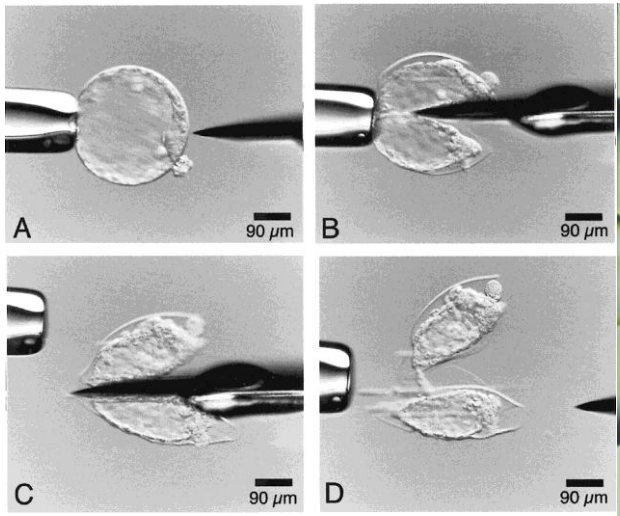
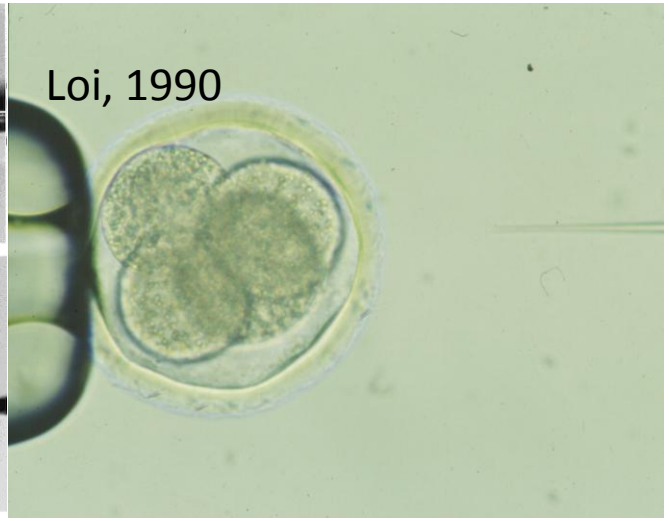
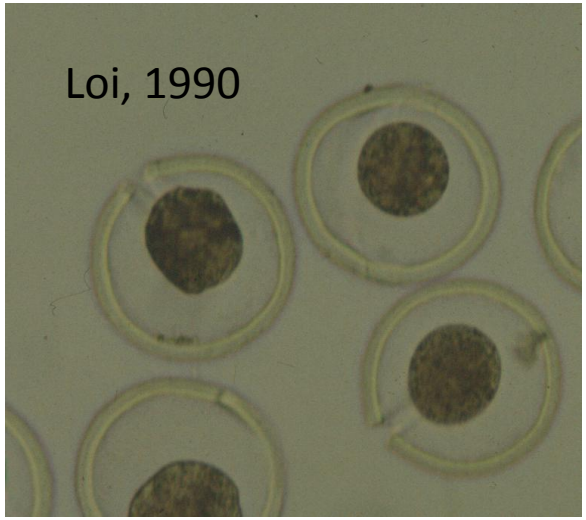
Splitting e separazione di blastomeri efficienza limitata, massimo 3, eccezionalmente 4 cloni

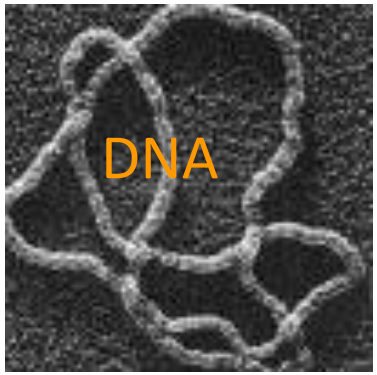


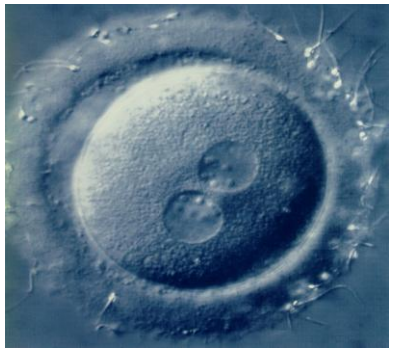


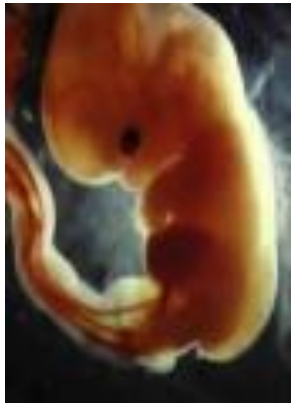


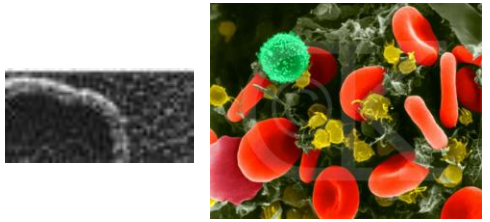
..altre opzioni?

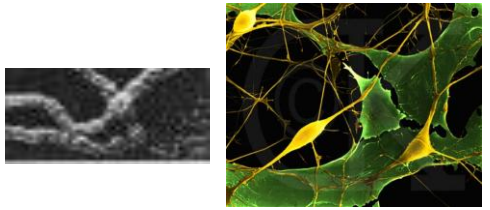
1900 – la differenziazione cellulare dovuta a perdita elettiva di “geni”

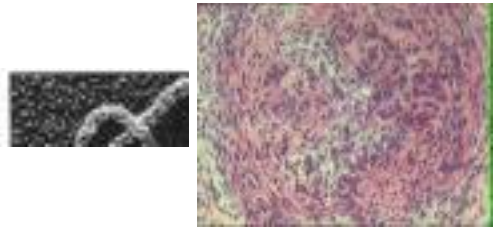


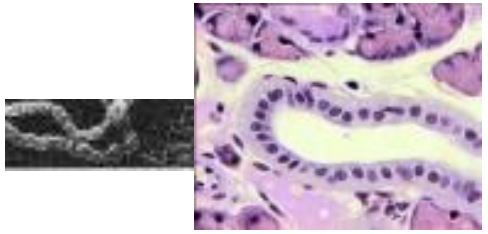






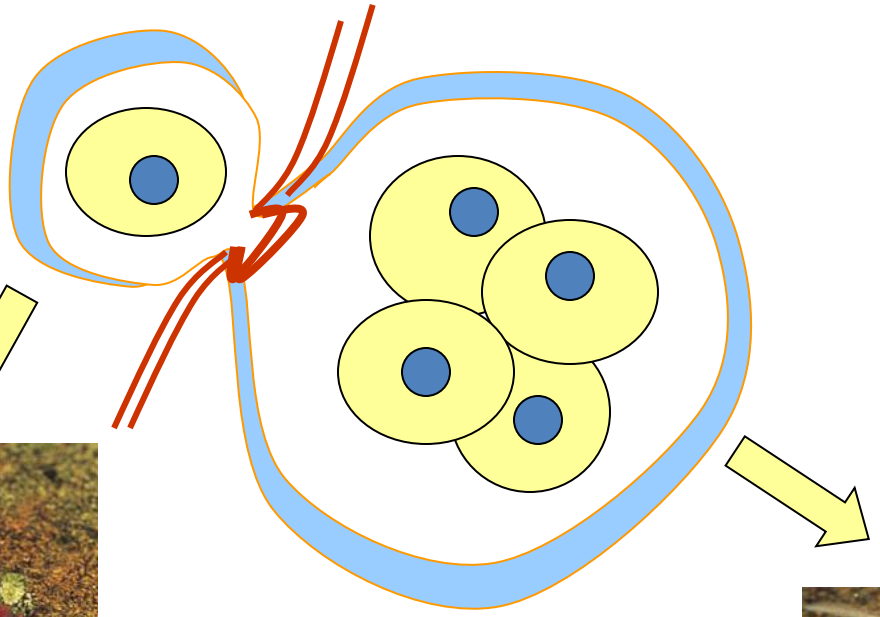






Perdita di DNA durante il processo differenziativo

Hans Spemann's experiment (1928)



Blastomeri prima della Attivazione genoma embrionario Sono totipotenti

L’esperimento “Fantastico” di Spemann

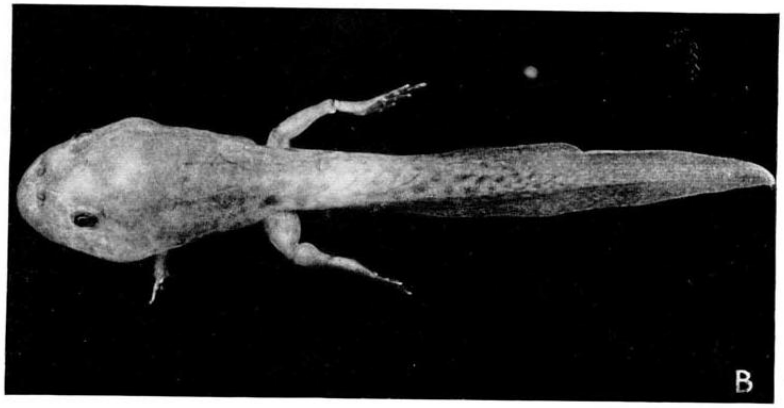
“Fecondare” un oocita con una cellula somatica

L'esperimento "fantastico" realizzato nel 1953 da Briggs & King

Transplantation of Living Nuclei of Late Gastrulae
into Enucleated Eggs of Rana pipiens
by THOMAS j . KING and ROBERT BRIGGS
1953

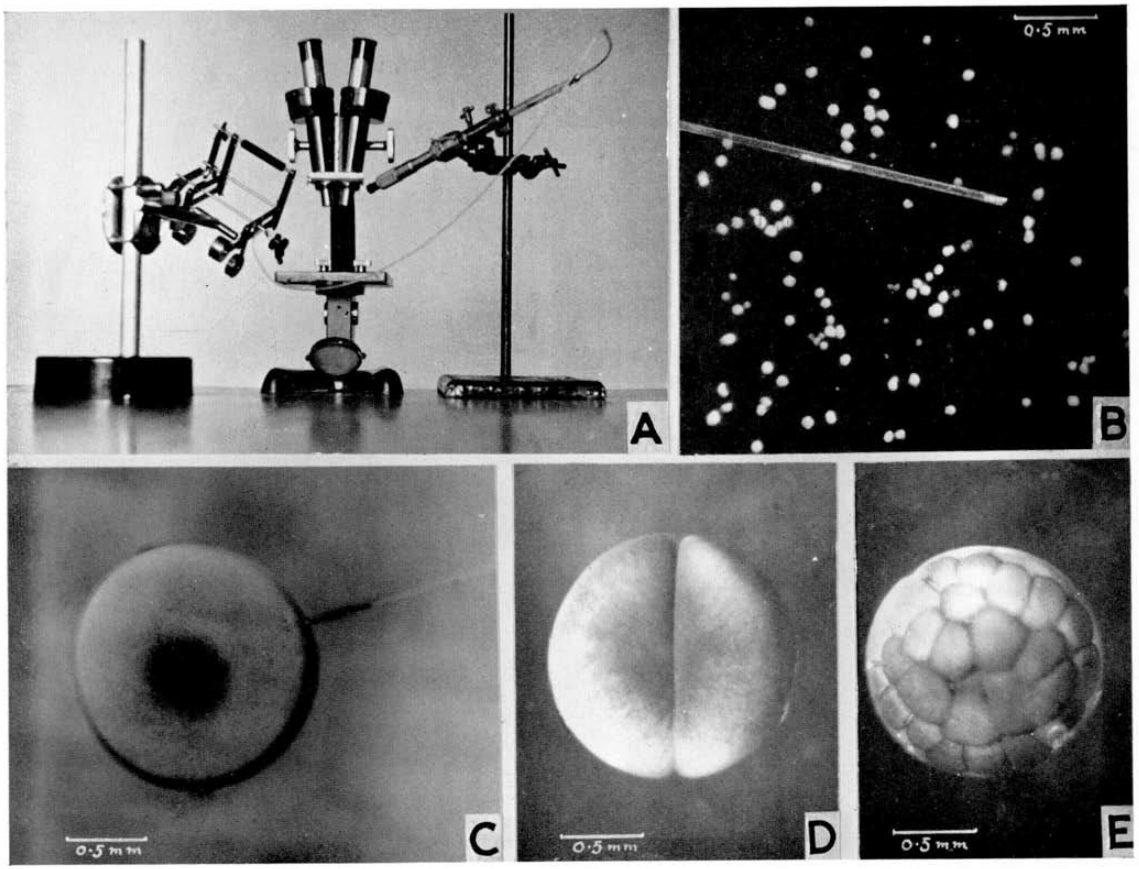
Robert Briggs (1911-1983)

Thomas King 1921-2000

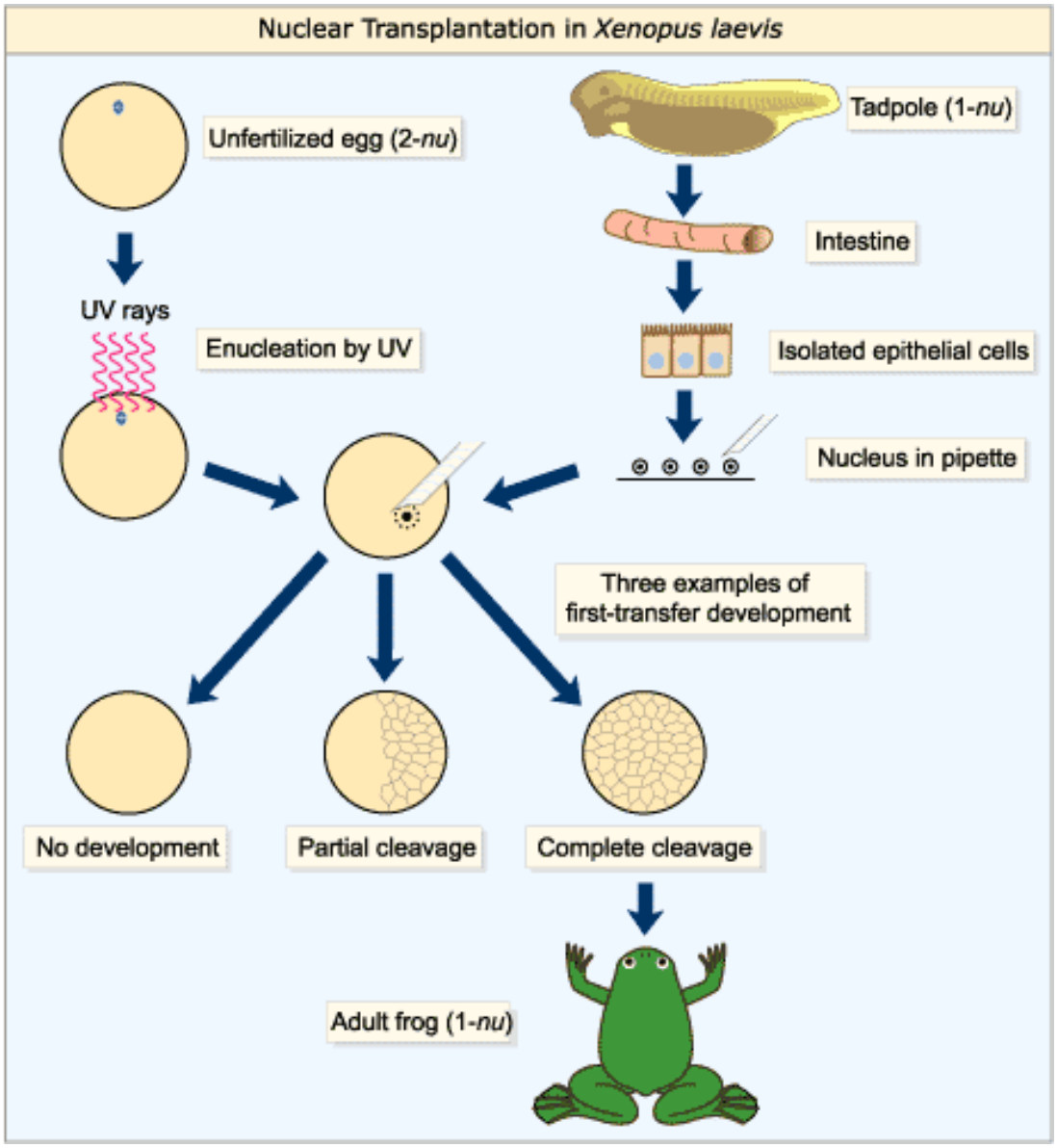


A Description of the Technique for Nuclear Transplantation in Xenopus laevis

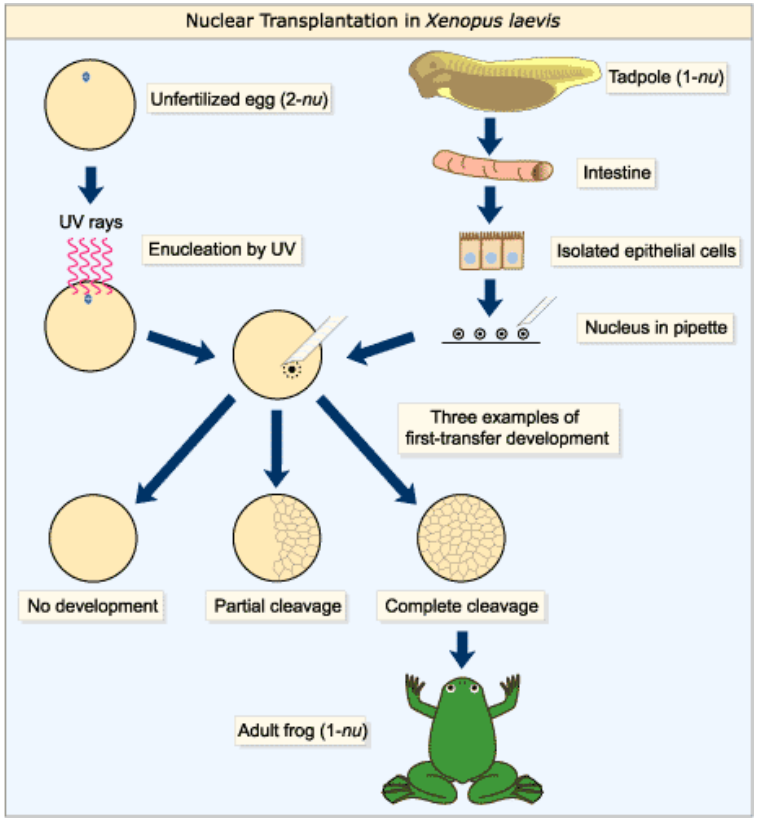
by T. R. ELSDALE, J. B. GURDON, and M. FISCHBERG
From the Embryology Laboratory, Department of Zoology and Comparative Anatomy, Oxford



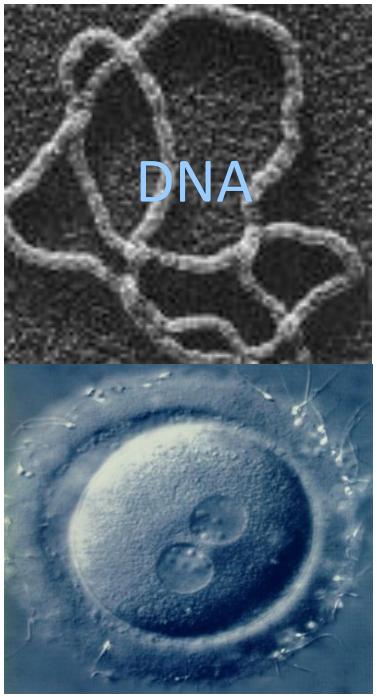
John Gurdon's experiment (1958)

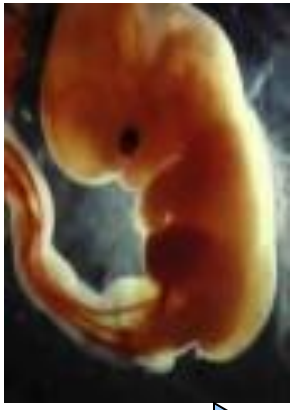


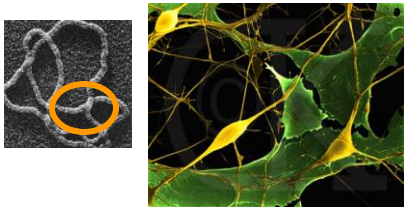
L'esperimento di John Gurdon
Ha stabilito la "totipotenza" del genoma in tutte le cellule

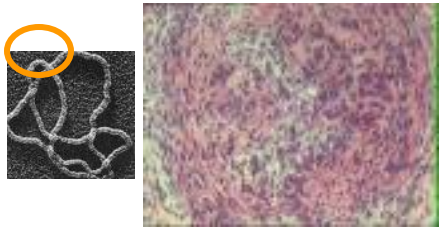


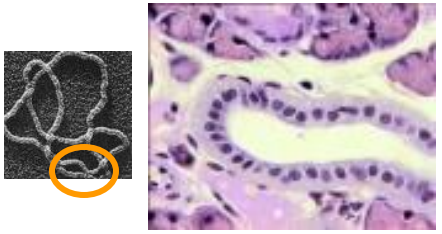
Moderna teoria della differenziazione cellulare







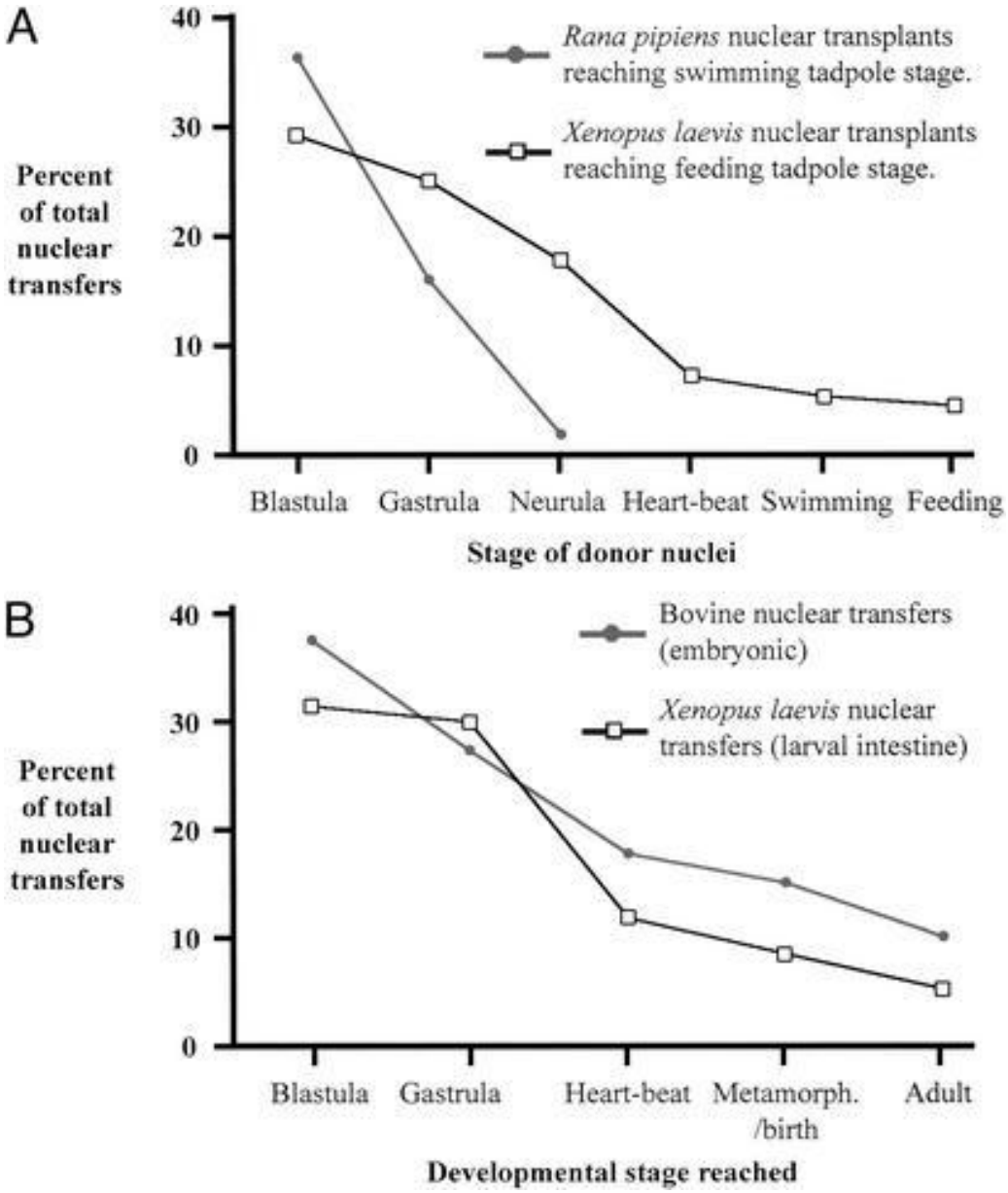




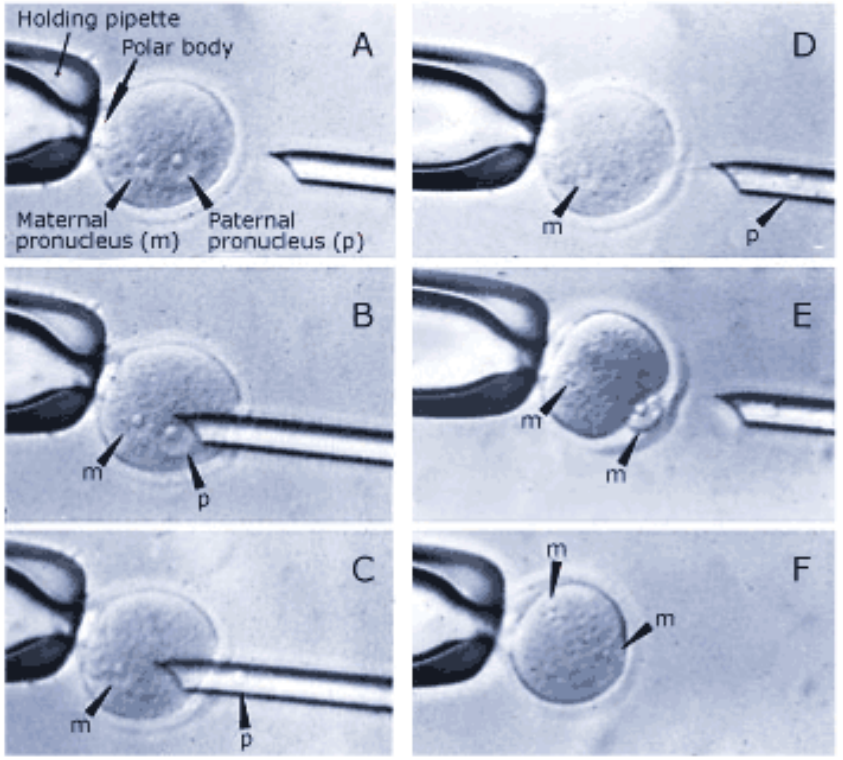
Il DNA non viene perso durante il processo Differenziativo, ma geni vengono espressi In modo diverso a seconda del tipo cellulare

Più avanzato il processo differenziativo, minore la riprogrammazione nucleare

Dati di Briggs and King, & Gurdon



McGrath and Solter applicano per la prima volta La tecnica del trapianto nucleare al topo (1983)

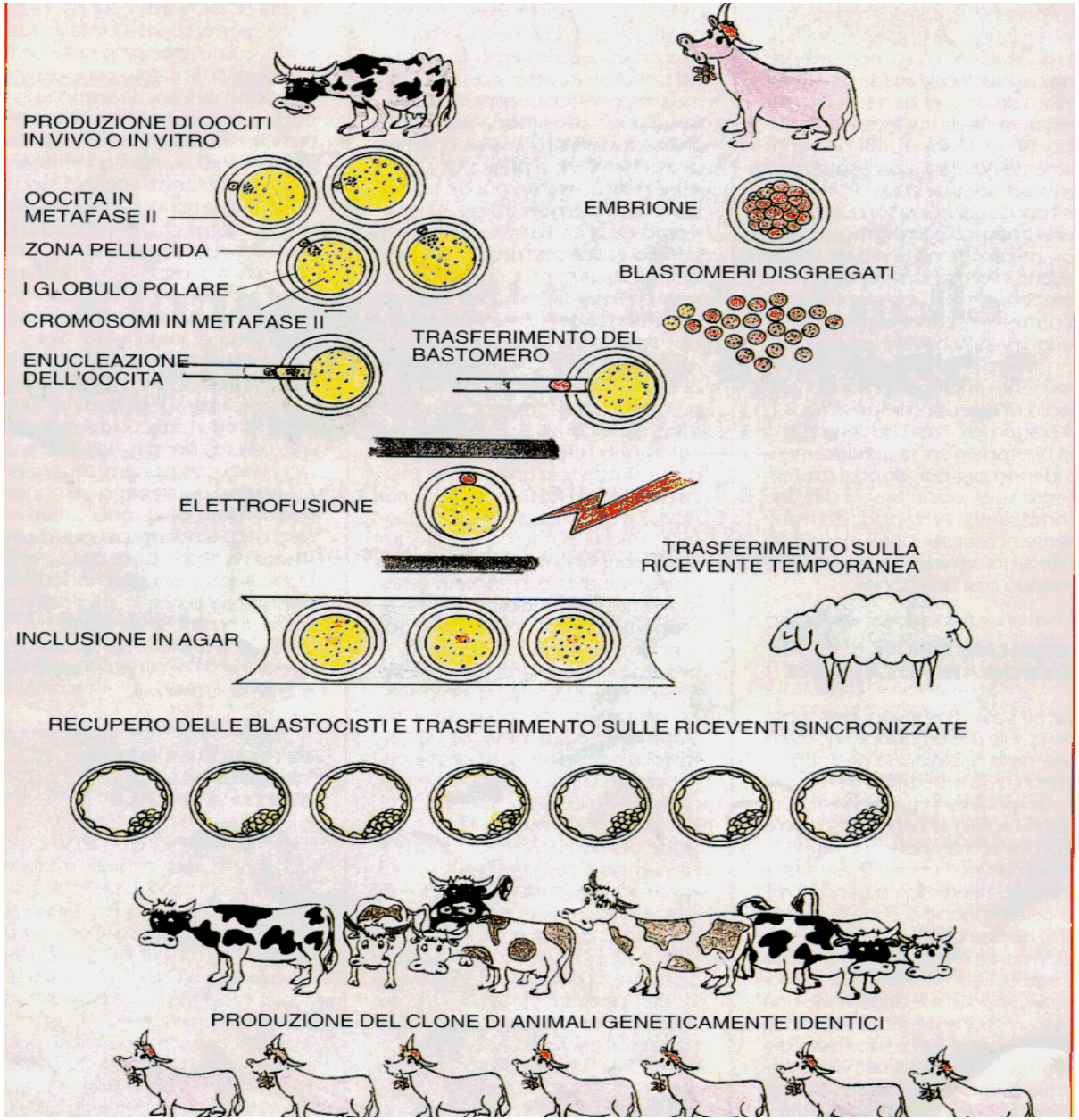


Clonazione attraverso trapianto nucleare Di specie di interesse zootecnico (pecora)

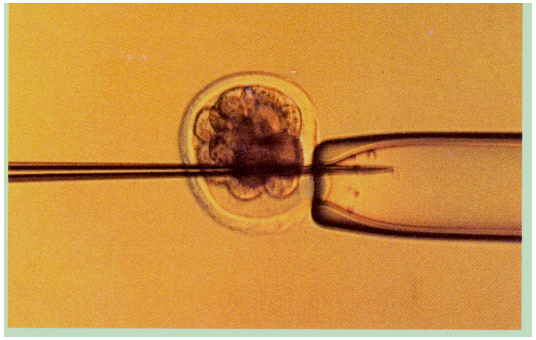
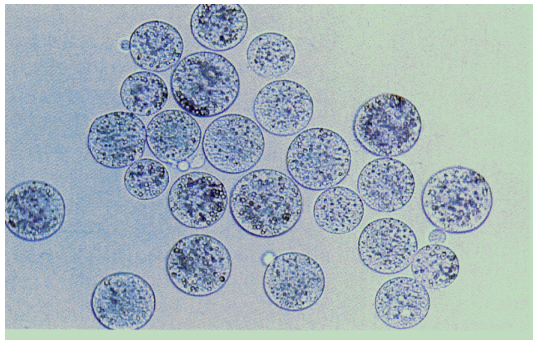
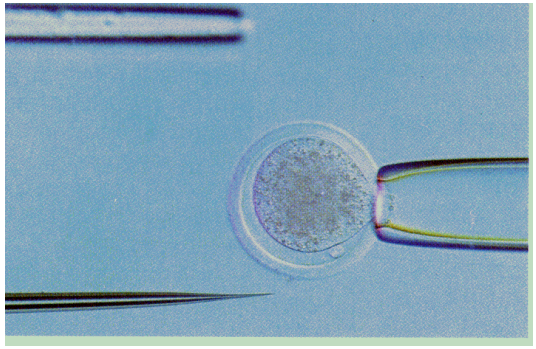
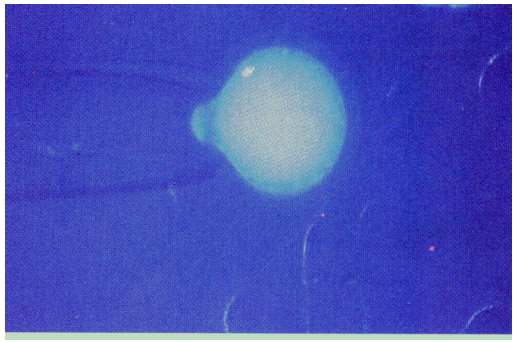
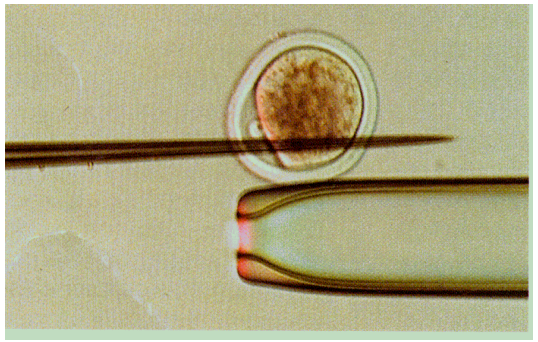
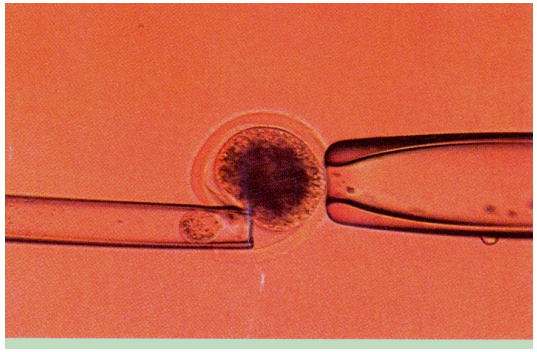
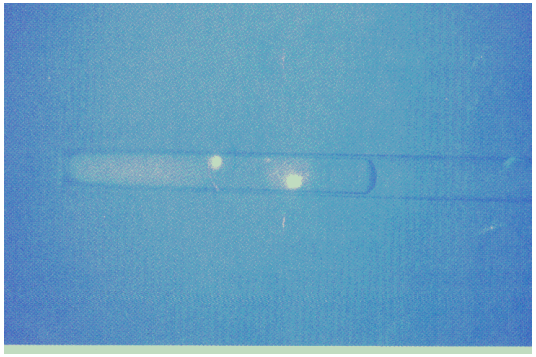
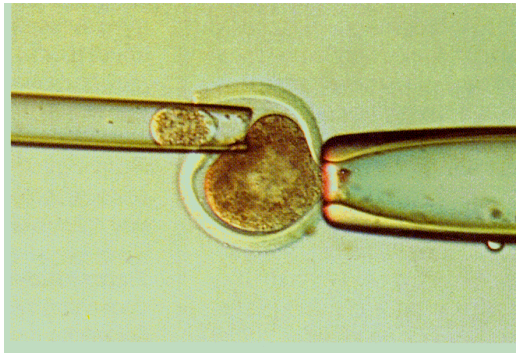
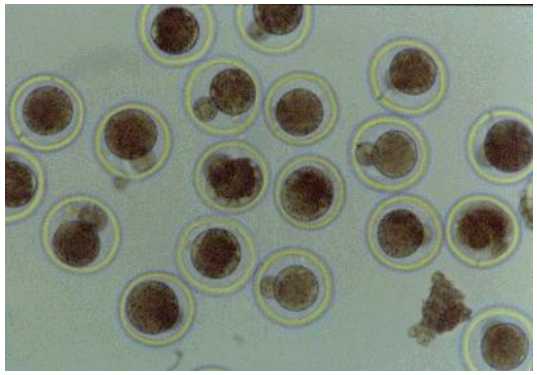
Steen Willadsen, 1943-

- **In seguito, numerose compagnie private iniziarono a "vendere" la clonazione come tecnica riproduttiva (Genus, Granada Genetics, Alta Genetics, BEMA, Animal Breeding Service, ArtTech, etc.)**

Schema di clonazione di embrioni attraverso il trapianto nucleare (Loi, 1991)



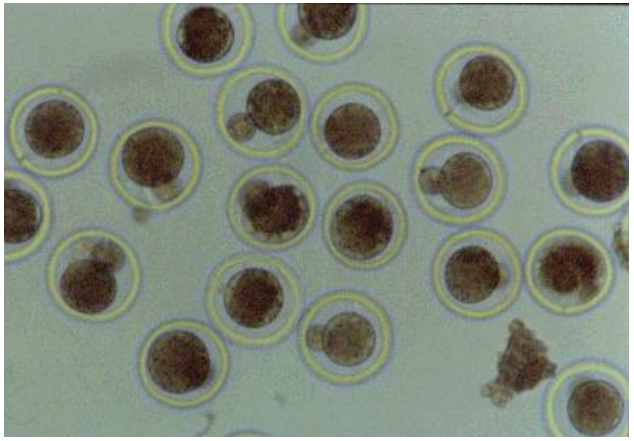
Fasi di micromanipolazione per il trapianto nucleare (Loi, 1992)

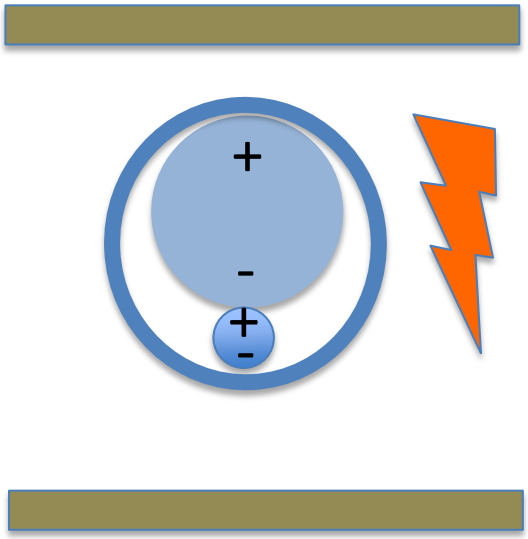
Fusione membrane indotta da:

Virus Sendai inattivo

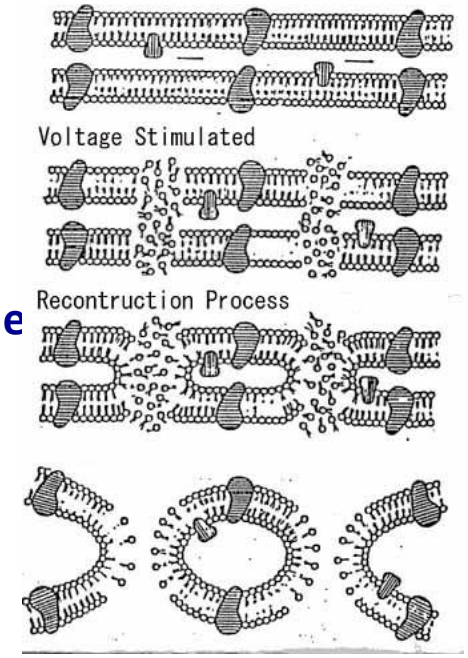
Glicole etilenico

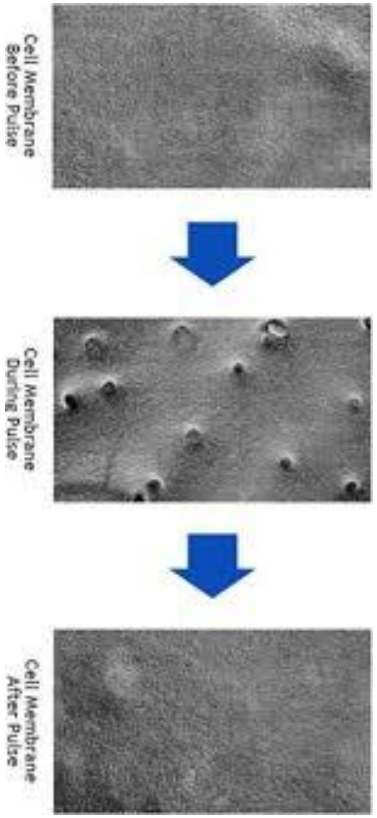


Pulse di corrente elettrica diretta (1200 kV/cm – 80-120 microsecondi) – medium non elettrolitico!!



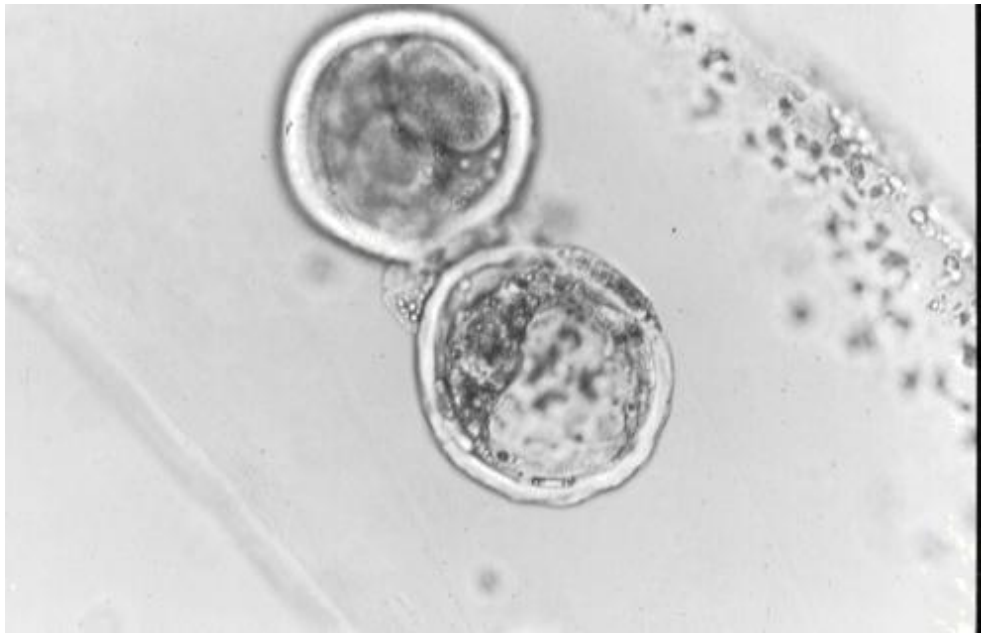
Dipolo rapido apre pori transitori Nelle due membrane contigue 10 minuti c.a.





Tuttavia, bassa efficianza della clonazione con

Cellule embrionali in quel periodo (Loi, 1992)



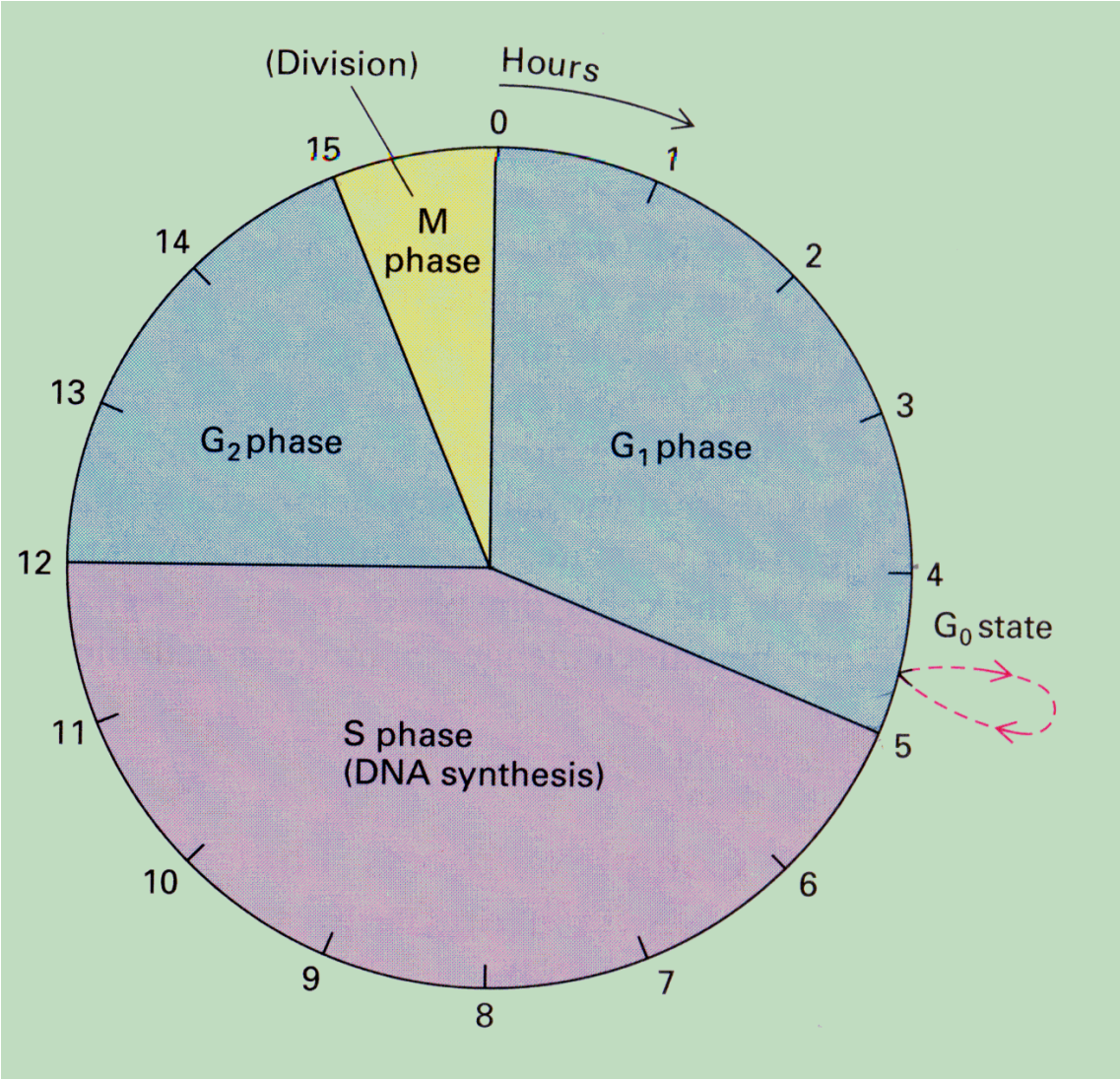
100 embrioni manipolati per produrre un agnello clonato !!!!

Fattori che influenzano l'efficienza della clonazione (con cellule embrionali)

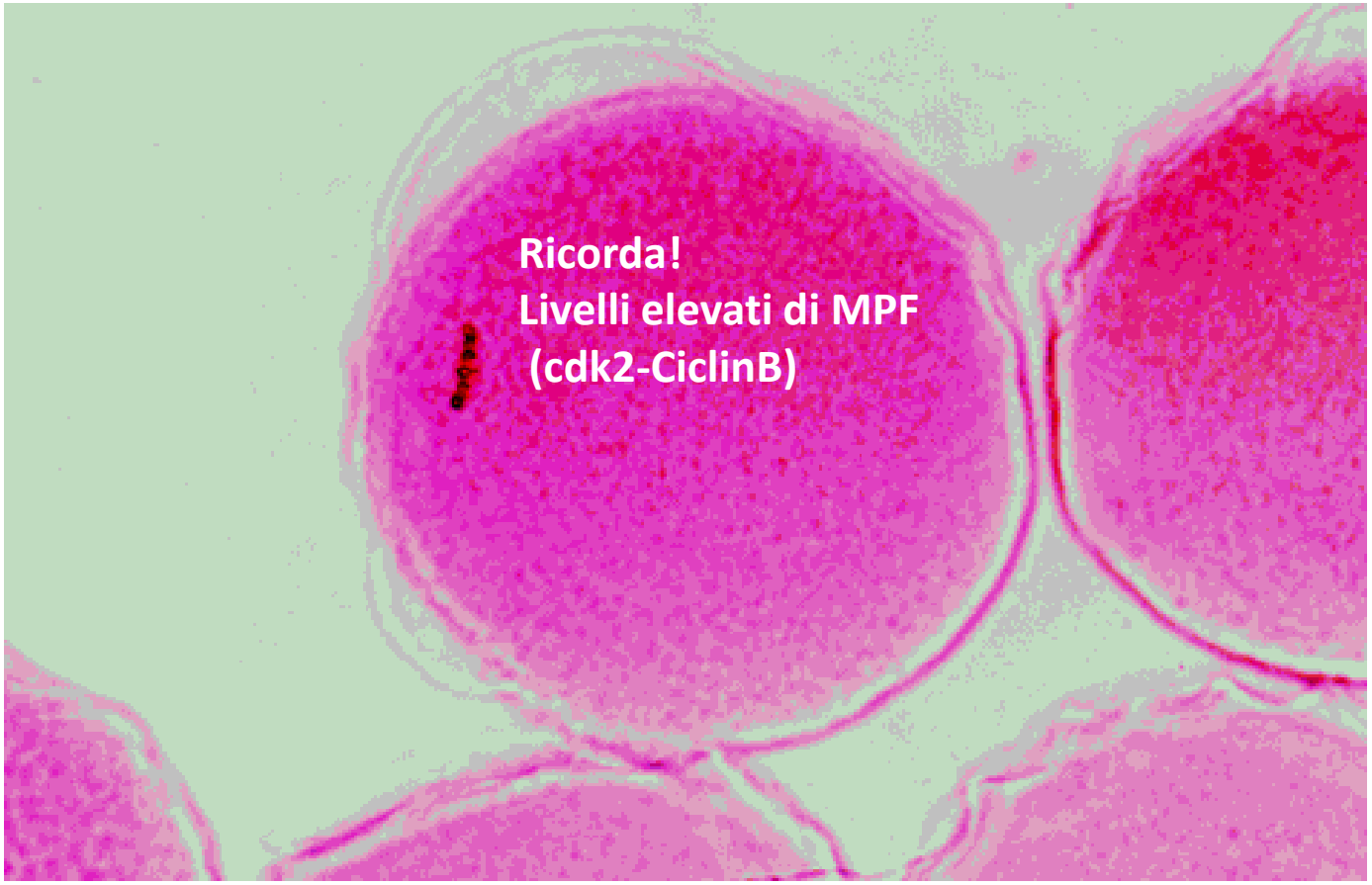
1) Attivazione dell'oocita

2) Compatibilità tra ciclo cellulare dell'oocita e cellula embrionale

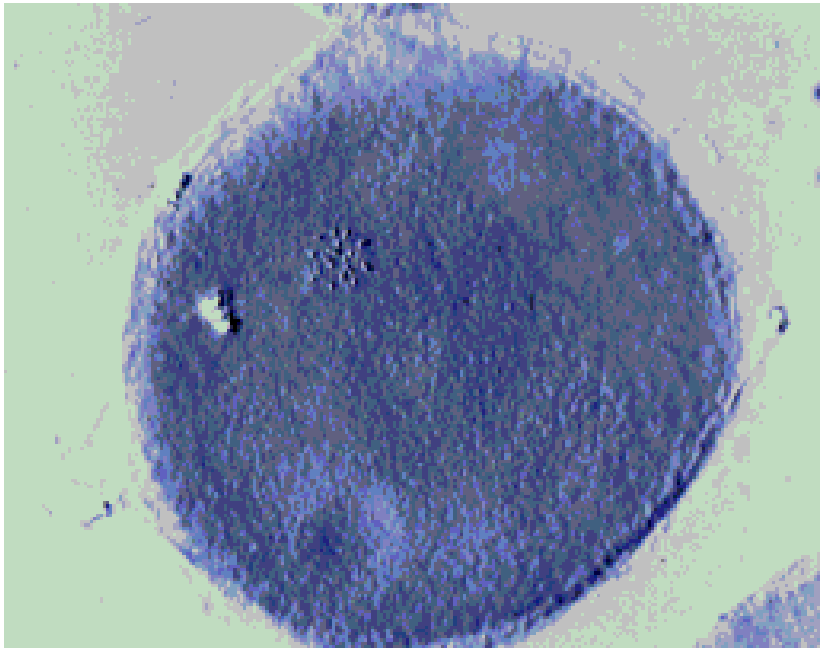
Cell cycle in eukaryotes



Gli oociti sui quali viene traferitop il nucleo sono sempre(!) in meiosi (II metafase)

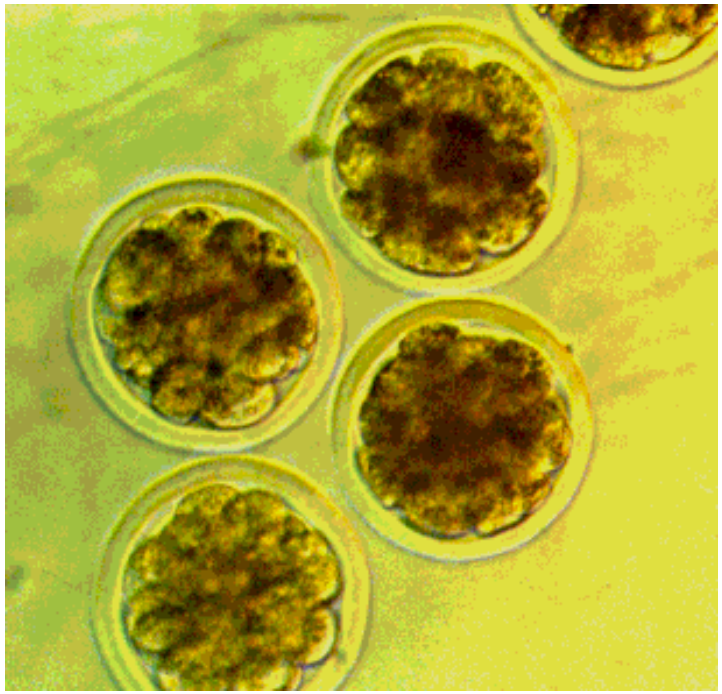


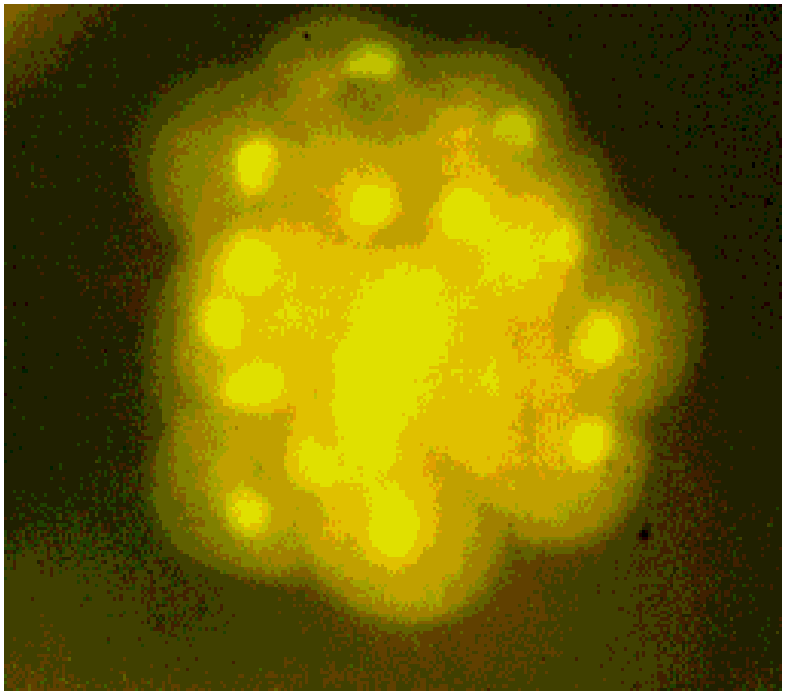
Quando trasferisco un nucleo in un oocita in metafase II il DNA si condensa rapidamente (30 minuti)



Fase M (Mitosi/Meiosi) dominante su tutte le altre fasi del ciclo cellulare

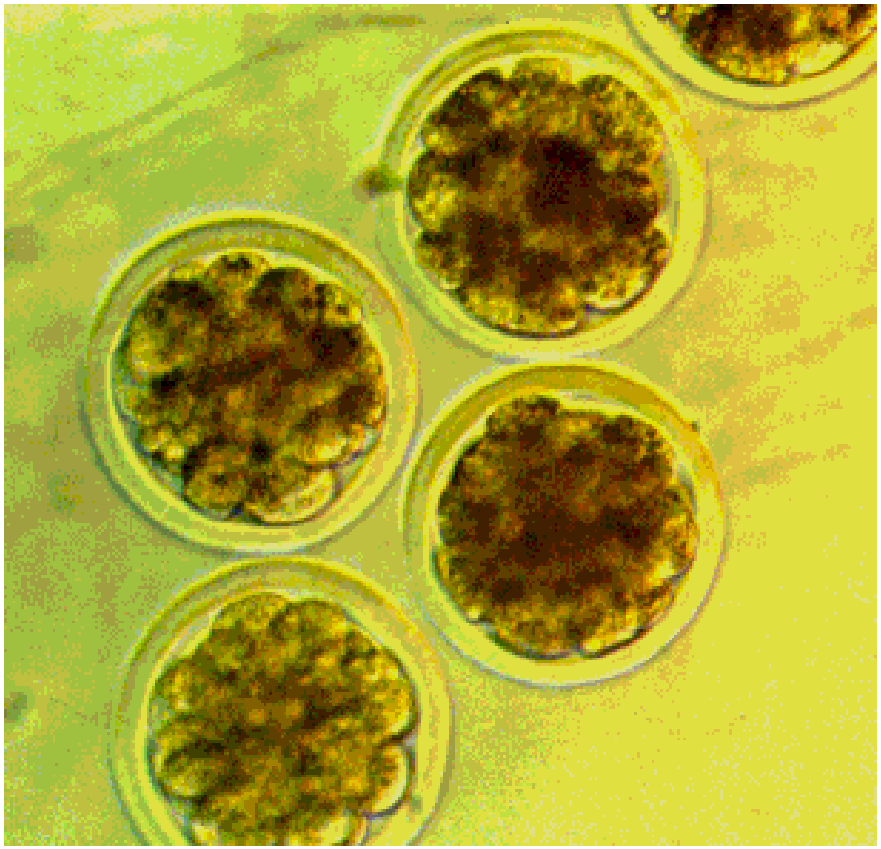
In un embrione preimpianto le cellule (blastomeri) sono quasi tutte (90%) in fase di duplicazione del DNA (fase S)





Seguite due strategie per indurre compatibilità tra oocita/nucleo

Sincronizzazione dei blastomeri in fase M
Trattamento degli embrioni prolungato con colcemid



Usare oociti pre-attivati (universal recipient – Campbell)

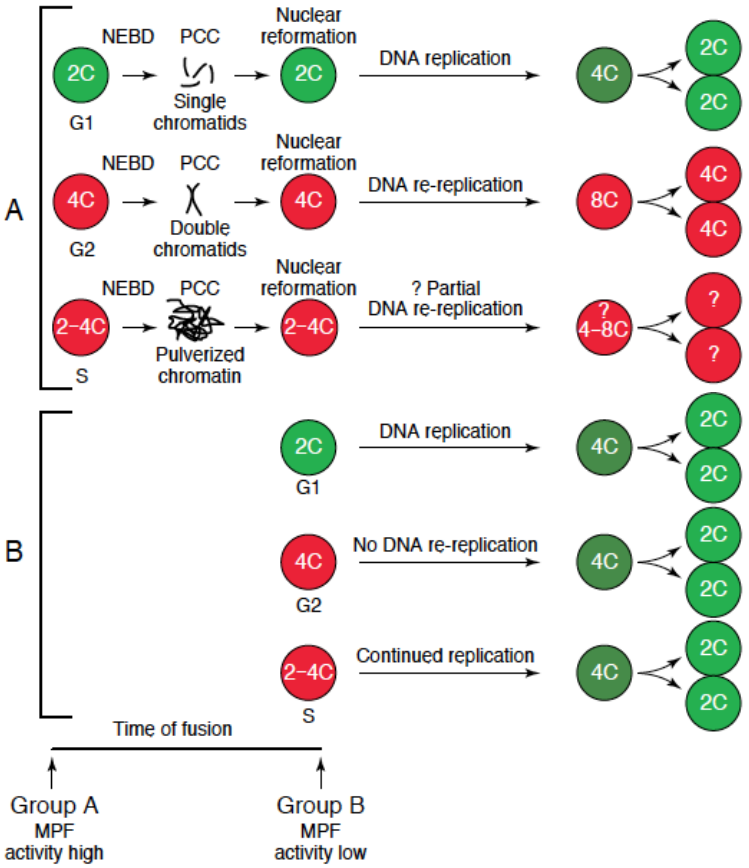
Reviews of Reproduction (1996) **1**, 40–46

Cell cycle co-ordination in embryo cloning by nuclear transfer

Keith H. S. Campbell, Pasqualino Loi*, Pedro J. Otaegui† and Ian Wilmut

Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, UK

Box 2. Effects of nuclear transfer of karyoplasts at defined cell cycle stages into cytoplasts with either high (group A) or low (group B) maturation-promoting factor (MPF) activity upon DNA synthesis during the first cell cycle and potential effects upon the ploidy of the reconstructed embryo.



All nuclei transferred at the time of activation (group A), when MPF activity is high, undergo nuclear envelope breakdown (NEBD), which is followed by premature chromosome condensation (PCC). The nuclear envelope is then reformed and DNA synthesis is observed in all nuclei. In this situation it is probable that unless the nucleus is in G1 phase at the time of transfer, re-replication of previously replicated DNA will occur and that at the end of the first cell cycle, the DNA content of the daughter nuclei will be incorrect. The increased amount of DNA present at the end of the first cycle may also adversely affect mitotis, resulting in unequal segregation or possible chromosomal abnormalities.

In contrast, when nuclei are transferred after the disappearance of MPF activity (group B), NEBD and PCC are not observed. Nuclei that are in G1 or S phase initiate or continue DNA synthesis; however, no DNA synthesis is observed in nuclei that are in the G2 phase at the time of transfer.

In this figure red circles represent nuclei that are 'out of phase' in terms of DNA content with the cell cycle stage of the recipient cytoplast; green circles represent nuclei that are 'in phase'.

BIOLOGY OF REPRODUCTION 50, 1385–1393 (1994)

Improved Development to Blastocyst of Ovine Nuclear Transfer Embryos Reconstructed during the Presumptive S-Phase of Enucleated Activated Oocytes

K.H.S. CAMPBELL,[1] P. LOI,[2] P. CAPPAI,[2] and I. WILMUT

AFRC Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, United Kingdom

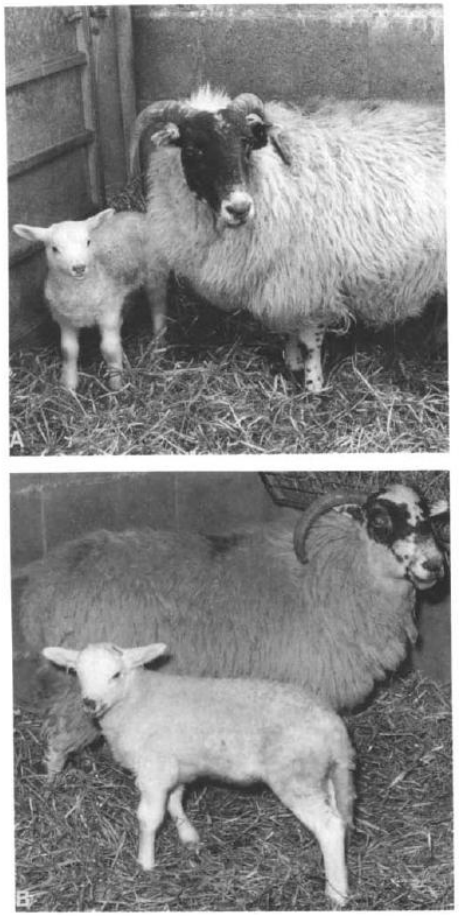
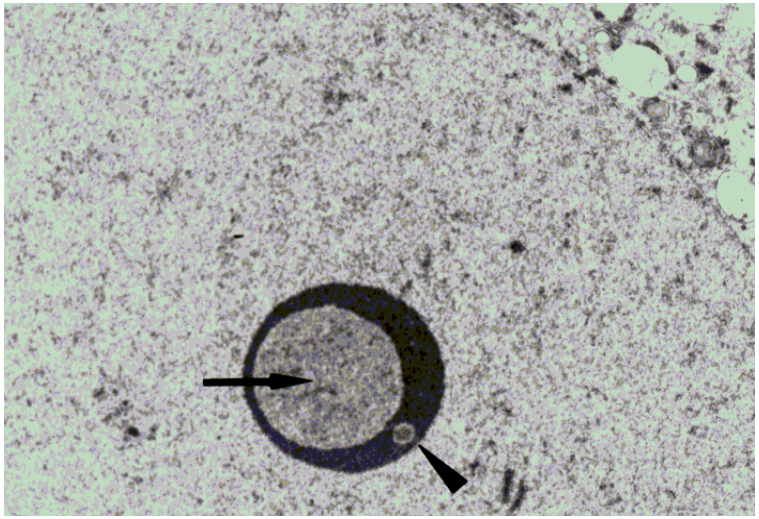
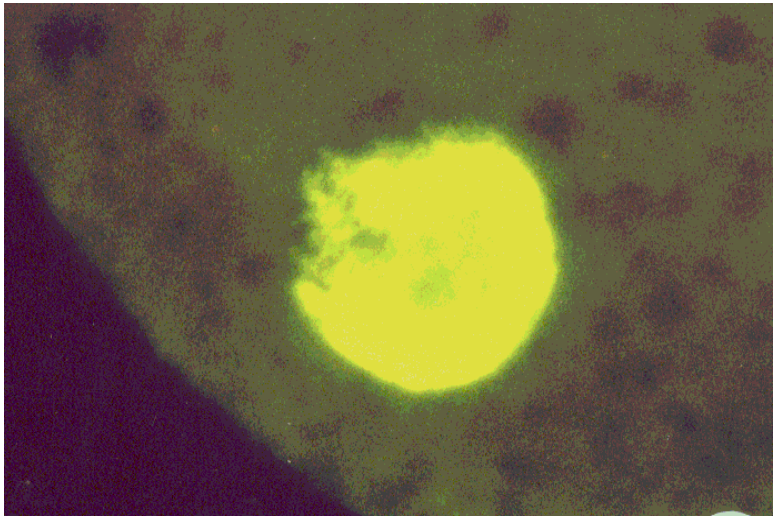


FIG. 4. Lambs born after transfer to final recipients of blastocysts derived from nuclear transfer reconstructed embryos. A) Lamb derived from the control group (0 hpa); B) lamb derived from an embryo reconstructed during the late S-phase (16–18 hpa) of enucleated activated MII oocytes.

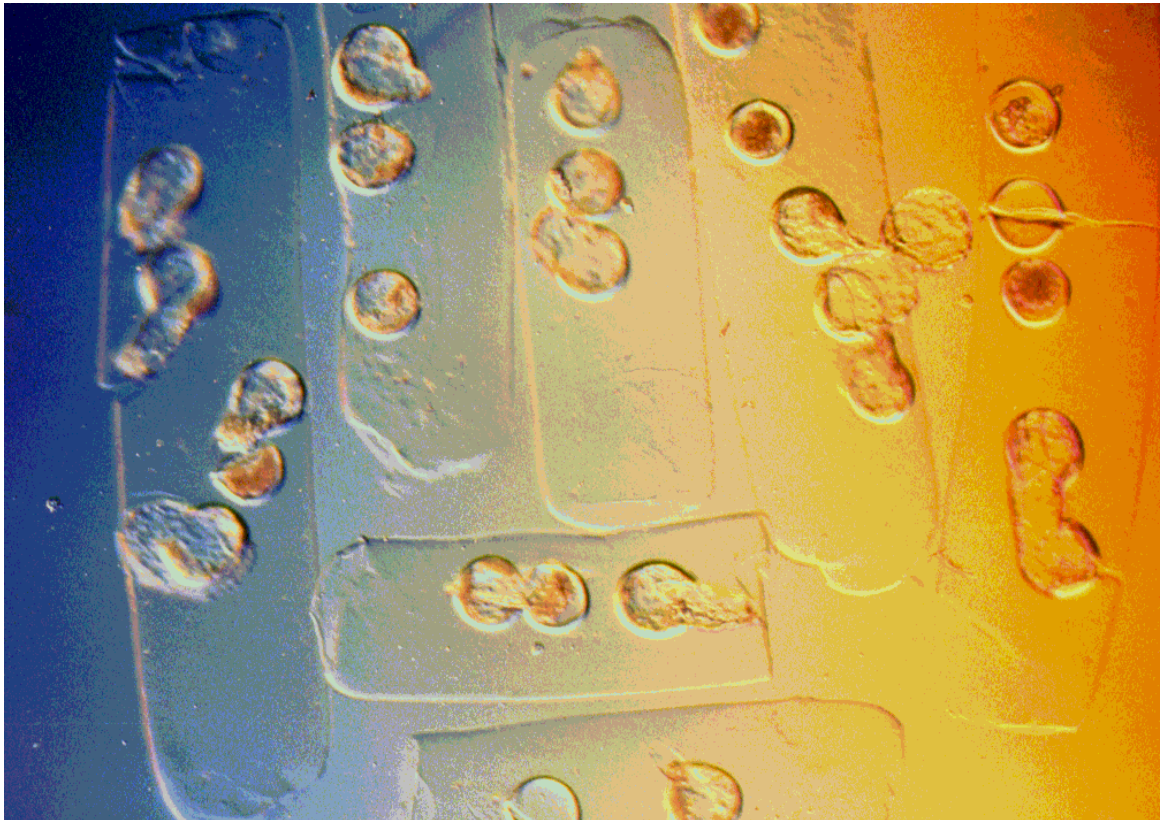
“Universal Recipient” efficiente ma non pratico –
necessaria una doppia manipolazione, tempi prolungati

Sintesi dei primi inibitori di protein kinase (6-Dymethyl-Amino-Purine 6-DMAP)
Controllo del ciclo cellulare!





6-dimethylaminopurine following ionomycin activation increased the proportion of blastocyst up to 80%…..(Loi et al., 1998)



BIOLOGY OF REPRODUCTION **58,** 1177–1187 (1998)

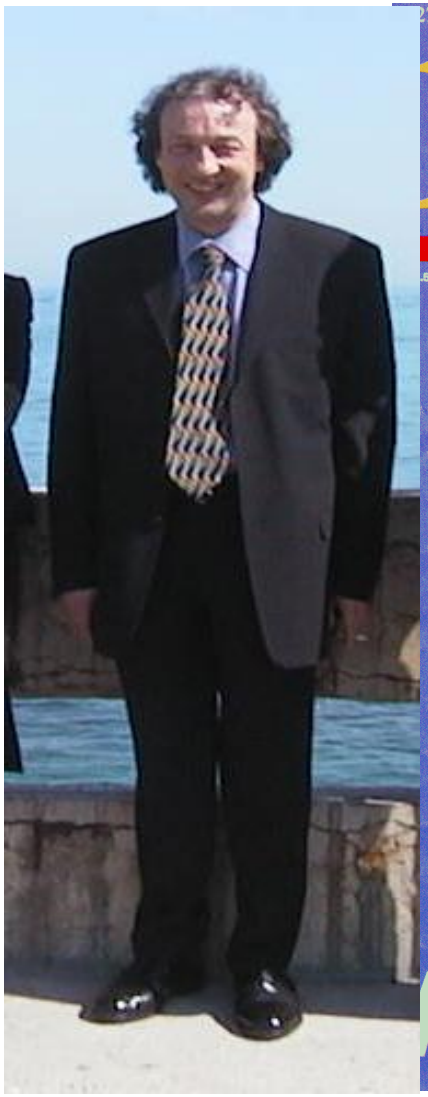
Development of Parthenogenetic and Cloned Ovine Embryos: Effect of Activation Protocols[1]

P. Loi,[2,3] S. Ledda,[4] J. Fulka, Jr.,[5] P. Cappai,[3] and R.M. Moor[6]

Istituto Zootecnico e Caseario per la Sardegna,[3] 07040 Olmedo, Italy
Dipartimento di Biologia Animale,[4] Sassari, Italy
Institute of Animal Production,[5] Prague, Czech Republic
The Babraham Institute,[6] Department of Development and Genetics, Babraham, Cambridge CB2 4AT, United Kingdom

Cloni di 7-15 animali normali ottenibili da un singolo embrione……

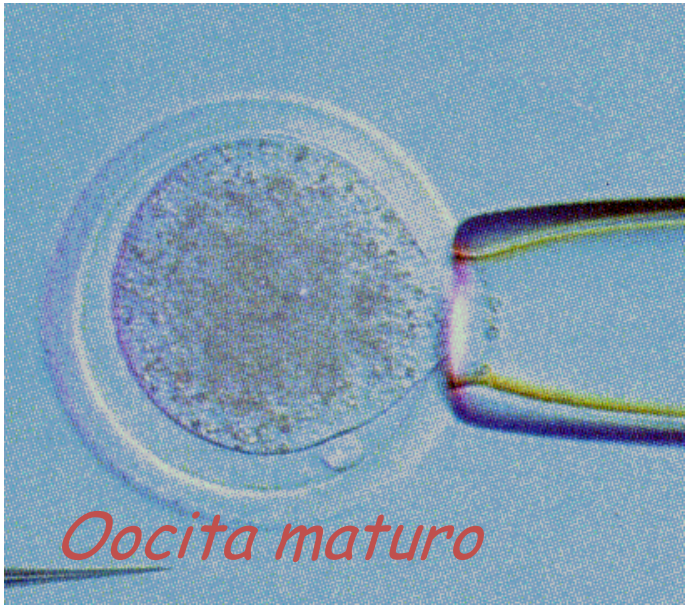
(Loi, et al. Biology of Reproduction 1998)

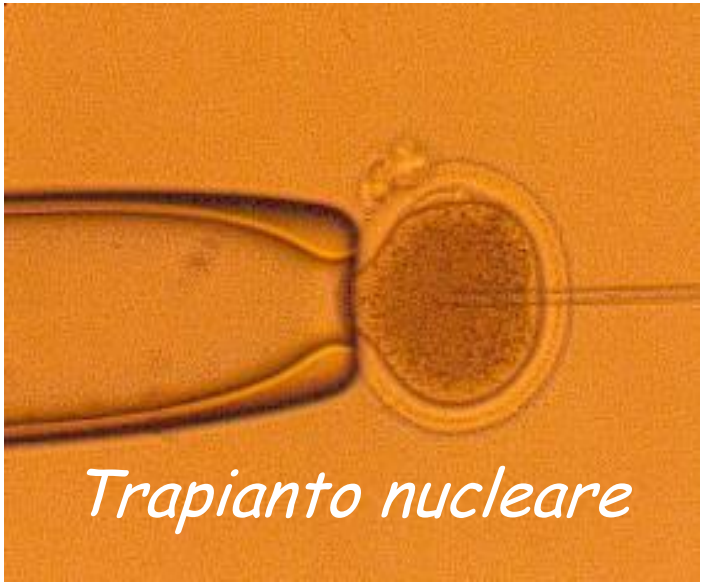


Keith Campbell, 1954-2012

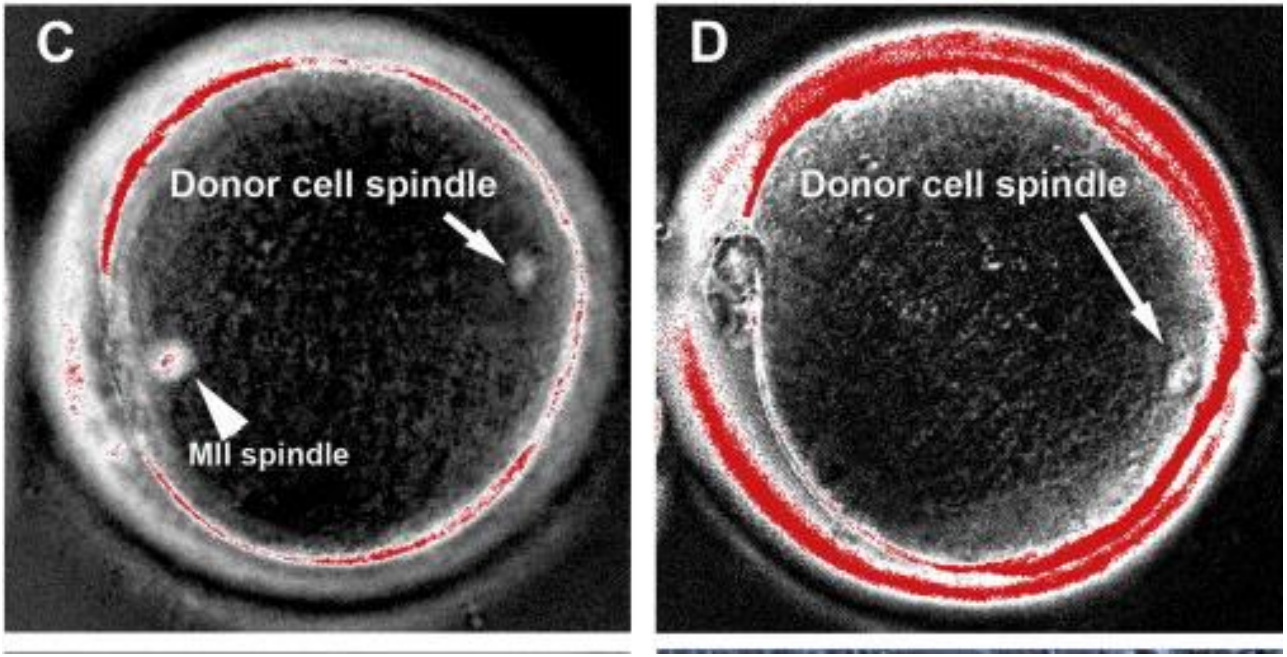
Ian Wilmut, 1943-

Clonazione con cellule somatiche





In oociti con citoplasma trasparente visualizzazione fuso meiotico con Microscopio a luce polarizzata (Poll-Scope)



La pecora Dolly

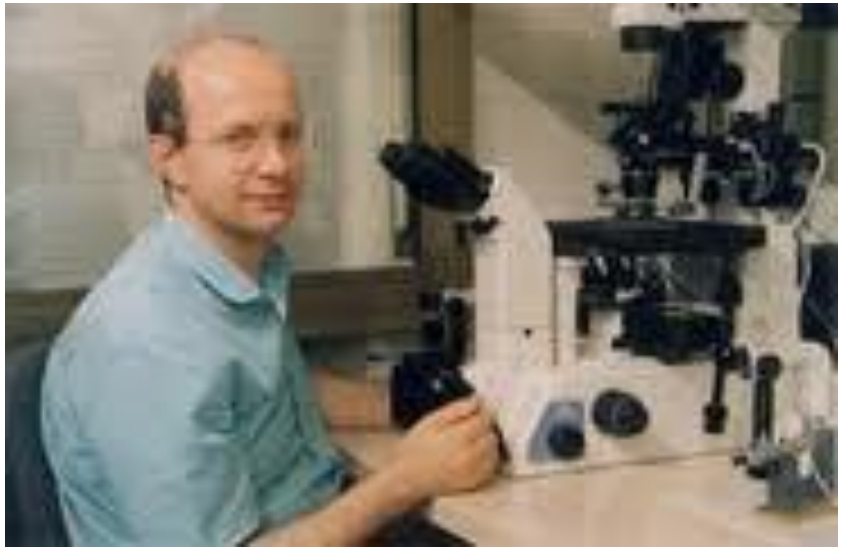
Dolly Patton, American folk singer……..


DER SPIEGEL
Wissenschaft auf dem Weg zum geklonten Menschen
DER


MARCH 10, 1997
TIME
FICTION BONUS: "CLONE ON THE RANGE" BY DOUGLAS COUPLAND
Will There Ever Be Another You?
A SPECIAL REPORT ON CLONING


Newsweek
March 10, 1997 | $2.95
THE LINCOLN BEDROOM
Was the White House for Sale?
Can We Clone Humans?
By Sharon Begley


SIX APPEAL: Sandown Park meets Jurassic Park as half-a-dozen cloned Cigars fight out a finish in a race of the future.
Fancy another Cigar?
Shock cloning plans for barren stallion
By Richard Palmer



Prof. Cesare Galli
Avantea and Univeristà di Bologna

Italian government 'arrests' cloned bull
By Associated Press

ROME -- Poor Galileo. Just a youngster and he's under arrest -- locked in a stall and accused of being a freak.

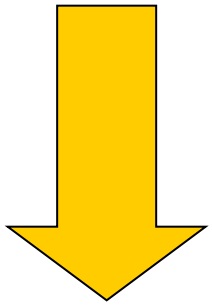
Dolly è stata imbalsamata
ed è esposta allo Scottish Museum di Edinburgo

Clonazione riproduttiva: appplicazioni in:

Riproduzione animale

Animali transgenici

Moltiplicazione di specie minacciate di estinzione



..però….bassa efficienza della clonazione 1-5%

Clonate praticamente tutte le specie da reddito e di affezione

1996

1998

1998

1999

2000

2001

2003

2003

2003

Clonati anche animali “non convenzionali”

Medaka Fish

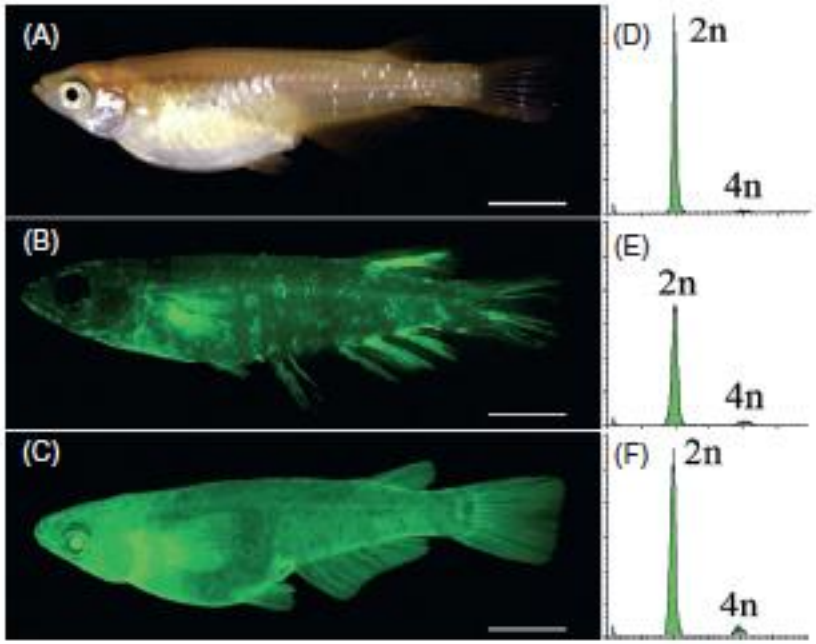


FIGURE 15.4 Three types of adult nuclear transplants generated in the second examination. (A) Donor clone, NT4. (B) Fluorescent image of chimeric fish, NT5. (C) Fluorescent image of NT fish originated from parthenogenesis of the recipient egg, NT1. (D–F) Ploidy analysis by flow cytometry showing the diploidy of adult fish: (D) NT4, (E) NT5, (F) NT1. Bars represent 5 mm. *From Bubenshchikova et al., (2008).*

zebrafish

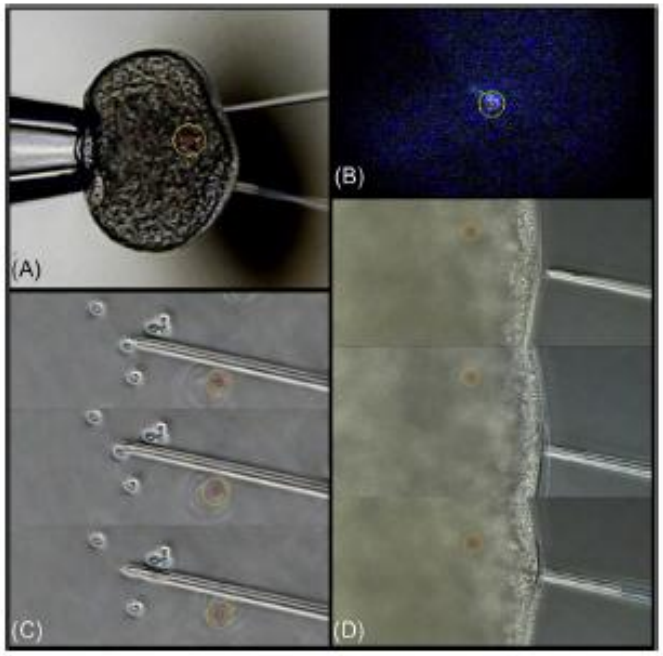


FIGURE 16.2 Method for somatic cell nuclear transfer in zebrafish. The egg micropyle is positioned facing the bottom of a Petri dish to visualize the metaphase plate (A). The Hoechst 33342-stained metaphase plate of the egg is ablated using the laser XY clone module (B). The areas under red and yellow circles, when the laser-pulse is introduced by a 40× laser-equipped objective lens, would be at 400°C and 100°C, respectively. An individual donor cell is picked up using a beveled-tip needle (C) and is then transferred through a micropyle to an animal pole of the egg (D).

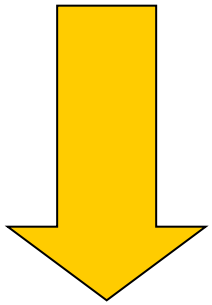
Clone di bovini (Nuova Zelanda)

Clonazione riproduttiva: appplicazioni in:

Riproduzione animale

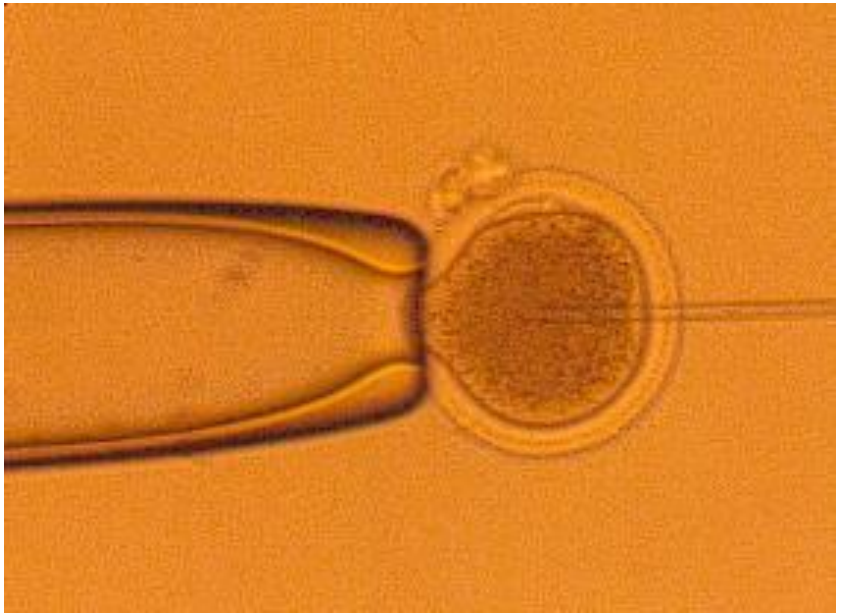
Animali transgenici

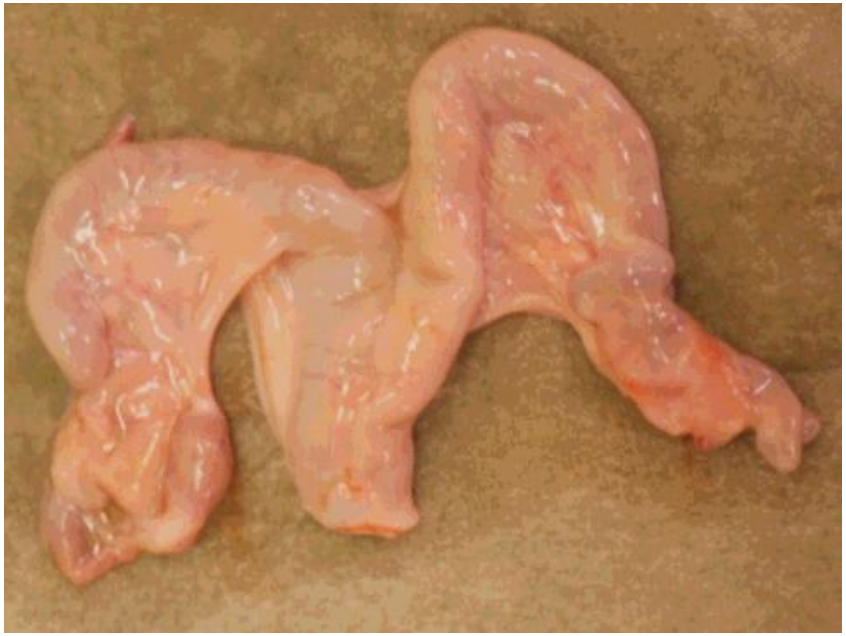
Moltiplicazione di specie minacciate di estinzione

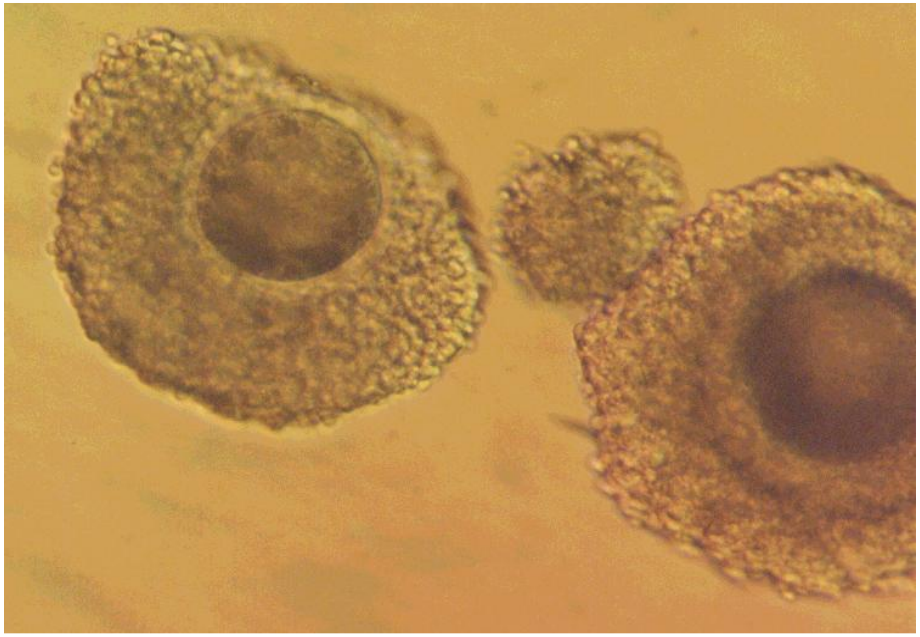


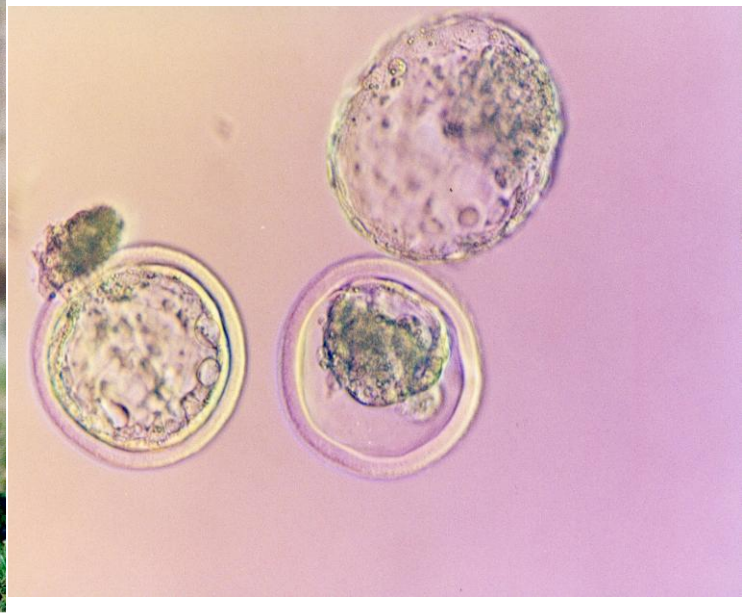
..però….bassa efficienza della clonazione 1-5%


A
B
C
D
E
F
G
H
I



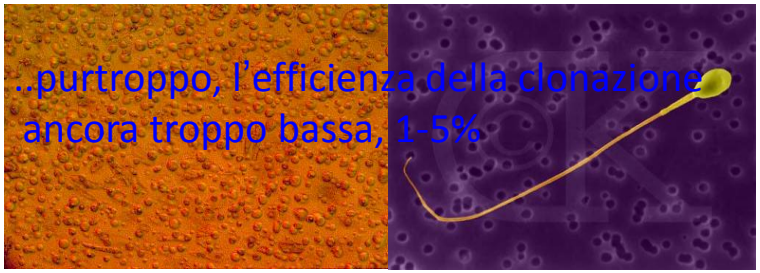




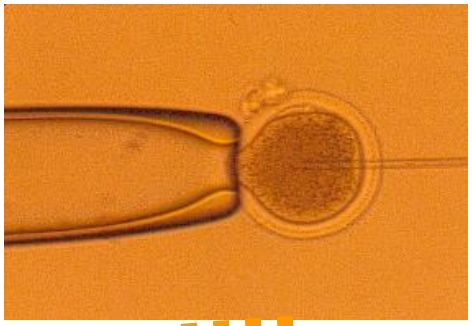


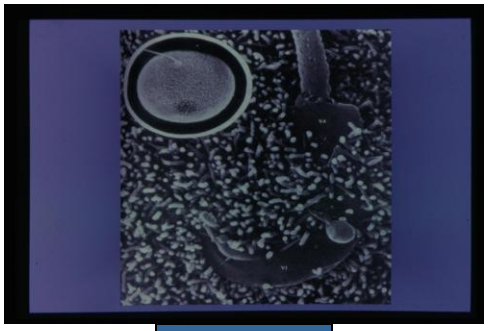
Genetic rescue of an endangered mammal by cross-species nuclear transfer using post-mortem somatic cells

Loi et al., Nature Biotechnology 2001



UNIVERSITÀ DEGLI STUDI DI TERAMO

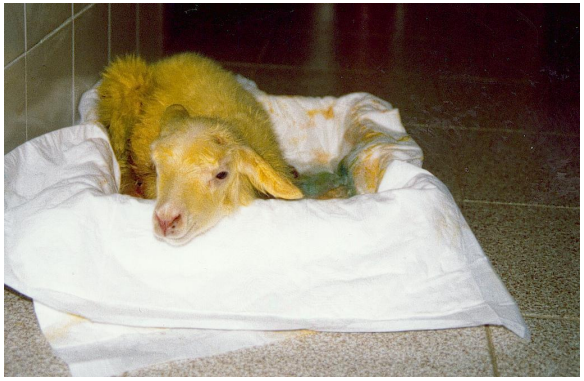




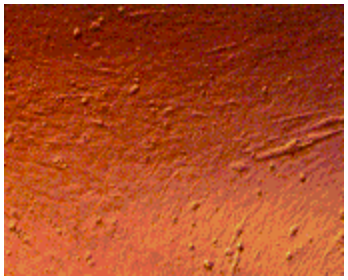
Sviluppo embrionale e fetale

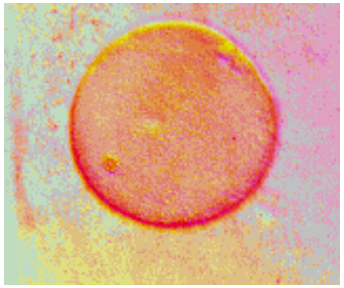
…Eventualmente…

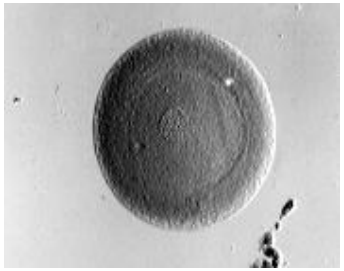
Elevata mortalità neonatale



Clonazione con cellule somatiche

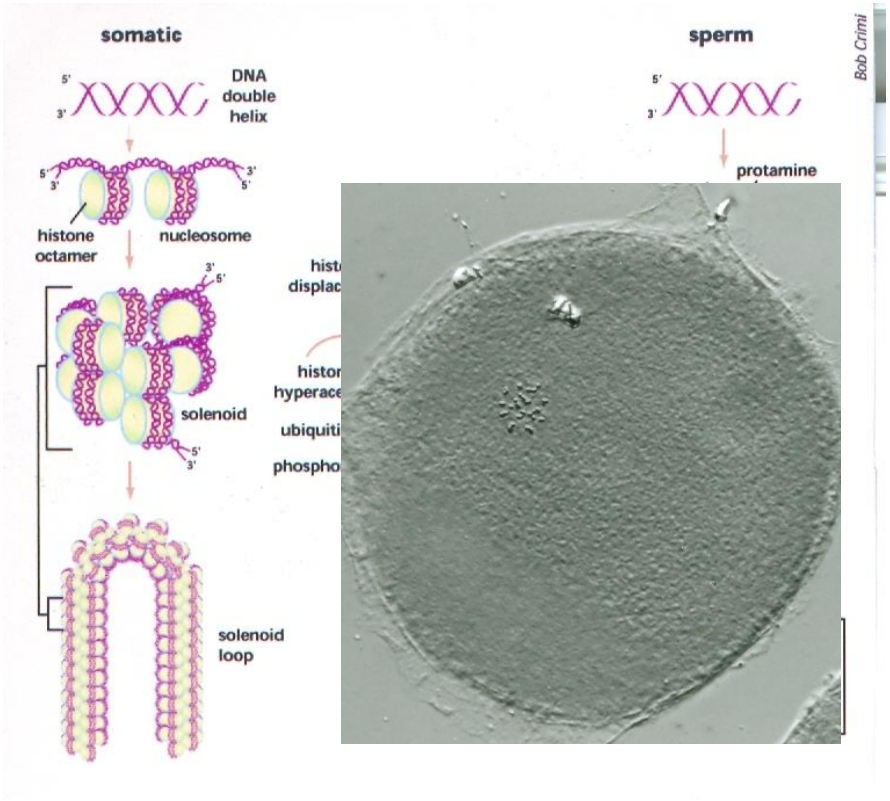






Normale sviluppo??? No

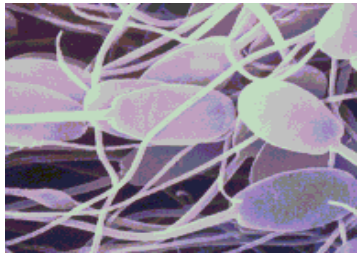
Rimodellamento nucleare

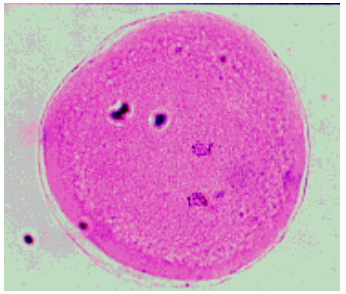


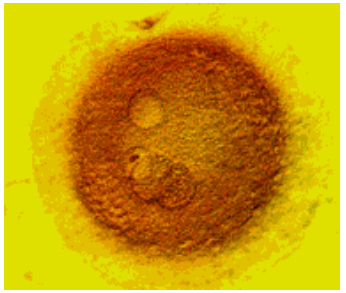
Principale problema del cloning

È l'insufficente riprogrammazione nucleare

Fertilizzazione

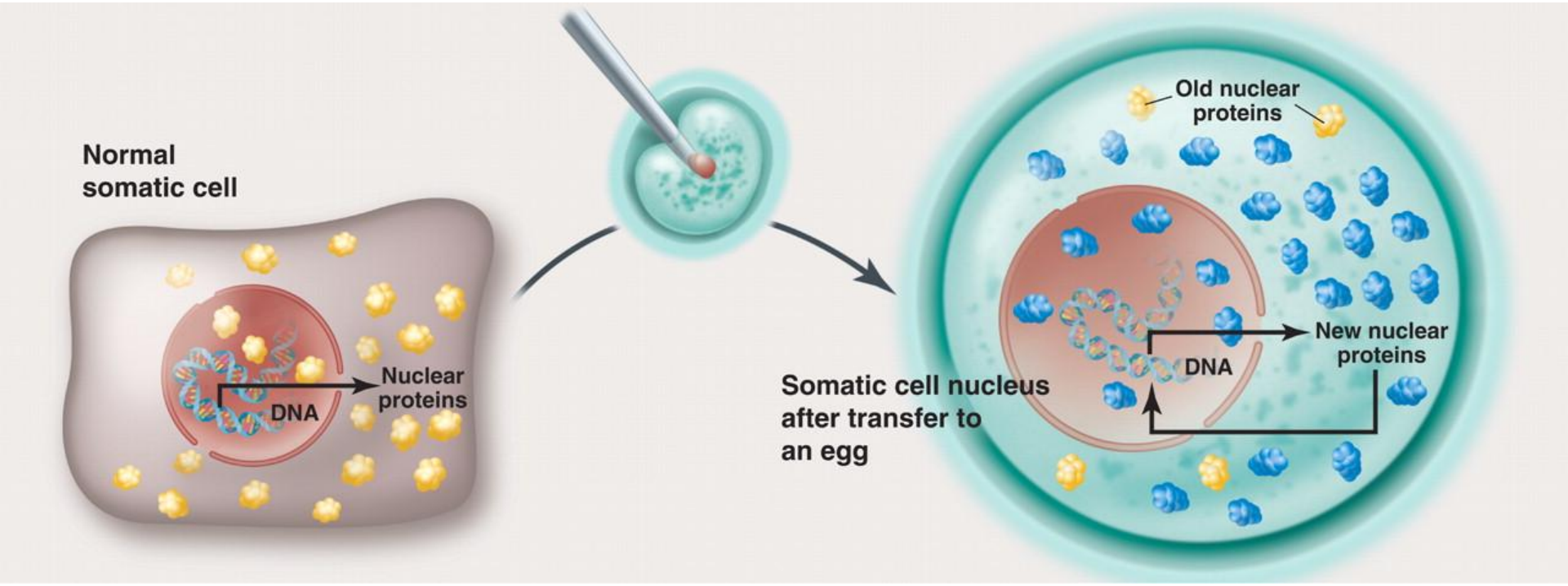






Normale sviluppo??? Si

Rimodellamento e riprogrammazione nucleare



Nucleo somatico (rimuovere la membrana!) si espande enormemente nell'oocita: nuclear swelling

Nuclear swelling espressione morfologica della riprogrammazione nucleare

Bassa efficienza: a prescindere dal tipo cellulare (Kato e Tsunoda, 2013)

TABLE 10.1 Donor Cell Types and Cell Cycle Combination of Successful Nuclear Transfer

Donor Cell Type			Species (Donor-Recipient)	Donor[a]:Recipient Cell Cycle(stage)
Somatic cells	adult	mammary gland	sheep	G0: MII
		oviduct	bovine	G0: MII
		cumulus	bovine, mice, goat, rabbit, cat, sand cat-domestic cat	G0:MII, ?:MII
		cultured cumulus	ferret, camel	G0: MII
		granulosa cell	pig, mouflon-sheep	G0: MII
		cultured granulosa	buffalo	G0: MII
		follicular epithelial cell	mouse	G0: MII
		tail tip	mouse	G0: MII
	bovine, gaur–bovine pig, horse, african wild cat-domestic cat, dog	fibroblasts	bovine, gaur–bovine pig, horse, african wild cat-domestic cat, dog, wolf-dog, Pyrenean Ibex-domestic goat	G0:MII, ?:MII
		fibroblast	rabbit	G0:S(2:cell)
		lymphocytes	mouse	?:MII
		anterior pituitary	goat	G0: MII
		bone marrow mesenchyma stem cell	bovine	G0: MII
		natural killer T cell	mouse	?:MII
		hematopoietic granulocytes	mouse	?:MII
		antler stem cell	red deer	G0: MII
		keratinocytes	mouse	?:MII
		iPS	mouse	M:MII
	newborn/young	fibroblast	bovine	G0: MII
		Sertoli	mouse	?:MII
		neural stem cell	mouse	?:MII
	fetus	fetal fibroblast	goat, pig, bovine, rat, mose, mule-horse	G0:MII, M:M
		genital ridge cells	pig	G0: MII
		neural cells, premature-early differentiated, differentiated	mouse	?:MII
		keratinocytes	mouse	?:MII
Embryonic	blastocyst	inner cell mass (ICM) cells	mouse	G1:MII
		mural trophectoderm (TE) cells	mouse	G1:MII
		embryonic disc	sheep	G0: MII
		cultured embyonic disc (TNT4)	sheep	G0: MII
	ES-cell		mouse	M:MII, M-M(zygote9, small size, large size:MII
	EC-cell		mouse	?:MII
	PGCs	day 10.5	mouse	G1:M
		day 15.5 male	mouse	G0: MII

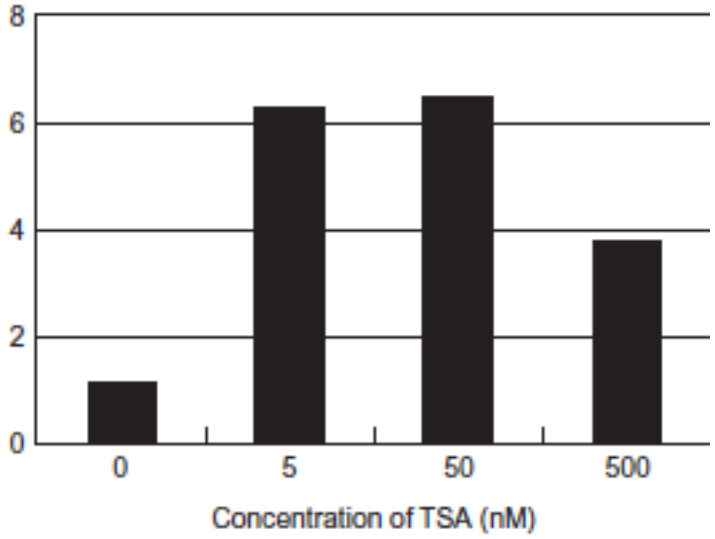
Strategie proposte per aumentare la riprogrammazione nucleare

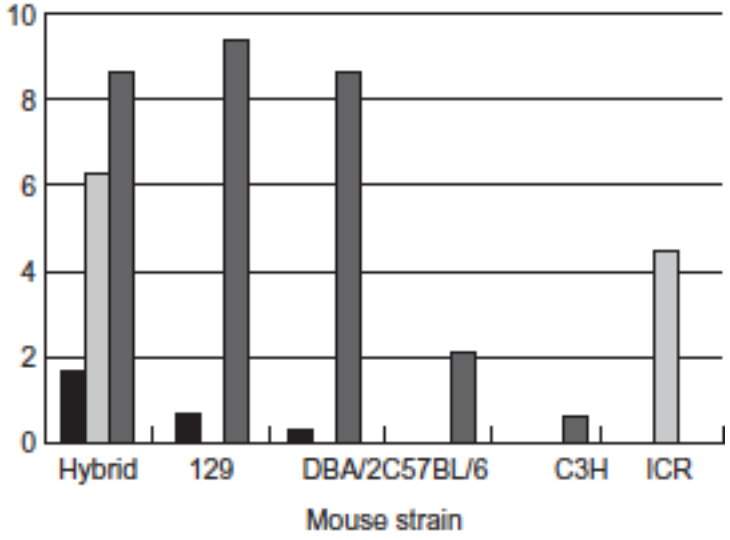
Sincronizzare le cellule somatiche in G0: 5 giorni a basse % di siero in coltura
Keith Campbell 1997 – allontana fattori trascrizione

Prolungata esposizione (4 ore) dei cromosomi somatici nell'oocita enucleato
Attivazione oocita posticipata – allontana fattori transcrizione
Yanagimachi 1998

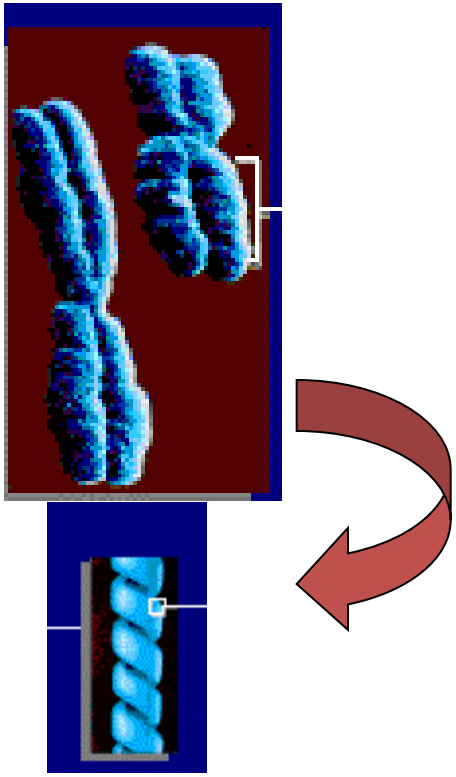
Esposizione delle cellule prima e dopo il trapianto nucleare a inibitori
Di istone de-acetilasi – apre la struttura della cromatina
Wakayama 2007

Strategie proposte per migliorare l'efficienza

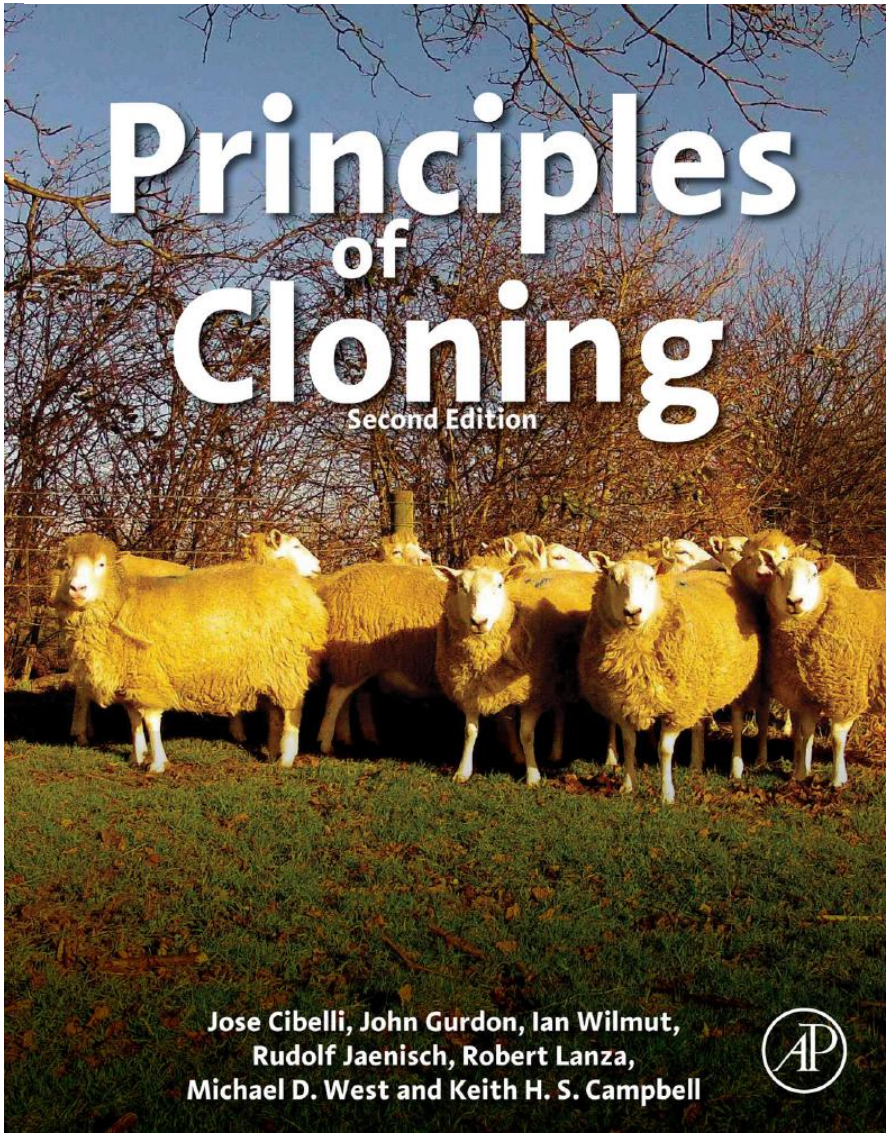




Loi e Coll…??????



Possiamo organizzare la cromatina
di una cellula somatica
Come quella dello spermatozoo?:



Clonare animali in via di estinzione

5485 animals specie animali in via di estinzione, 180 sono mammiferi

3500 razze locali minacciate di estinzione (FAOglobal survey 2005)

Current Biology 2002

News focus

Can cloning save endangered species?

SCIENCE'S COMPASS

POLICY FORUM: ECOLOGY

DNA Banks for Endangered Animal Species

Oliver A. Ryder, Anne McLaren, Sydney Brenner, Ya-Ping Zhang, Kurt Benirschke

Azoto liquido golden standard per cryostoccaggio

FREEZE DRYING

Peptidi – vaccini liofilizzati di uso ordinario

“semplici” genomi conservati liofilizzati
Batteri-lieviti

Spermatozoi liofilizzati mantengono la potenzialità di generare normali individui (topi) Wakayama & Yanagimachi 1998

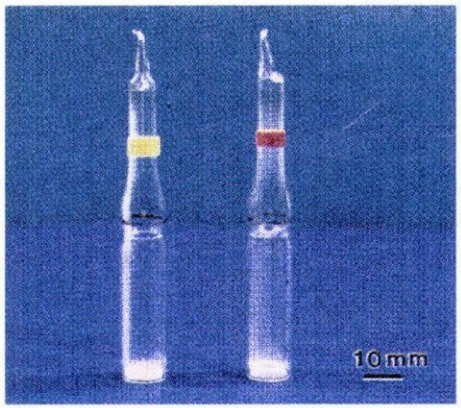
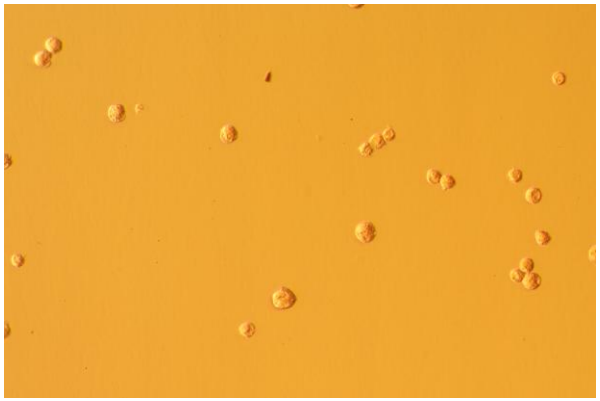


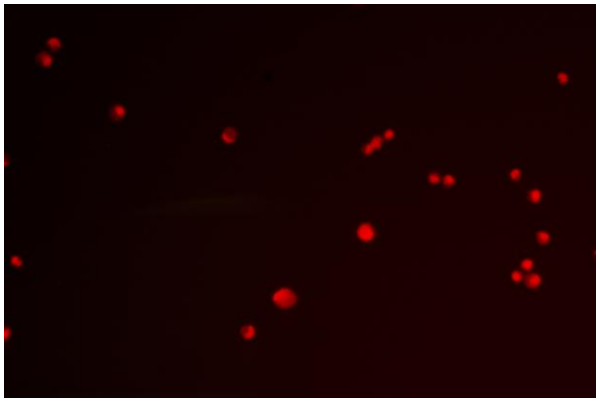
Figure 1. Vacuum-sealed ampules with freeze-dried mouse spermatozoa. The white powder at the bottom of each ampule is the dried CZB medium containing spermatozoa.

Figure 3. Offspring with a CD-1 (albino) foster mother. These three young developed from B6D2F1 oocytes injected with B6D2F1 spermatozoa that had been kept at room temperature for 1 month after freeze-drying.

…e le cellule somatiche?

Miei primi esperimenti di trapianto nucleare (1999-2000)





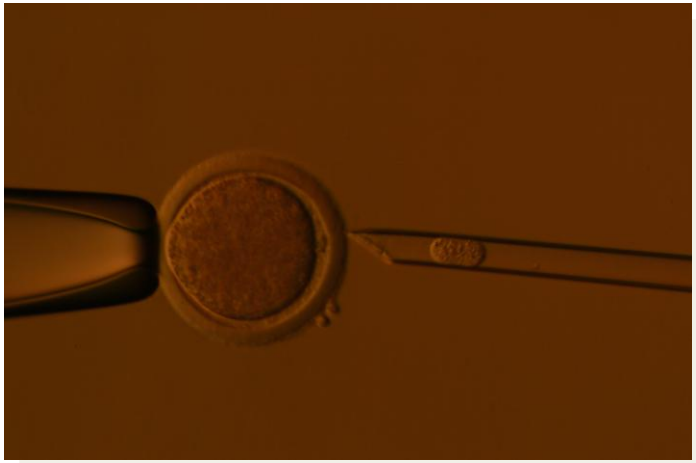
Ma si possono usare cellule non vitali per il nuclear transfer?

BIOLOGY OF REPRODUCTION 67, 126–132 (2002)

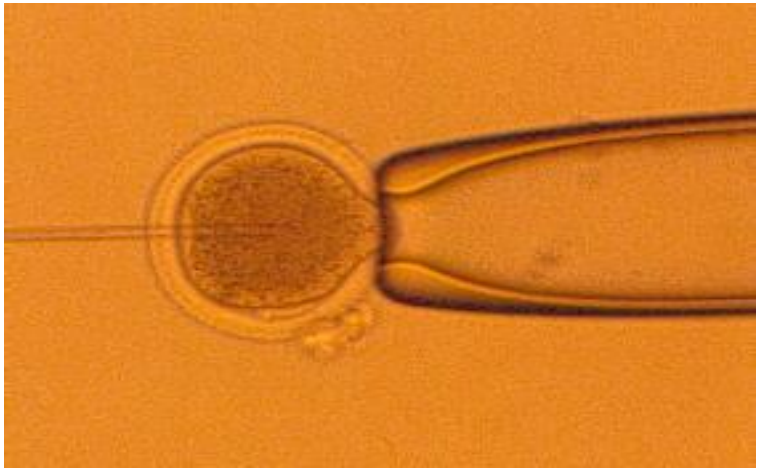
Nuclei of Nonviable Ovine Somatic Cells Develop into Lambs after Nuclear Transplantation

Pasqualino Loi,[2,3] Michael Clinton,[4] Barbara Barboni,[3] Josef Fulka, Jr.,[5] Pietro Cappai,[6] Robert Feil,[7] Robert M. Moor,[8] and Grazyna Ptak[3,9]

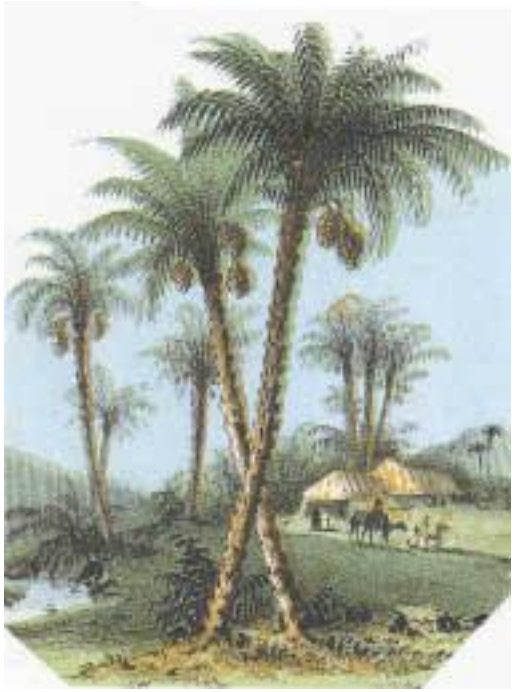
Fusione cellulare non praticabile con cellule non vitali

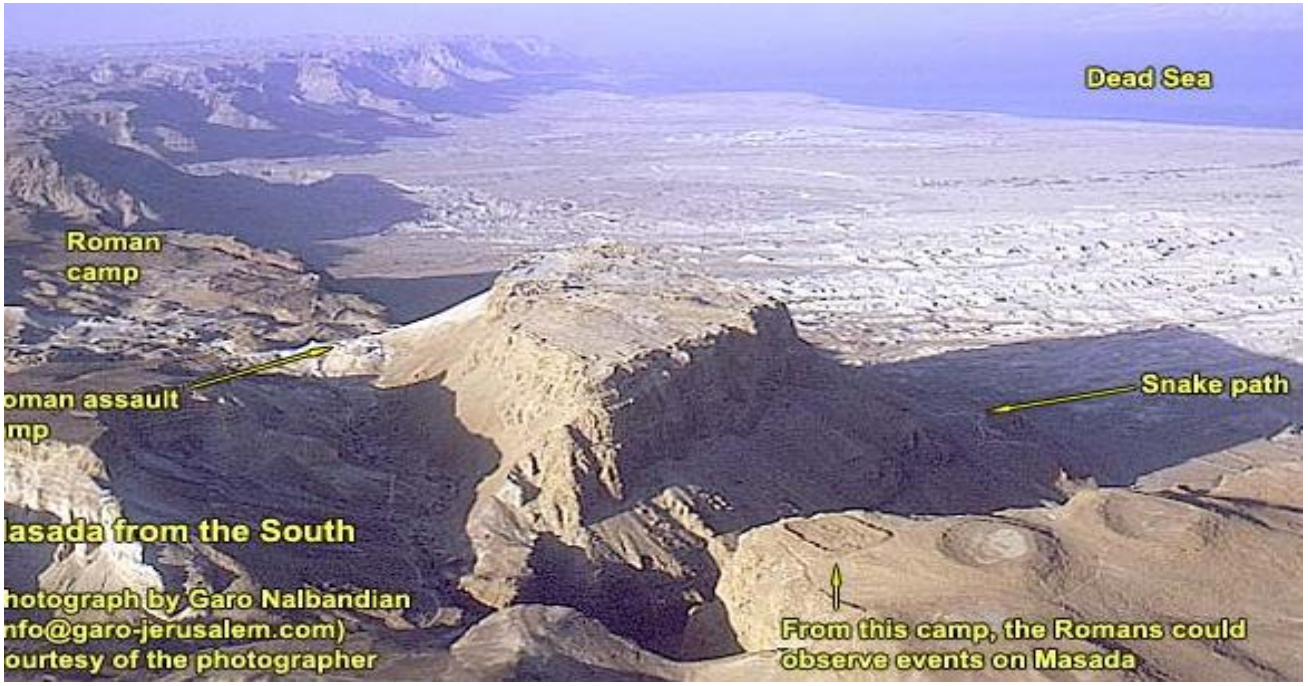


..iniezione diretta in oociti enucleati



Donor nuclei	n. injected	n. Cleaved (%)	Blastocysts (%)
Freeze dried	461	-	-
control	320	268 (84)	119 (37)

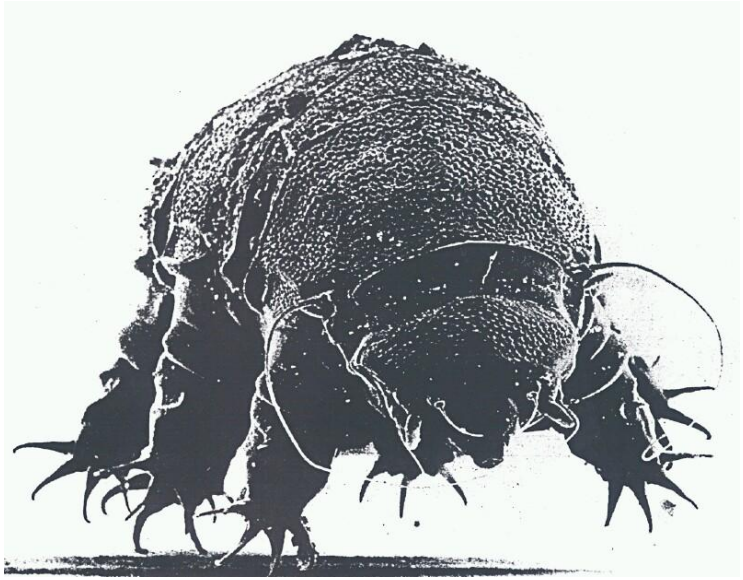


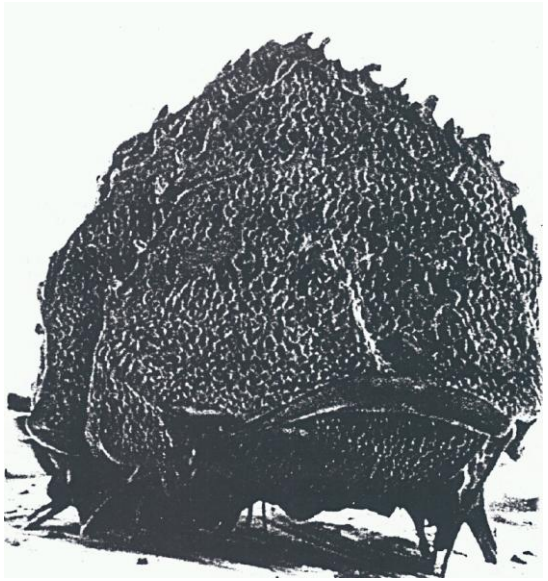


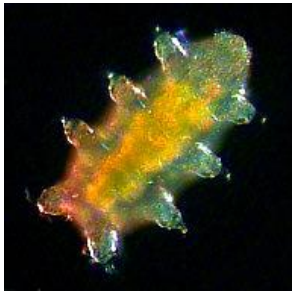
הנבטת זרע עץ התמר

- זרעי התמר התגלו בחפירות במצדה בשנות ה-70
- הזרעים הם משנת 35-65 לספירה - בתקופת המצור על מצדה
- החוקרים טוענים שמדובר בזרע הקדום ביותר שעד כה הצליחו להנביט
- אחת החוקרות: "הסיכויים שהצמח יניב פירות - 50%"

Lezioni dalla Natura:
trealosio sintetizzato da organismi anidrobionti





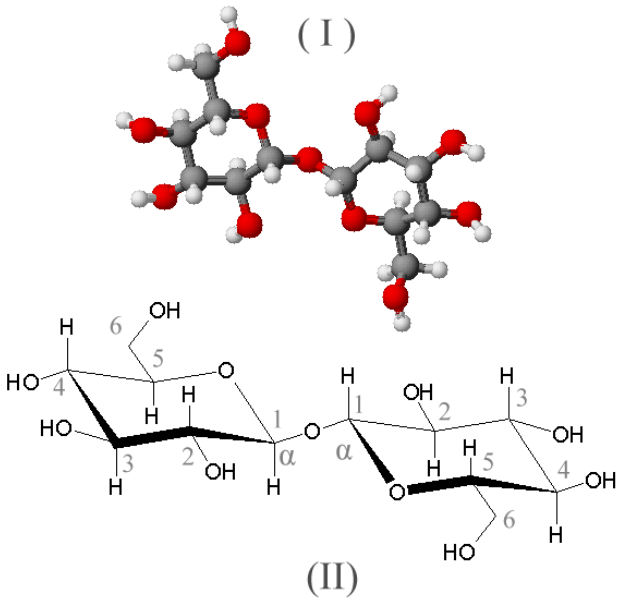


Active Tardigrade

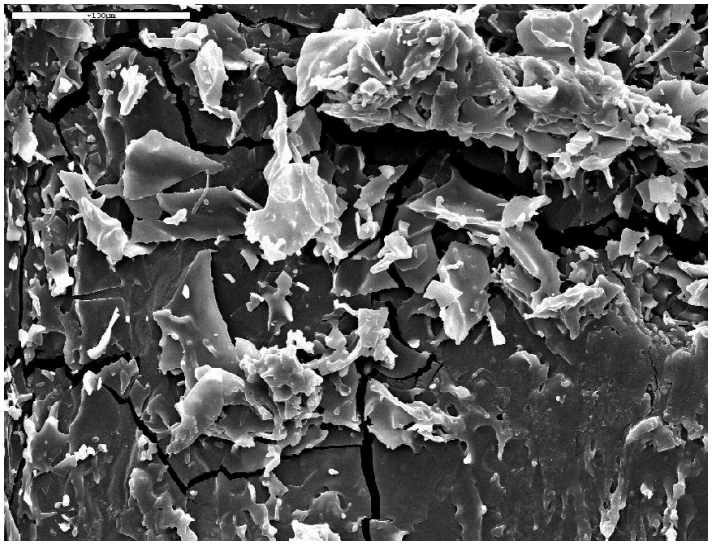
Dried Tardigrade

Crowe and Cooper. 1971. Scientific American

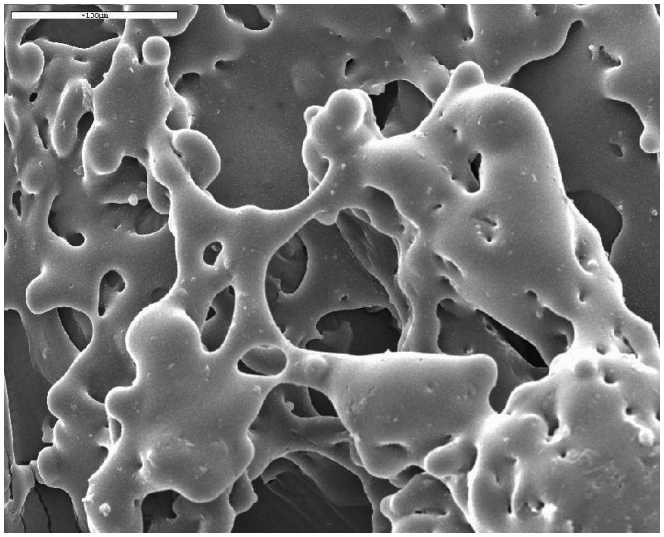
Thehalose (0.1M in Hepes) inserito nel medium di congelamento



Effetti del trealosio sulle cellule allo stato anidro

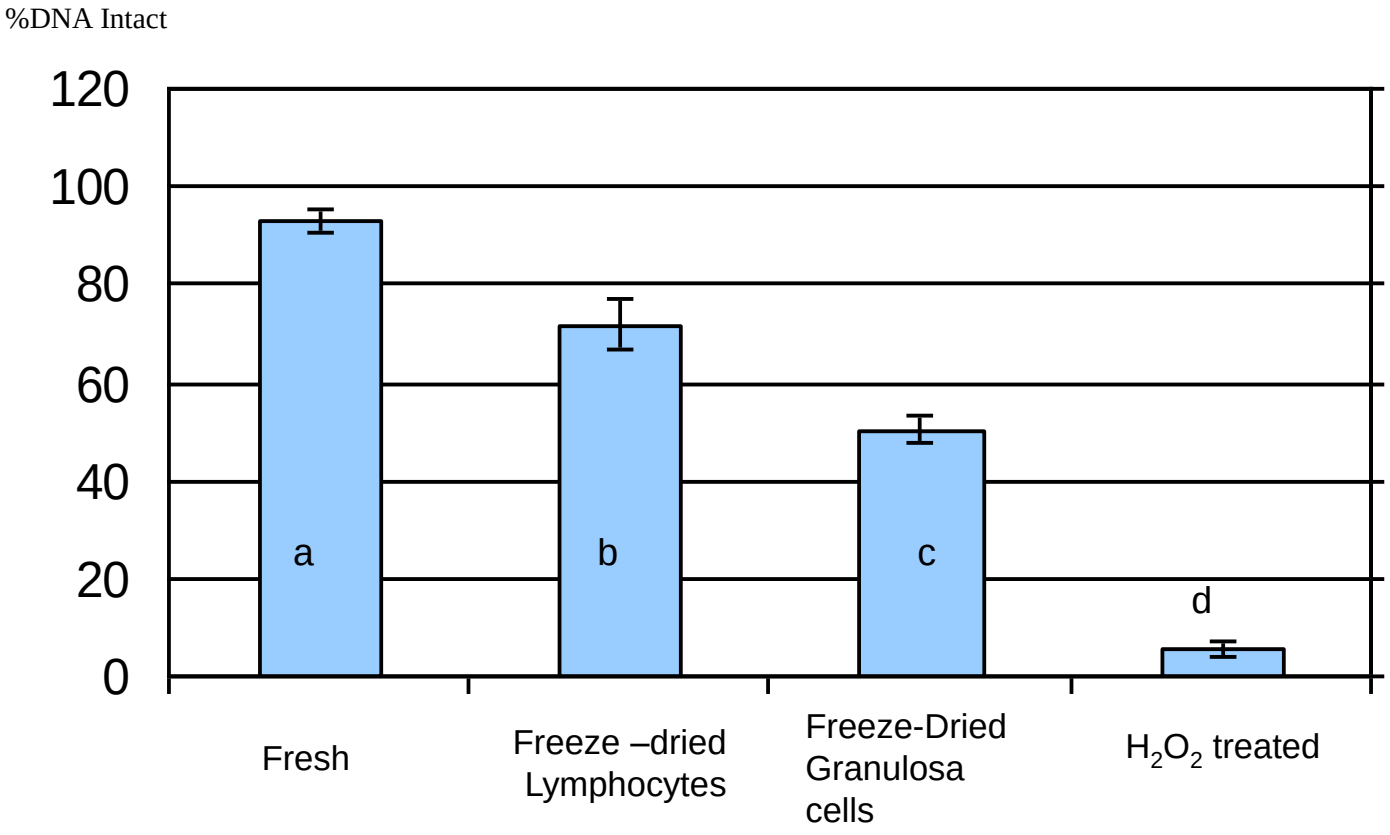


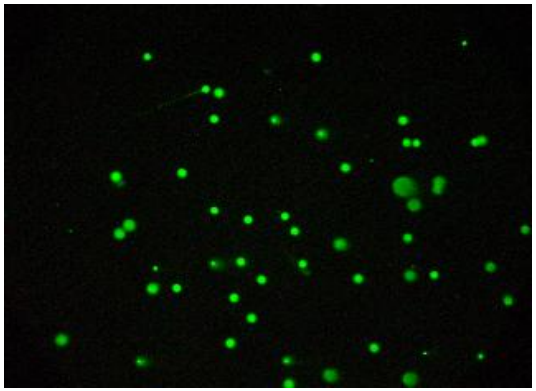
Trehalose - SEM x 150



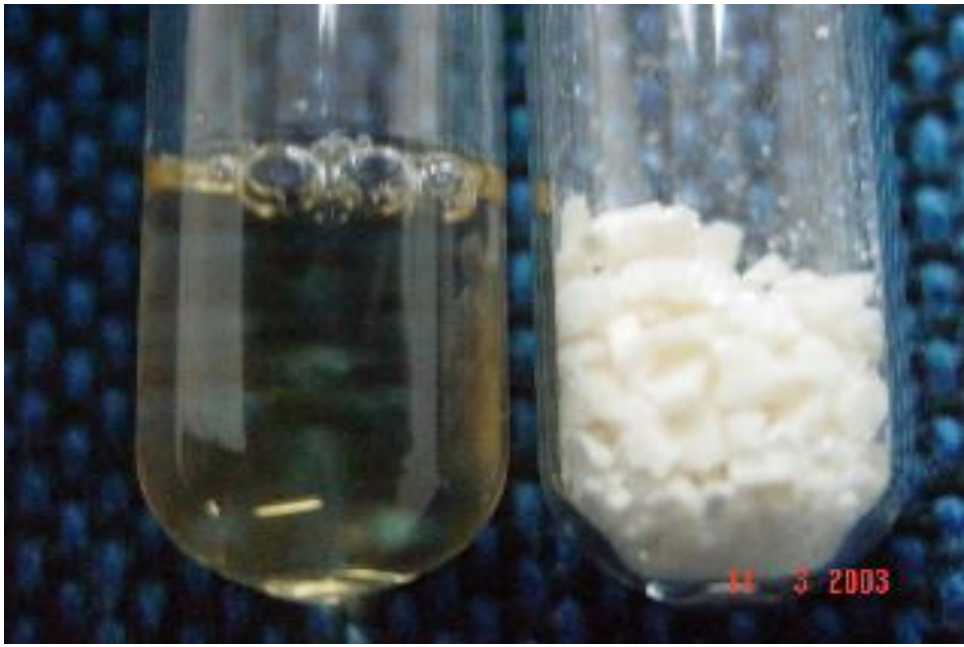
Trehalose + SEM x 150

Danno cellulare rilevato con Comet assay



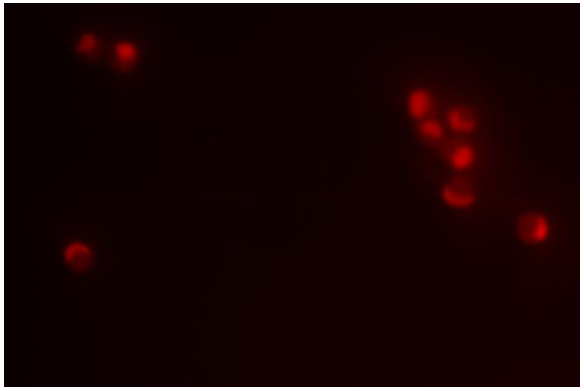


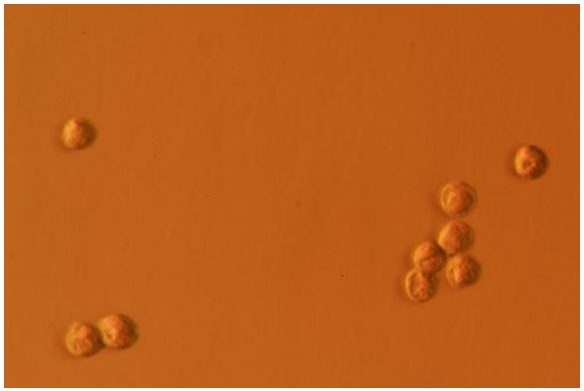
Cellule della Granulosa liofilizzate

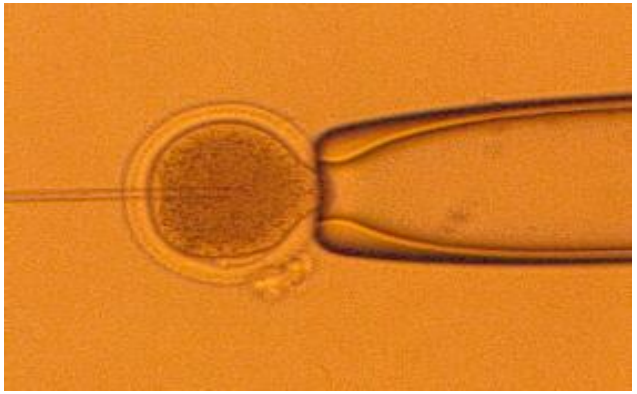


Cellule della granulosa di pecore di razza Awassi liofilizzate e spedite da Tel Aviv a Teramo

Trapianto nucleare di cellule liofilizzate







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PLoS one

Freeze-Dried Somatic Cells Direct Embryonic Development after Nuclear Transfer

Pasqualino Loi[1]*, Kazutsugu Matsukawa[1¤], Grazyna Ptak[1], Michael Clinton[2], Josef Fulka, Jr.[3], Yehudith Nathan[4], Amir Arav[4]

1 Department of Comparative Biomedical Sciences, Teramo University, Teramo, Italy, 2 Roslin Institute and Royal (Dick) School of Veterinary Studies, Edinburgh, United Kingdom, 3 Institute of Animal Production, Prague, Czech Republic, 4 Institute of Animal Science, Agricultural Research Organization, The Volcani Centre, Bet Dagan, Israel

Abstract

The natural capacity of simple organisms to survive in a dehydrated state has long been exploited by man, with lyophylization the method of choice for the long term storage of bacterial and yeast cells. More recently, attempts have been made to apply this procedure to the long term storage of blood cells. However, despite significant progress, practical application in a clinical setting is still some way off. Conversely, to date there are no reports of attempts to lyophilize nucleated somatic cells for possible downstream applications. Here we demonstrate that lyophilised somatic cells stored for 3 years at room temperature are able to direct embryonic development following injection into enucleated oocytes. These remarkable results demonstrate that alternative systems for the long-term storage of cell lines are now possible, and open unprecedented opportunities in the fields of biomedicine and for conservation strategies.

Journal of Reproduction and Development, Vol. 54, No. 6, 2008

—Full Paper—

Nuclear Transfer Preserves the Nuclear Genome of Freeze-Dried Mouse Cells

Tetsuo ONO[1,2], Eiji MIZUTANI[1], Chong LI[1,3] and Teruhiko WAKAYAMA[1–3]

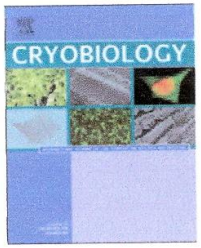
[1]Laboratory for Genomic Reprogramming, RIKEN Center for Developmental Biology, Kobe 650-0047, [2]Department of Medical Science, Graduate School of Medicine, Kyoto University, Kyoto 606-8502 and [3]Department of Bioscience, Graduate School of Science and Technology, Kwansei Gakuin University, Sanda 669-1337, Japan

Cryobiology 61 (2010) 220–224

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo



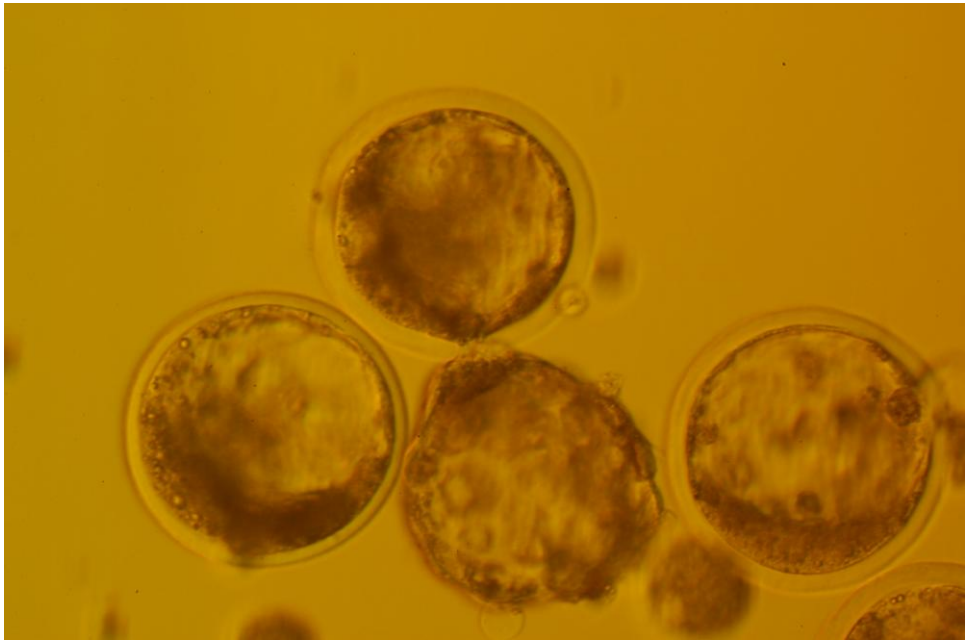
Lyophilized somatic cells direct embryonic development after whole cell intracytoplasmic injection into pig oocytes ☆

Ziban Chandra Das [a], Mukesh Kumar Gupta [b,*], Sang Jun Uhm [b], Hoon Taek Lee [b,**]

[a] *Department of Bioscience and Biotechnology, Animal Resources Research Center/Bio-Organ Research Center, Institute of Biomedical Science and Technology, Konkuk University, Seoul 143 701, South Korea*

[b] *Department of Animal Biotechnology, Animal Resources Research Center/Bio-Organ Research Center, Institute of Biomedical Science and Technology, Konkuk University, Seoul 143 701, South Korea*

Eseguiti due trasferimenti di embrioni clonati da cellule liofilizzate la nascita di un normale individuo prova fondamentale



UNIVERSITÁ DEGLI STUDI DI TERAMO

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PLoS one

Freeze-Drying of Mononuclear Cells Derived from Umbilical Cord Blood Followed by Colony Formation

Dity Natan[1]*, Arnon Nagler[2], Amir Arav[3]

1 Core Dynamics Ltd., Ness-Ziona, Israel, 2 Hematology Division, BMT and Cord Blood Bank, Chaim Sheba Medical Center, Israel, 3 Institute of Animal Science, Agricultural Research Organization (ARO), Bet Dagan, Israel

Abstract

Background: We recently showed that freeze-dried cells stored for 3 years at room temperature can direct embryonic development following cloning. However, viability, as evaluated by membrane integrity of the cells after freeze-drying, was very low; and it was mainly the DNA integrity that was preserved. In the present study, we improved the cells' viability and functionality after freeze-drying.

Methodology/Principal Findings: We optimized the conditions of directional freezing, i.e. interface velocity and cell concentration, and we added the antioxidant EGCG to the freezing solution. The study was performed on mononuclear cells (MNCs) derived from human umbilical cord blood. After freeze-drying, we tested the viability, number of $CD34^+$-presenting cells and ability of the rehydrated hematopoietic stem cells to differentiate into different blood cells in culture. The viability of the MNCs after freeze-drying and rehydration with pure water was 88%–91%. The total number of $CD34^+$-presenting cells and the number of colonies did not change significantly when evaluated before freezing, after freeze-thawing, and after freeze-drying ($5.4\times10^4\pm4.7$, $3.49\times10^4\pm6$ and $6.31\times10^4\pm12.27$ cells, respectively, and 31 ± 25.15, 47 ± 45.8 and 23.44 ± 13.3 colonies, respectively).

Conclusions: This is the first report of nucleated cells which have been dried and then rehydrated with double-distilled water remaining viable, and of hematopoietic stem cells retaining their ability to differentiate into different blood cells.

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PLoS one

Preservation of Differentiation and Clonogenic Potential of Human Hematopoietic Stem and Progenitor Cells during Lyophilization and Ambient Storage

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1 Center for Pharmaceutical Biotechnology and Department of Pharmaceutical Sciences, University of Colorado, Aurora, Colorado, United States of America, **2** Summit Toxicology, Superior, Colorado, United States of America

Abstract

Progenitor cell therapies show great promise, but their potential for clinical applications requires improved storage and transportation. Desiccated cells stored at ambient temperature would provide economic and practical advantages over approaches employing cell freezing and subzero temperature storage. The objectives of this study were to assess a method for loading the stabilizing sugar, trehalose, into hematopoietic stem and progenitor cells (HPC) and to evaluate the effects of subsequent freeze-drying and storage at ambient temperature on differentiation and clonogenic potential. HPC were isolated from human umbilical cord blood and loaded with trehalose using an endogenous cell surface receptor, termed P2Z. Solution containing trehalose-loaded HPC was placed into vials, which were transferred to a tray freeze-dryer and removed during each step of the freeze-drying process to assess differentiation and clonogenic potential. Control groups for these experiments were freshly isolated HPC. Control cells formed 1450±230 CFU-GM, 430±140 BFU-E, and 50±40 CFU-GEMM per 50 μL. Compared to the values for the control cells, there was no statistical difference observed for cells removed at the end of the freezing step or at the end of primary drying. There was a gradual decrease in the number of CFU-GM and BFU-E for cells removed at different temperatures during secondary drying; however, there were no significant differences in the number of CFU-GEMM. To determine storage stability of lyophilized HPC, cells were stored for 4 weeks at 25°C in the dark. Cells reconstituted immediately after lyophilization produced 580±90 CFU-GM (~40%, relative to unprocessed controls $p<0.0001$), 170±70 BFU-E (~40%, $p<0.0001$), and 41±22 CFU-GEMM (~82%, $p=0.4171$), and cells reconstituted after 28 days at room temperature produced 513±170 CFU-GM (~35%, relative to unprocessed controls, $p<0.0001$), 112±68 BFU-E (~26%, $p<0.0001$), and 36±17 CFU-GEMM (~82%, $p=0.2164$) These studies are the first to document high level retention of CFU-GEMM following lyophilization and storage for 4 weeks at 25°C. This type of flexible storage stability would potentially permit the ability to ship and store HPC without the need for refrigeration.

L'Arca di Noè delle banche di cellule liofilizzate

http://www.nationalgeographic.com/deextinction

Recipe for a Resurrection
Scientists could try to clone a mammoth. Should they?

Vol. 14: 227–233, 2011
doi: 10.3354/esr00366

ENDANGERED SPECIES RESEARCH
Endang Species Res

Published online September 23

OPEN ACCESS

REVIEW

Biological time machines: a realistic approach for cloning an extinct mammal

Pasqualino Loi[1,*], Teruhiko Wakayama[2], Joseph Saragustry[3], Josef Fulka Jr[4], Grazyna Ptak[1]

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[2]RIKEN Centre for Developmental Biology, 2-2-3 Minatojima-minamimachi, Kobe 650-0047, Japan
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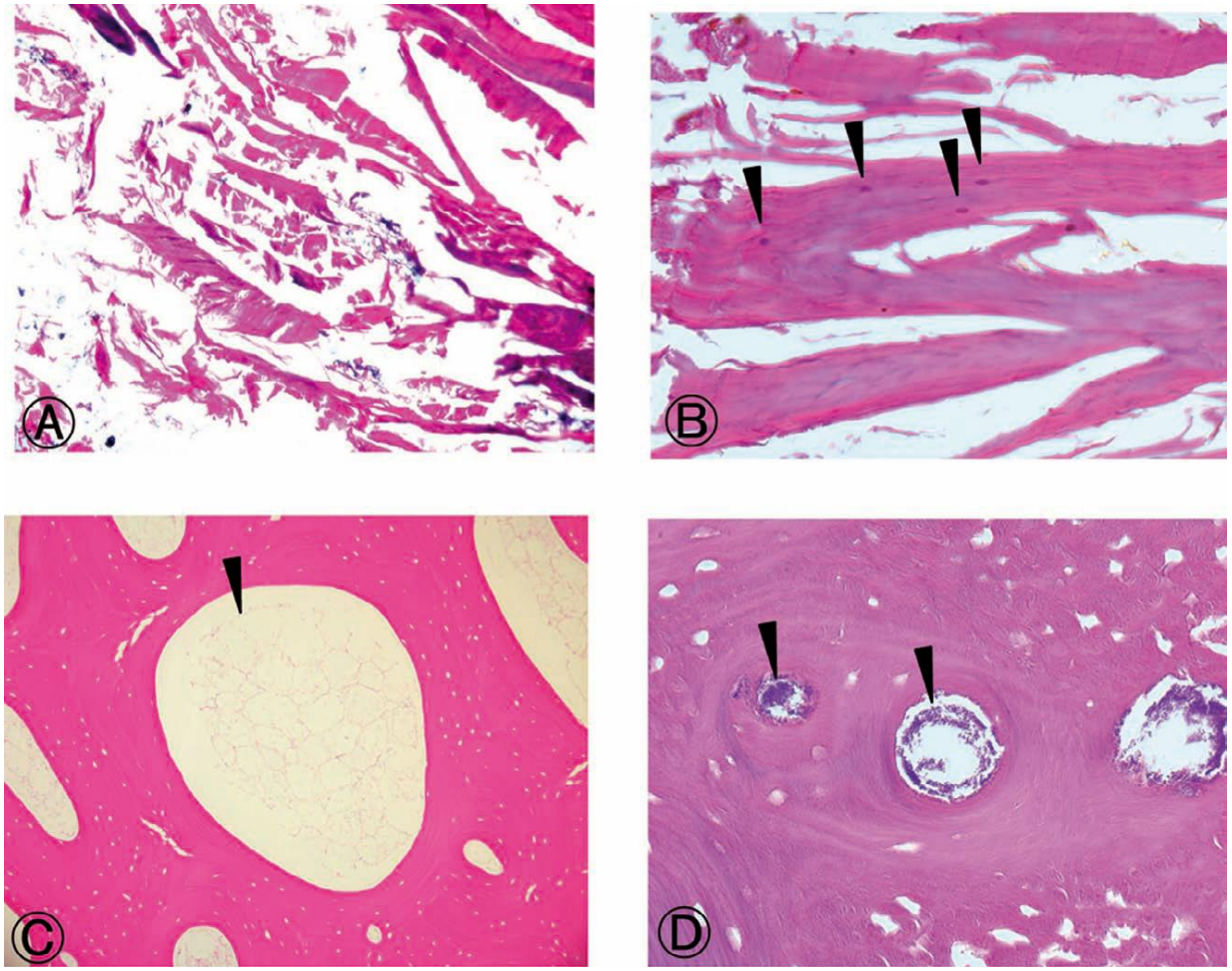
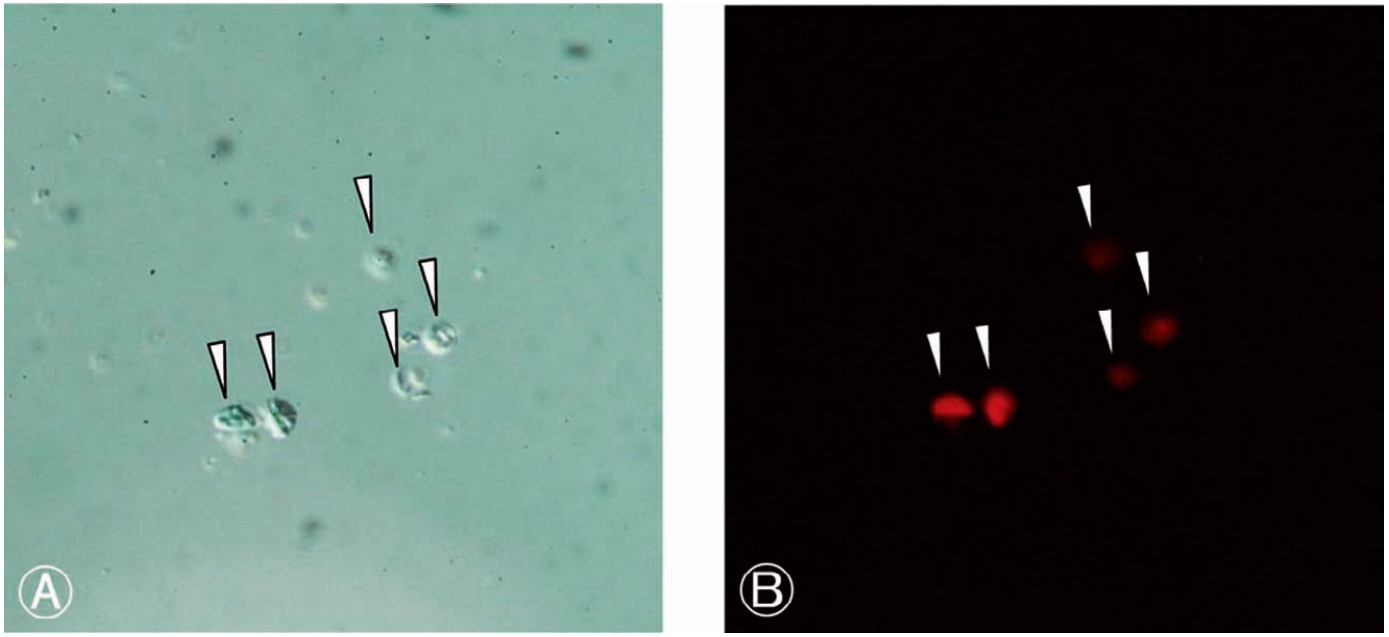
Possiamo clonare il Mammut

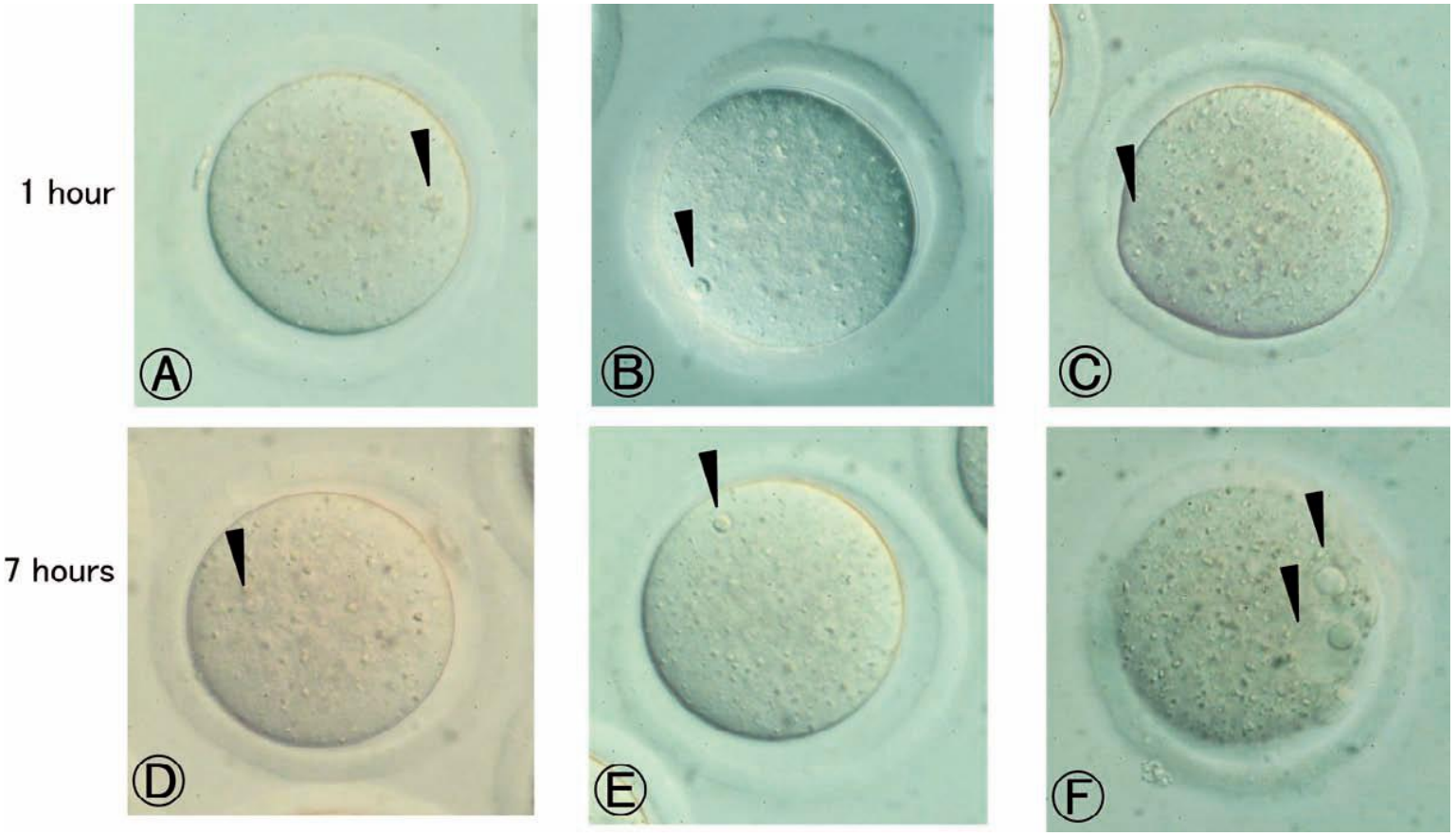
Sezioni istologiche tessuti (arto anteriore) di un Mammoth di 15000 anni trovato in Siberia

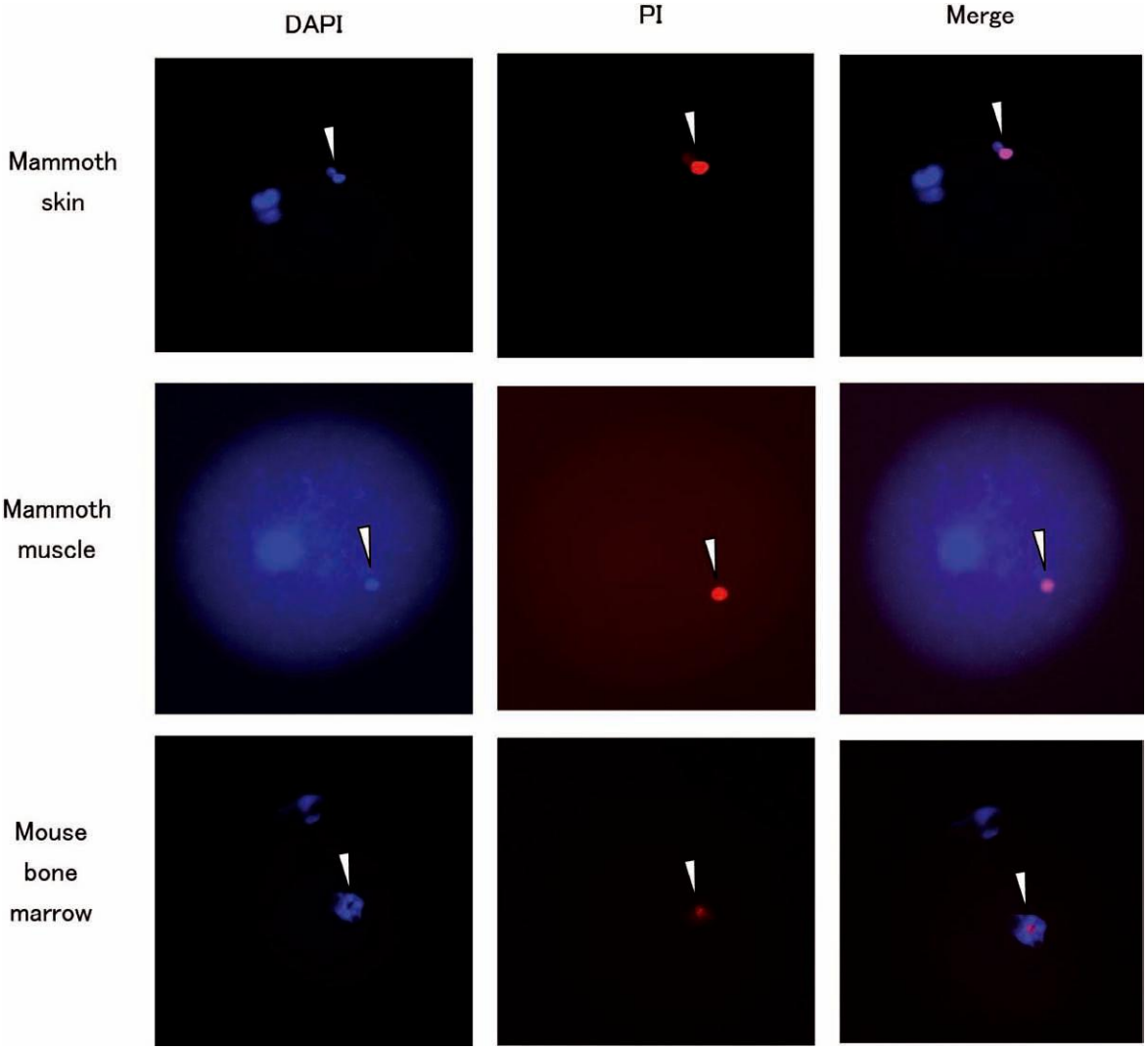


Nuclei isolati dai tessuti di mammut



Trapianto nucleare di nuclei di mammut in oociti enucleati di topo



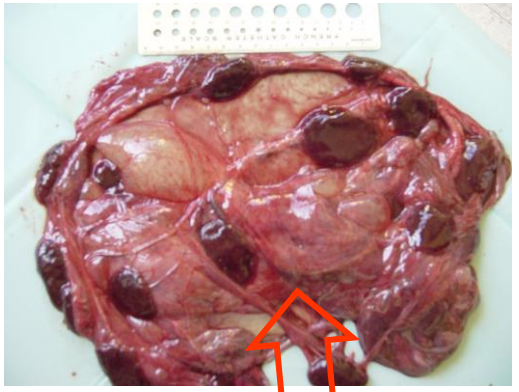

DAPI
PI
Merge
Mammoth skin
Mammoth muscle
Mouse bone marrow

Altro problema: Disponibilità di femmine riceventi per l'embryo transfer

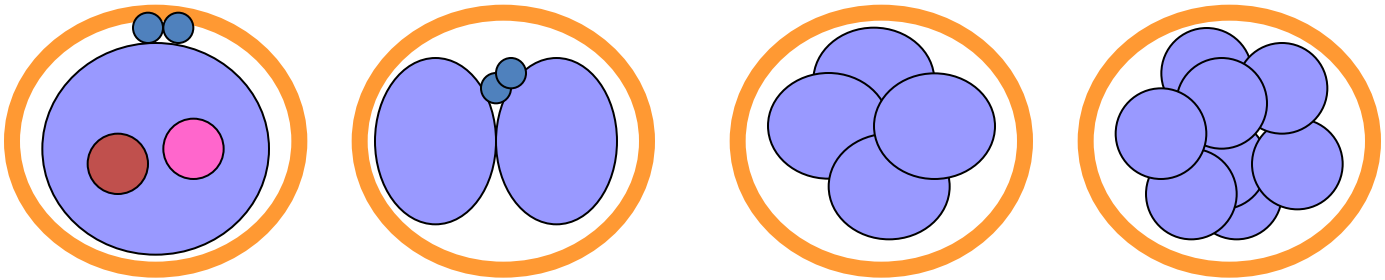
Semplice nel caso pecora-muflone……..

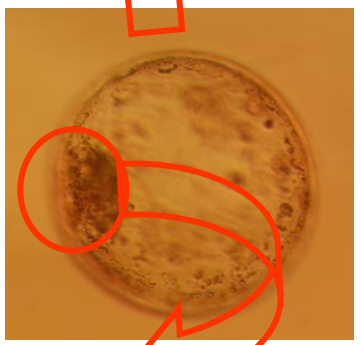
…….si può evitare il rigetto immunologico nel caso di trapianto embrionale intraspecifico?.....

Trofoblasto dà origine
alla placenta

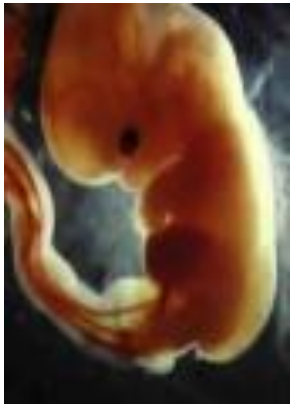


Sviluppo embrionale giorni 0-7

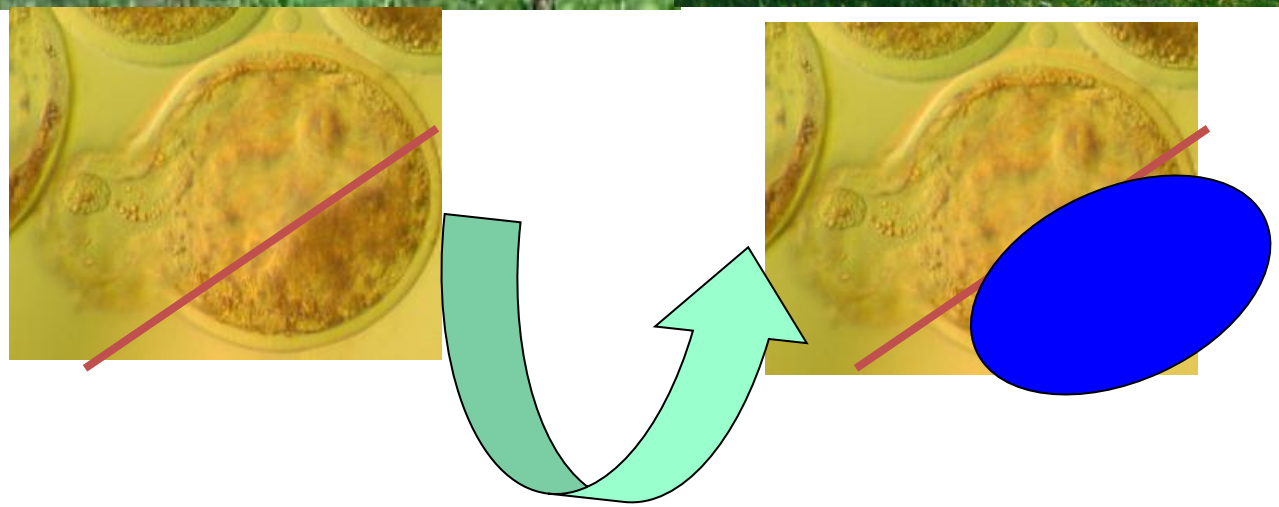




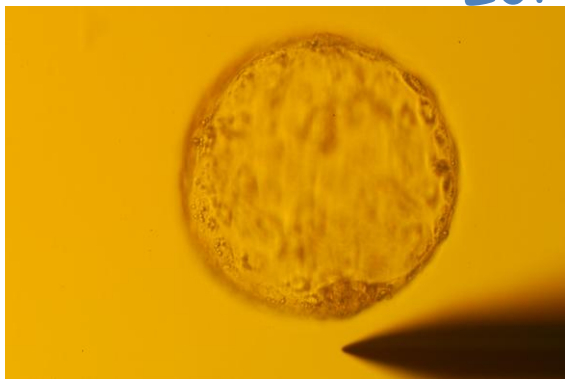
Bottone embrionario
dà origine al feto

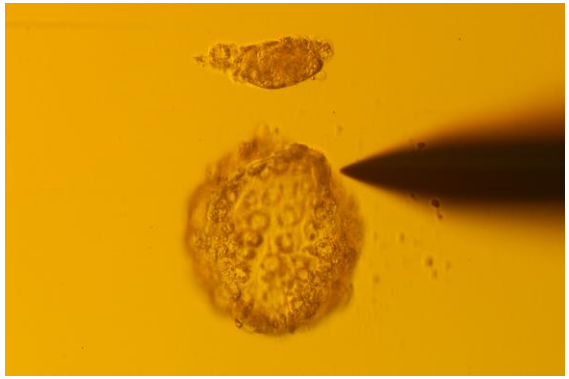


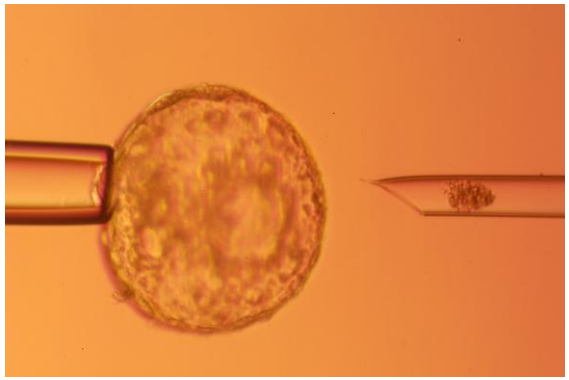
Si può trasferire un embrione di daino Nell'utero di una pecora?

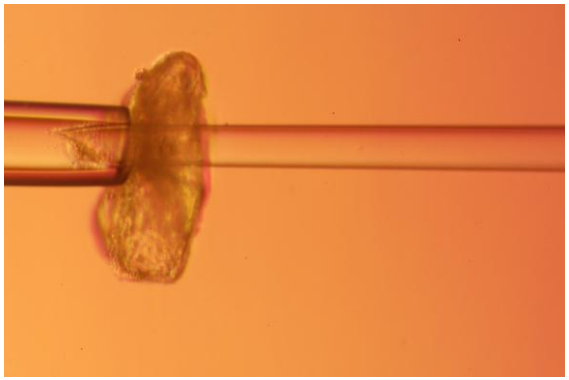


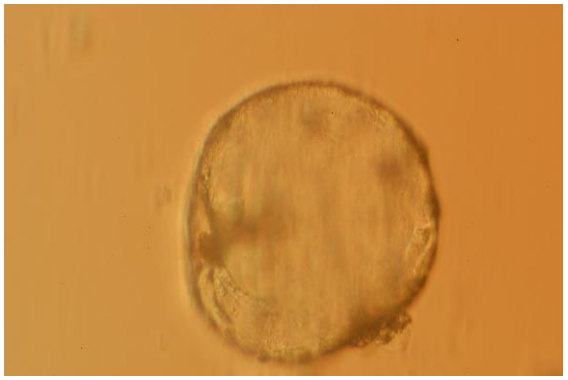
Microchirurgia per il trapianto del bottone embrionario
Loi P. et al., Trends in Biotechnology 2007

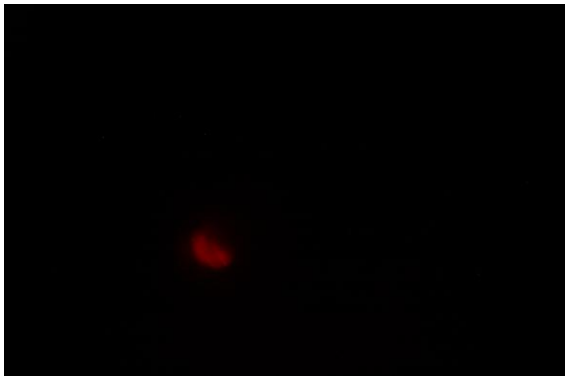






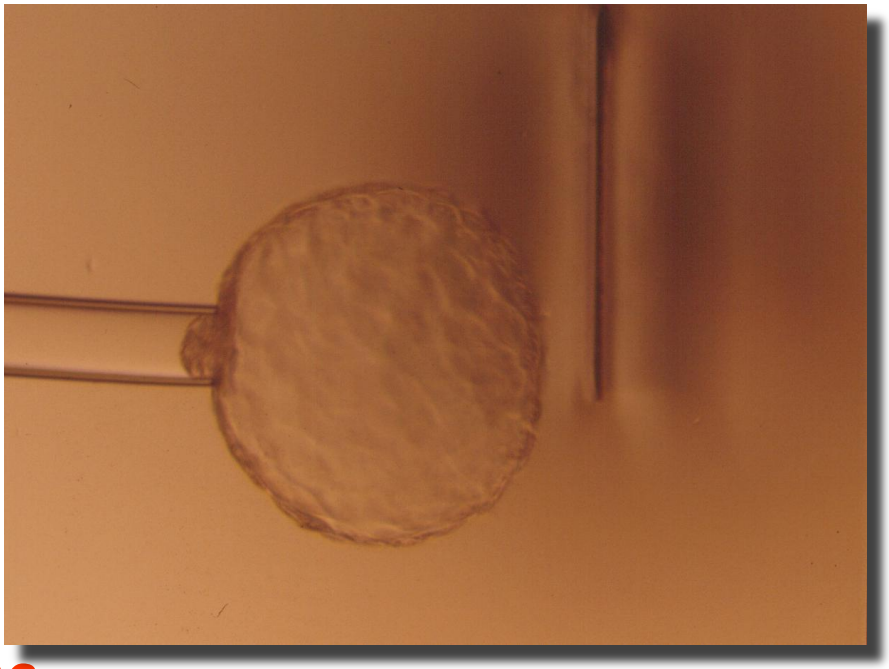






Scambio di trofoblasto tra blastocisti di pecora

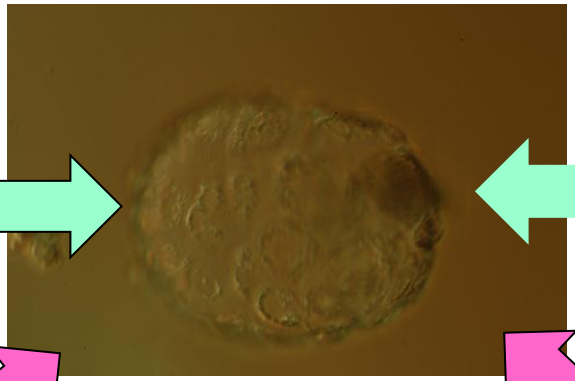
Loi P. et al., submitted



7 blastocisti ricostruite trasferite su riceventi sincrone

3 agnelli nati

Blastocisti al giorno 7 di sviluppo



…..il sogno…….

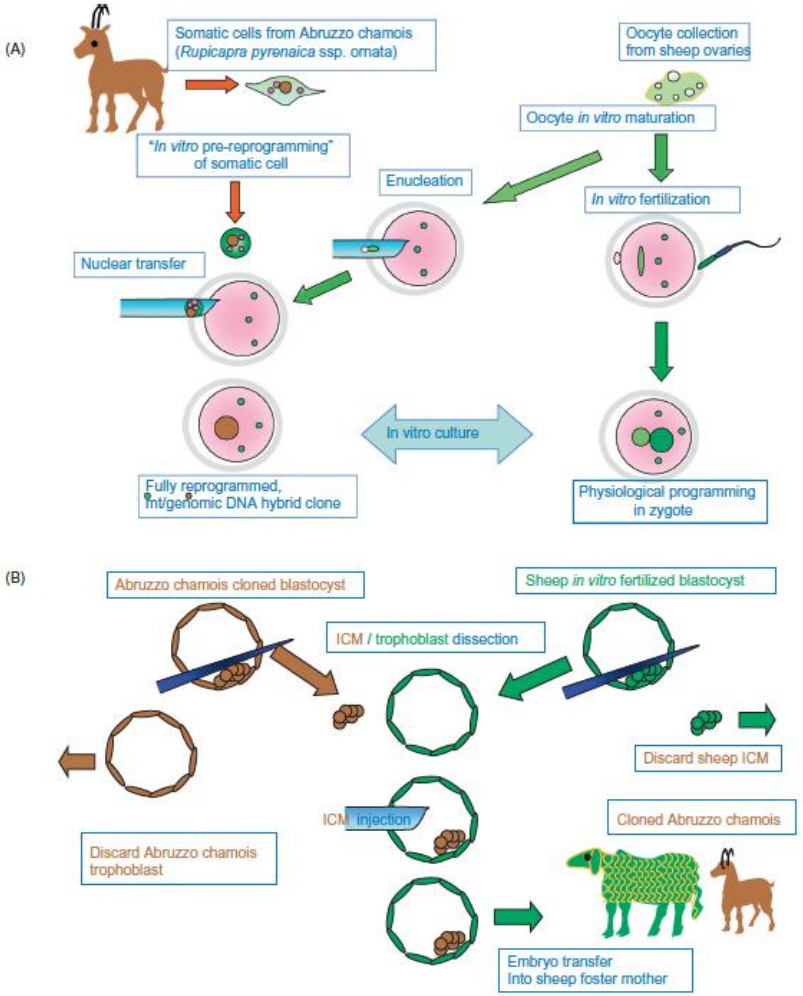


FIGURE 28.2 "Idealized" procedure for cloning endangered animals (A) Step 1: nuclear transfer. (B) Step 2: ICM–trophoblast exchange and interspecies embryo transfer. ICM, inner cell mass.

ACKNOWLEDGEMENTS

This work was supported by the program "Ideas" of the European Research Council, project "Angioplace," to G. Ptak; and the 7 FP EU project "NextGen," PRIN 2009 Miur, Tercas Foundation to P. Loi.

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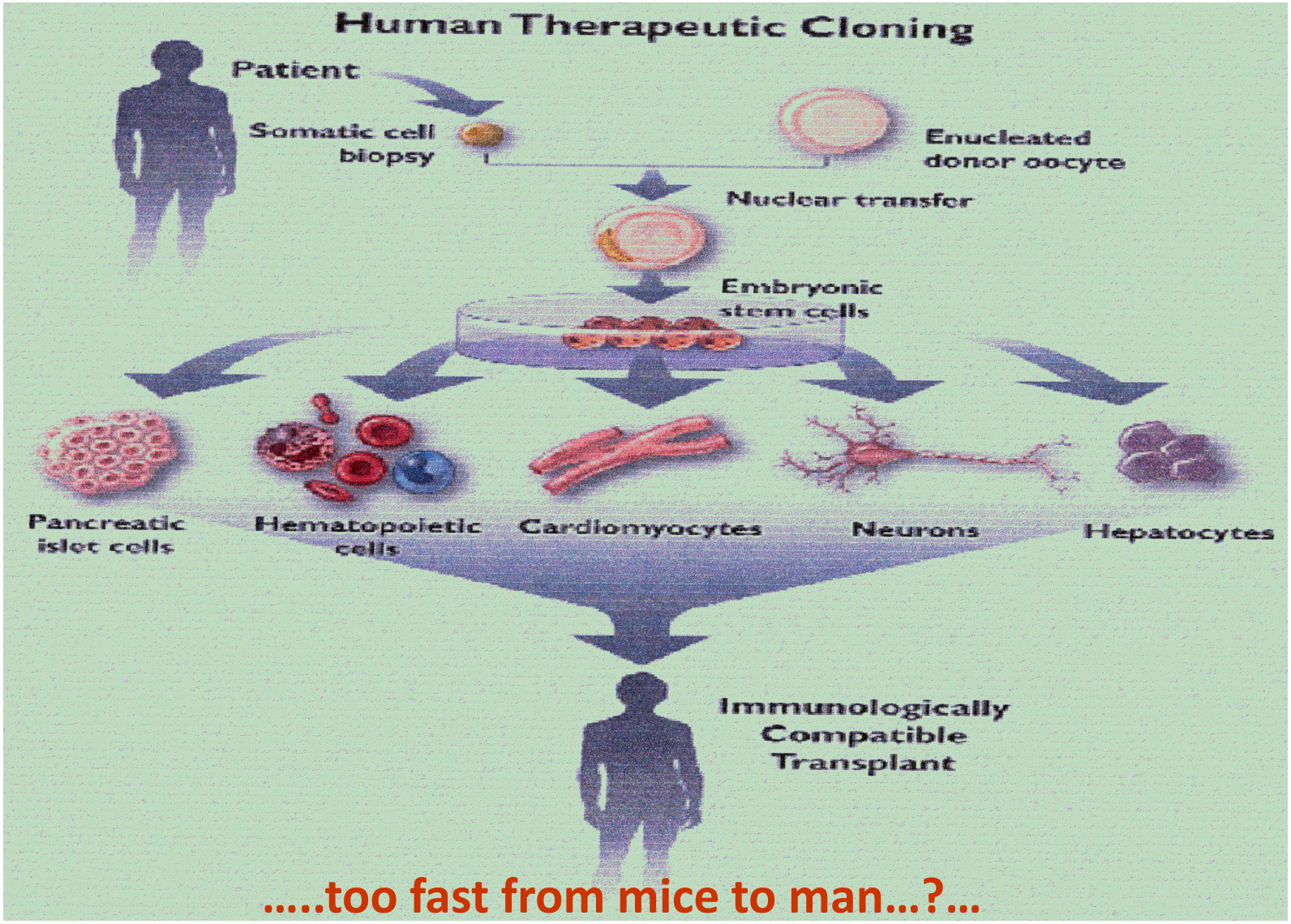
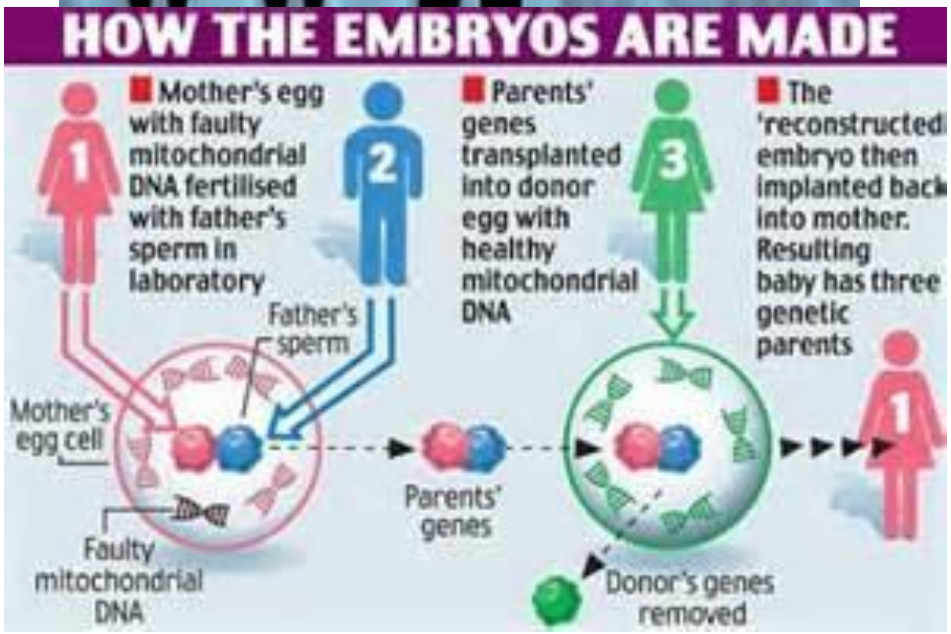


…..too fast from mice to man…?…

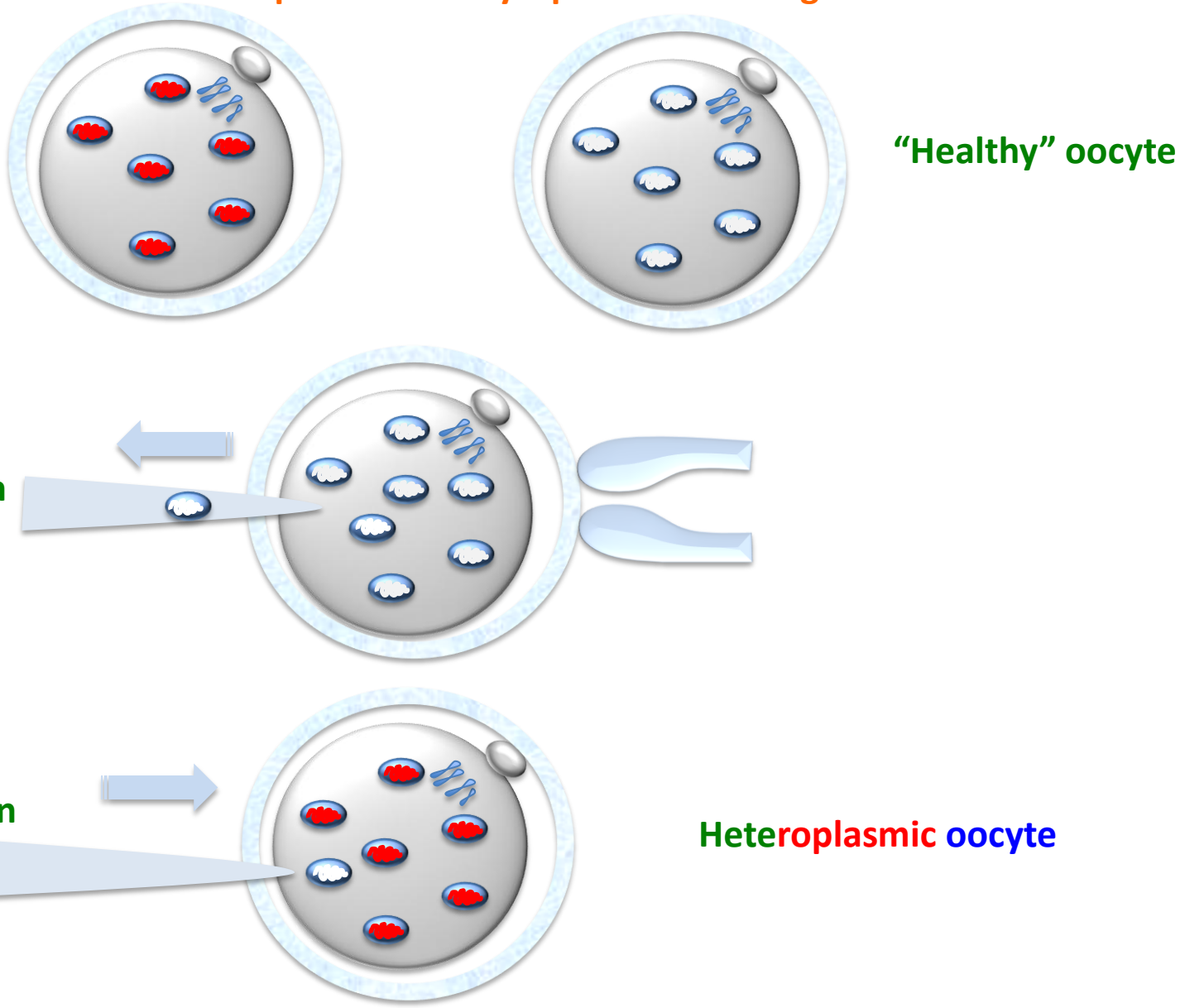
From: Lanza et al., Nature Medicine 5, 975-977 (1999)

“Three parents” babies….?
Prevenzione patologie dovute a mutazioni del DNA mitocondriale

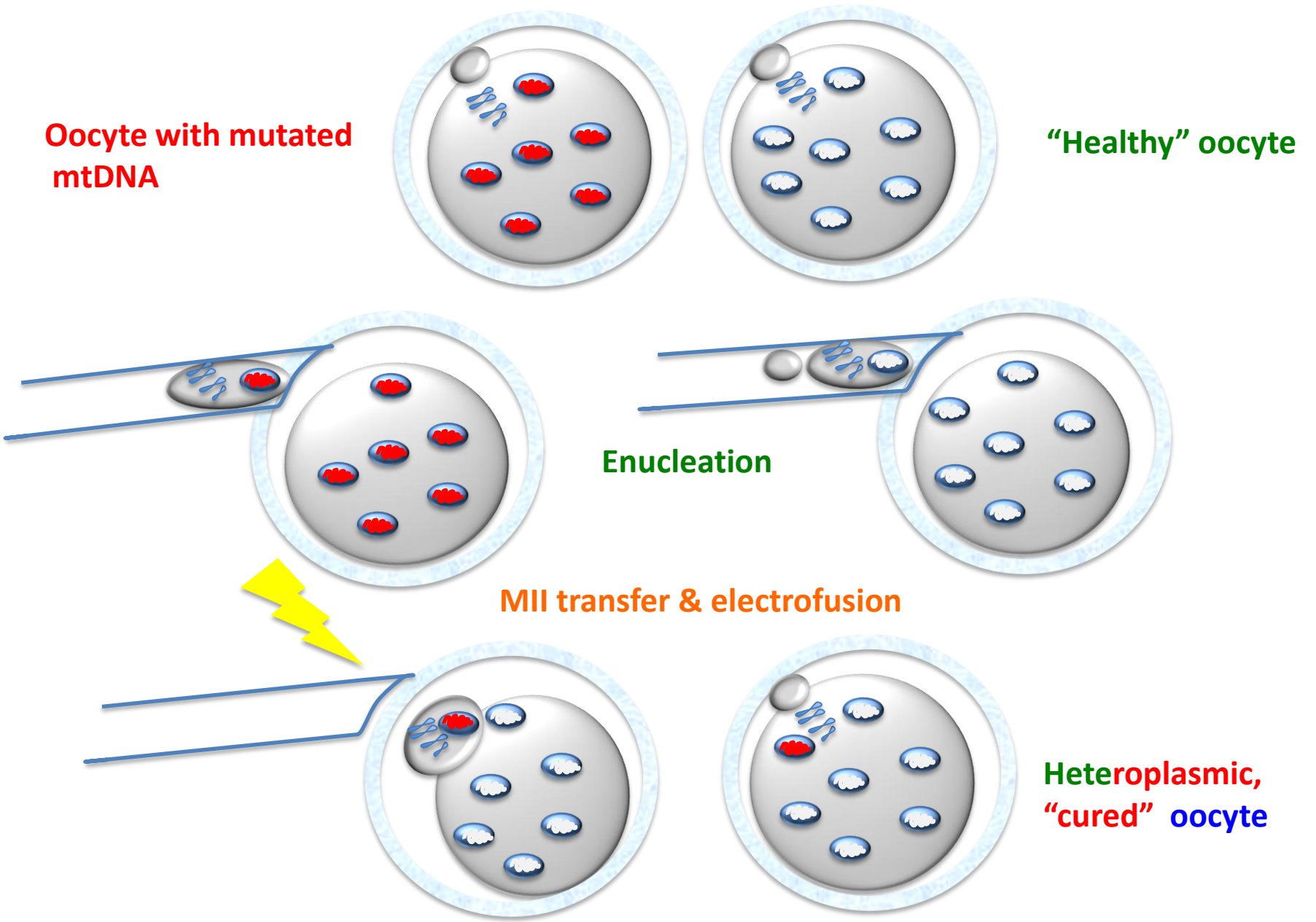


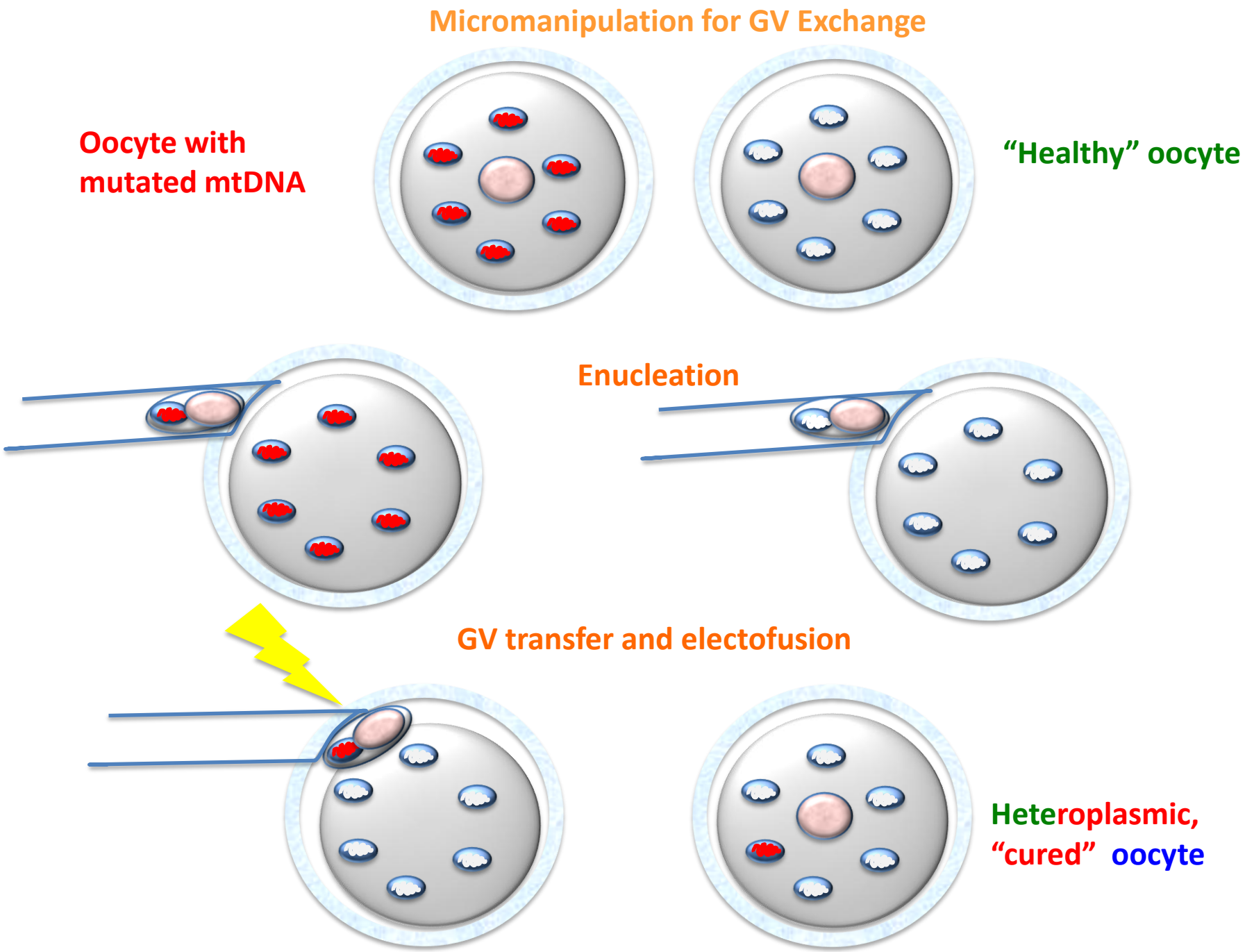
Lynsey Cree & Pasqualino Loi, Molecular Human Reproduction, in press

Micromanipulation for Cytoplasmic exchange



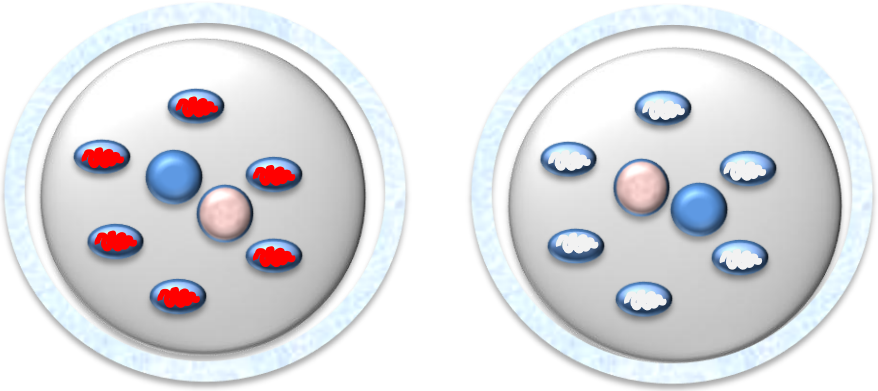
Micromanipulation for MII transfer




Micromanipulation for GV Exchange
Oocyte with mutated mtDNA
"Healthy" oocyte
Enucleation
GV transfer and electofusion
Heteroplasmic, "cured" oocyte

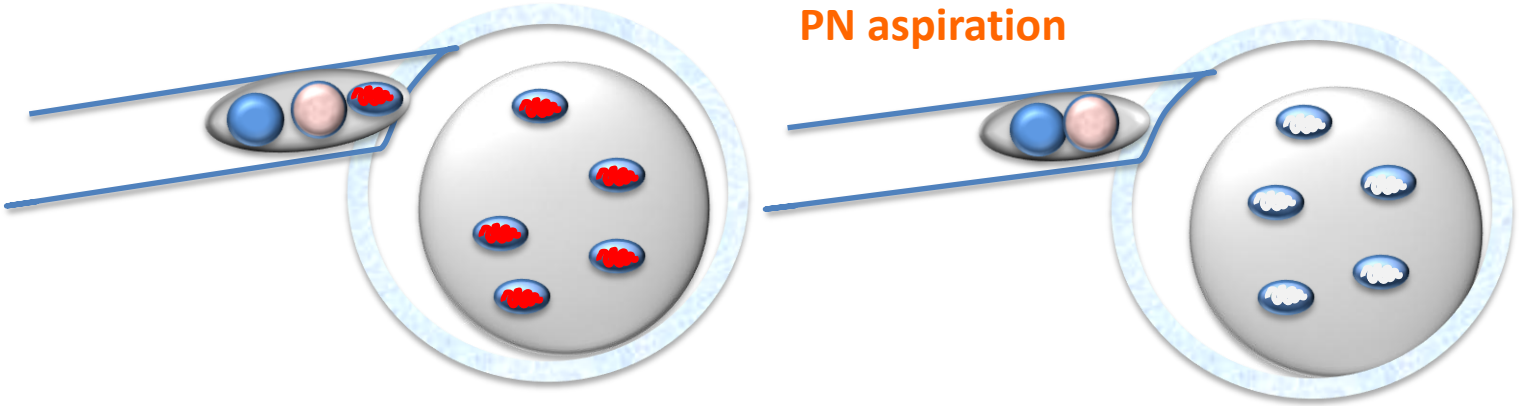
Micromanipulation for PN exchange

Zygote with mutated mtDNA

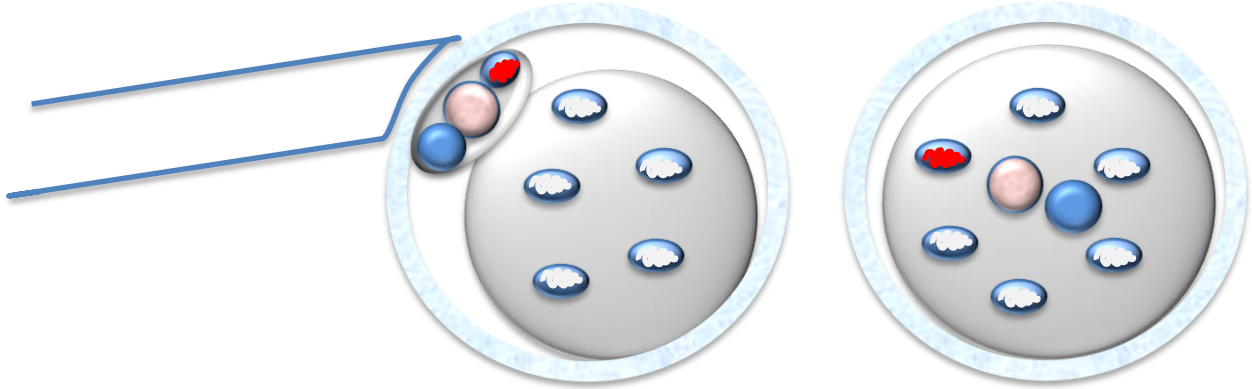


"Healthy" zygote

PN aspiration



PN transfer & electrofusion



Heteroplasmic, "cured" zygote

Altre opzioni per indurre la riprogrammazione nucleare (nuclear remodelling)

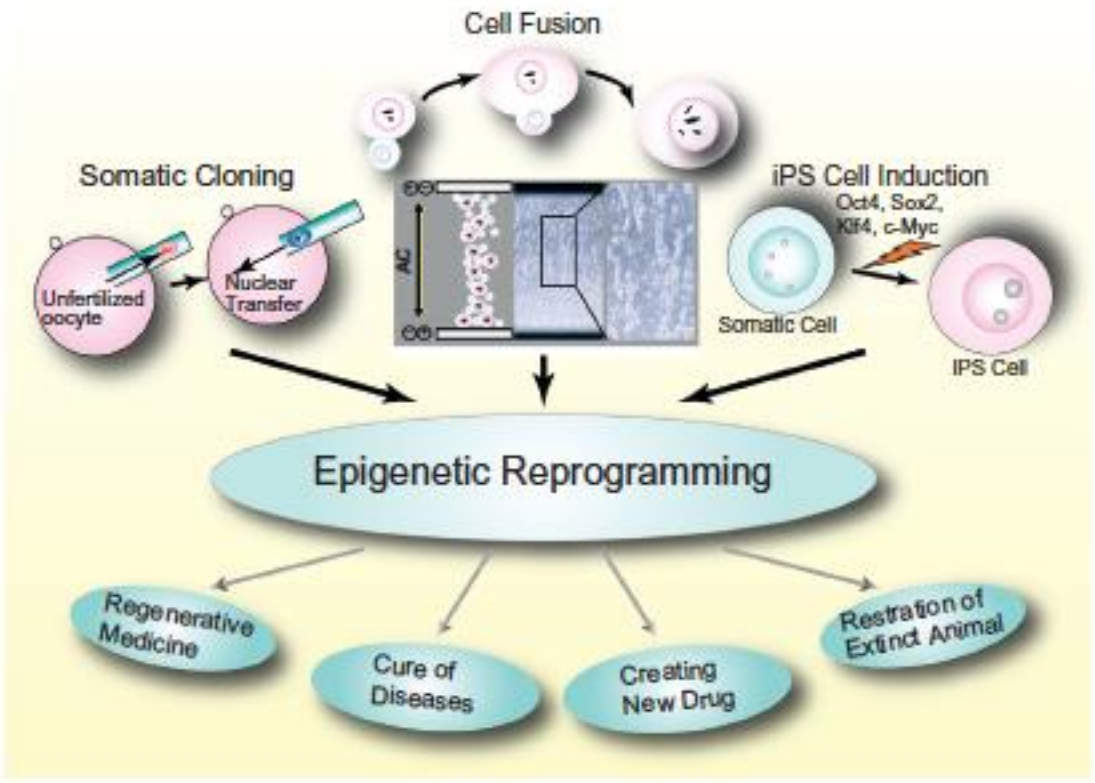
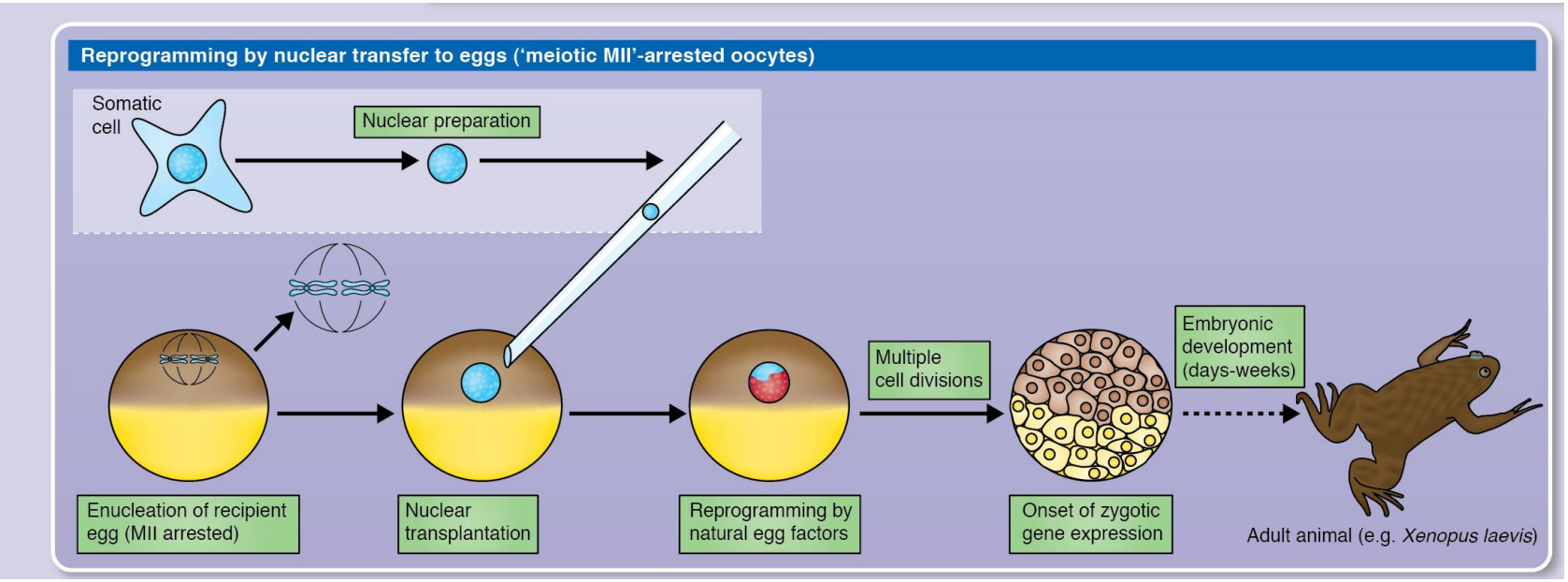


FIGURE 2.1 Epigentic reprogramming by somatic cloning, cell fusion, and iPS cell induction. Somatic cloning is a technique for generating cloned animals, while iPS cell induction is a technique for generating pluripotent stem cells *in vitro*. The technology of epigenetic reprogramming can contribute not only to regenerative medicine but also to many other subjects, including the cure of diseases, diagnosis of diseases, screening of drugs, and restoration of extinct animals.

Riprogrammazione con trapianto nucleare su oocita (canonico)



Riprogrammazione indotta da fattori fisiologici dell'oocita: alta efficienza

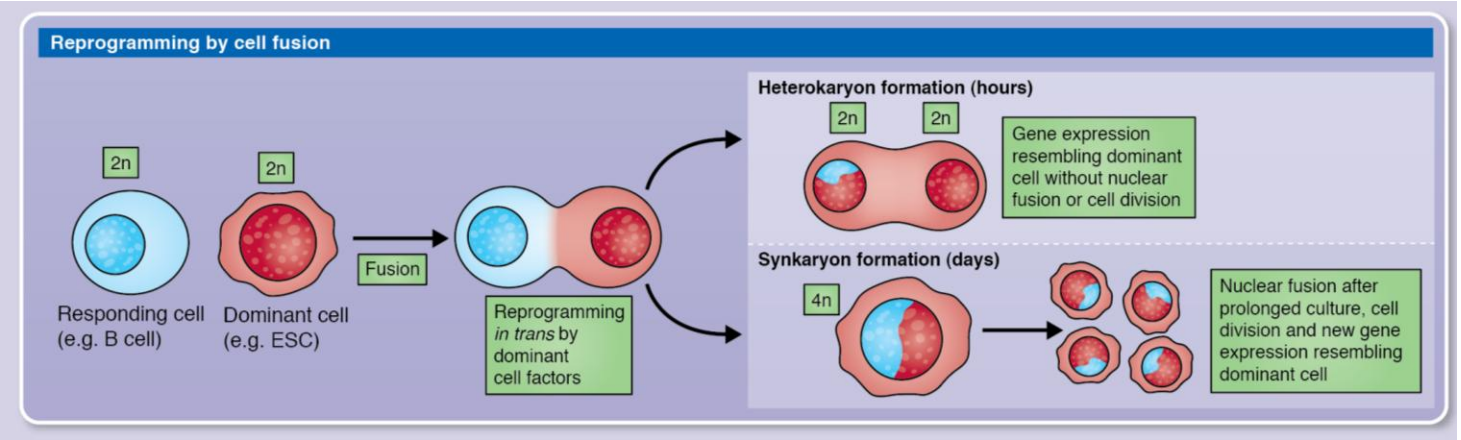
Riprogrammazione sino a sviluppo embrionale, comprese ICM cells (stem cells): alta

Riprogrammazione sino a sviluppo a termine (nati): bassa, alta incidenza anomalie epigenetiche

Impegno tecnico e costi: basso

Applicazioni : clonazione riproduttiva, clonazione terapeutica (isolamento stem cells Per terapia rigenerativa), modelli sperimentali

Riprogrammazione mediate fusione cellulare: stem cell + cellula differenziata

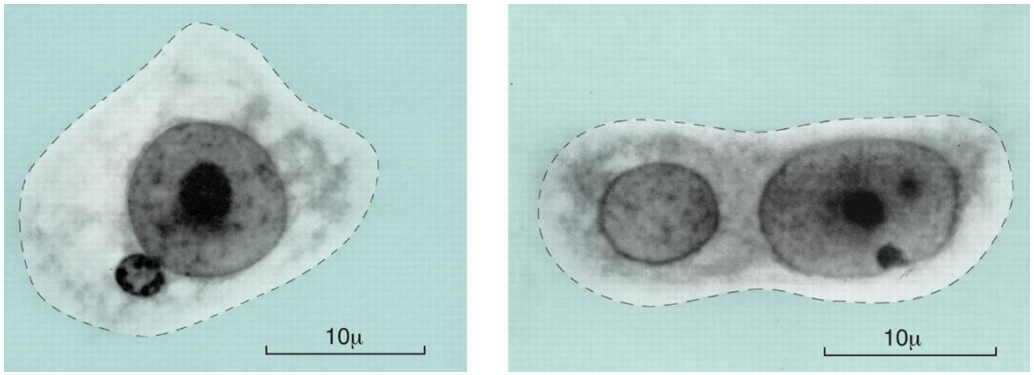


Fusione indotta con glicole etilenico

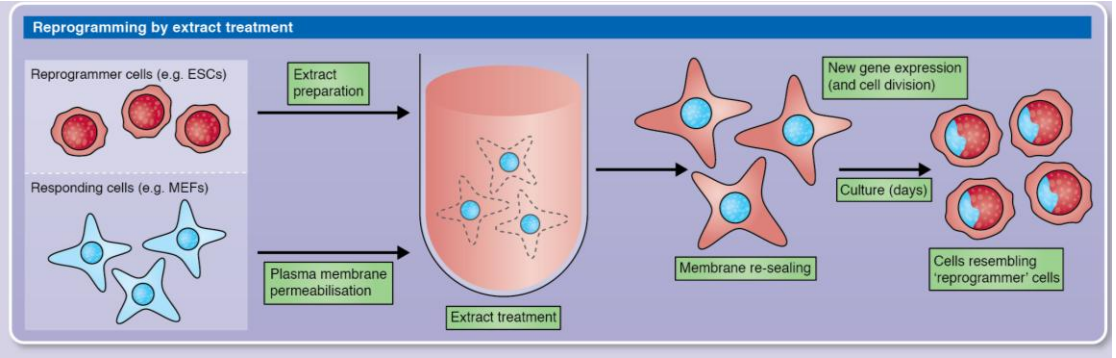
Riprogrammazione in trans da fattori di staminalità della stem cells

Impegno tecnico e costi: basso

Applicazioni: cellule tetraploidi alla fine del processo, solo ricerca di base



Riprogrammazione con estratti cellulari (Philippe Collas, 2006)



Esposizione di cellule differenziate (previa permeabilizzazioone membrane con streptolisina O) a estratti di stem cells

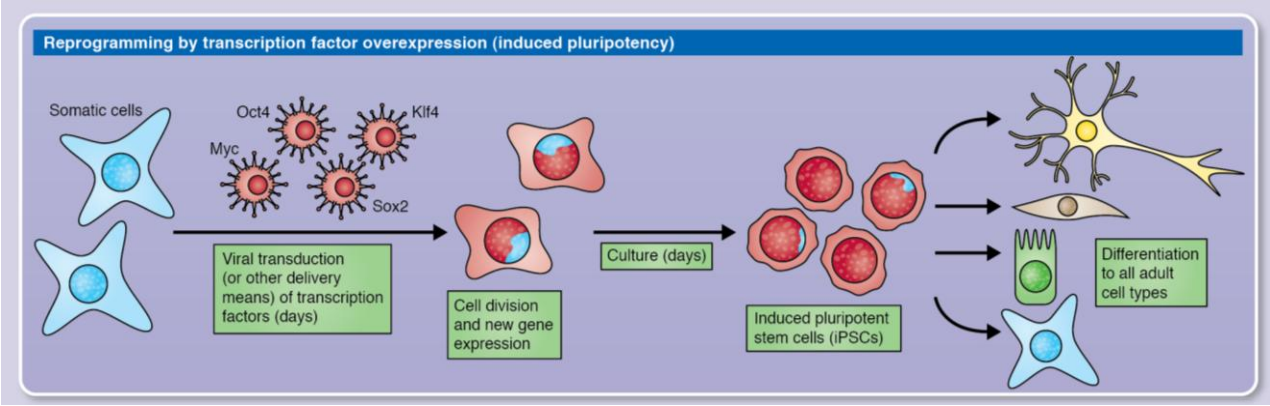
Riprogrammazione in trans dovuta a mRNA di geni plutipotenti e loro prodotti

Efficienza: bassa

Impegno tecnico e costi: tecnicamente molto complessa, basso costo

Applicazioni: ricerca di base

Inducible plutipotent cells (IPS); Yamanaka, 2006



Riprogrammazione indotta da geni di pluripotenza: Oct4, Sox2, C-Myc, Kfl4

Efficienza di riprogrammazione: bassa

Riprogrammazione sino a generare nati: si, solo con chimerismo, molto bassa

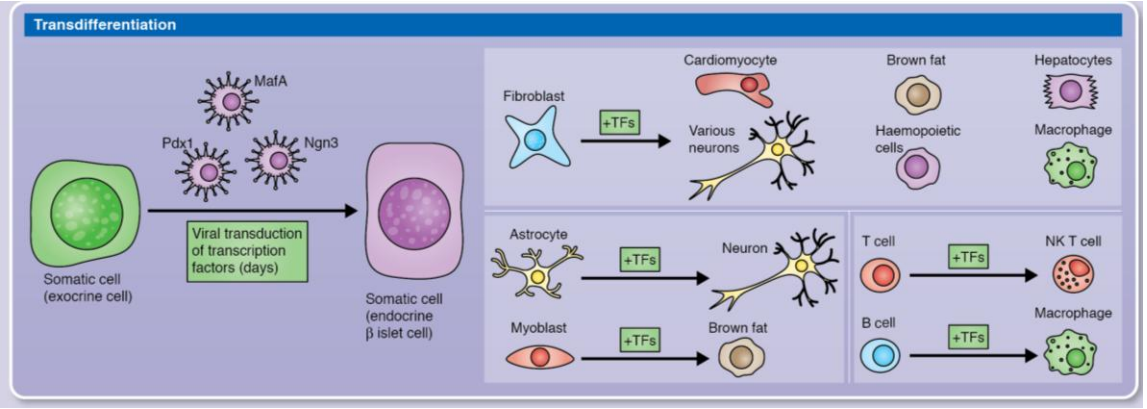
Impegno tecnico: alto

Rischi: C-Myc potenziale carginogeno, le IPS integrano i quattro vettori nel genoma, Alterazioni della metilazione

Applicazioni: terapia rigenerativa (***), drug screening, modelli di patologia

Per terapia degenerativa: nessun transgene, si usa mRNA o i prodotti dei geni Oct4, Sox2, C-Myc, Kfl4

Riprogrammazione per “Trans-differenziazione”



Transformazione di una cellule differenziata in un’altra di derivazione embrionale Diversa mediante transfezione di costrutti (virali) codificanti per fattori di trascrizione Tessuto-specifici

Efficienza: abbastanza alta

Impegno tecnico e costi: alto

Applicazioni: medicina rigenerativa, ricerca di base

Premio Nobel 2012 per la medicina a John Gurdon e Yamanaka

IPS

Nuclear Transfer

http://www.youtube.com/watch?v=cPvidAvzmx0