India



Dr. Mahesh Sahare

Scientist D, **Head,** Mouse Genome Engineering Facility (MGEF), inStem, Bangalore, India

MGEF - Outreach & Training Programs

Mouse Cryobiology workshop

CRISPR/Cas9 genome editing workshop

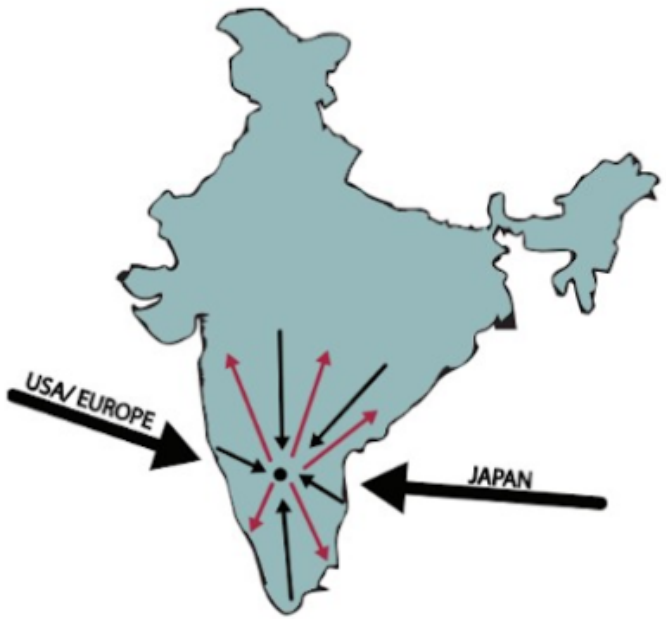
Mice model generation training for internal users

Certification courses
Mice model generation: Microinjection technician certificate course (SIX months)

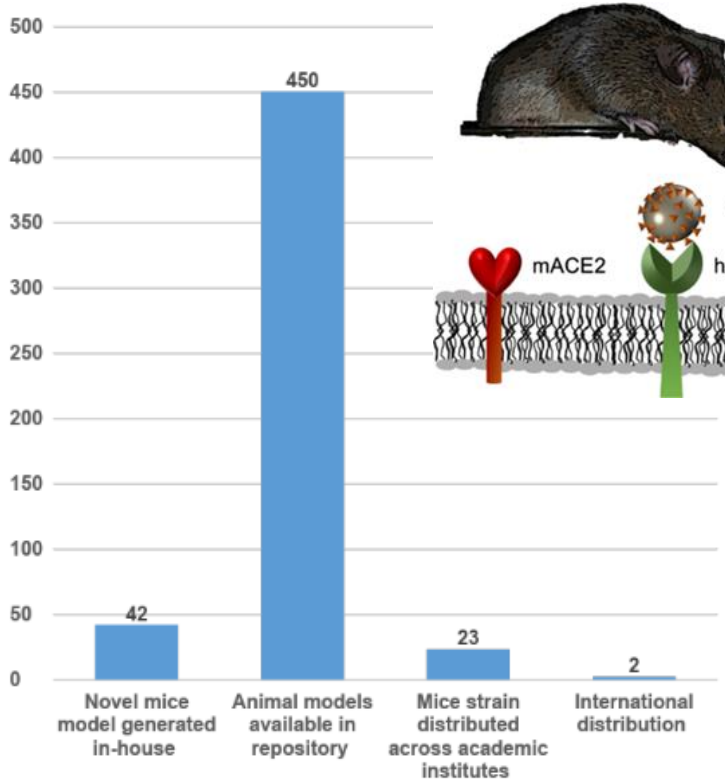
- Up coming workshop :

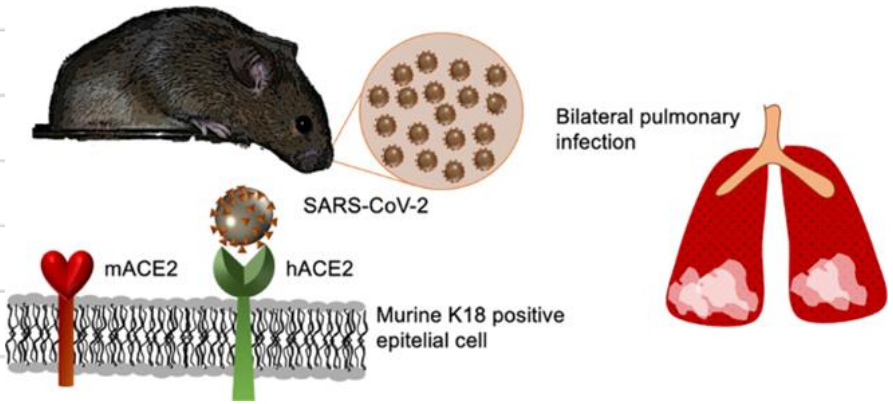
August 26, to August 29, 2024	Cryobiology and Assisted Reproductive Technologies (ARTs) in the Laboratory Mouse
November 18, 2024 to November 22, 2024	Hands on workshop on :CRISPR Genome Editing: Generating Mouse Models for Human Therapeutics

National Repository for Mouse Model Cryo-Archiving



Nationwide Distribution of mice models





The National Facility for Gene Function in Health and Disease (NFGFHD) was established at ISER, Pune. India in 2016. They have well-equipped functional facility for animal experimentation and the generation of transgenic mice models. **They provide nationwide services related to animal models generation, Assisted Reproductive Technologies and Space renting.**

In addition to this routine work, we also offer services such as the generation of **transgenic rodent models** using the CRISPR/Cas9 technology, embryo/sperm cryopreservation, and the re-derivation of rodent strains from cryopreserved embryos.

Knock-Out Models, Knock-In Models , Conditional Knock-Out Models.ROSA26 Knock-In Models
Reporter Knock-In Models,Humanized Models,CRISPR/Cas9 Based Construct Designing, Genotyping Services

Center for Cellular and Molecular Biology (CCMB), Hyderabad, India

Transgenic and Gene Knockout
A National Facility for Transgenic and Gene Knock-out Mice sponsored by the Department of Science and Technology (DST), Government of India has been set up at the CCMB with the following aims:

P. Chandra Shekar*, SandeepGoel*, S. DeepaSelvi Rani, D. ParthaSarathi, Jomini Liza Alex, Shashi Singh, and Satish Kumar; K-Casein-deficient mice fail to lactate. Proc. Natl. Acad. Sci. USA.2006,103,8000-8005

Transgenic Goats/ attempts in buffalo

National Dairy Research Institute

- Vats etal. Production of Transgenic Handmade Cloned Goat (Capra hircus) Embryos by Targeted Integration into Rosa 26 Locus Using Transcription Activator-like Effector Nucleases. CELLULAR REPROGRAMMING.Volume 23, Number 4, 2021.DOI: 10.1089/cell.2021.0011

- The NDRI is working on the project to generate human insulin in milk (in buffalo)

National Institute of Animal Biotechnology, Hyderabad

Attempting generation of Transgenic goat for producing therapeutic proteins and also myostatin knock down animals for more meat (my own group).

ZEBRA FISH TRANSGENESIS

Institute for Genomic and Inigrative Biology, New Delhi, India

Transgenic Zebra fish facility : Using genetic manipulations such as transgenics, antisense knockdowns and CRISPR-based mutations to perturb gene functions.

SHIV NADIR UNIVERSITY. Shiv Nadar IoE has set up state-of-the-art facility - Centre for Integrative and Translational Research (CITRES). The facility is equipped to import, maintain, serve as a repository and generate in-house transgenic/knock-out mice and rats as well as zebrafish and serve as a National Facility.