Ventidue anni dopo Dolly: come usiamo la tecnologia della clonazione?

FESTIVAL DELLA SCIENZA MEDICA
BOLOGNA MEDICINA

CESARE GALLI

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WWW.1BILLIONHUNGRY.ORG

1 BILLION PEOPLE LIVE IN CHRONIC HUNGER AND I'M MAD AS HELL.

CLICK HERE TO SIGN THE PETITION TO END HUNGER **1 611 469** ALREADY SIGNED.

Pope, Diouf: "United Against Hunger"

"Responding properly to the hunger problem requires urgent, resolute and concerted action by all relevant actors and at all levels. It calls for the need for all of us to be united," said FAO Director General Jacques Diouf at World Food Day celebrations in Rome. [more...]

UNITED AGAINST HUNGER

16 October 2010 **World Food Day**

Fruit Trees for Haiti

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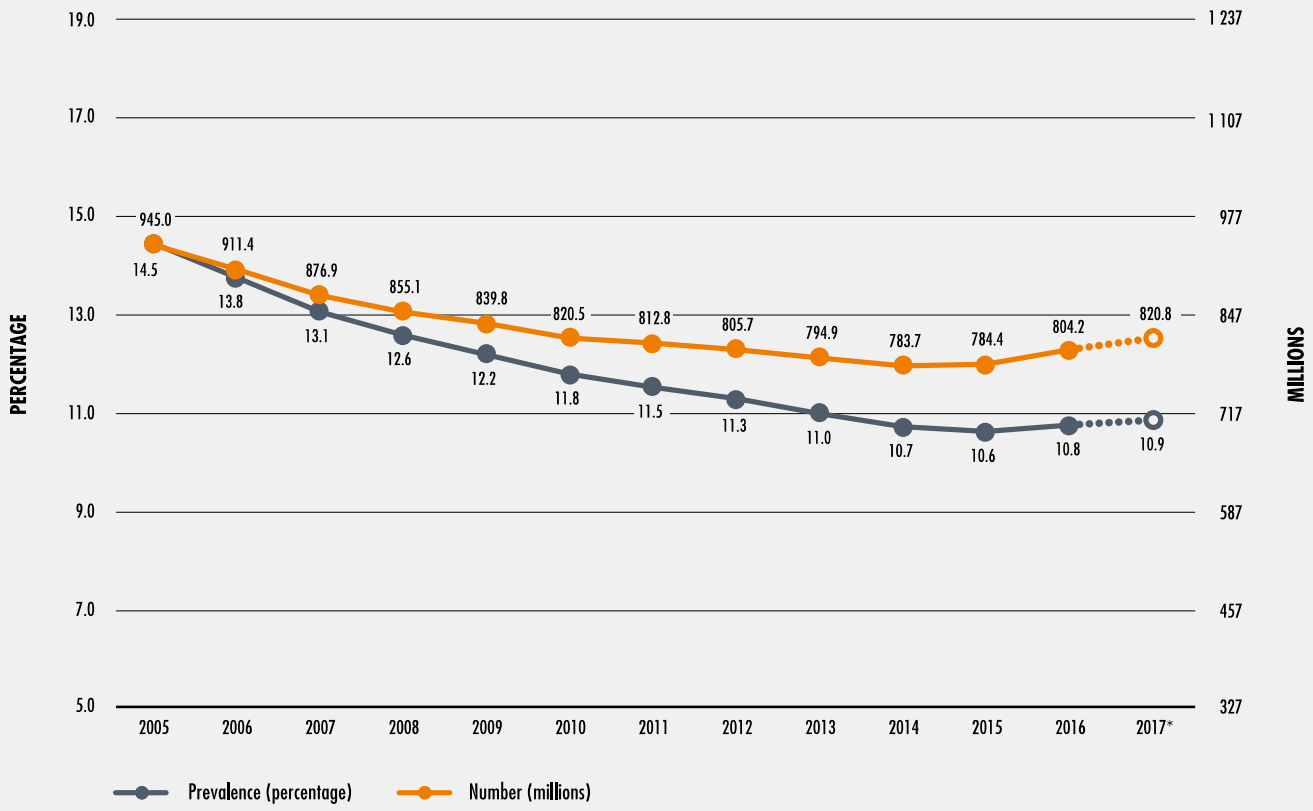
FIGURE 1

THE NUMBER OF UNDERNOURISHED PEOPLE IN THE WORLD HAS BEEN ON THE RISE SINCE 2014, REACHING AN ESTIMATED 821 MILLION IN 2017



* Projected values, illustrated by dotted lines and empty circles.
SOURCE: FAO.

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Consistenza mondiale della popolazione dei grossi animali d'allevamento (in milioni)

bovini			**1.371**
bufali			**200**
pecore			**1.024**
capre			**768**
maiali			**956**
cavalli			**55**

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La selezione passa attraverso la riproduzione per le future generazioni

1950
produzione 40 q
per lattazione

2010
produzione 90 q
per lattazione

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Generazioni di tecnologie riproduttive

- **Inseminazione artificiale (prima generazione anni ‘50)**
- **MOET superovulazione ed embryo transfer (anni ‘70)**
- **Produzione di embrioni in vitro (anni ‘80)**
- **Clonazione (anni ‘90)**
- **Ingegneria Genetica (anni ‘00)**

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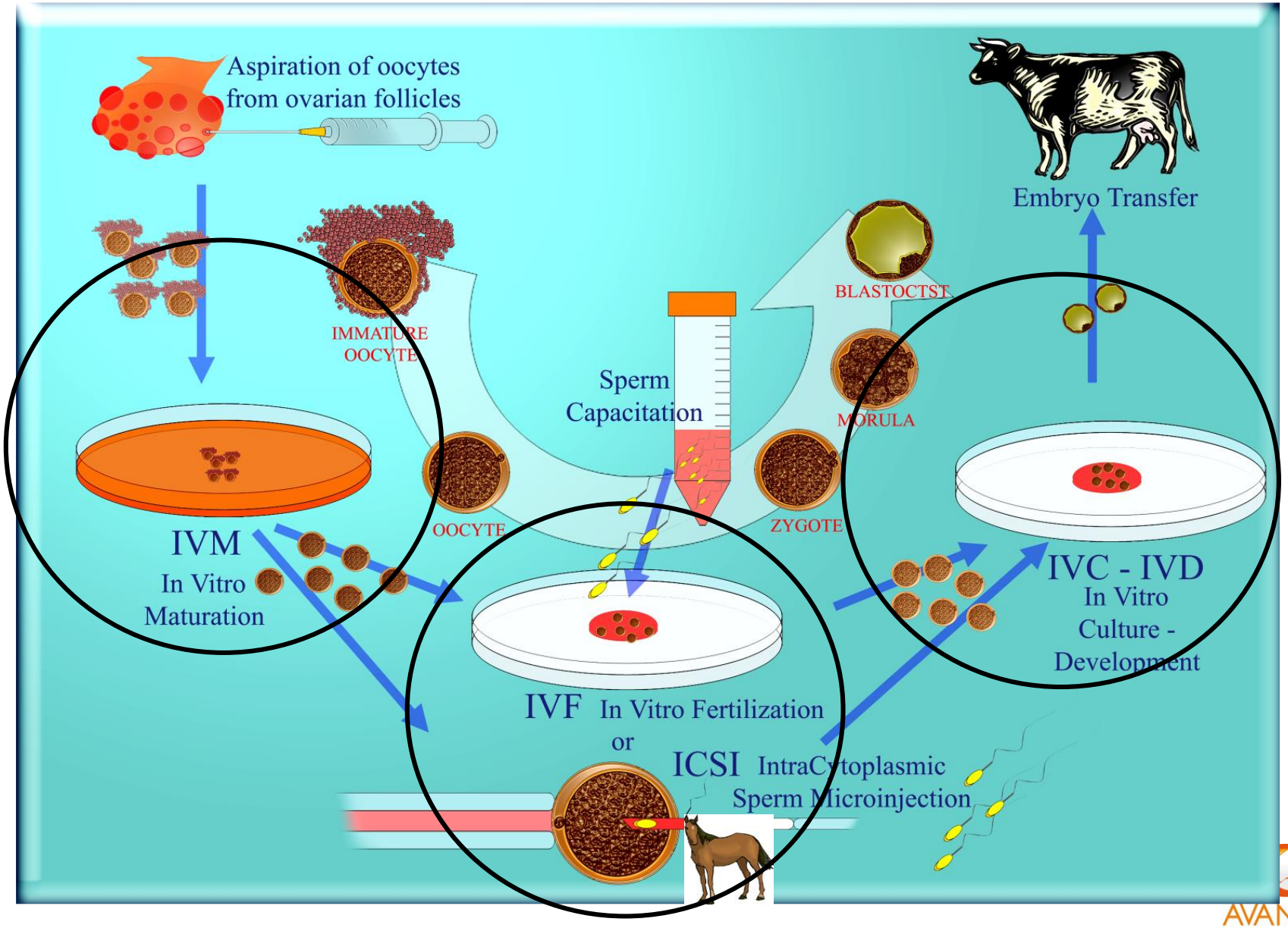
Produzione di embrioni *In vitro*



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SOMATIC CELL NUCLEAR TRANSFER (SCNT) (clonazione)

COME LE TALEE PER RIPRODURRE LE PIANTE!

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COME SI FA UNA TALEA?

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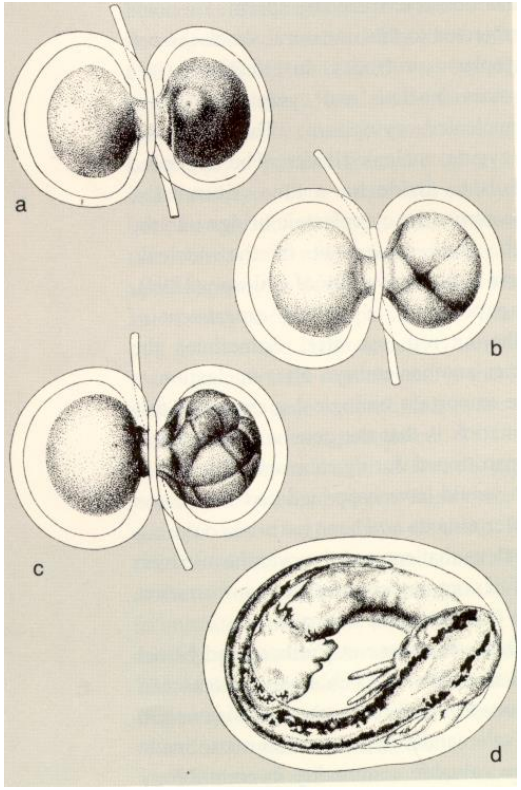
Storia della clonazione animale

Hans Spemann, 1928

- salamanders



Briggs & King, 1952

- frogs

Alcuni girini ma mai adulti

Willadsen, 1986

- sheep

McGrath & Solter, 1983

- sentenziavano che fosse impossibile

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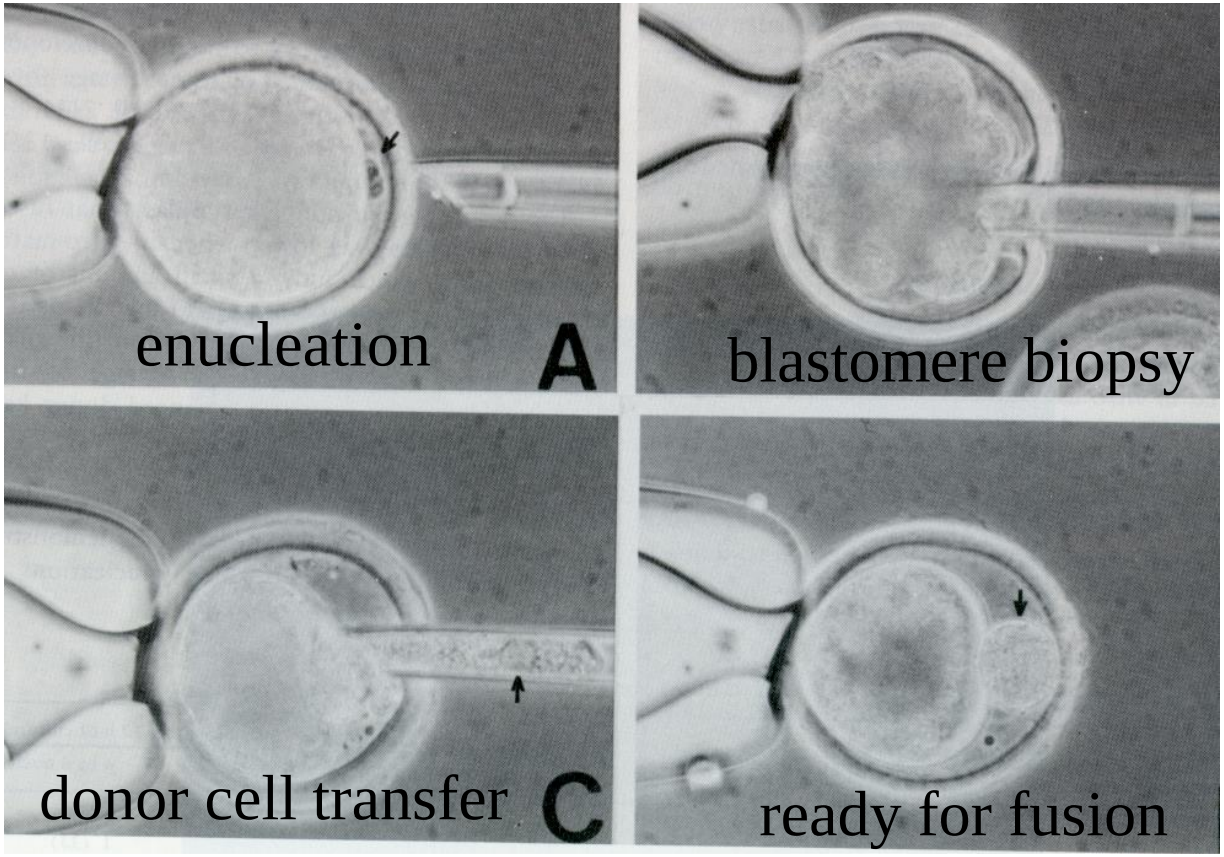
Clonazione da embrioni

Steen Willadsen, 1986: ottenne degli agnelli sando le cellule di un embrione di 8 cellule trasferito in un ovocita enucleato



"The most obvious practical aim of such experiments will be to define conditions which will offer large-scale cloning of domestic animals."

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La Pecora Dolly (1996-2003)

La pecora Dolly è stata il primo mammifero a essere stato clonato con successo da una cellula somatica

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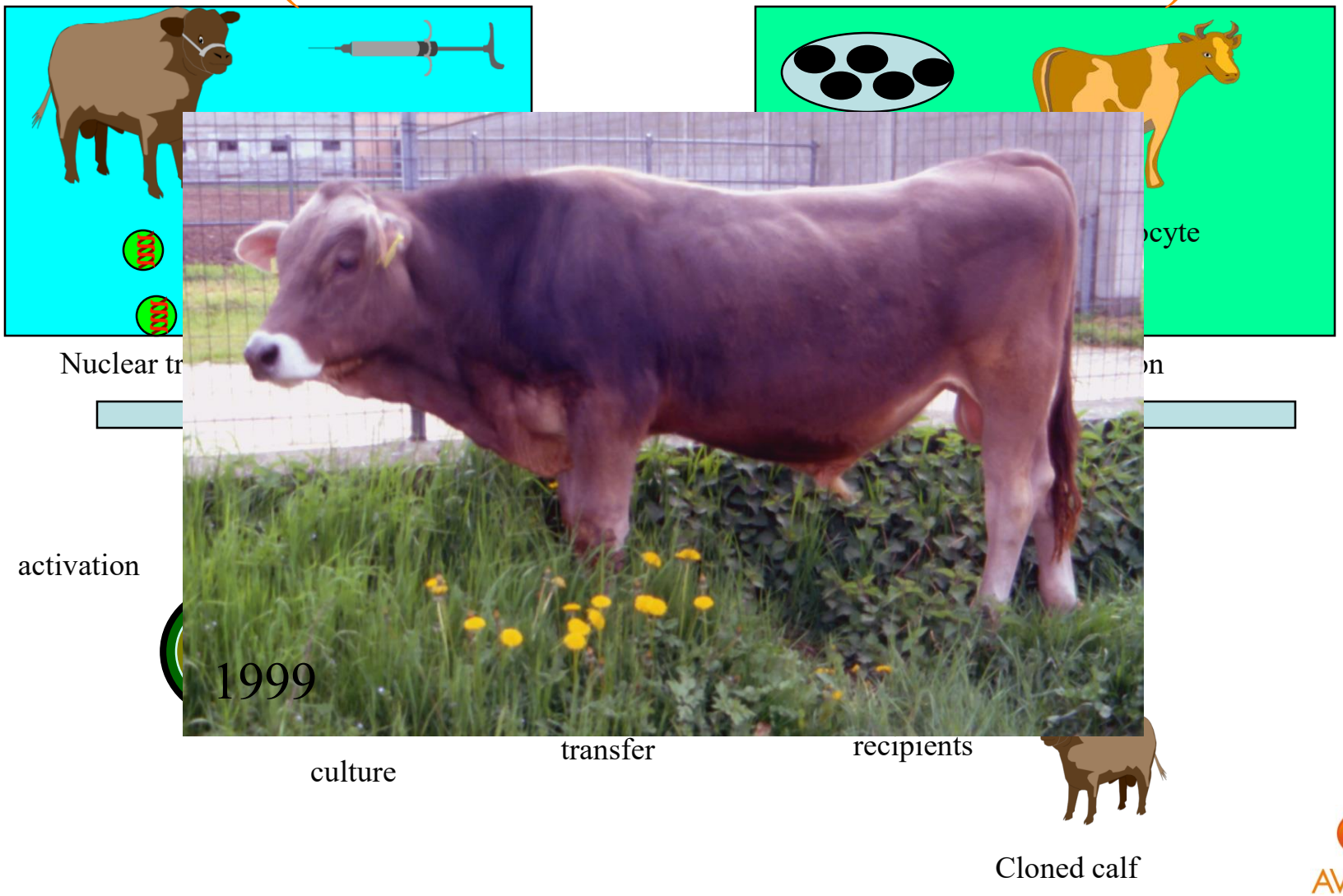
Somatic Cell Nuclear Transfer SCNT (Tecnica di Clonazione)



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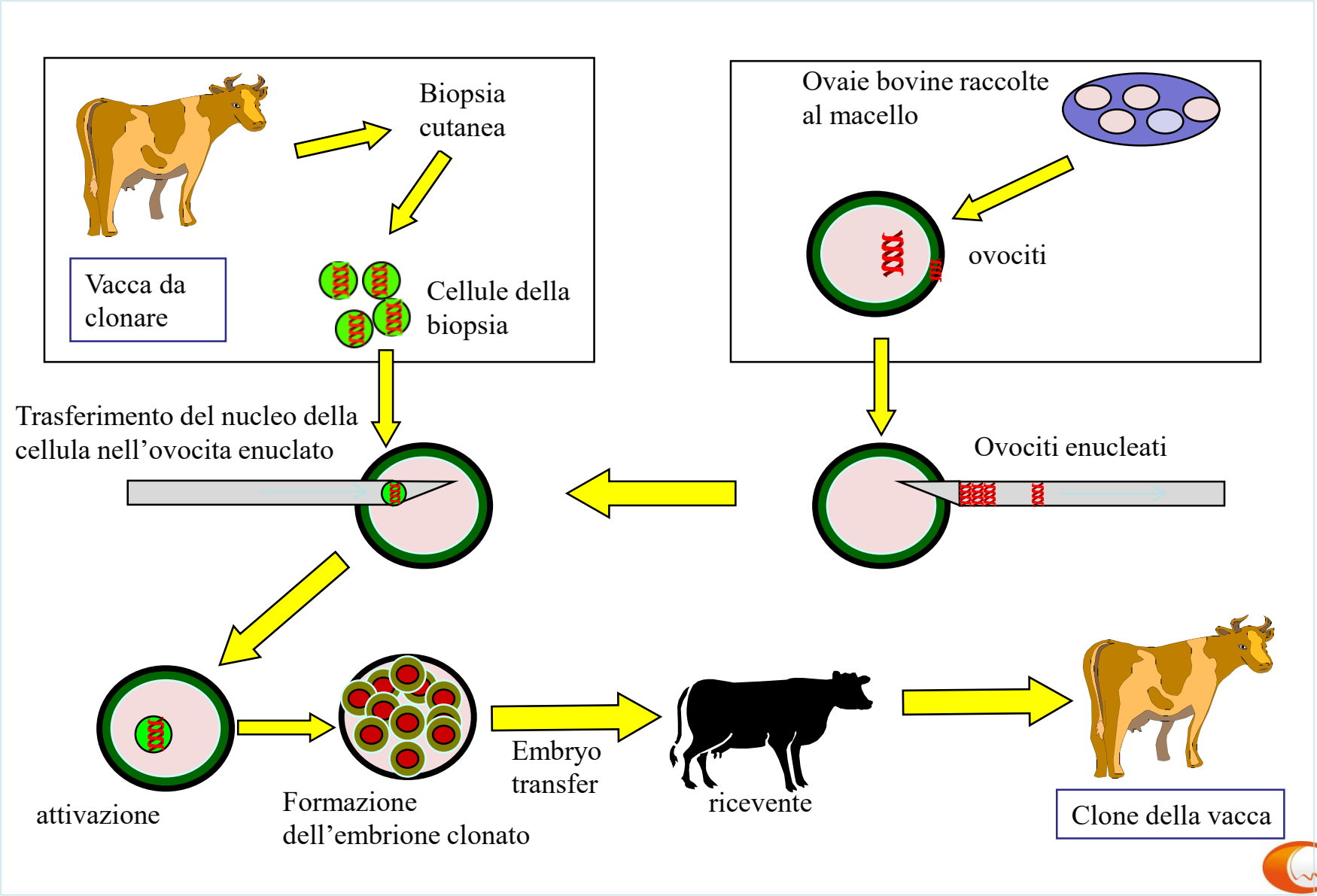
Clonazione animale



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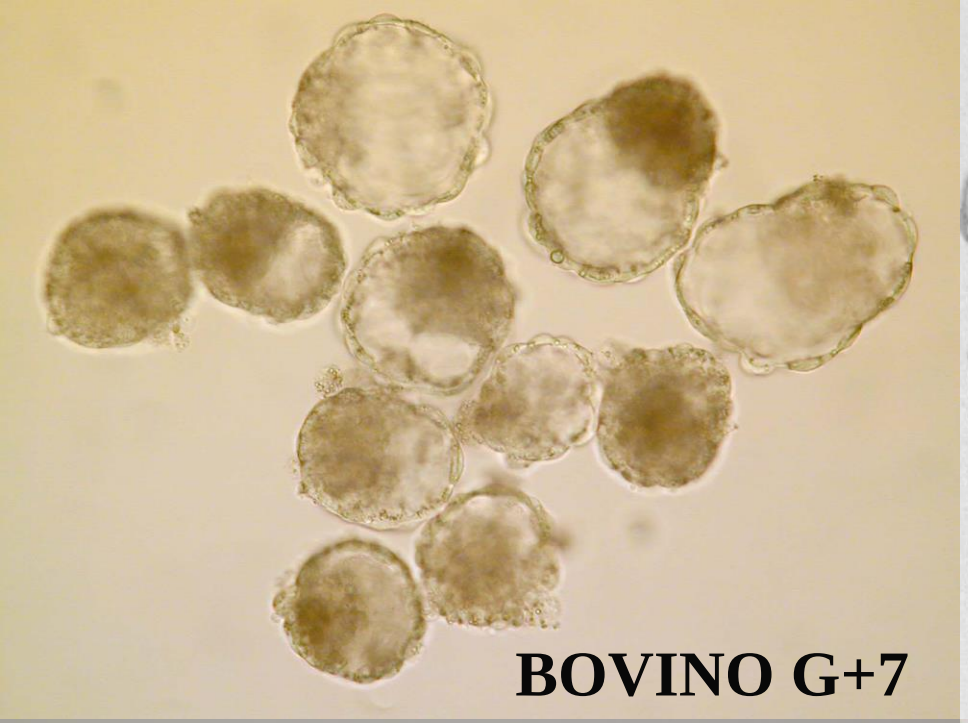
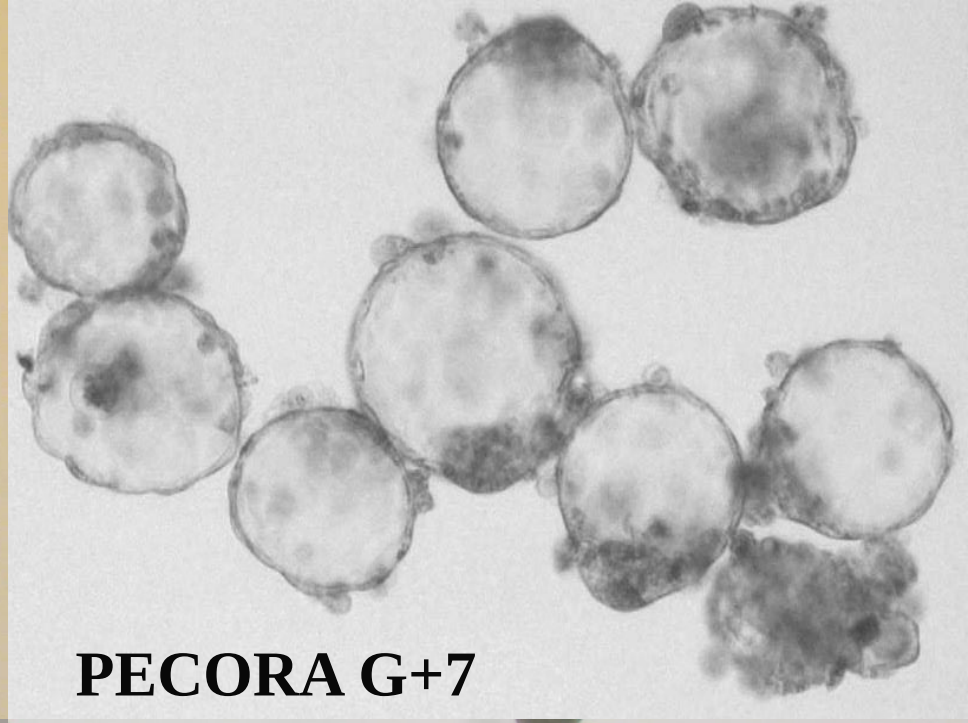
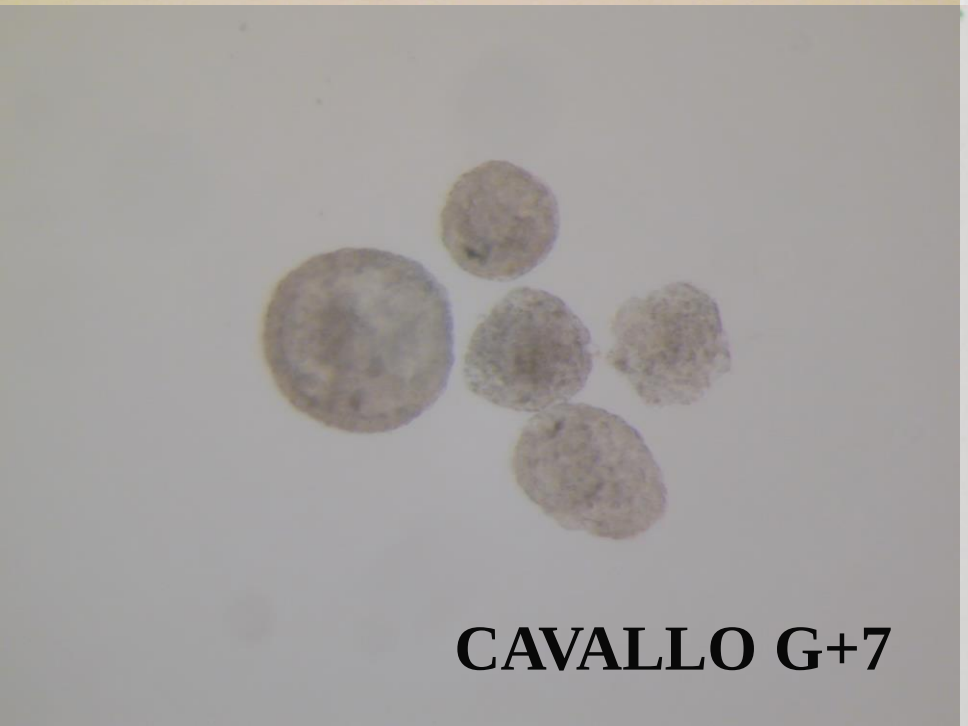
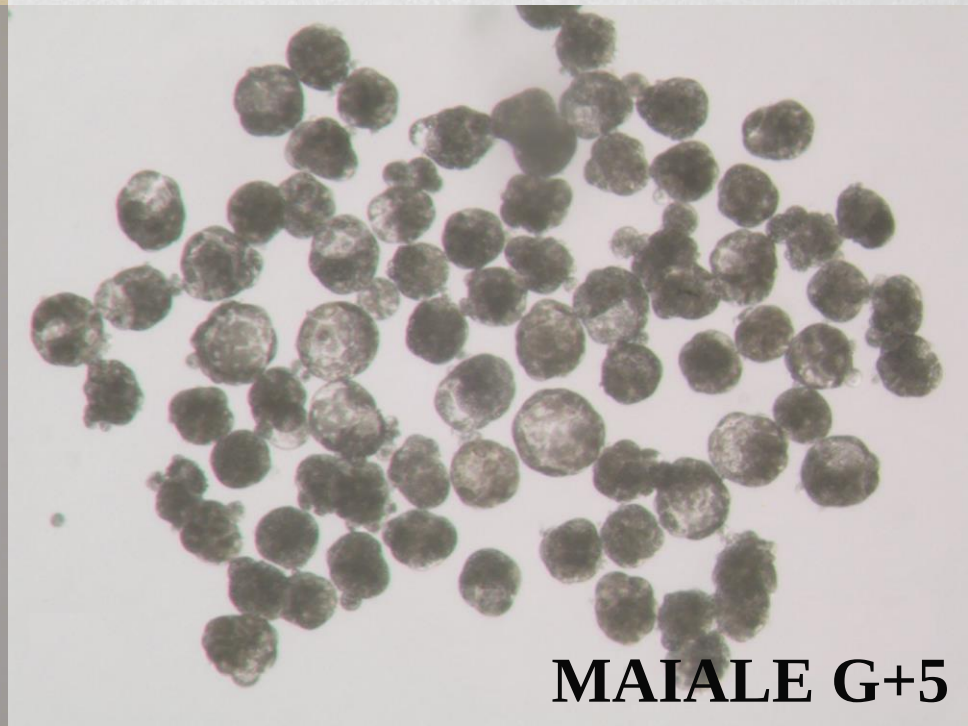
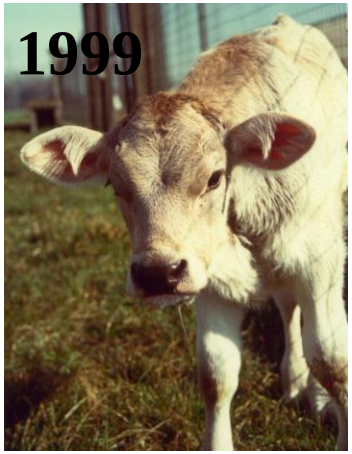

BOVINO G+7

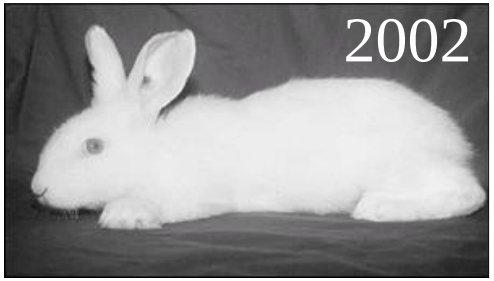

PECORA G+7

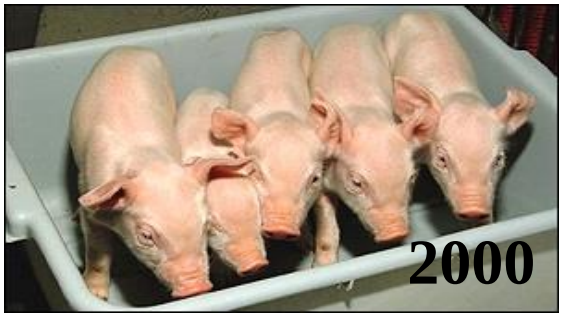

CAVALLO G+7


MAIALE G+5

ALCUNE SPECIE DI MAMMIFERO OTTENUTE CON SCNT







PROGENIE DEI CLONI

Pegaso nato a marzo 2008
da Prometea da
inseminazione artificiale

Piearazade du Vialaret
Figlio di Pieraz

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Carol Prelude Mtoto clone 1-2-3

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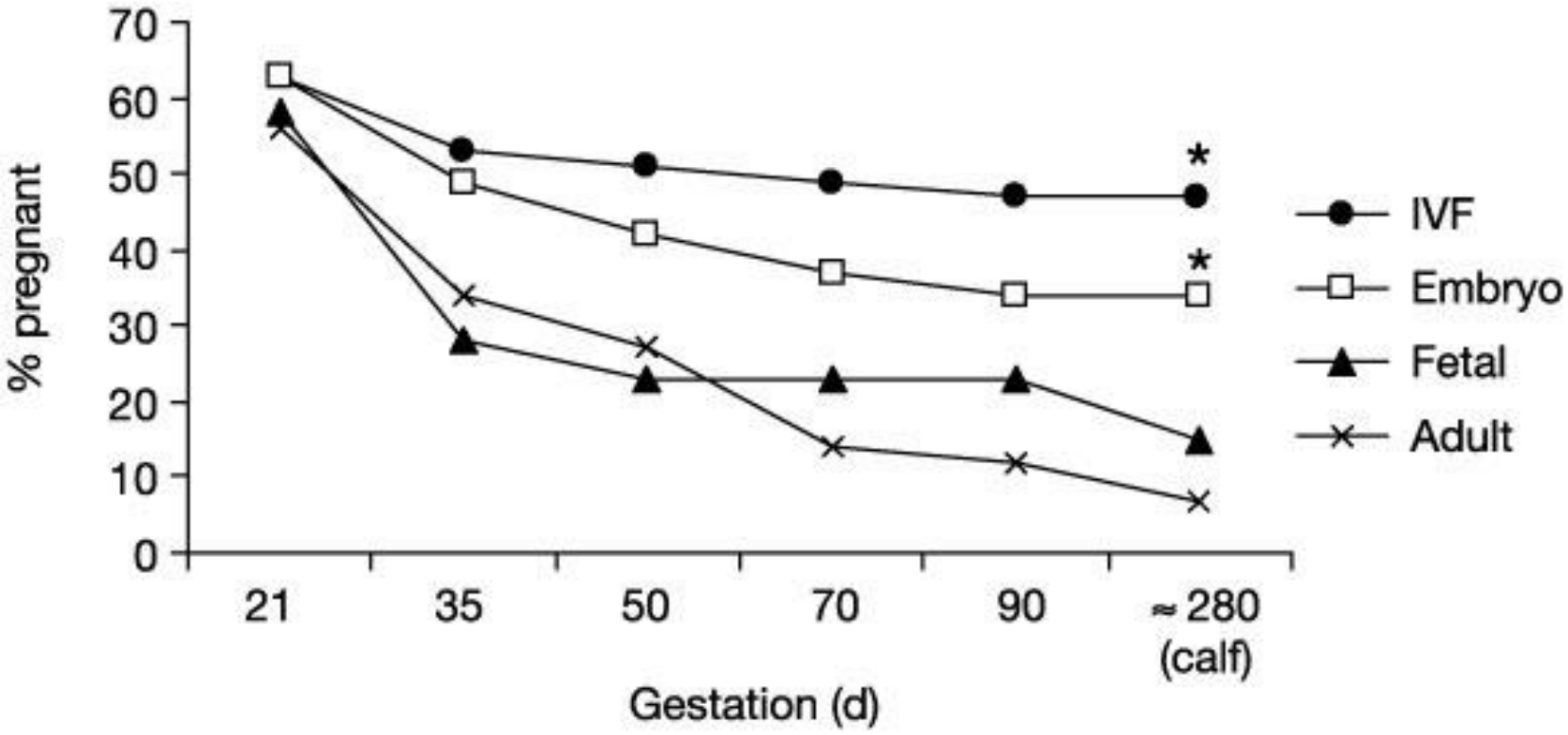
Efficienza della clonazione somatica nella specie bovina



Heyman, Y. *et al.* Frequency and occurrence of late-gestation losses from cattle cloned embryos. *Biol. Reprod.* **66**, 6-13 (2002)

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Efficienza della clonazione somatica nella specie suina

Type of pig (cell lines)	N embryos	MC %	N recipients	Pregnant %	To term %	N piglets	%of N & % of MC *
minipigs							
Basic lines	7661	41±5	27	41 a	26	22 (16 stillb.)	0,5 & 1,3
Transg. lines	3387	33±6	11	9 b	9	2 (stillb.)	0,2 & 0,7
Commercial							
Basic lines	6429	31±9	27	74 c	50*	21 (3 stillb.)	1,7 & 5,1
Transg. lines	1222	45±2	4	75 c	50	12 (5 stillb.)	3 & 6,9

* - counted for donor cell lines that were able to develop to term.
MC - compact morula D5.
a,b,c - values with different letters within columns differ significantly. Chi square-test, $P<0.05$.

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SPECIE DI MAMMIFERO OTTENUTE CON SCNT

Year of Publication	Donor Cell		Recipient Oocyte	
	Species	Cell Type	Species	References
Interspecies				
1996	sheep	differentiated embryonic cell line	sheep	(Campbell et al., 1996b)
1997	sheep	adult mammary epithelium	sheep	(Wilmut et al., 1997)
1998	cow	fetal fibroblasts (transgenic)	cow	(Cibelli et al., 1998)
1998	cow	adult cumulus and oviductal cells	cow	(Kato et al., 1998)
1998	mouse	adult cumulus cells	mouse	(Wakayama et al., 1998)
1999	goat	fetal fibroblasts (transgenic)	goat	(Baguisi et al., 1999)
2000	pig	fetal fibroblasts	pig	(Onishi et al., 2000)
2000	pig	cultured adult granulosa cells	pig	(Polejaeva et al., 2000)
2002	rabbit	adult transgenic cumulus cells	rabbit	(Chesné et al., 2002)
2002	cat	adult cumulus cells	cat	(Shin et al., 2002)
2003	mule	fetal fibroblasts	horse	(Woods et al., 2003)
2003	horse	adult skin fibroblasts	horse	(Galli et al., 2003)
2003	rat	fetal fibroblasts	rat	(Zhou et al., 2003)
2005	dog (Afghan hound)	adult skin fibroblasts	dog (golden retriever)	(Lee et al., 2005)
2006	ferret	adult cumulus cells	ferret	(Li et al., 2006)
2007	red deer	adult antlerogenic cells	deer	(Berg et al., 2007)
2007	buffalo	fetal fibroblasts and adult granulosa cells	buffalo	(Shi et al., 2007)
2010	camel	adult cumulus cells	camel	(Wani et al., 2010)
2018	cynomolgus monkey	fetal fibroblast	cynomolgus monkey	(Liu et al., 2018b)

Cell Stem Cell *23*, October 4, 2018 © 2018 Elsevier Inc.

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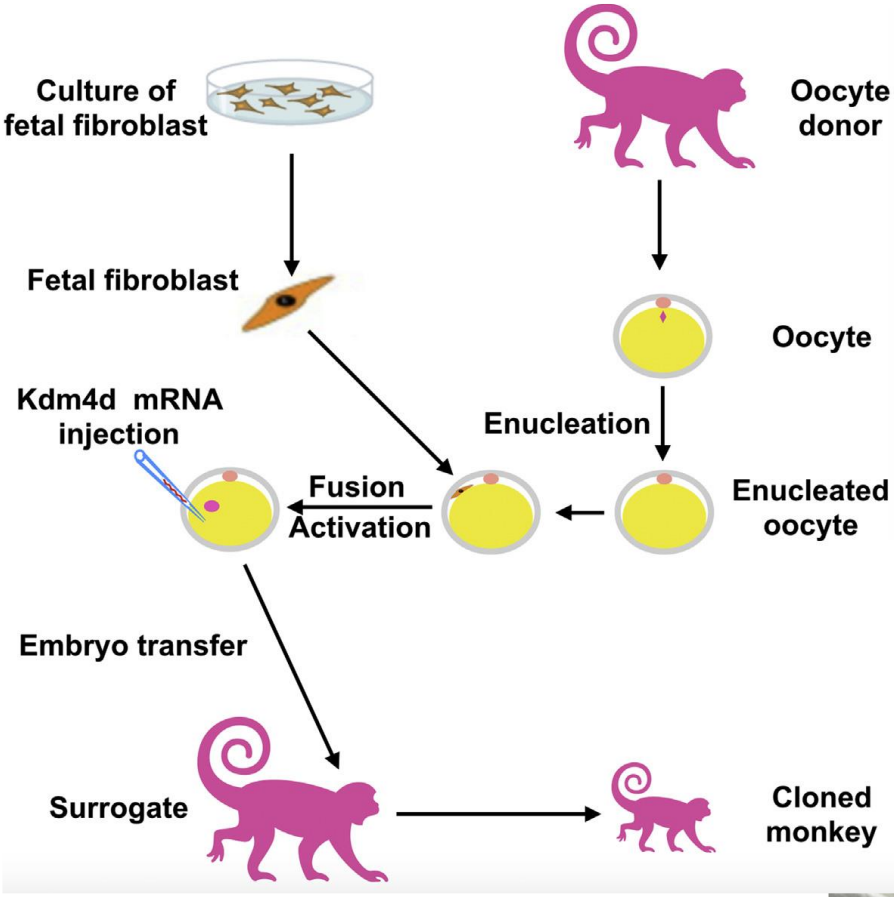
Primati clonati mediante SCNT



C

Monkey/Donor cell	D6S2741	D16S409	D18S72
Cloned monkey "ZZ"	250/277	135/148	200/217
Cloned monkey "HH"	250/277	135/148	200/217
Fibroblast donor cell (0523)	250/277	135/148	200/217

Cell *172*, 881–887, February 8, 2018

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Efficienza della clonazione somatica nei primati

tipo cellulare	n embrioni trasferiti	n madri surrogate	n gravid.	n nati vivi	n sopravv.	% nati / embrioni
fibroblasti	79	21	6	2	2	2,5
cumulo oof.	181	42	22	2	0	0
totale	260	63	28	4	2	0,77

Cell *172*, 881–887, February 8, 2018

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Prospettive applicative della clonazione somatica: quali animali si clonano e a che scopo?

In campo zootecnico (tecnica di riproduzione)

- Clonazione di animali con caratteristiche desiderate
- Clonazione di animali geneticamente modificati

Conservazione delle risorse genetiche

- Stoccaggio di cellule somatiche delle specie a rischio
- Clonazione di razze in estinzione

In campo biomedico

- Animali genetic. modificati per la produzione di farmaci
- Animali genetic. modificati per xenotrapianti
- Prevenzione di malattie degenerative dei mitocondri
- Modelli animali di malattie su base genetica

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Animale clonato da una specie in via di estinzione

Loi et al, 2001 Nat. Biotech

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SPECIE DI MAMMIFERO OTTENUTE CON SCNT INTERSPECIFICO

2000	gaur (*Bos gaur*)	cryopreserved adult skin cells	cow (*Bos taurus*)	(Lanza et al., 2000)
2001	mouflon (*Ovis orientalis musimon)*	adult granulosa cells	sheep (*Ovis aries)*	(Loi et al., 2001)
2001	zebu (*Bos indicus*)	morula stage blastomere	cow (*Bos taurus*)	(Meirelles et al., 2001)
2004	African wildcat (*Felis lybica)*	adult skin fibroblasts	cat (*Felis catus)*	(Gómez et al., 2004)
2004	banteng (*Bos javanicus)*	cryopreserved adult fibroblasts	cow (*Bos taurus*)	(Janssen et al., 2004)
2007	gray wolf (*Canis lupus)*	adult ear fibroblasts	dog (*Canis familiaris)*	(Kim et al., 2007)
2009	Pyrenean ibex (*Capra pyrenaica pyrenaica*)	cryopreserved skin fibroblasts	goat (*Capra aegagrus hircus)*	(Folch et al., 2009)
2013	coyote (*Canis latrans*)	neonatal/adult fibroblasts	dog (*Canis familiaris)*	(Hwang et al., 2013)
2017	Bactrian camel (*Camelus bactrianus)*	adult skin fibroblasts	dromedary camel (*Camelus dromedaries)*	(Wani et al., 2017)

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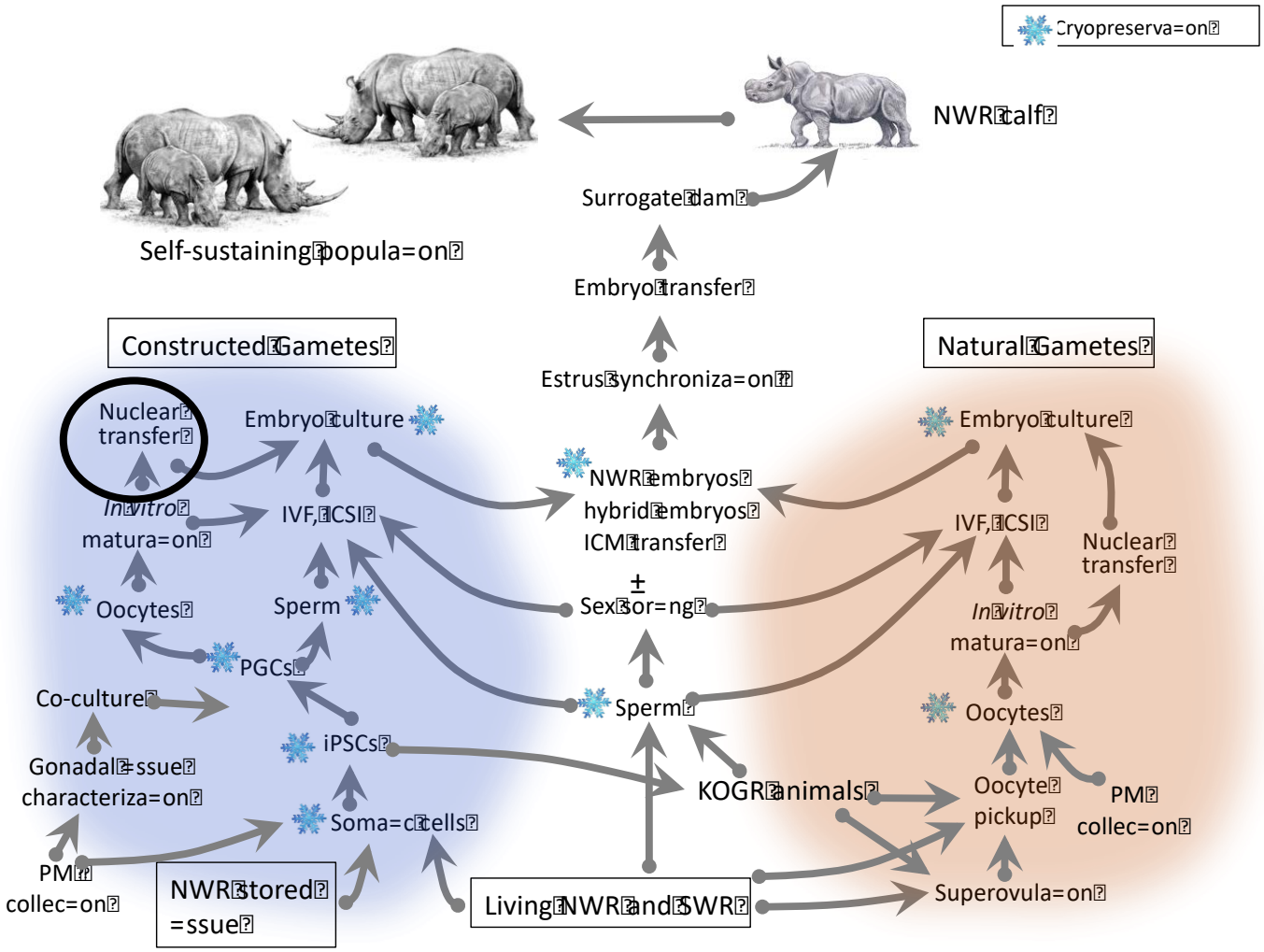
Rewinding the process of mammalian extinction



(Saragusty et al 2015, Zoo Biology)

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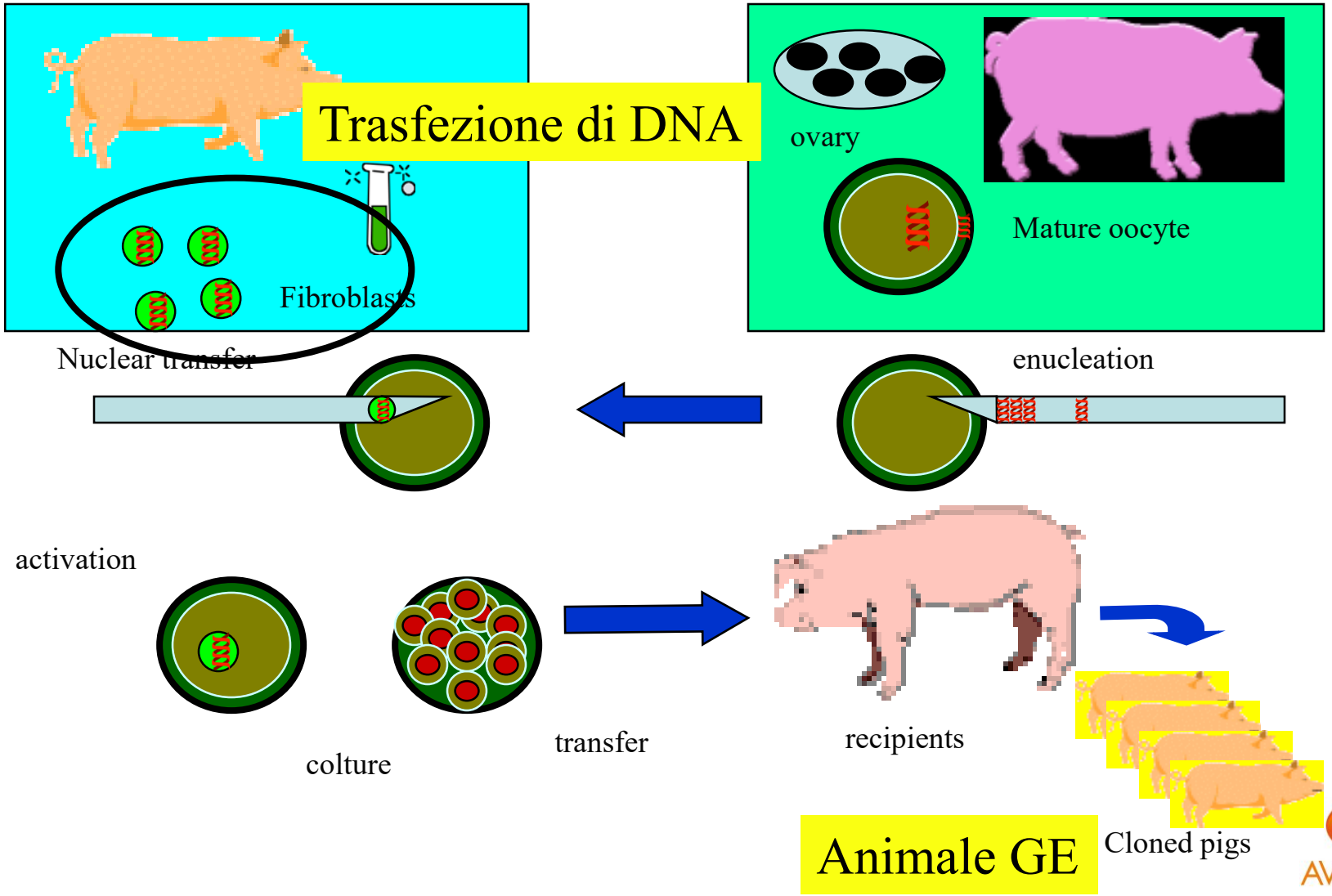
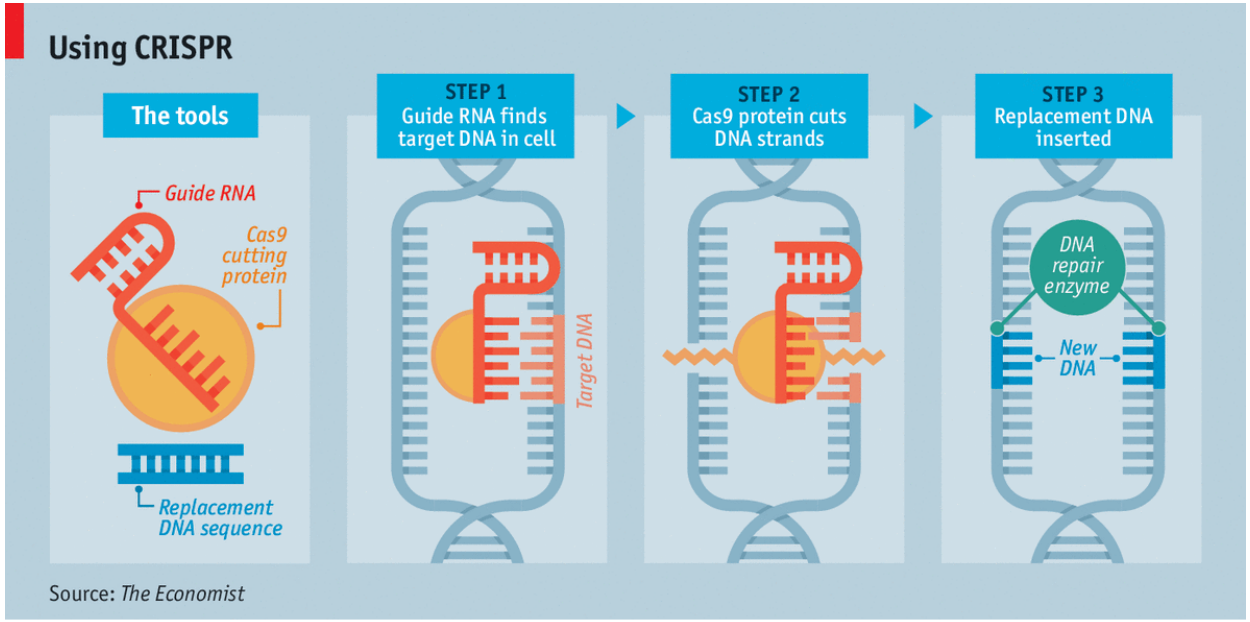
Tecnica di clonazione per generare animali con genoma editato



CRISPR/CAS9 system in S. pyogenes – I

clustered regularly interspaced short palindromic repeats



Economist.com

Ingegneria Genetica di precisione /editing del genoma

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Bovini a cui non crescono le corna

Gene edited hornless cow improve animal welfare but regulatory fate unclear

Nicholas Staropoli | May 11, 2016 | Genetic Literacy Project

PRINTER FRIENDLY

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Critics have called it the "hornless Frankencow," but scientists and animal welfare advocates have a different view of the latest innovation in genetic engineering.

Polled livestock—animals without horns in normally horned species—have been around for ages. While farmers have been able to crossbreed beef cattle, such as Aberdeen Angus, so they do not have dangerous horns, they've had less luck in the most productive dairy breeds, particularly Holsteins, the world's highest producing dairy cow.

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Maiali resisenti al virus della sindrome respiratoria e riproduttiva (PRRS)

PLOS | PATHOGENS

RESEARCH ARTICLE

Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function

Christine Burkard[1], Simon G. Lillico[1], Elizabeth Reid[2], Ben Jackson[2], Alan J. Mileham[3], Tahar Ait-Ali[1], C. Bruce A. Whitelaw[1], Alan L. Archibald[1]*

1 The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, United Kingdom, **2** The Pirbright Institute, Ash Road, Pirbright, Woking, United Kingdom, **3** Genus plc, DeForest, Wisconsin, United States of America

* alan.archibald@roslin.ed.ac.uk

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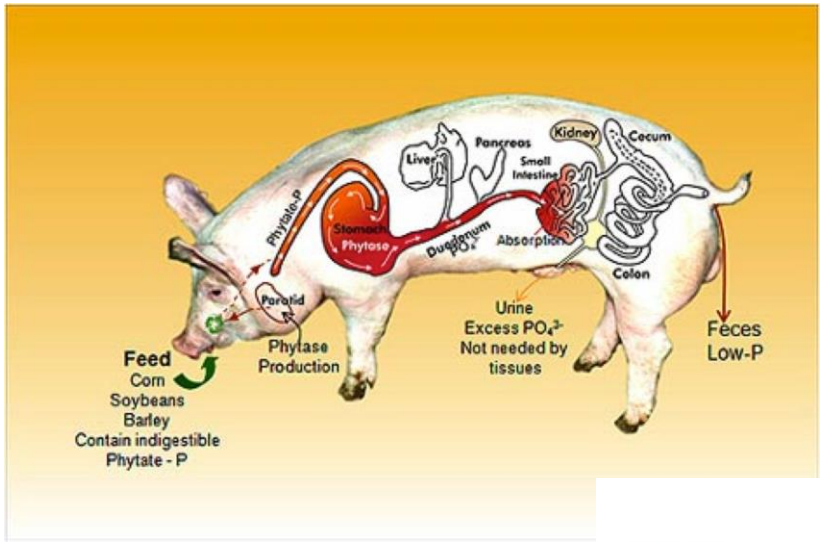

http://www.uoguelph.ca/enviropig/

nature biotechnology

BRIEF COMMUNICATIONS

Generation of cloned transgenic pigs rich in omega-3 fatty acids

Liangxue Lai[1,2,8], Jing X Kang[5,8], Rongfeng Li[1], Jingdong Wang[5], William T Witt[6], Hwan Yul Yong[1], Yanhong Hao[1], David M Wax[1], Clifton N Murphy[1], August Rieke[1], Melissa Samuel[1], Michael L Linville[3], Scott W Korte[4], Rhobert W Evans[7], Thomas E Starzl[6], Randall S Prather[1,2] & Yifan Dai[6]

p http://www.nature.com/naturebiotechnology

Meat products are generally low in omega-3 (*n*-3) fatty acids, which are beneficial to human health. We describe the

such as the *fat-1* gene found in the roundworm *C. elegans*[3]. Earlier work in transgenic mice carrying the *fat-1* gene has suggested the feasibility of creating *fat-1* transgenic livestock capable of producing *n*-3 fatty acids from the corresponding *n*-6 fatty acids[4]. Here we report the cloning of *fat-1* transgenic pigs that produce high levels of *n*-3 fatty acids in their tissues and organs.

An h*fat-1* expression vector, pCAGGS-h*fat-1*, which contains a humanized *fat-1* cDNA (with modification of codon usage) driven by the cytomegalovirus enhancer and chicken β-actin promoter, has been described previously[4]. A pgk-neo expression cassette as a selection marker was inserted into pCAGGS-h*fat-1* to generate pST103, which was transfected into early-passage male primary porcine fetal fibroblast cells, pCFF4-3[5], by electroporation; the transfected cells were selected with 250 μg/ml G418. The G418-resistant colonies were pooled. Gas chromatographic analysis showed that pCFF4-3/pST103 cells contained higher amounts of *n*-3 fatty acids and lower amounts of *n*-6 fatty acids compared with the nontrans-

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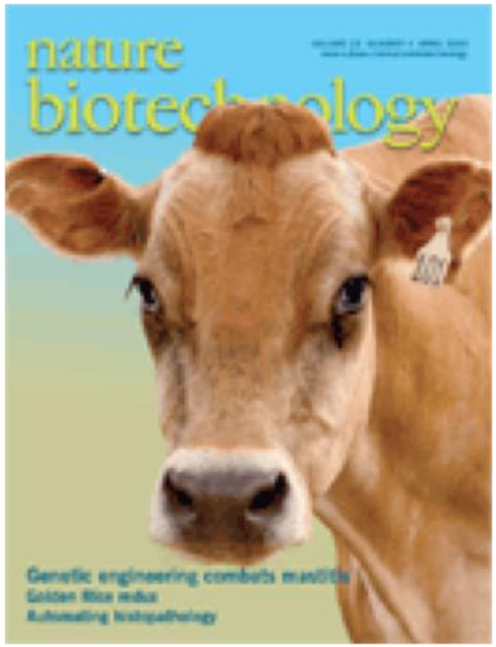
RESEARCH ARTICLE

Cloned transgenic cattle produce milk with higher levels of β-casein and κ-casein

Brigid Brophy[1], Grant Smolenski[1], Thomas Wheeler[1], David Wells[1], Phil L'Huillier[1,2], and Götz Laible[1*]

Published online 27 January 2003; doi:10.1038/nbt783

To enhance milk composition and milk processing efficiency by increasing the casein concentration in milk, we have introduced additional copies of the genes encoding bovine β- and κ-casein (*CSN2* and *CSN3*, respectively) into female bovine fibroblasts. Nuclear transfer with four independent donor cell lines resulted in the production of 11 transgenic calves. The analysis of hormonally induced milk showed substantial expres-



ARTICLES

nature biotechnology

Genetically enhanced cows resist intramammary *Staphylococcus aureus* infection

Robert J Wall[1], Anne M Powell[1], Max J Paape[2], David E Kerr[3], Douglas D Bannerman[2], Vernon G Pursel[1], Kevin D Wells[4], Neil Talbot[1] & Harold W Hawk[1]

Mastitis, the most consequential disease in dairy cattle, costs the US dairy industry billions of dollars annually. To test the feasibility of protecting animals through genetic engineering, transgenic cows secreting lysostaphin at concentrations ranging

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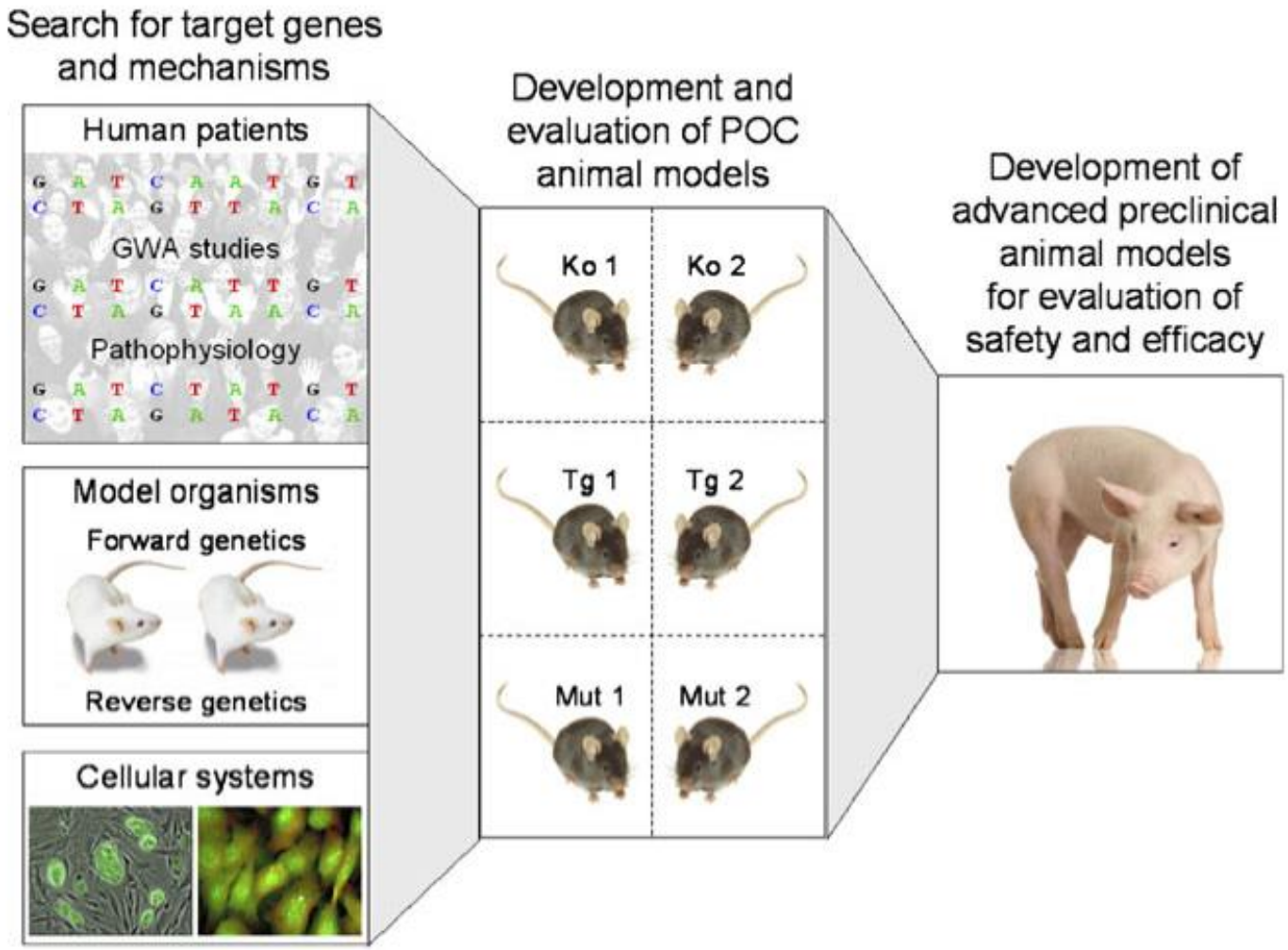
Strategia per lo sviluppo di modelli di grande animale per studi preclinici



Aigner et al. J Mol Med (2010) 88:653–664

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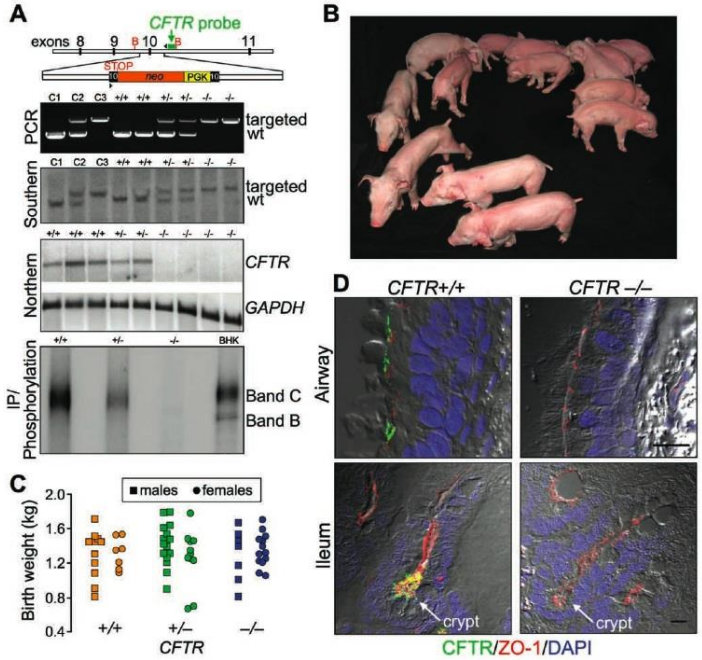
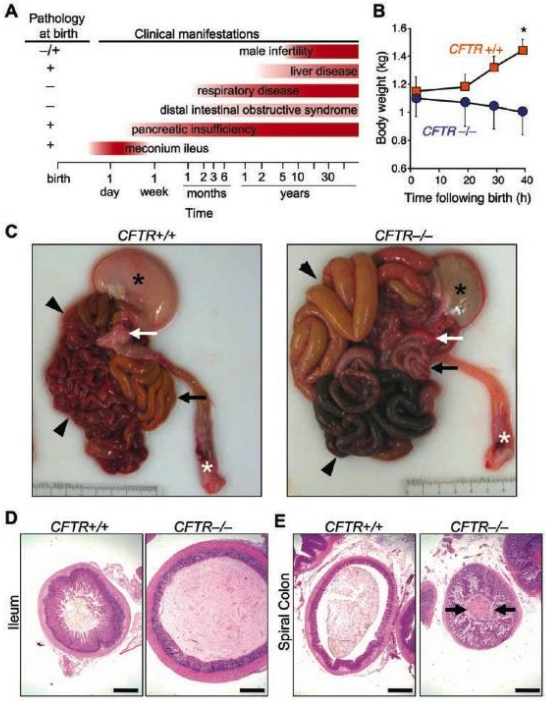
Disruption of the *CFTR* Gene Produces a Model of Cystic Fibrosis in Newborn Pigs

Christopher S. Rogers,[1*] David A. Stoltz,[1*] David K. Meyerholz,[2*] Lynda S. Ostedgaard,[1] Tatiana Rokhlina,[1] Peter J. Taft,[1] Mark P. Rogan,[1] Alejandro A. Pezzulo,[1] Philip H. Karp,[1,3] Omar A. Itani,[1] Amanda C. Kabel,[1] Christine L. Wohlford-Lenane,[4] Greg J. Davis,[1] Robert A. Hanfland,[5] Tony L. Smith,[5] Melissa Samuel,[6] David Wax,[6] Clifton N. Murphy,[6] August Rieke,[6] Kristin Whitworth,[6] Aliye Uc,[4] Timothy D. Starner,[4] Kim A. Brogden,[7] Joel Shilyansky,[5] Paul B. McCray Jr.,[4] Joseph Zabner,[1] Randall S. Prather,[6] Michael J. Welsh[1,3,8†]





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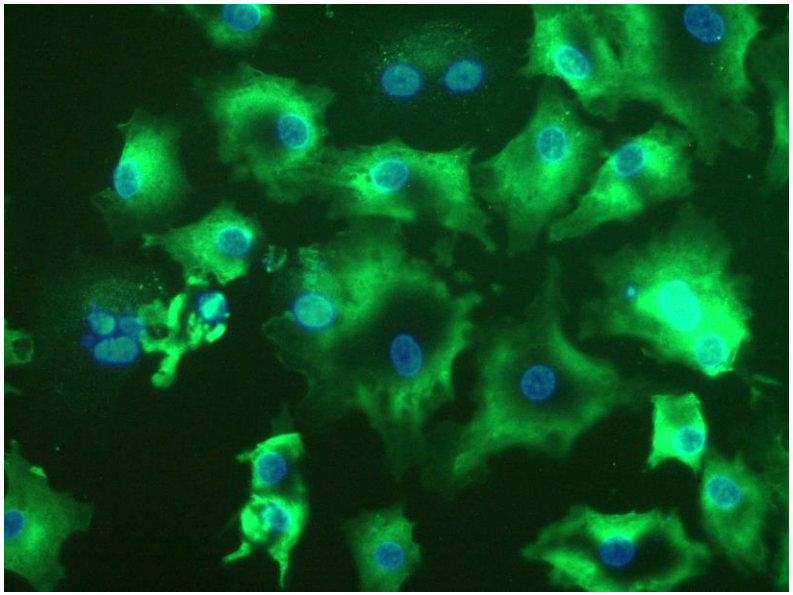
Modello suino di SLA

ICC PafYuc-pCAGGS-SodG93A
(Mutated SOD1 human)



Chieppa et al. 2013
Crociara et al, 2019

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DEL PIEMONTE, LIGURIA E VALLE D'AOSTA - L. ALTARA

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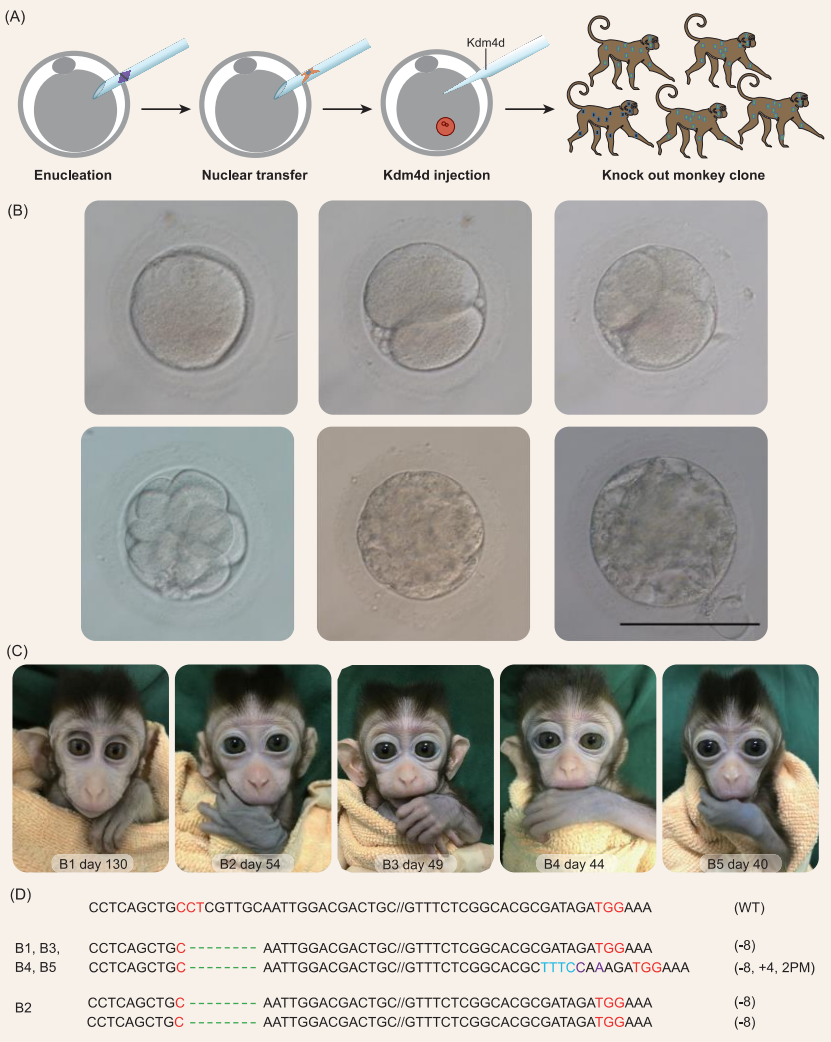
PRIMATI CON GENOMA EDITATO (macaque monkey)



National Science Review
6: 101–108, 2019
doi: 10.1093/nsr/nwz003
Advance access publication 24 January 2019

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TECHNICAL REPORTS

nature genetics

Sequential targeting of the genes encoding immunoglobulin-μ and prion protein in cattle

Yoshimi Kuroiwa[1,2], Poothappillai Kasinathan[3], Hiroaki Matsushita[3], Janaki Sathiyaselan[3], Eddie J Sullivan[3], Makoto Kakitani[2], Kazuma Tomizuka[2], Isao Ishida[2] & James M Robl[3]

http://www.nature.com/naturegenetics

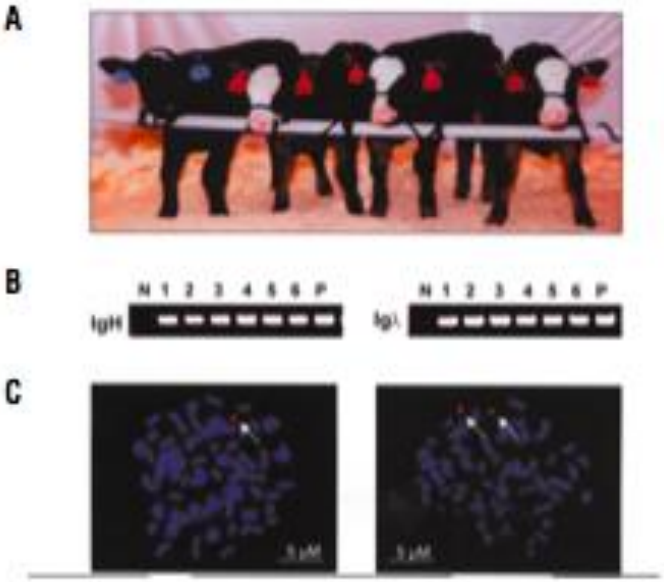
Gene targeting is accomplished using embryonic stem cells in the mouse but has been successful, only using primary somatic

of homologous recombination[5,8] and correctly targeted cells can be easily selected by having the targeted gene promoter drive expression

Cloned transchromosomic calves producing human immunoglobulin

Yoshimi Kuroiwa[1], Poothappillai Kasinathan[2], Yoon J. Choi[3], Rizwan Naeem[4], Kazuma Tomizuka[1], Eddie J. Sullivan[2], Jason G. Knott[2], Anae Duteau[3], Richard A. Goldsby[3], Barbara A. Osborne[5], Isao Ishida[1]*, and James M. Robl[2]*

Published online: 12 August 2002, doi:10.1038/nbt727



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Primo farmaco approvato nella UE prodotto da animali GE

NEWS

Nature Biotechnology - **24**, 877 (2006)
Published online: 3 August 2006; | doi:10.1038/nbt0806-877

Belated approval of first recombinant protein from animal

Charlie Schmidt

Portland, Maine

In June, the London-based European Medicines Agency (EMEA) announced approval of the first drug produced in an animal bioreactor: GTC Biotherapeutics' ATryn, four months after its initial rejection. The drug—a recombinant form of human antithrombin produced in goats—prevents blood clots in patients who lack the natural anticoagulant protein. Industry insiders believe that EMEA altered its position, after a more detailed review of the data, because it wanted to convey its support to industry for this bioprocessing method.

The first recombinant protein produced in goat bioreactor Atryn received a belated approval from the European Union in June after an initial rejection.

EMEA's positive opinion, announced on June 2, 2006, overturns the agency's rejection of ATryn last February. Atryn is indicated for

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Xenotrapianto

NEW LIFE FOR

Gene-editing technologies h
languishing field of xe

BY SARA RE

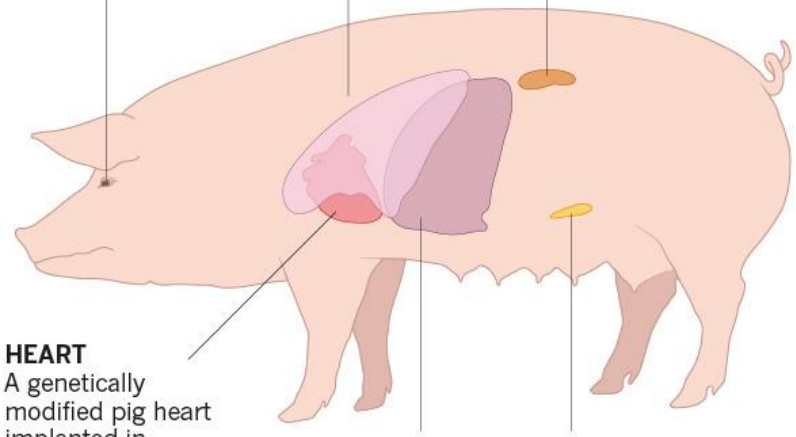
CHOICE CUTS

Researchers are looking to source an increasing variety of living tissues, including solid organs, from pigs. Many are attempting to genetically engineer the animals to reduce the risk of rejection and infection in humans.

CORNEA
Pig corneas were approved for marketing in China in April.

LUNG
A factory farm is being designed to produce 1,000 pig lungs per year.

KIDNEY
A kidney with six genetic modifications supported a baboon's life for 4 months.



HEART
A genetically modified pig heart implanted in a baboon's abdomen survived for 2.5 years.

LIVER
Livers could be engineered to produce their own antibodies against primate immune cells.

PANCREAS
Phase III clinical trials of insulin-producing islet cells are under way.

154 | NATURE | VOL 527 | 12 NOVEMBER 2015

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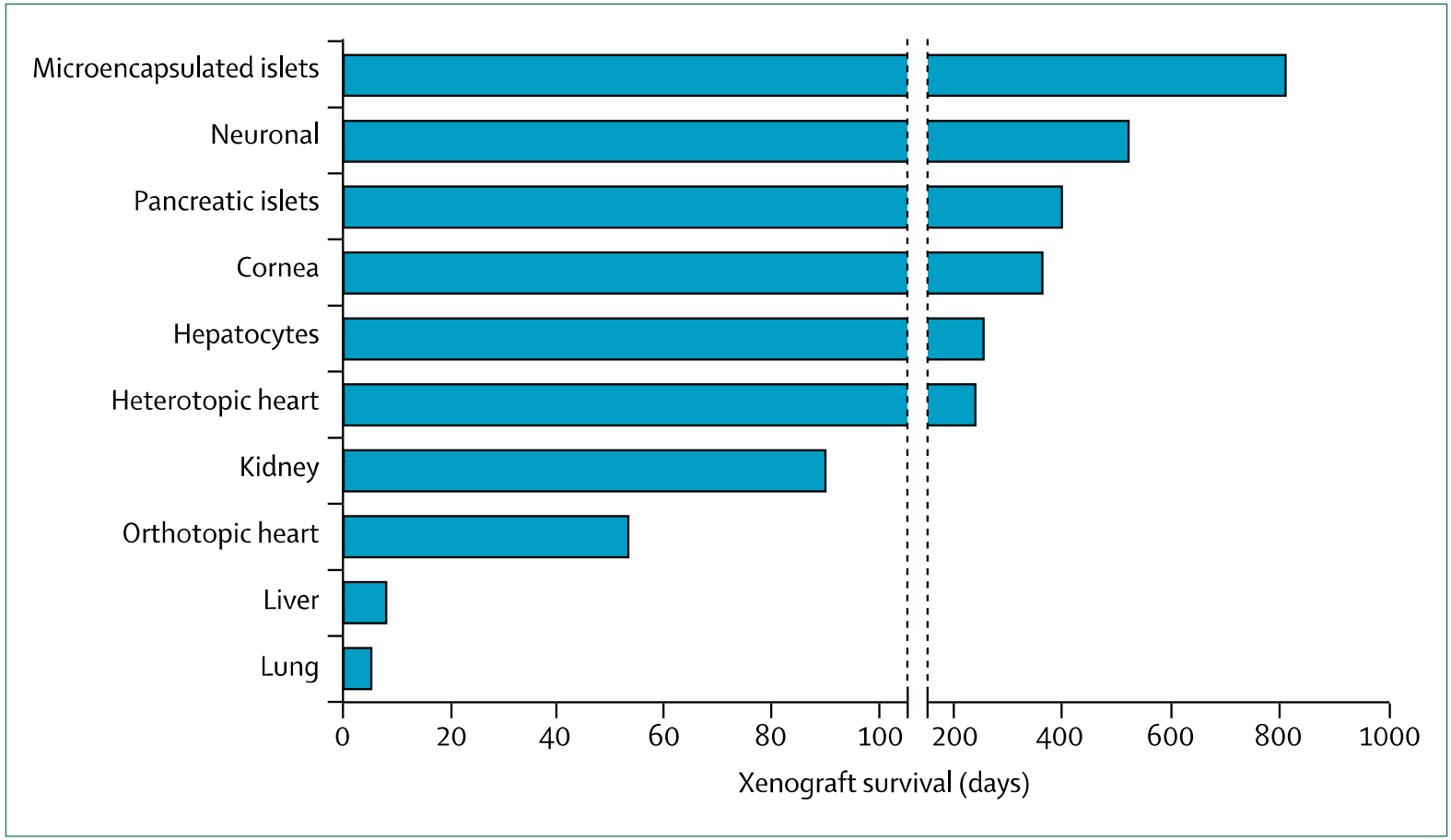
Massimi tempi di sopravvivenza di organi o cellule trapiantati in primate non umani



Ekser et al. 2012, The Lancet

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CONCLUSIONI

- La clonazione è una tecnica di riproduzione
- Ha bassa efficienza ma sufficiente per specifiche applicazioni
- Potrebbe avere importanti ricadute in campo zootecnico
- Attualmente è molto importante nella ricerca biomedica
- Gli animali clonati sono uguali a quelli non clonati

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GRAZIE PER L'ATTENZIONE

www.fondazioneavantea.org
CF:3050960199

Articolo 2 Statuto
(scopi e attività)

a) prevenzione, diagnosi e cura di tutte le patologie dell'essere umano
b) produzione di nuovi farmaci e vaccini per uso umano e veterinario
c) metodi e sistemi per aumentare la sicurezza nella categoria agroalimentare e nell'ambiente a tutela della salute pubblica