Biotechnology opportunities and challenges in livestock production

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Aim

- How biotechnologies can work together in order to enhance livestock production
  - Reproductive biotechnologies
    - In vitro embryo production (*in vitro fertilization*) and associated technologies
  - Genome editing

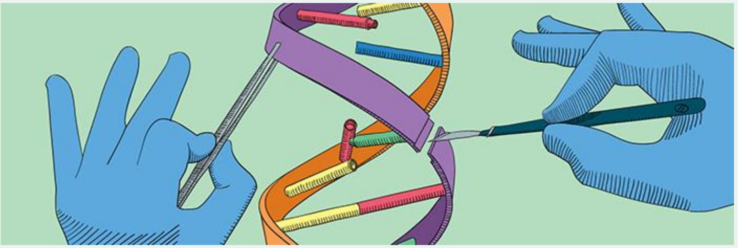

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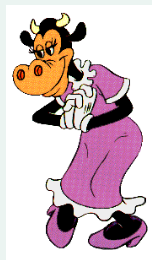
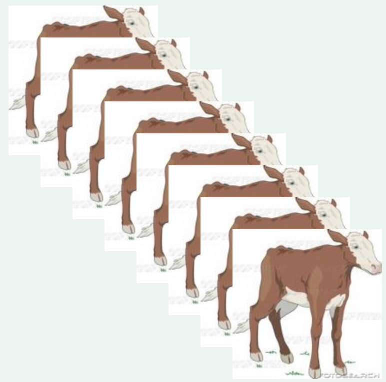
Image source: https://www.genomicseducation.hee.nhs.uk/news/item/336-how-genome-editing-could-enter-mainstream-medicine/

The in vitro embryo production (IVEP)

Source: JHM Viana

IVEP - multiplication power

Conventional (In vivo) embryo transfer (ET)

 X  = 

IVEP

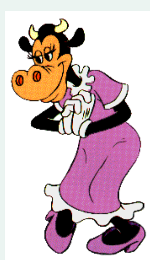
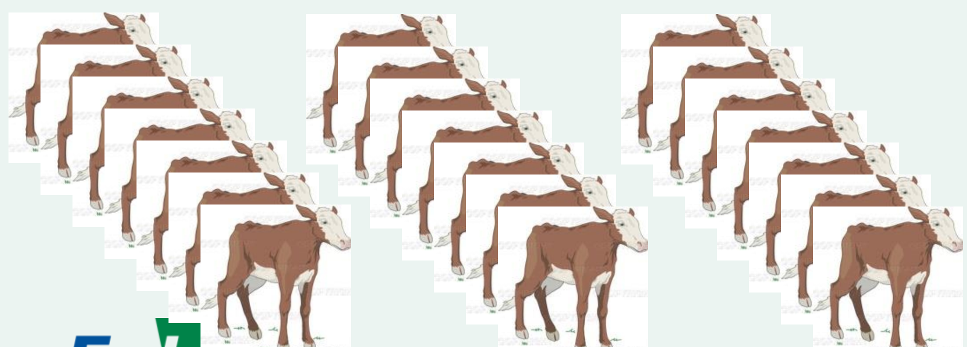
 X  = 

Image Source: LS Camargo

IVEP + sex-sorted semen

- Sex-sorting semen: straws 3-4x more expensive than conventional semen
  - IVEP: one straws can be used for >3 cows – and produce several female embryos per cow/ovum pick up session - reasonable pregnancy rates
    - 35-45% pregnancy rate

Table 1
Mean ± SEM number of oocytes, embryos and pregnancy rate obtained from Gir (*B. indicus*), Holstein (*B taurus*) and *indicus–taurus* donors submitted to OPU – IVP.

Type of donor	Total oocytes/ OPU (n)	Viable oocytes/ OPU (n)	Embryos/total oocytes % (n)	Embryos/ OPU-IVP (n)	Pregnancy/ OPU-IVP* (n)	Pregnancy rate (%)* (n)
Gir	17.1 ± 4.5^{a} (64617/3778)	12.1 ± 3.9^{a} (45838/3778)	18.9 (12243/64617)	3.2^{a} (12243/3778)	1.2^{a} (3113/2670)	40 (3113/7763)
Holstein	11.4 ± 3.9^{b} (12977/1138)	8.0 ± 2.7^{b} (9082/1138)	18.7 (2426/12977)	2.1^{b} (2426/1138)	0.7^{b} (604/822)	36 (604/1698)
¼ Holstein ¾ Gir	20.4 ± 5.8^{c} (5457/267)	16.8 ± 5.0^{c} (4472/267)	18.9 (1033/5457)	3.9^{ac} (1033/267)	1.3^{ac} (137/103)	37 (137/368)
½ Holstein ½ Gir	31.4 ± 5.6^{d} (7035/224)	24.3 ± 4.7^{d} (5434/224)	17.4 (1222/7035)	5.5^{c} (1222/224)	1.7^{c} (82/47)	37 (82/220)
Total	16.7 ± 6.3 (90086/5407)	12.0 ± 4.4 (64826/5407)	18.8 (16924/90086)	3.1 (16924/5407)	1.1 (3936/3642)	39 (3936/10049)

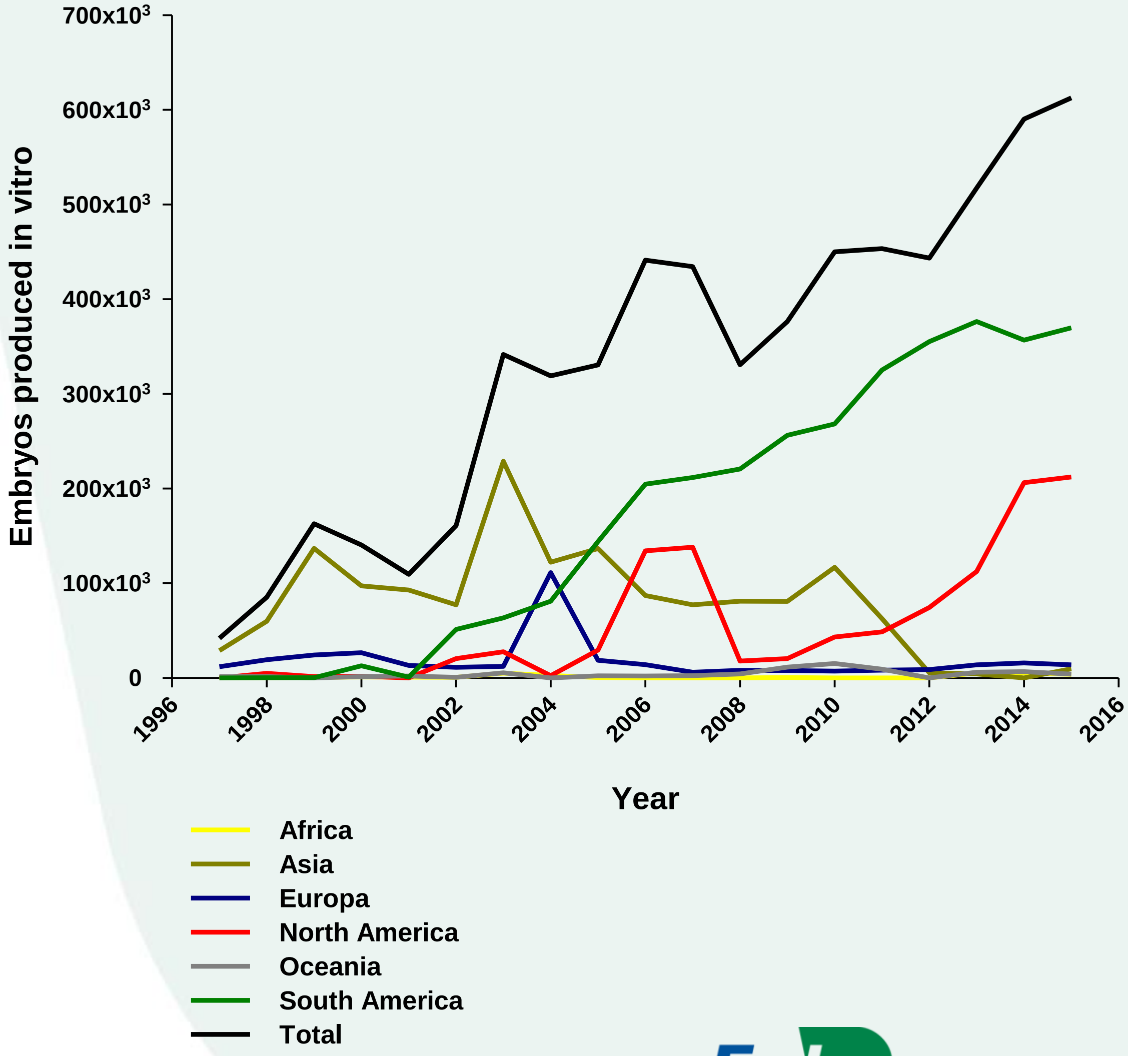
a–d Within a column, means without a common superscript differ ($P < 0.05$).
* Pregnancy data of 10 049 embryos from the total of 16 924 transferred (results of 6 876 transferred embryos were not available).

ELSEVIER
Theriogenology 74 (2010) 1349–1355
www.theriojournal.com

Large-scale *in vitro* embryo production and pregnancy rates from *Bos taurus*, *Bos indicus*, and *indicus-taurus* dairy cows using sexed sperm

J.H.F. Pontes[a,b], K.C.F. Silva[a], A.C. Basso[b], A.G. Rigo[b], C.R. Ferreira[b,c], G.M.G. Santos[a], B.V. Sanches[b], J.P.F. Porcionato[b], P.H.S. Vieira[b], F.S. Faifer[b], F.A.M. Sterza[a], J.L. Schenk[d], M.M. Seneda[a,]*

IVEP in the world – 1997-2015



Country	IVEP	%
Brazil	353,539	52.7
USA	198,618	29.6
Total	552,157	82.3

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Source: JHM Viana

IVEP + prepubertal heifers

- Young heifers (or even calves) can be used as oocyte donors:
  - Important for breeds that reach puberty later in life (zebu breeds)
  - Reduce generation interval

Table 2
Embryo development of in vitro fertilized oocytes derived from 9- to 14-month-old prepubertal *B. indicus* crossbred heifers and mature cows

Source of oocyte (group)	Total oocytes	Cleavage at 68–72 hpi % (*n*)[a]			Blastocysts % (*n*)[a]			Hatching % (*n*)[a]
		2–8 cells	9–16 cells	Total	168 hpi	192 hpi	Total	
Prepubertal heifer	189	47.0 (89)	17.5 (33)	64.5 (122)	22.2 (42)	1.6 (3)	23.8 (45)	18.5 (35)
Cow	74	40.5 (30)	20.3 (15)	60.8 (45)	31.1 (23)	1.3 (1)	32.4 (24)	18.9 (14)

Hpi: hours post insemination.

[a] No significant difference was found between embryos derived from prepubertal heifers and cows (Chi-square test; $P > 0.05$).

ELSEVIER Animal Reproduction Science 85 (2005) 53–59
www.elsevier.com/locate/anireprosci

Developmental competence of oocytes from prepubertal *Bos indicus* crossbred cattle

L.S.A. Camargo[a,*], J.H.M. Viana[a], W.F. Sá[a], A.M. Ferreira[a], V.R. Vale Filho[b]

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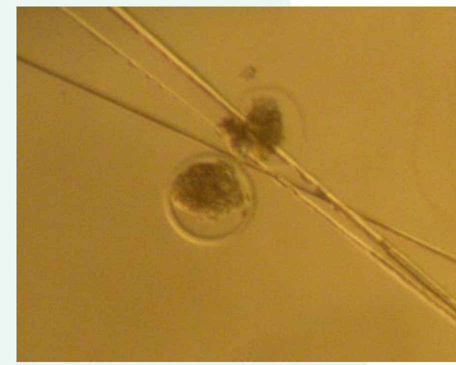
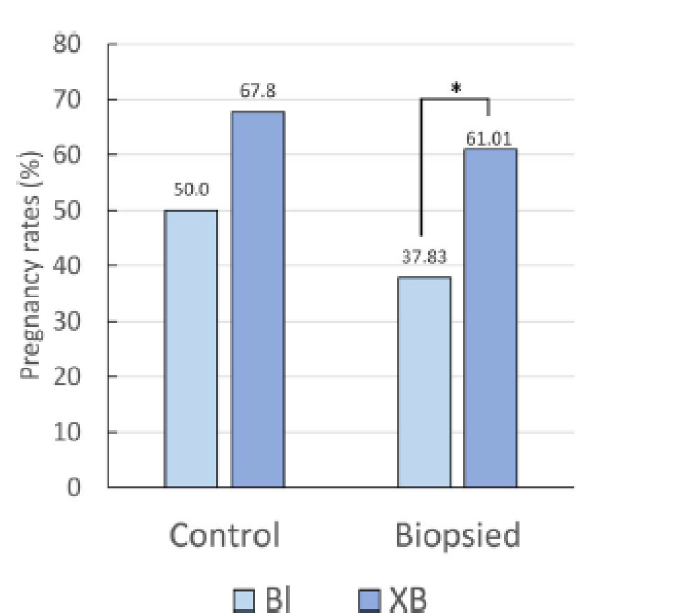
IVEP + embryo biopsy + genomic selection

- In vitro produced embryos (blastocysts) can be biopsied and samples submitted to genomic evaluation
  - Genomic estimated breeding value (GEBV) of every embryo can be calculated before transferring to recipients
  - No significant effect on pregnancy rate





- Save cost with recipients
- Only calves with high GEBV are born
  - Reduction of culling rate

Animal Reproduction Science 184 (2017) 51–58
Contents lists available at ScienceDirect
ELSEVIER
Animal Reproduction Science
journal homepage: www.elsevier.com/locate/anireprosci

Post implantation development reveals that biopsy procedure can segregate "healthy" from "unhealthy" bovine embryos and prevent miscarriages

Clara Slade Oliveira[a,*], Carolina Capobiango Romano Quintão[b], Celio de Freitas[a], Agostinho Jorge dos Reis Camargo[c], Raquel Varella Serapião[c], Luiz Sérgio de Almeida Camargo[b]

In short

- With IVEP and associated technologies we can now:
  - Produce a large amount of female (or male) embryos of a cow (or prepubertal heifer) with high genetic merit
  - Select the best embryos based on the GEBV

- Generate calves with high potential to produce meat or milk from cows or heifers:
  - increase the intensity of selection;
  - reduce the generation interval
- New opportunity:

IVEP + Genome editing

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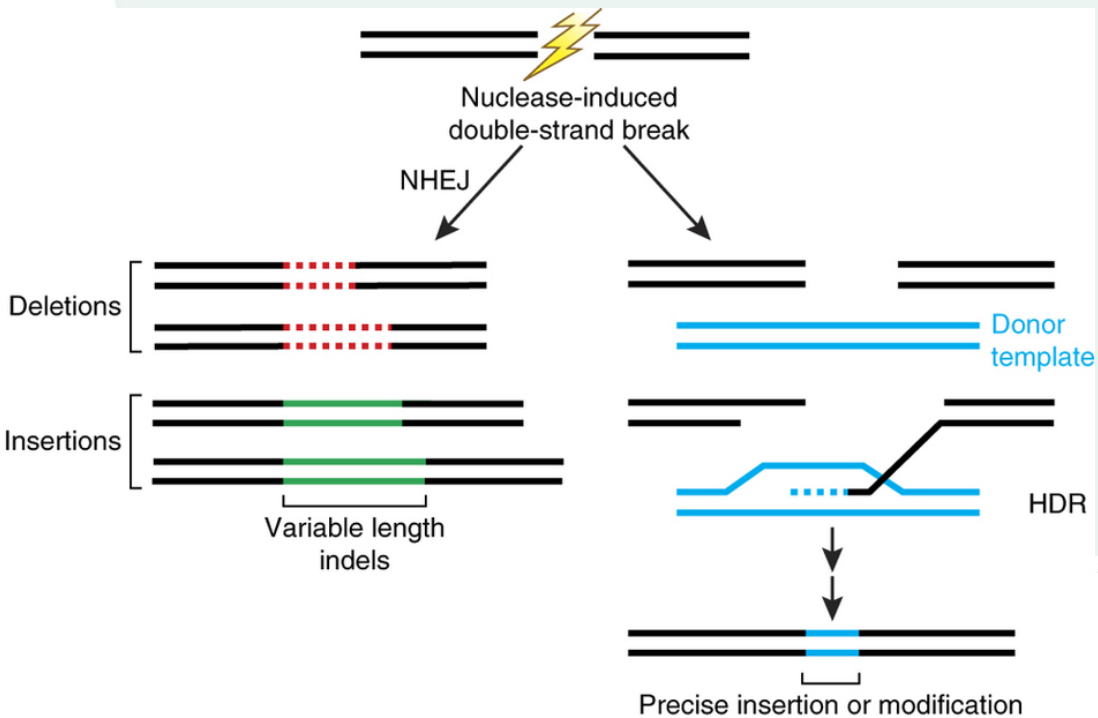
Genome editing

- Allow a more precise genome manipulation:
  - DNA sequence can be deleted, inserted or modified; mutations can be induced
  - Nucleases break the DNA double strand in a specific site of the genome. Broken strands can be repaired by non-homologous-end-joining (NHEJ) that promote Indels or by homologous recombination (homology-directed repair – HDR)



Journal of Animal Breeding and Genetics
J. Anim. Breed. Genet. ISSN 0931-2668

EDITORIAL

Promotion of alleles by genome editing in livestock breeding programmes

nature biotechnology

CRISPR-Cas systems for editing, regulating and targeting genomes

Jeffry D Sander[1,2] & J Keith Joung[1,2]

Potential issues that can be addressed by genome editing in livestock

- Heat stress - increase the frequency of alleles/mutations that promote heat tolerance

- Diseases and parasite infestations - Screwworm, horn flies, ticks and ticks born-diseases, foot-and-mouth disease, etc.

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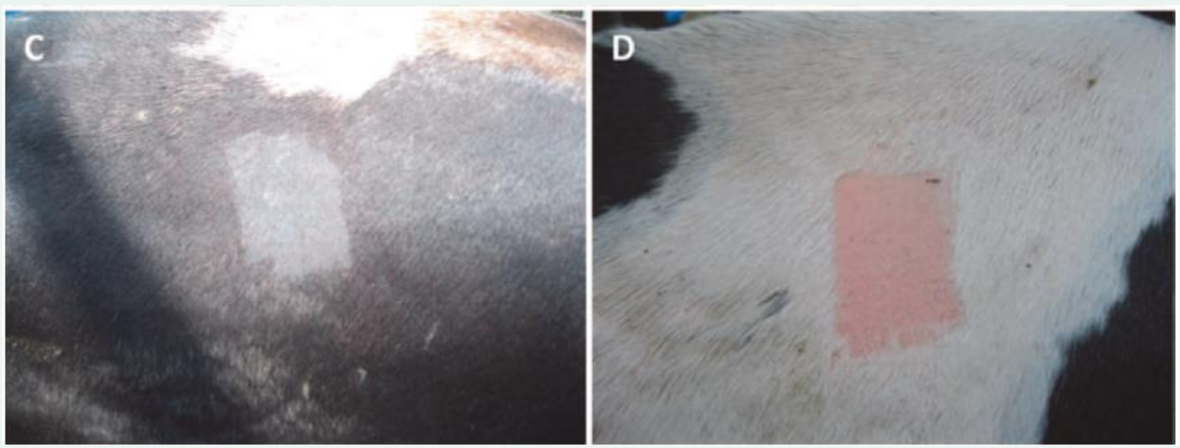
Image: http://m.milkpoint.com.br/radar-tecnico/bemestar-e-comportamento-animal/vantagens-do-manejo-do-estresse-calorico-em-gado-leiteiro-86072n.aspx

Increase the thermotolerance

- Mutations in prolactin receptor (PRLR) – genetic effect on hair length and coat structure: smooth coats, short hair (slick hair)



  – Found in some taurine breeds in the Americas (Senepol, Carora, Limonero)

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J. Dairy Sci. 97:5508–5520
http://dx.doi.org/10.3168/jds.2014-8087

The *SLICK* hair locus derived from Senepol cattle confers thermotolerance to intensively managed lactating Holstein cows

S. Dikmen,* F. A. Khan,†[1] H. J. Huson,‡[2] T. S. Sonstegard,‡ J. I. Moss,† G. E. Dahl,† and P. J. Hansen†[3]
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frontiers in Genetics
ORIGINAL RESEARCH
published: 23 February 2018
doi: 10.3389/fgene.2018.00057

Convergent Evolution of Slick Coat in Cattle through Truncation Mutations in the Prolactin Receptor

Laercio R. Porto-Neto[1], Derek M. Bickhart[2], Antonio J. Landaeta-Hernandez[3], Yuri T. Utsunomiya[4,5], Melvin Pagan[6], Esbal Jimenez[6], Peter J. Hansen[7], Serdal Dikmen[8], Steven G. Schroeder[9], Eui-Soo Kim[10], Jiajie Sun[11], Edward Crespo[3], Norman Amati[3], John B. Cole[9], Daniel J. Null[9], Jose F. Garcia[4,5,12], Antonio Reverter[1], William Barendse[1] and Tad S. Sonstegard[10]*

PRLR mutations

- Slick hair: different mutations according to the breed

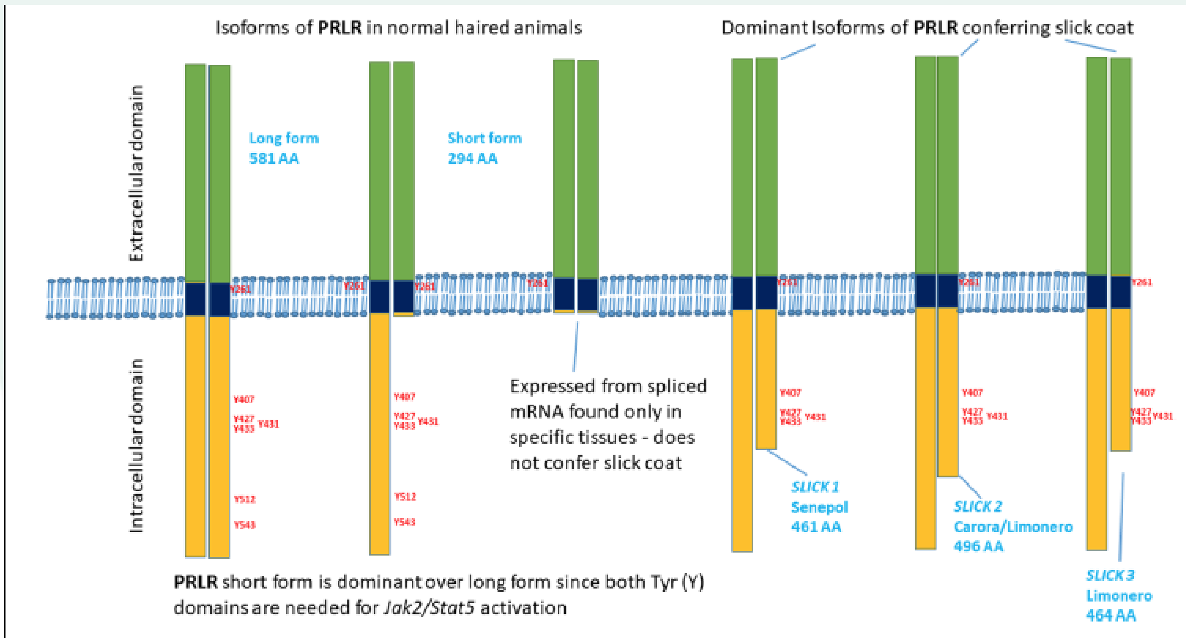
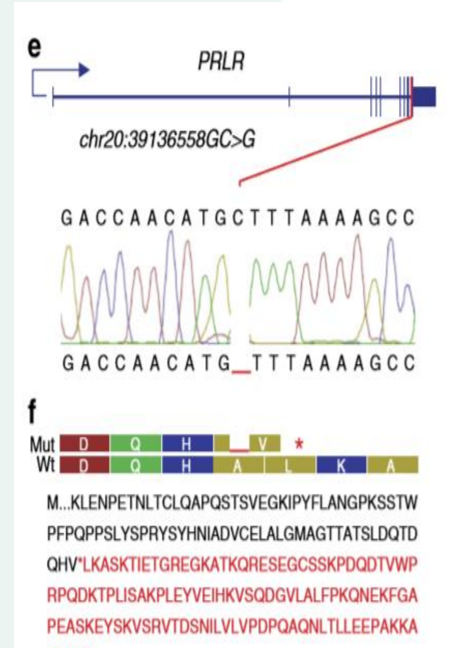


FIGURE 3 | Schematic diagram of the truncated cytoplasmic domains of the PRLR mutations associated with slick coats.



Littlejohn et al. Nat. Comm. 2014

TABLE 2 | Observed variants on the bovine chromosome 20 at the *PRLR* gene.

Position	A1	A2	Consequence		Reference
39136497[a]	T	A	Stop gained, nonsense	p.C440*	rs468544332, dbSNP (release 150)
39136518	C	A	Synonymous coding	p.A447	rs110971500, dbSNP (release 150)
39136558	GC	G	Frameshift	p.A461L	rs517047387, dbSNP (release 150)
39136571[b]	C	A	Stop gained, nonsense	p.S465*	This article
39136666[b]	C	T	Stop gained, nonsense	p.R497*	This article

[a]*SNP monomorphic in the validation sample.* [b]*Polymorphic SNP in the validation samples, and used on further analyses.*

Brazil: Caracu breed

- Data from few Caracu cows showed mutation on PRLR – similar to other breeds originated from Iberian peninsula
  - Some animals with slick hair phenotype

GLTCR1: T/T GLTCR2: C/T

GLTCR3: T/T GLTCR4: C/T

GLTCR5: C/T GLTCR10: T/T

GLTCR15: C/C GLTCR19: T/T

GLTCR33: T/T GLTCR46: T/T

T: Slick variant, frequency= 0.75

C: Reference, frequency = 0.25

Image Source: LS Camargo

Source data: Tad Sonstegard

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Introgression of PRLR mutation in thermo-sensitive breeds

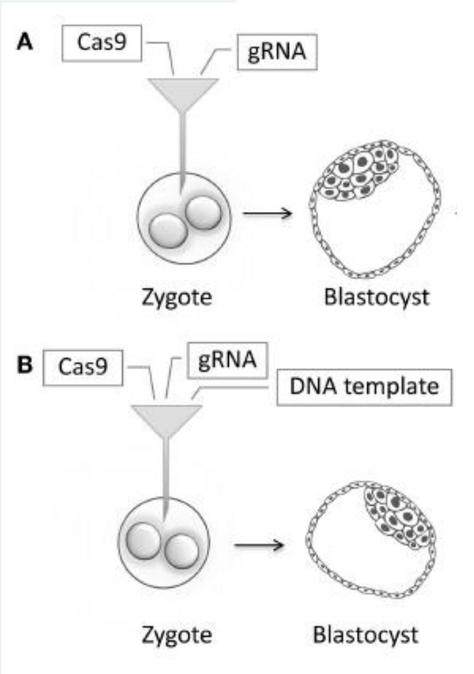

frontiers in Immunology

REVIEW

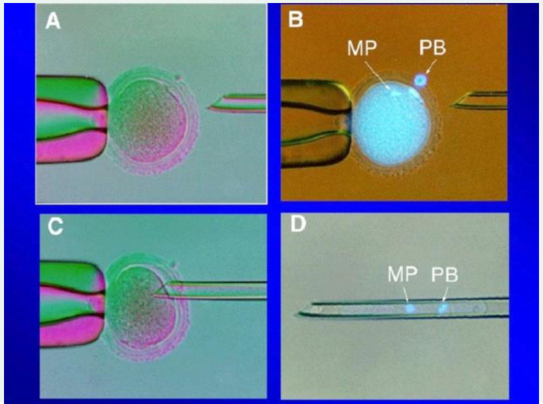
Novel genome-editing tools to model and correct primary immunodeficiencies

*Lisa M. Ott de Bruin[1,2], Stefano Volpi[3,4] and Kiran Musunuru[5]**

- It can by done by different platforms: TALENs or CRISPR/Cas9 systems



- Requirement:
  - Zygotes produced by IVEP
    - » Injection of gRNA/Cas9 or TALENs mRNA into cytoplasm of zygotes
  - Somatic cell nuclear transfer (SCNT)
    - » Enucleated oocytes reconstructed with transfected somatic cells



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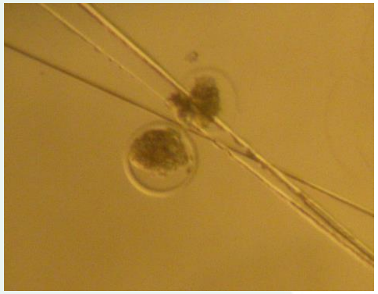
IVEP + genome editing + genomic selection

- IVEP to produce female (or male) zygotes;
  - Zygotes injected for genome editing (to promote a specific trait)

  - Blastocysts are biopsied



  - Mutation is detected and GEBV is calculated for every embryo
  - Only embryos with the target mutation and with high GEBV are transferred to recipients

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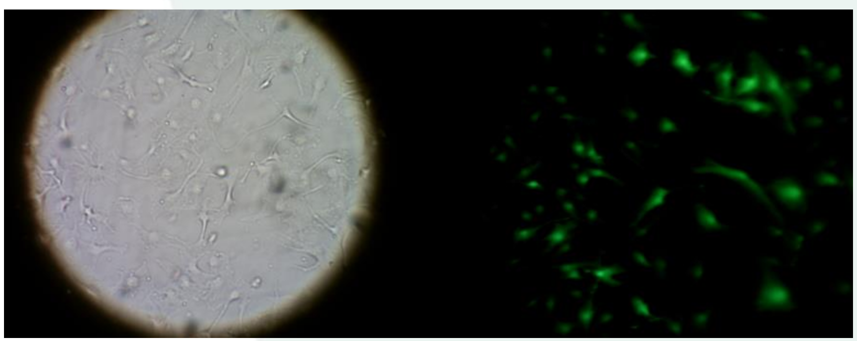
Image Source: LS Camargo

SCNT + genome editing

- Somatic cells from high genetic merit sires are transfected with CRISPR/Cas9 (gRNA/Cas9) or TALENs systems (mRNA or vectors);
  - Cells can be selected and propagated during in vitro culture: only genome edited cells are used as nucleus donor for cloning;
  - The SCNT embryos will keep the genetic modification



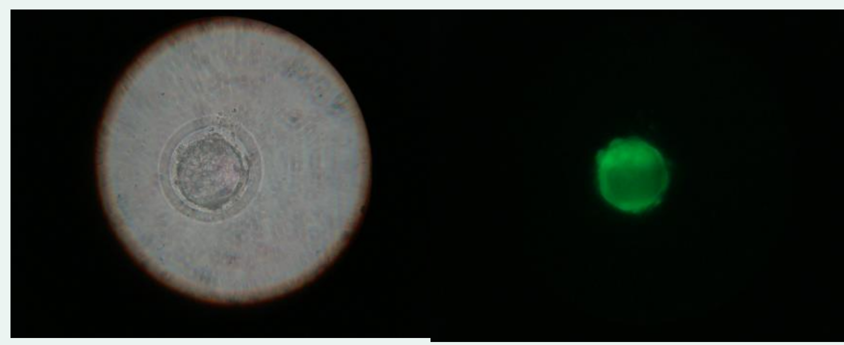


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Image Source: LS Camargo

IVEP # SCNT

- IVEP:
  - not all embryos will have the genetic modification (20-30%)
    - But delivery rate of viable offspring is between 30-40%
  - Several commercial IVEP labs around the world (>50 labs in Brazil)
- SCNT:
  - all offspring will have the genetic modification
    - But the procedure is inefficient: delivery rate of viable offspring is <5%
    - Animal welfare concerns: higher abortion and stillbirth rate
  - Only few companies are able to produce cloning animals (only 2 in Brazil) - costly procedure

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Image Source: LS Camargo

Challenges

- Increase the genome editing efficiency
  - Increase the rate of HDR: about 10% of DNA double breaks are repaired by homologous recombination only;
  - Increase the efficiency zygote transfection: news procedures to replace cytoplasm injection

- Control or avoid (functional) off-targets modifications
  - Concerns (contested) about *in vivo* off-targets and theirs effects on animals
    - May require improvements of gRNA design and/or use of new high fidelity nucleases

NATURE METHODS | CORRESPONDENCE

Unexpected mutations after CRISPR–Cas9 editing *in vivo*

Kellie A Schaefer, Wen-Hsuan Wu, Diana F Colgan, Stephen H Tsang, Alexander G Bassuk & Vinit B Mahajan

Nature Methods 14, 547–548 (2017) doi:10.1038/nmeth.4293
Published online 30 May 2017 Updated online 14 June 2017

To the Editor:

REVIEW ARTICLE WILEY Reproduction in Domestic Animals

Basics of genome editing technology and its application in livestock species

Bjoern Petersen

Concerns

- International trade of IVEP embryos
  - Need a well established sanitary protocol among countries
- GM Animals regulation:
  - Clear and reasonable regulations by governmental agencies
    - Should the regulatory requirements for crops and livestock not be the same?
- Public perception and acceptance of animals with an edited genome for food production
  - Public concern: Is there any health risks for consumers?
  - Animal welfare: is there any animal suffering?

Acknowledges

CNPq

FAPEMIG

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Thank you

Mercy beaucoup

Muito obrigado

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