

Università di Roma
Tor Vergata

Rigenerazione e Cellule staminali

Gargioli Cesare

Perchè tanta attenzione nei media?

Le staminali contro il Parkinson
Un trapianto di cellule e il paziente non ha più tremori

Per il piccolo Roberto, 11 anni, la vita è un miracolo. Il Parkinson, una malattia che ha colpito il bimbo, è stato sconfitto. Il trapianto di cellule staminali ha fatto sì che il piccolo non abbia più tremori e possa vivere una vita normale. Il caso è stato riportato dalla stampa internazionale, attirando l'attenzione dei media.

Torino, miracolo staminali per un bimbo paralizzato

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WOODY ALLEN
and
DIANE KEATON
in
Sleeper



Giulio Bizzozzero:

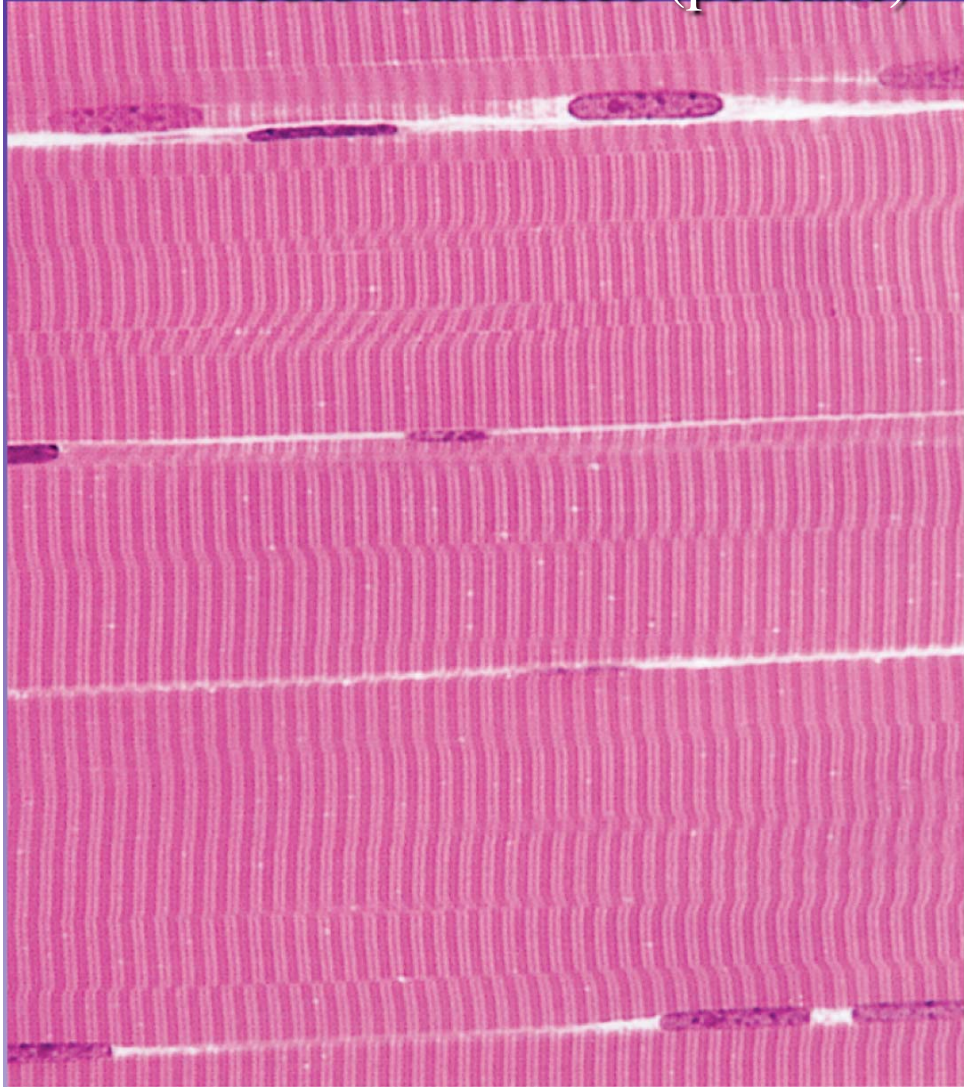
cellule labili, stabili e perenni

- **Giulio Bizzozzero** (1846-1901, Professore di Patologia all' Università di Torino) scoprì che l'eritropoiesi/leucopoiesi ha luogo nel midollo osseo [1868]; scoprì le piastrine. Per di più, **Bizzozzero** comprese che il rinnovamento cellulare varia nei diversi tessuti che conseguentemente classificò in:
 - **labili** (es. midollo osseo, epidermide)
 - **stabili** (es. fegato)
 - **perenni** (es. tessuto nervoso, cuore)

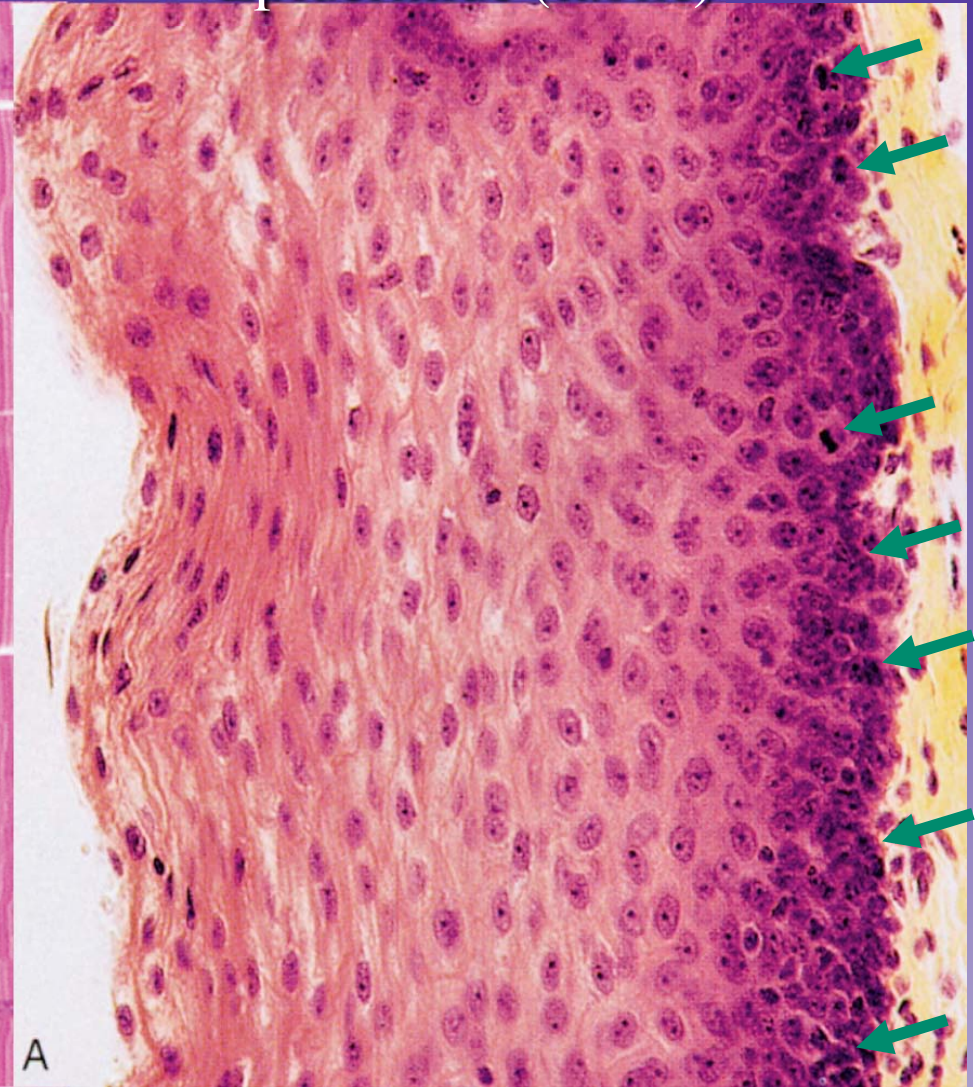


In alcuni tessuti si notano cellule in mitosi (labili), in altri no (perenni),
in altri ancora cellule in mitosi compaiono solo dopo un danno (stabili)

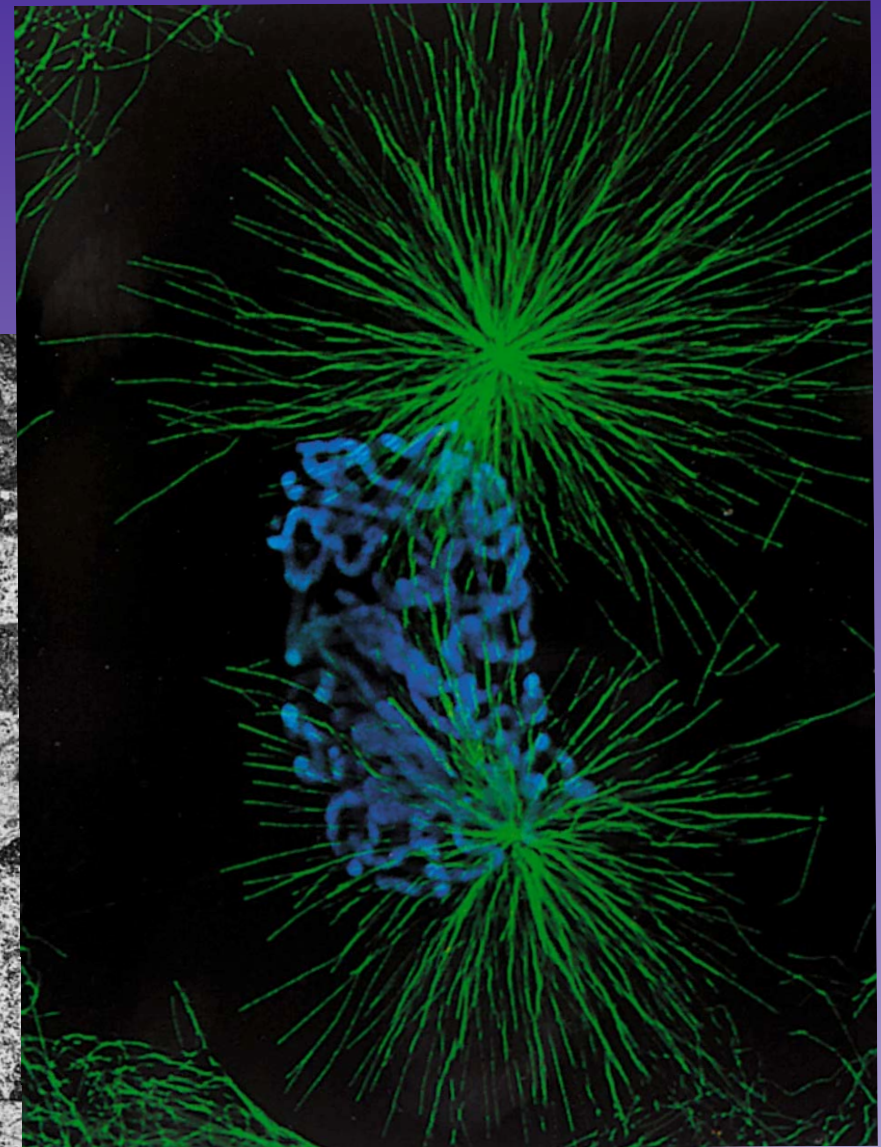
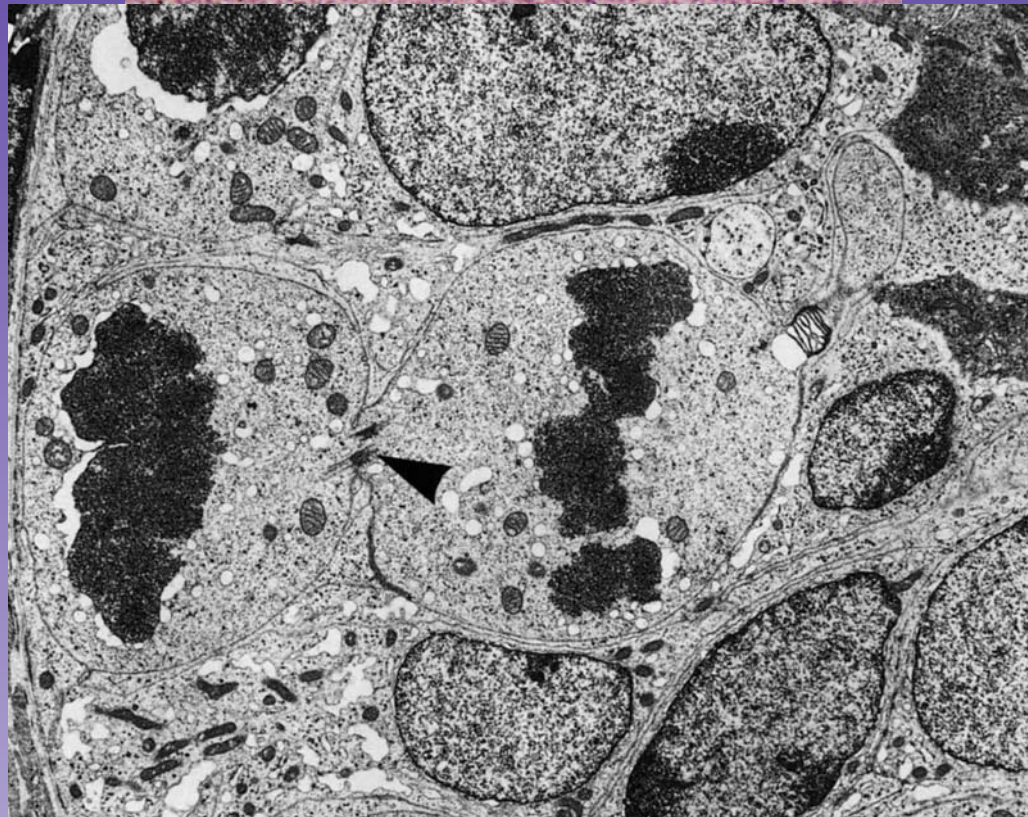
Muscolo scheletrico (perenne)



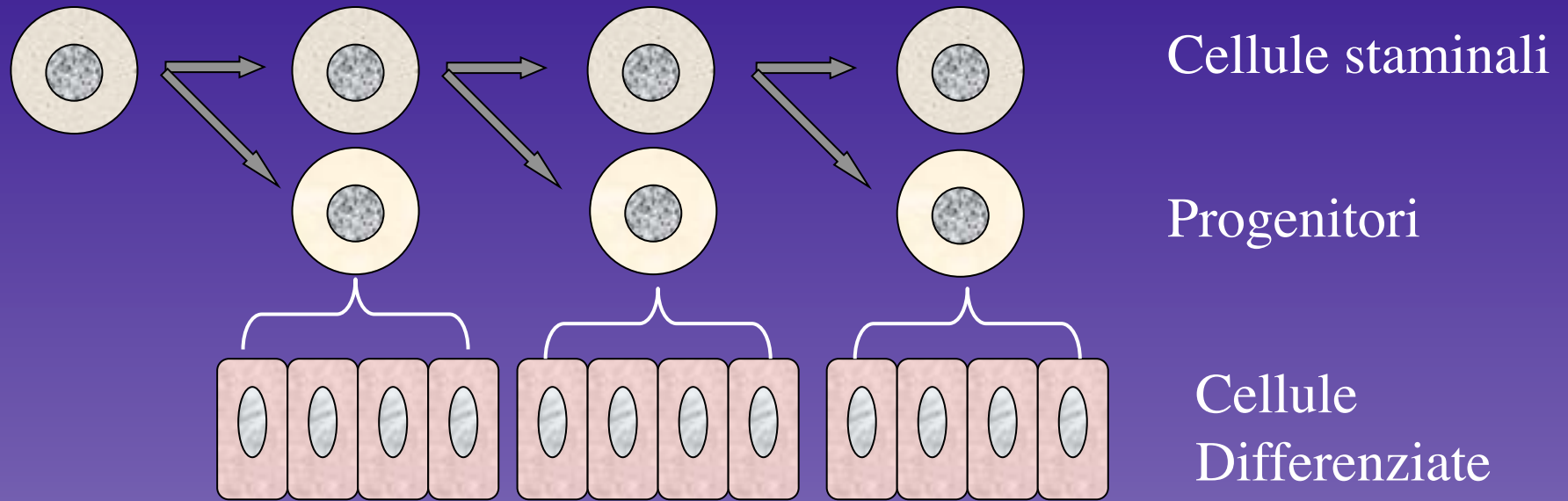
Epidermide (labile)



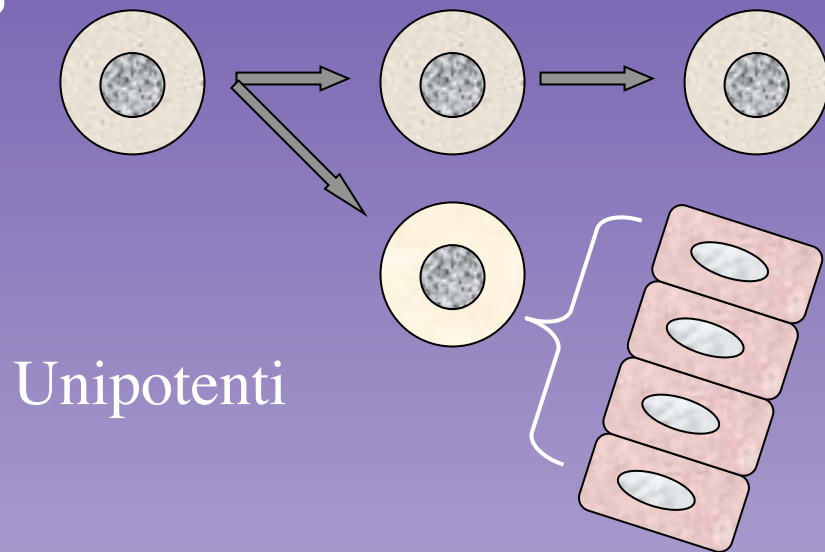
Oggi è facile osservare le cellule mentre si dividono



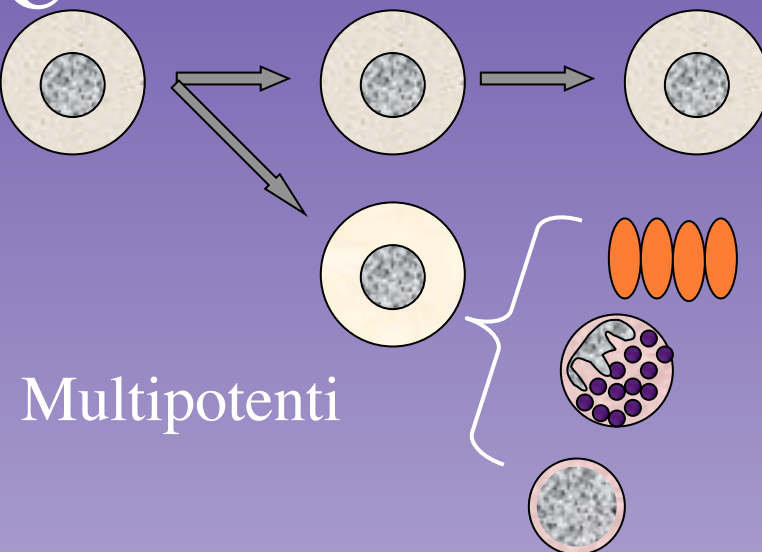
A



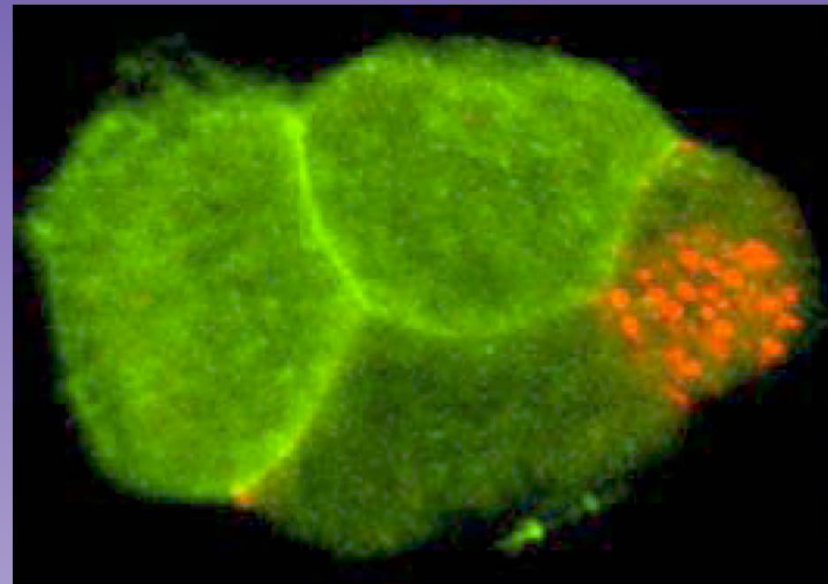
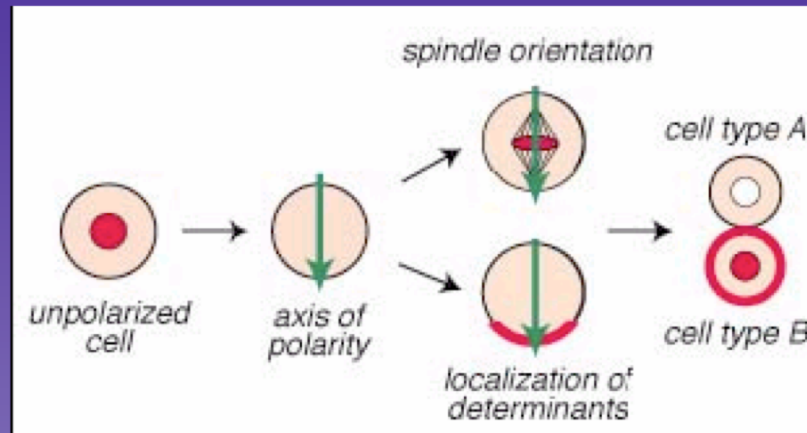
B



C



Divisioni cellulari simmetriche e asimmetriche



TWO TYPES OF STEM CELLS:

1. ADULT

(Also include fetal, extra-embryonic and cord blood stem cells)

They raise no ethical concerns but for certain tissues (and their diseases) their self-renewal and differentiation potency may not be adequate

2. EMBRYONIC

Derived from the blastocyst inner cell mass. Unlimited self-renewal and differentiation potency. There are still technical problems to be solved. However **they raise ethical concerns** and their use is made difficult or prohibited in certain countries.

The potency of stem cells

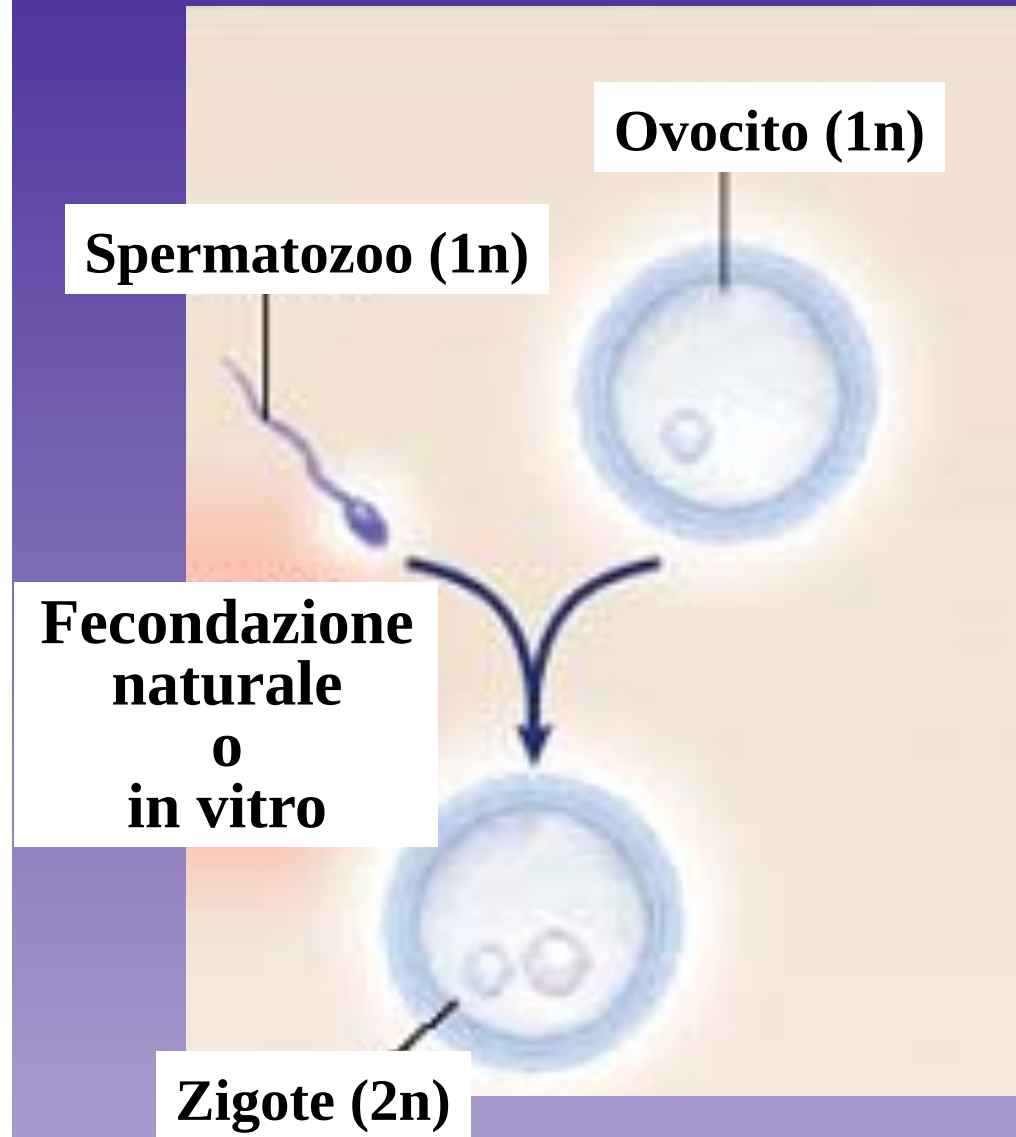
1. Totipotent: all embryonic and extra-embryonic tissues (blastomer)
2. Pluripotent: all embryonic tissues (ICM)
3. Multipotent: many different tissues (usually of same germ layer, after gastrulation)
4. Oligopotent: few tissues of one germ layer
5. Unipotent: only one tissue

Jonathan Slack: “Egg & Ego”

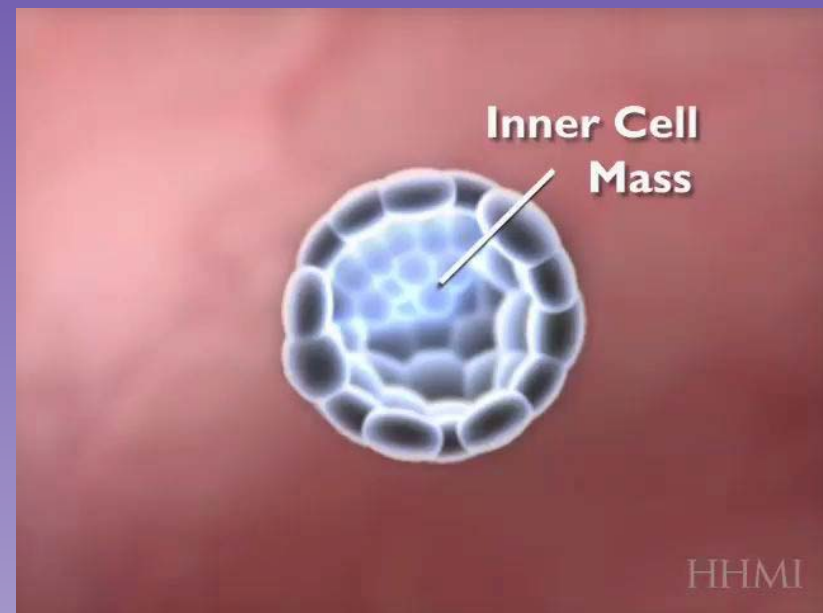
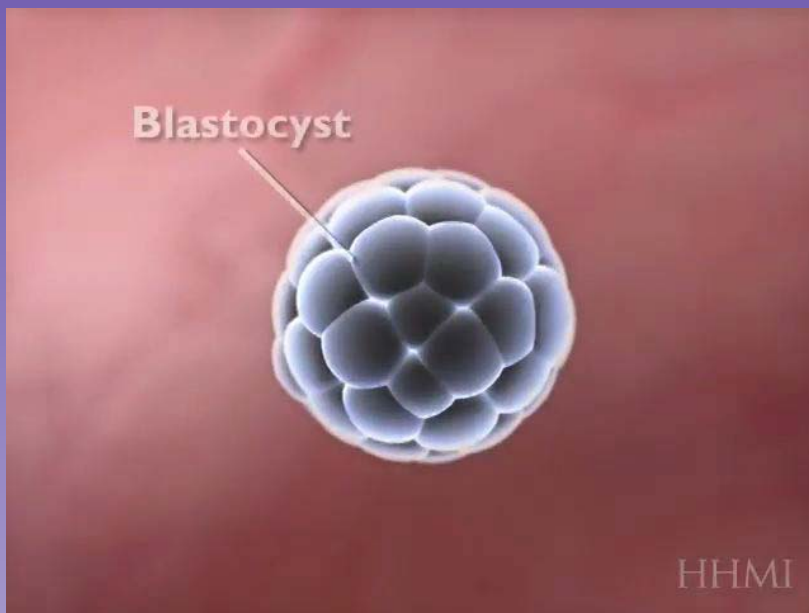
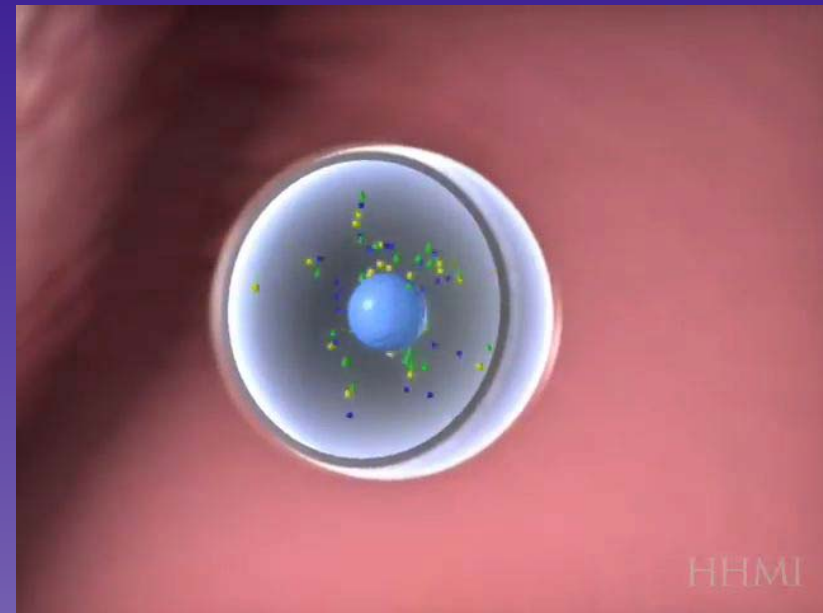
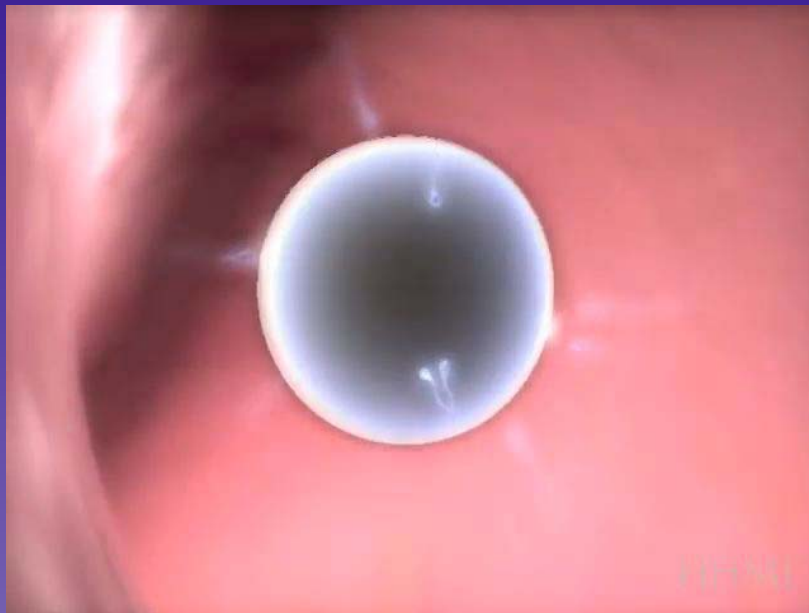
Springer, 2001

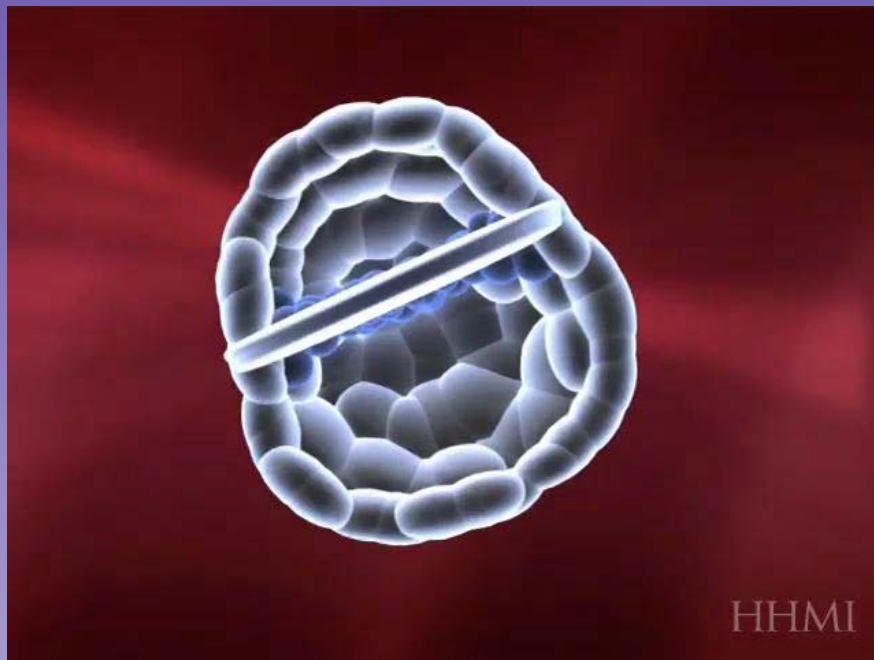
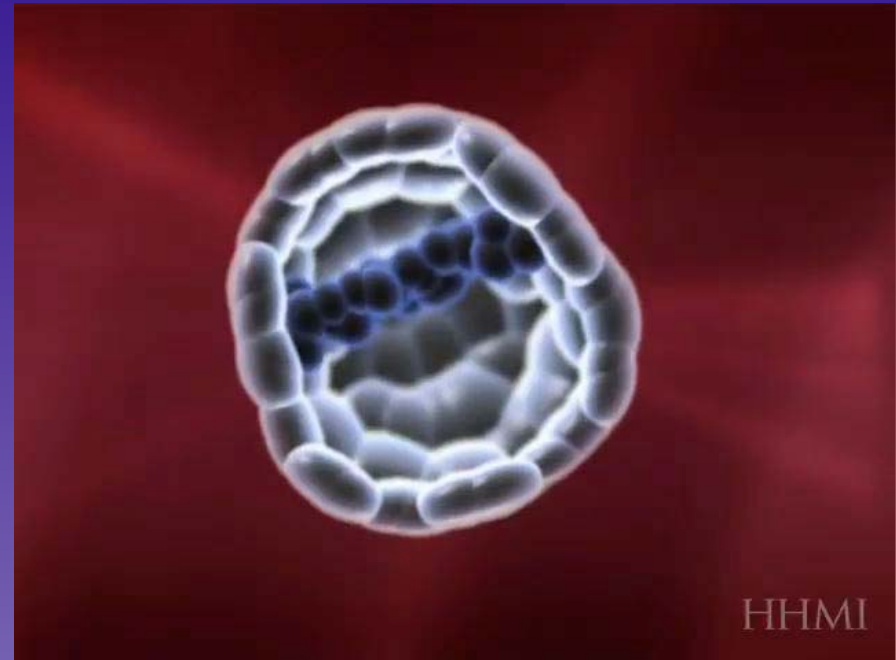
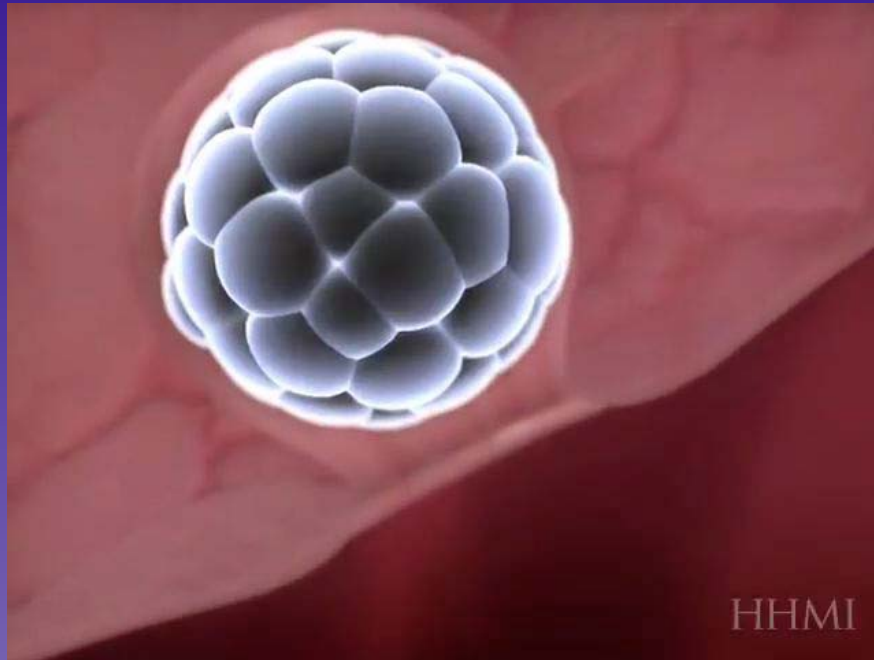
You can't fix it if you do not
know how it was put together
in the first place

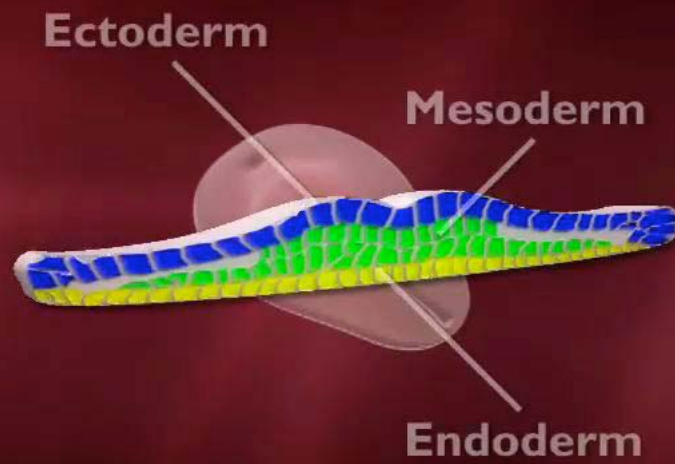
Fecondazione e formazione dello Zigote



Zigote (2n)







HHMI



HHMI

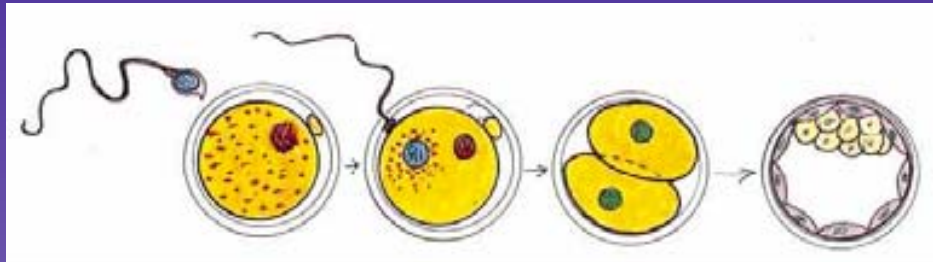


HHMI



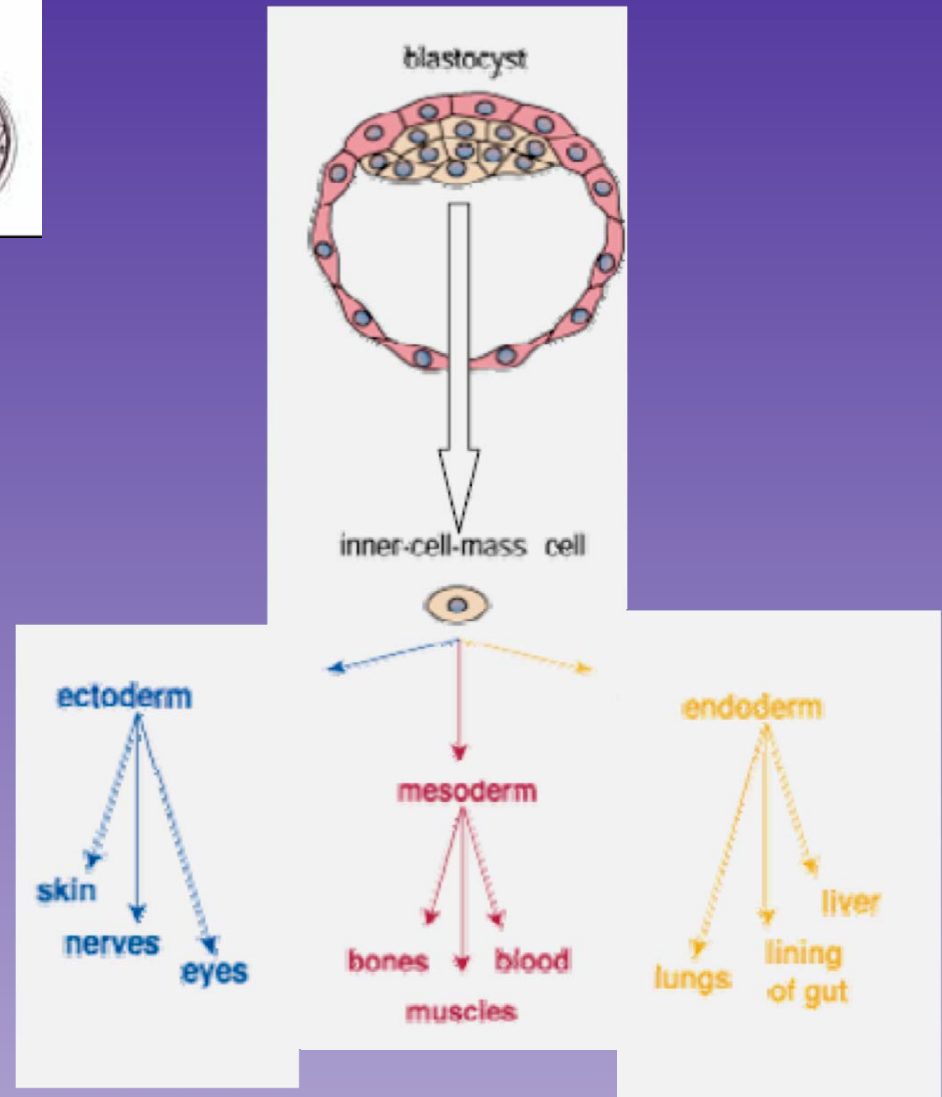
HHMI

Cellule staminali embrionali (ESC)



Box 3. Characteristics of embryonic stem cells

- Non-transformed
- Indefinite proliferative potential
- Stable diploid karyotype
- Clonality
- Formation of multi-differentiated tumours (teratomas) on ectopic transplantation
- Multi-lineage differentiation *in vitro*
- Permissive for genetic manipulation
- Incorporation in chimaeras (not practical for human)



Zigote

2 cellule

4 cellule

8 cellule

morula

blastocisti



0



24 ore



48 ore



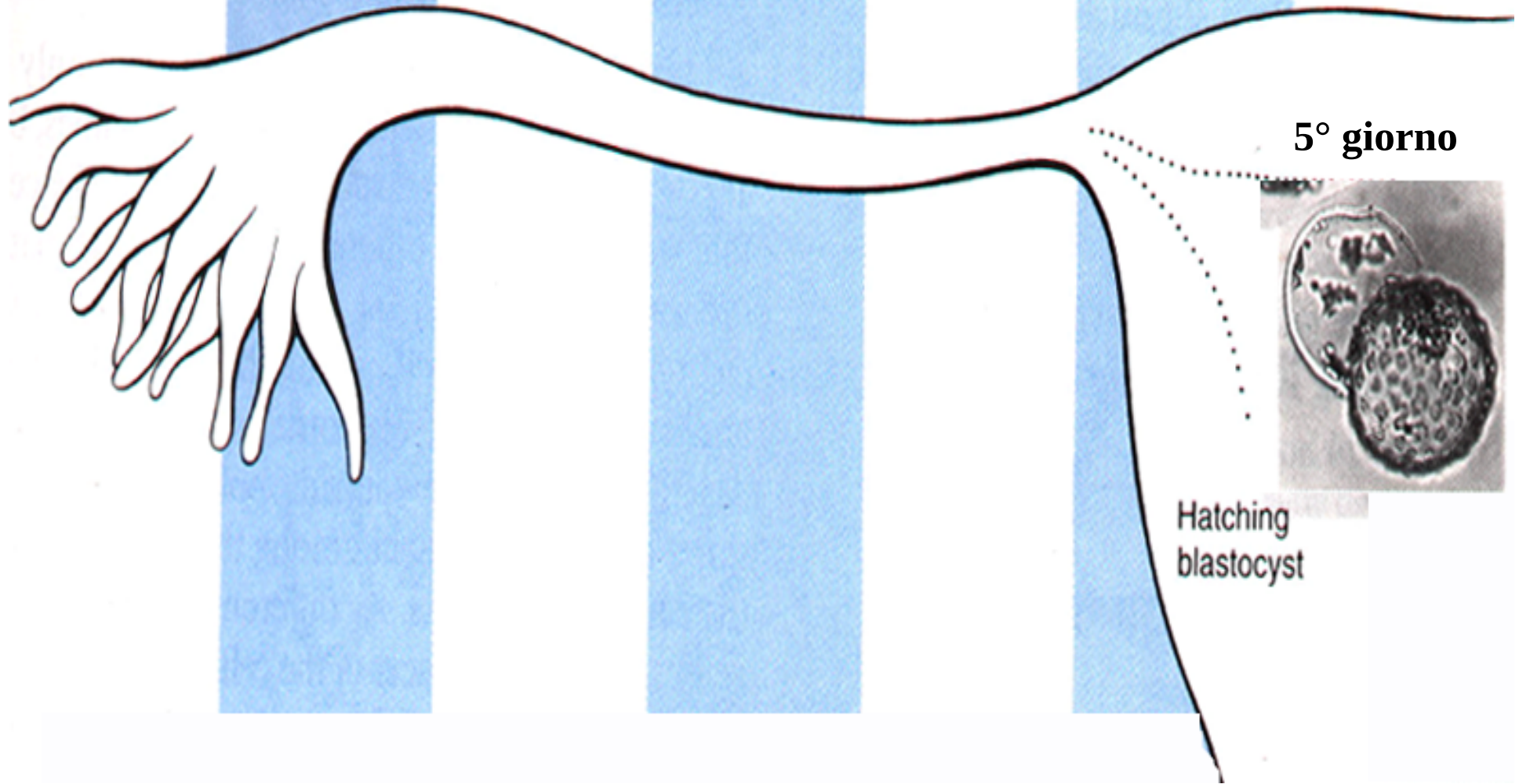
72 ore



96 ore



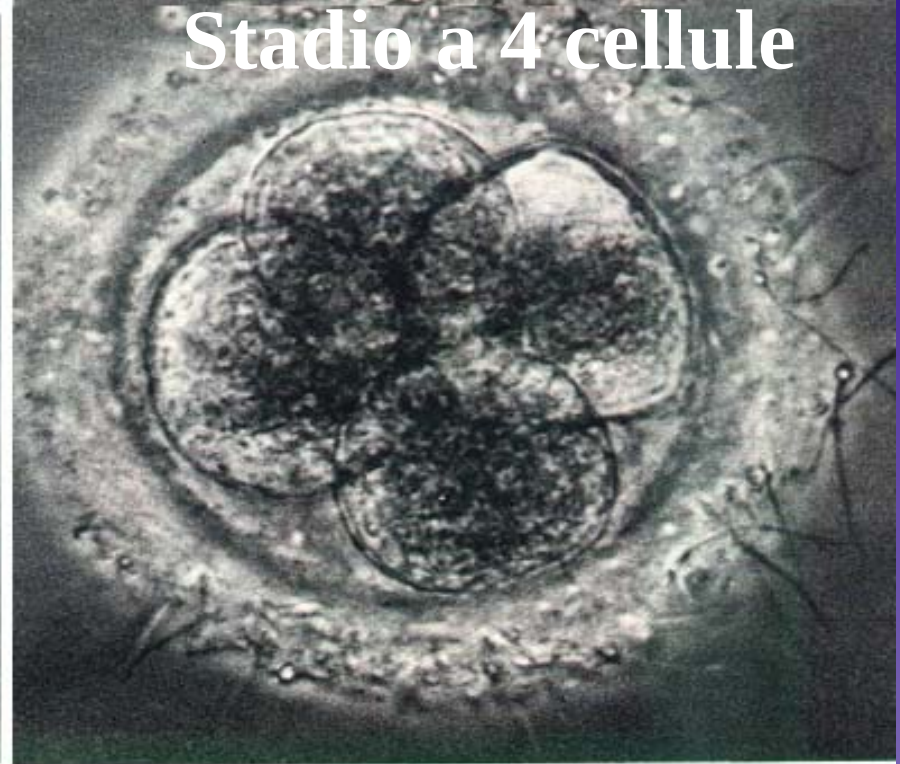
120 ore



Stadio a 2 cellule



Stadio a 4 cellule



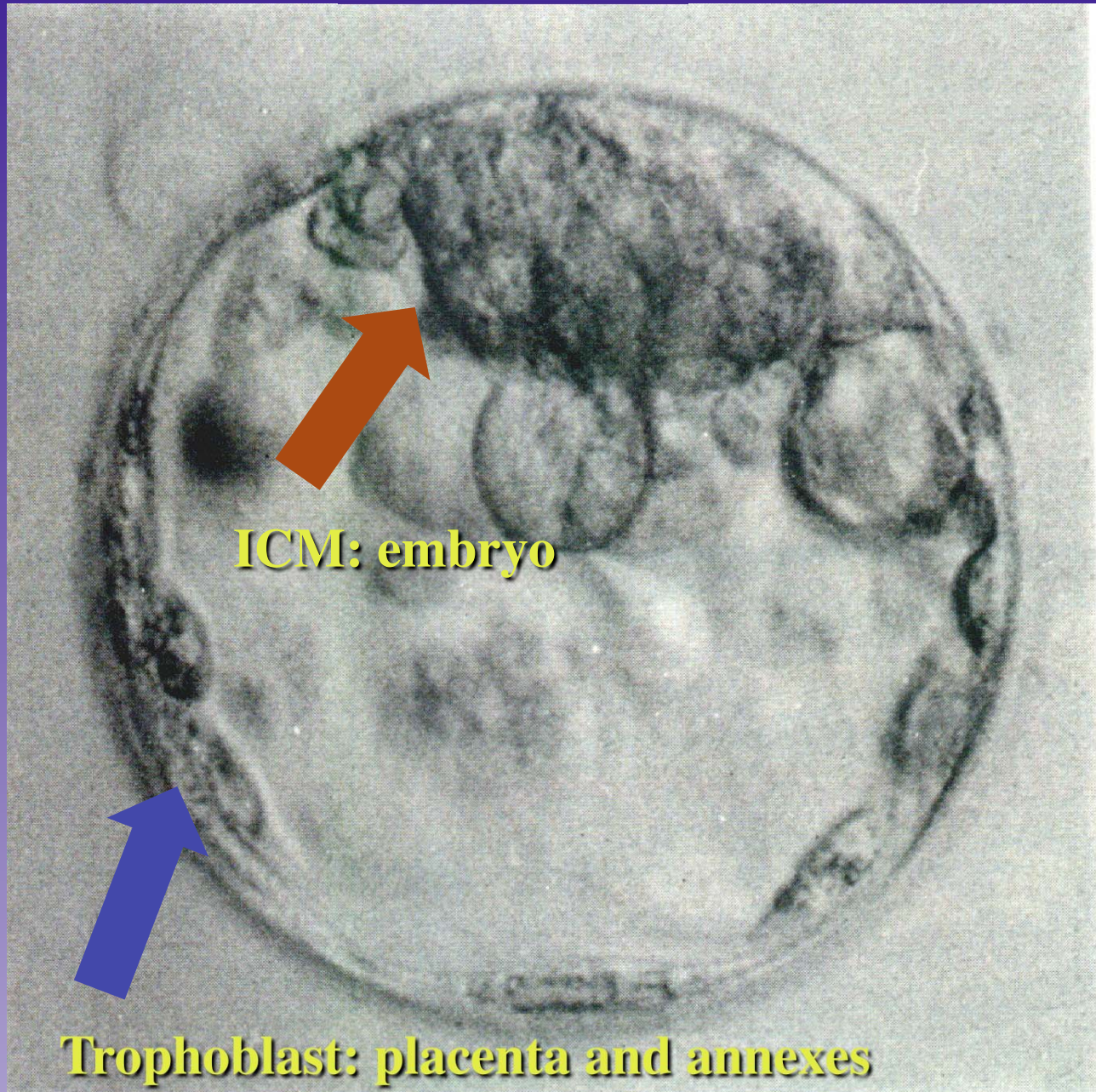
Stadio a 8 cellule



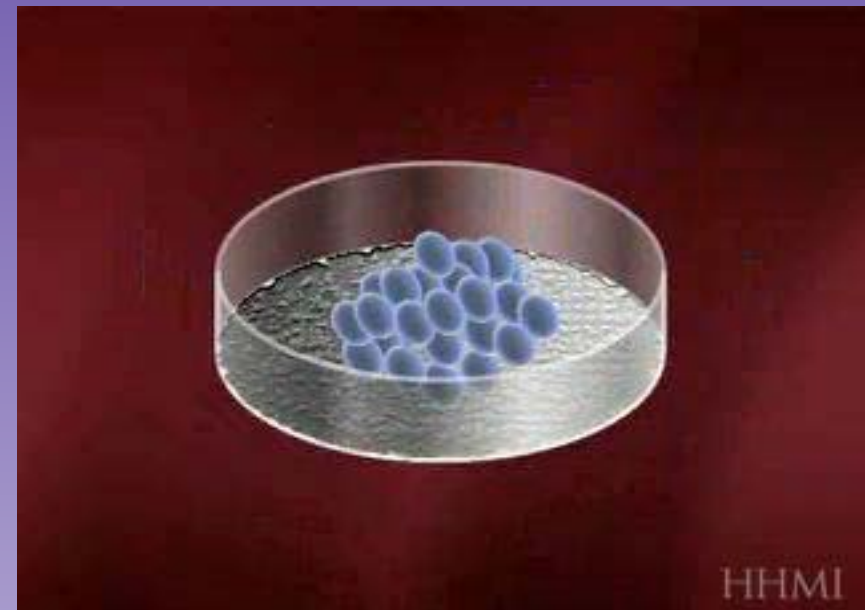
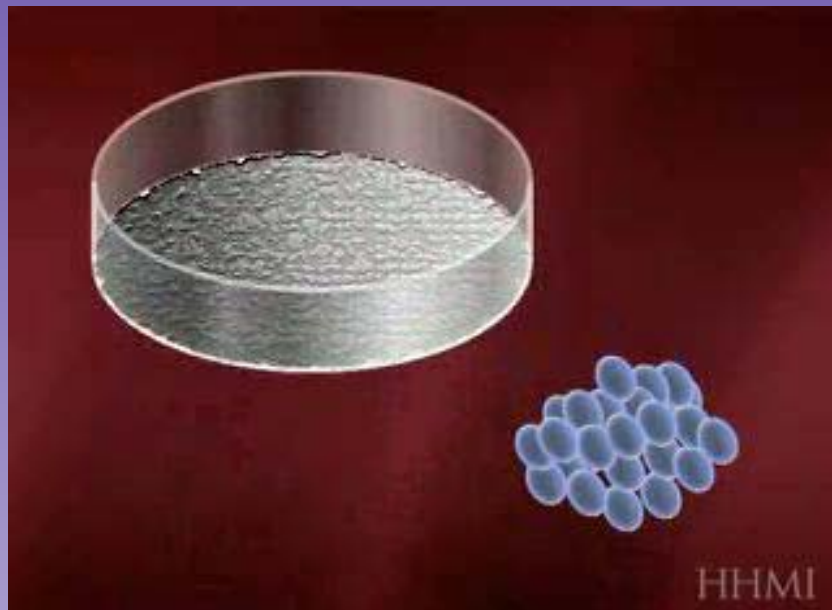
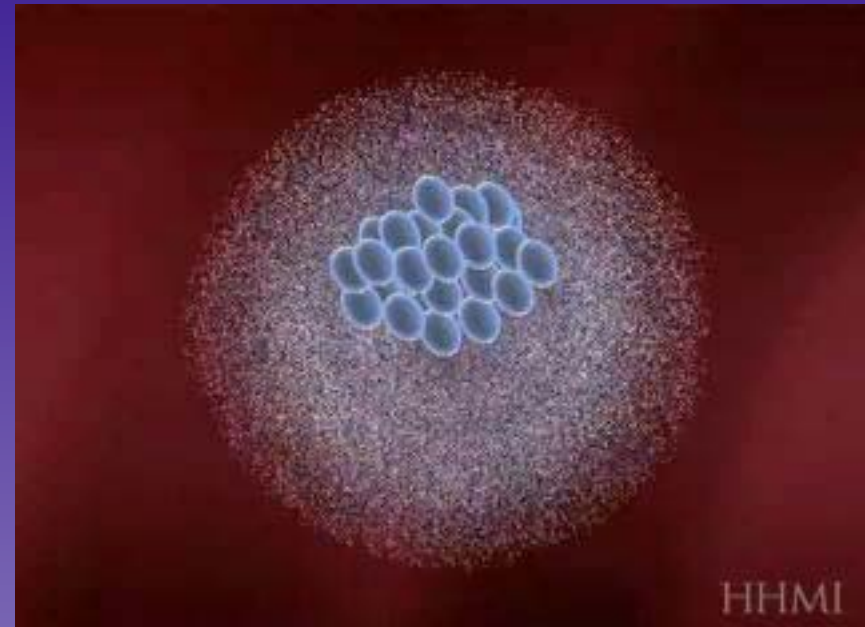
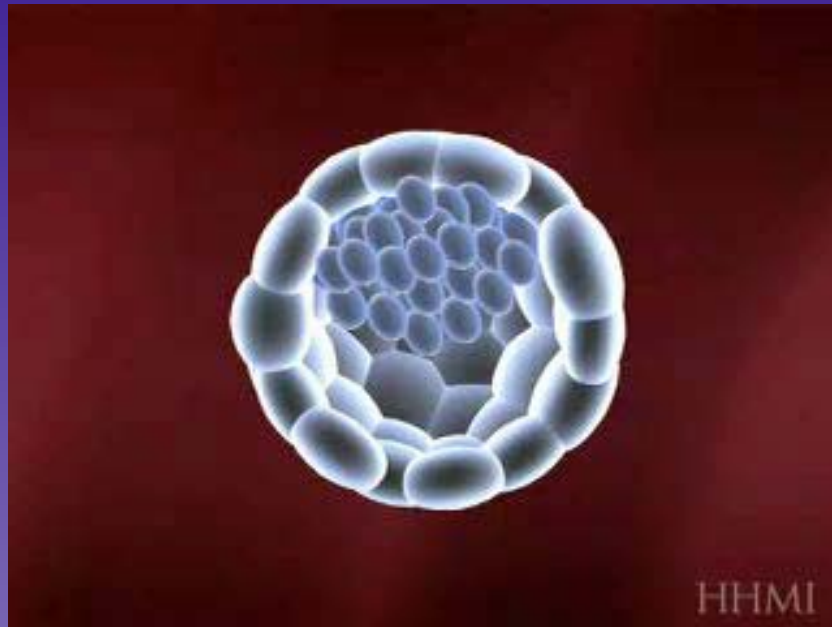
Stadio a 16 cellule

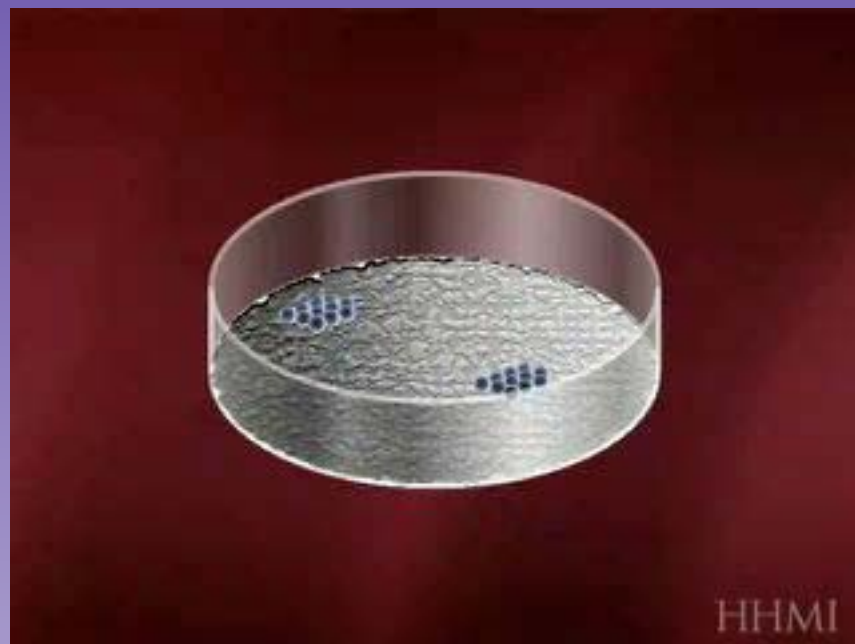
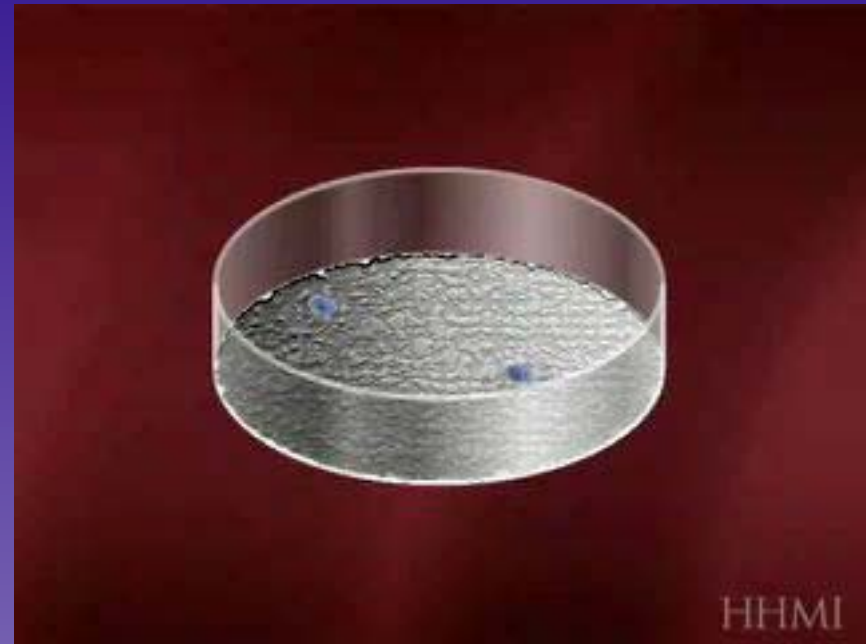


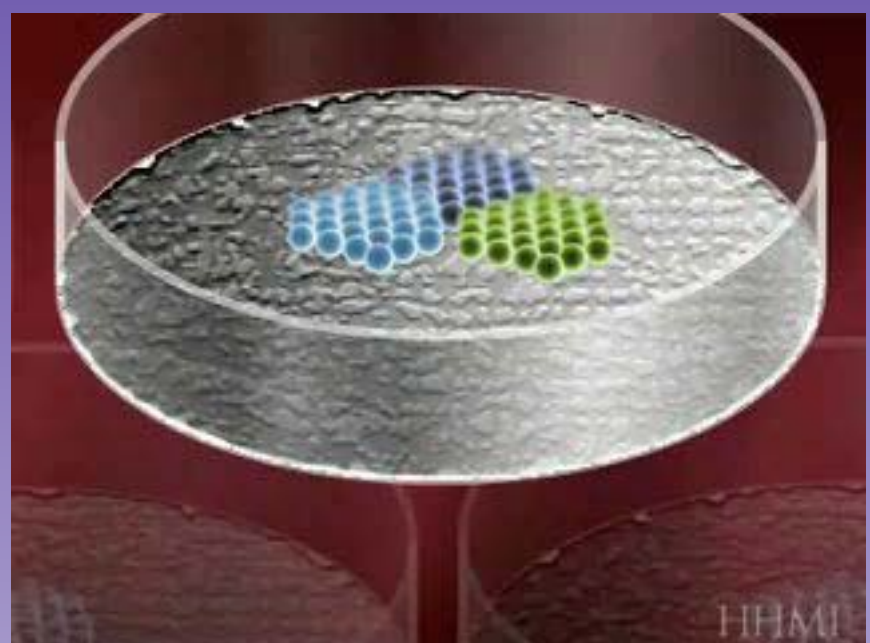
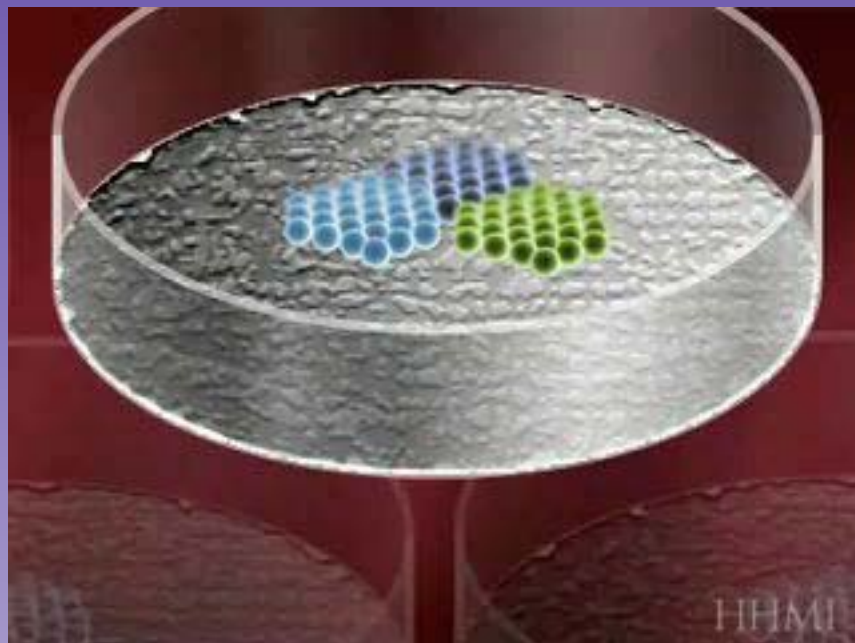
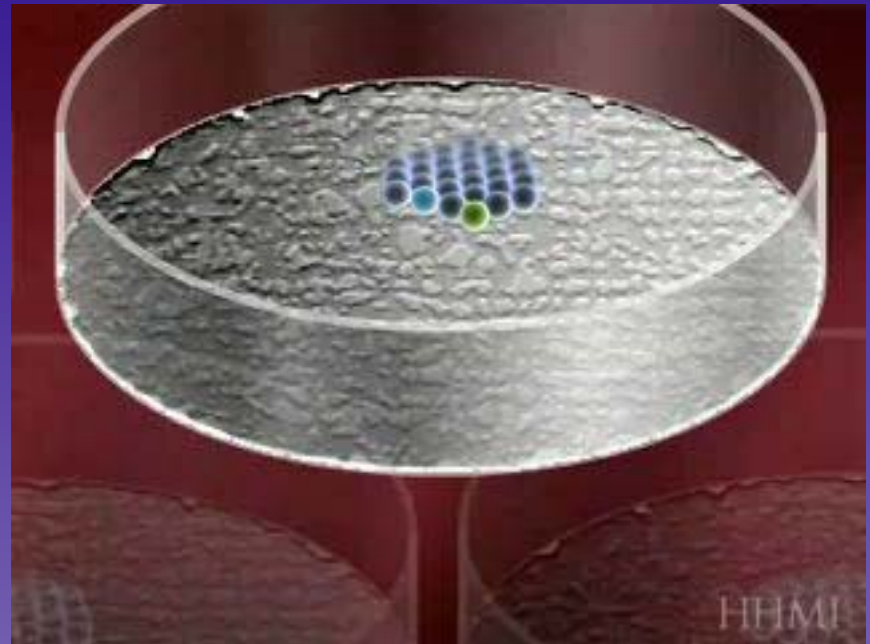
Blastocist

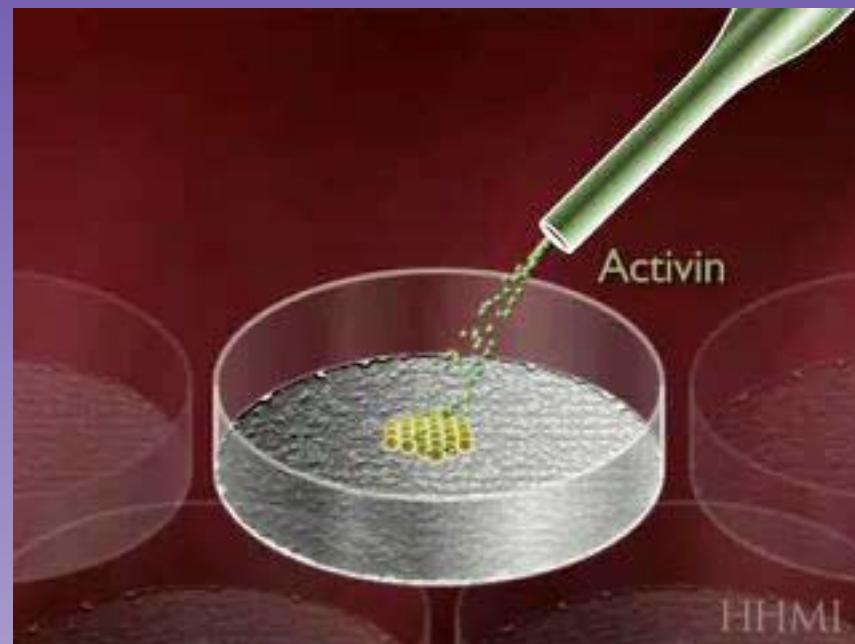
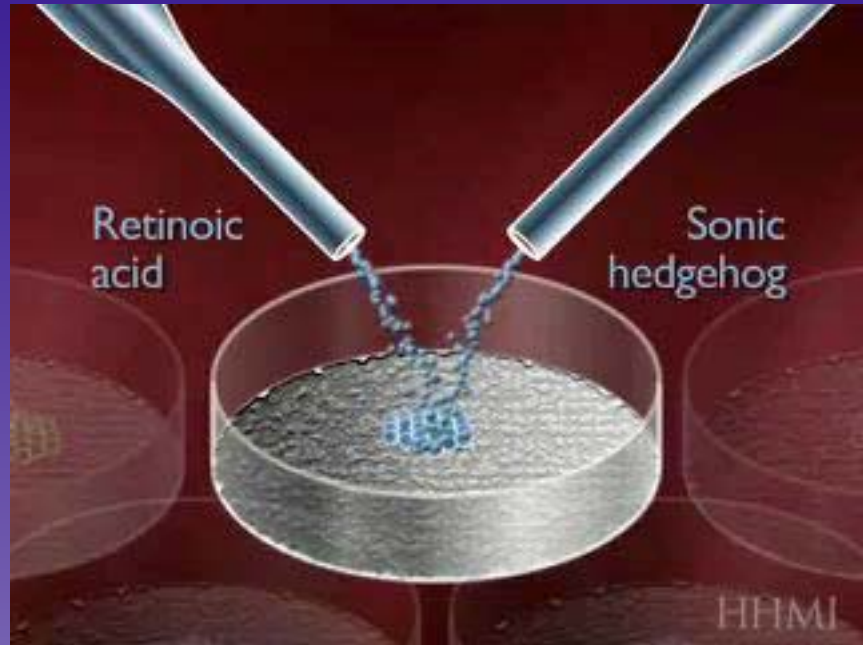
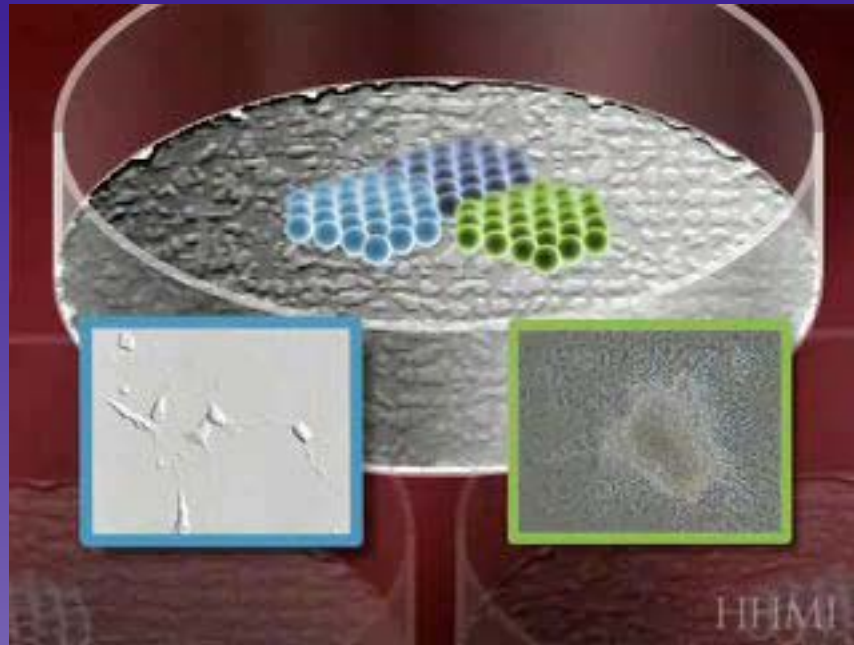


ES isolation

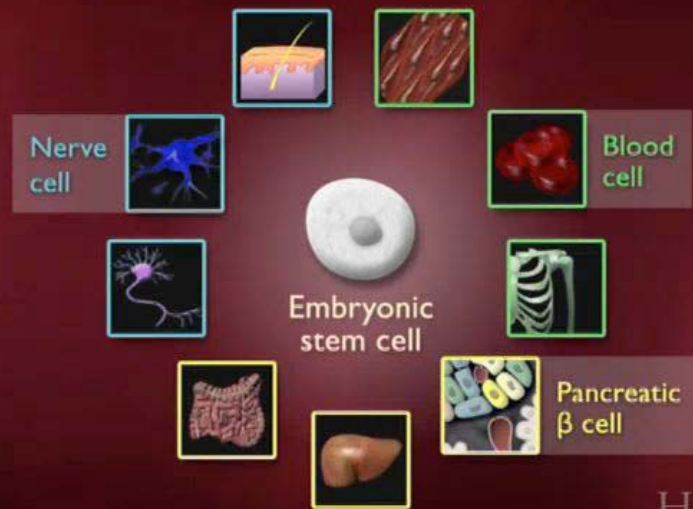








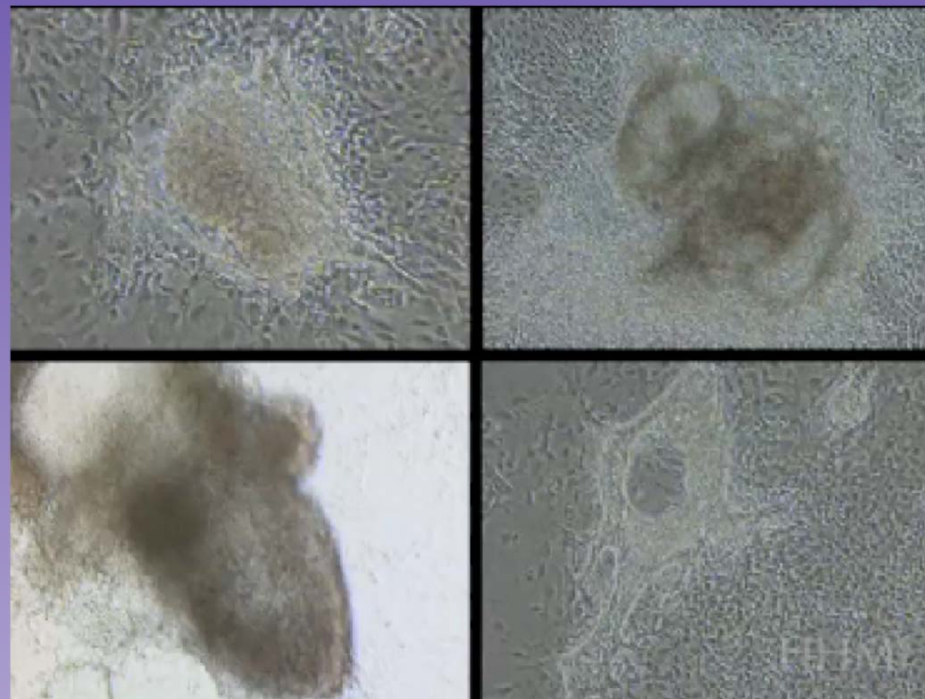
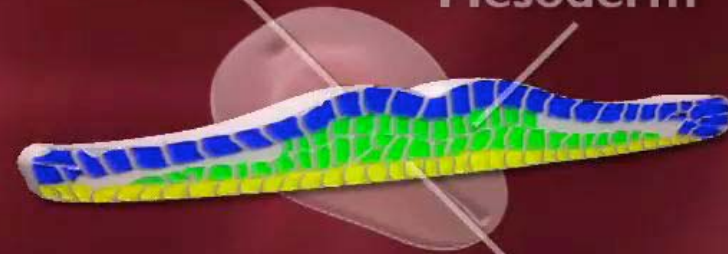
ES Cells Make All Cell Types



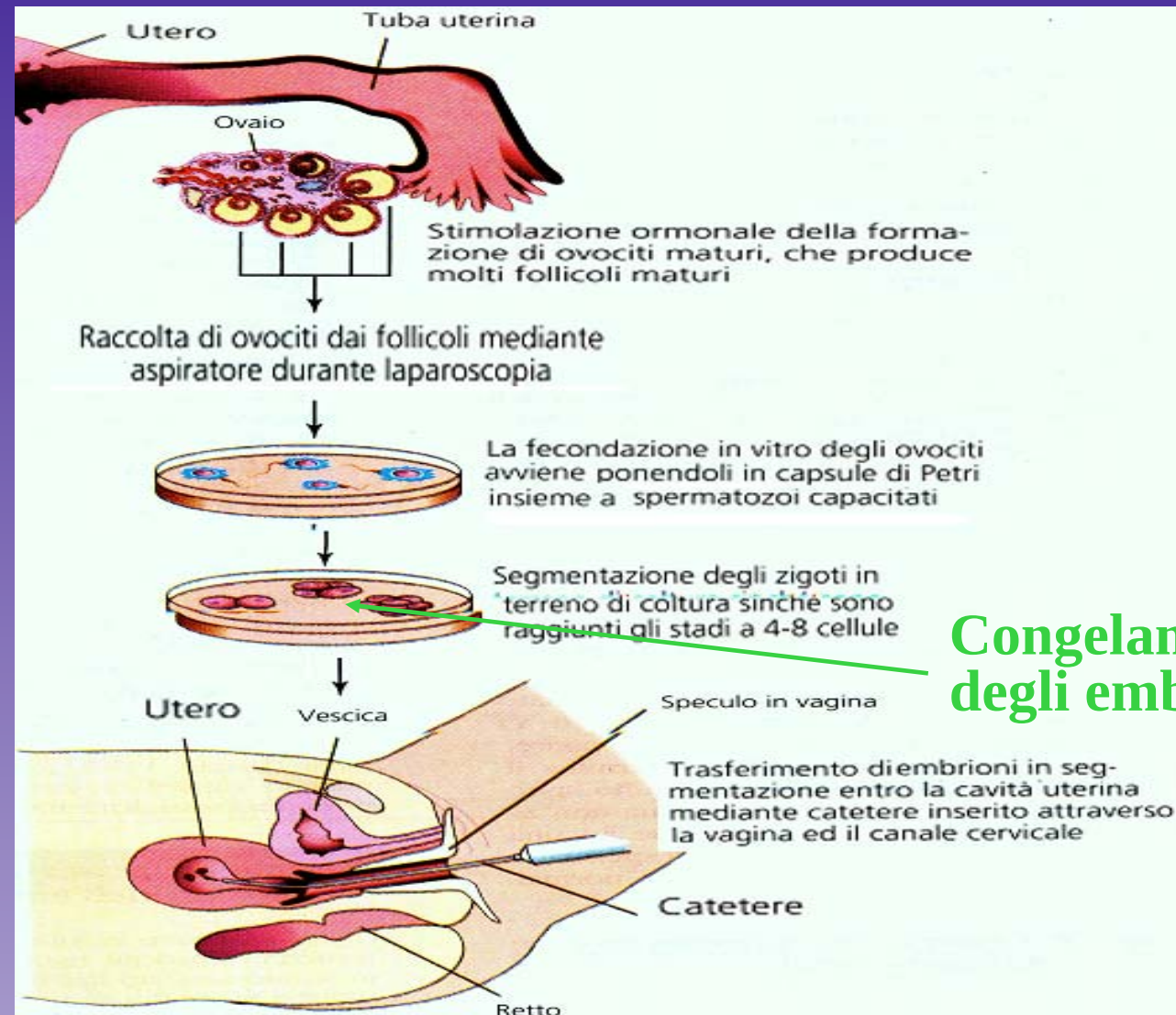
Ectoderm

Mesoderm

Endoderm



Fecondazione *in vitro*



**Diagnosi
Preimpianto**

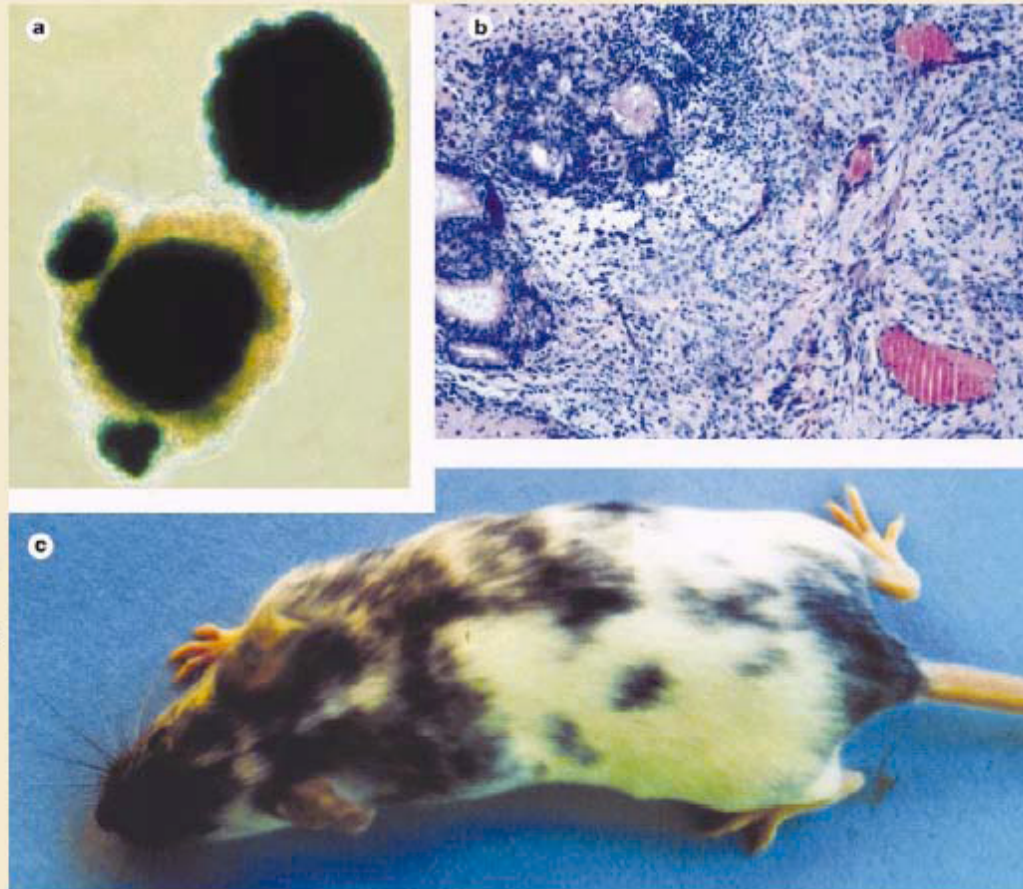
**Congelamento
degli embrioni**

- Edwards (1967): da blastocisti di coniglio vengono ottenute cellule che, coltivate *in vitro*, formano aggregati (*embryoid bodies*) o monostrati che possono essere propagati per diverse generazioni e congelati.

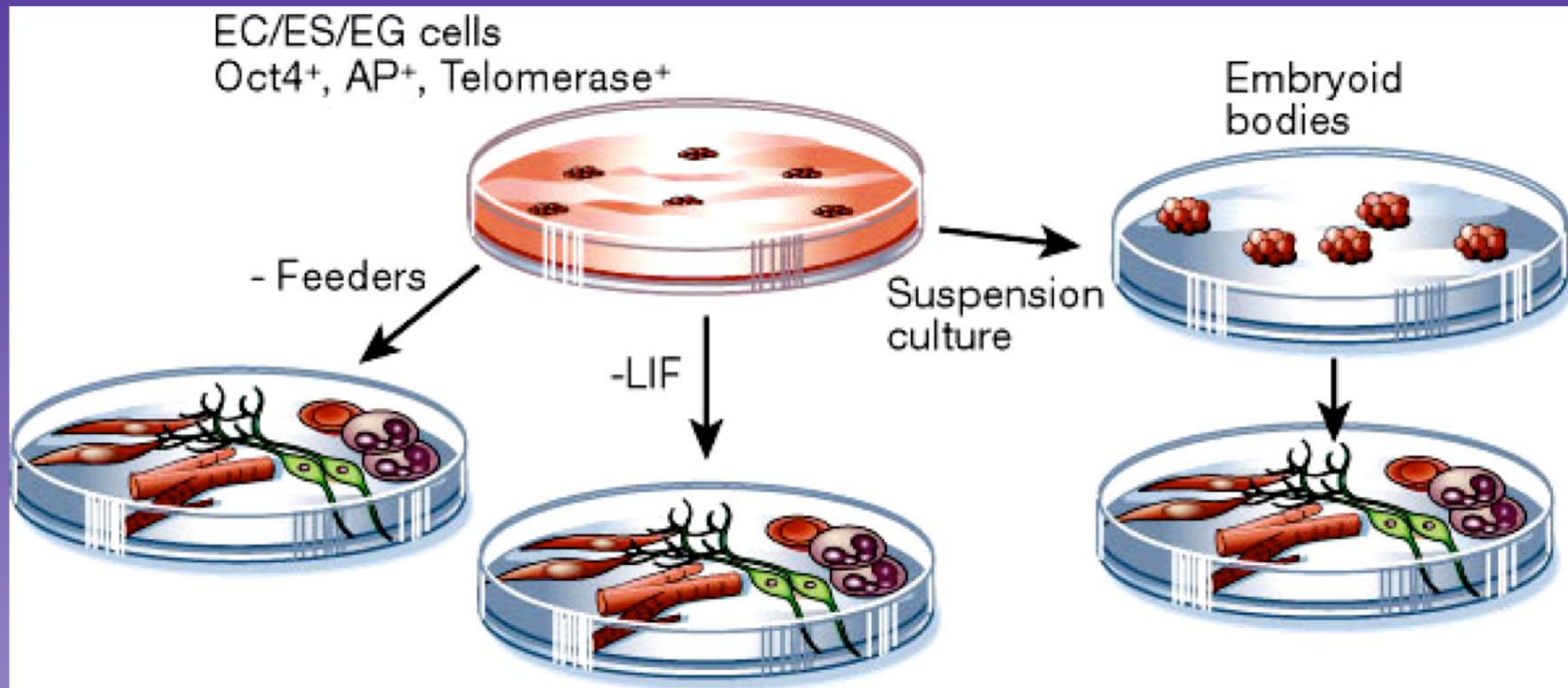
- Evans e Kaufman (1981): coltivano blastocisti di topo, ottenendo ESC pluripotenti.

Figure 1 Pluripotency of mouse embryonic stem (ES) cells.

a, Aggregates of mouse ES cells forming embryoid bodies. The dark staining shows expression of Sox2 in the less differentiated cells, whereas the ring of differentiated endoderm is unstained. (Image courtesy of A. Avilion). **b**, Histological section of a teratocarcinoma derived from mouse ES cells. Many different cell types are found, all formed from the ES cells, including representatives of all three germ layers. (Image courtesy of M. Parsons.) **c**, The ultimate test of pluripotentiality: a chimaera made by injecting ES cells into a blastocyst. The pigmented areas reveal the contribution of ES cell derivatives to the skin, but all tissues are composed of a mixture of ES and host embryo derivatives. Even a single ES cell can give a chimaera like this.

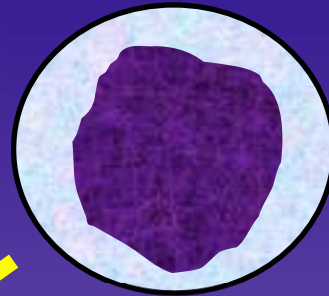


Le cellule staminali embrionali possono essere cresciute indefinitamente in vitro e, a seconda delle condizioni di coltura, possono generare diversi tipi di cellule differenziate



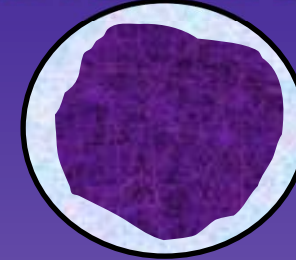
Soprattutto, neuroni, cardiociti, endotelio e cellule del sangue

PRECURSORI



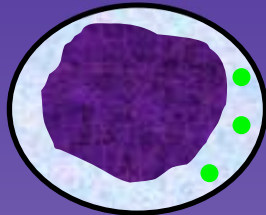
CD34

MEGACARIOCITO



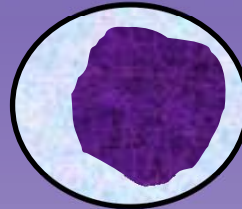
CD41/42/61

MIELOIDE



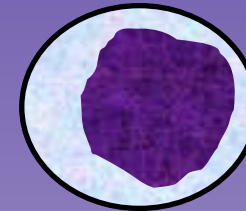
cyMPO/CD33

LINFOIDE T



cyCD3/CD7

LINFOIDE B



cyCD79A/CD19

MARCATORI DI LINEAGE EMPOIETICI

MATURAZIONE MIELOIDE

cyMPO / CD33

CD11b

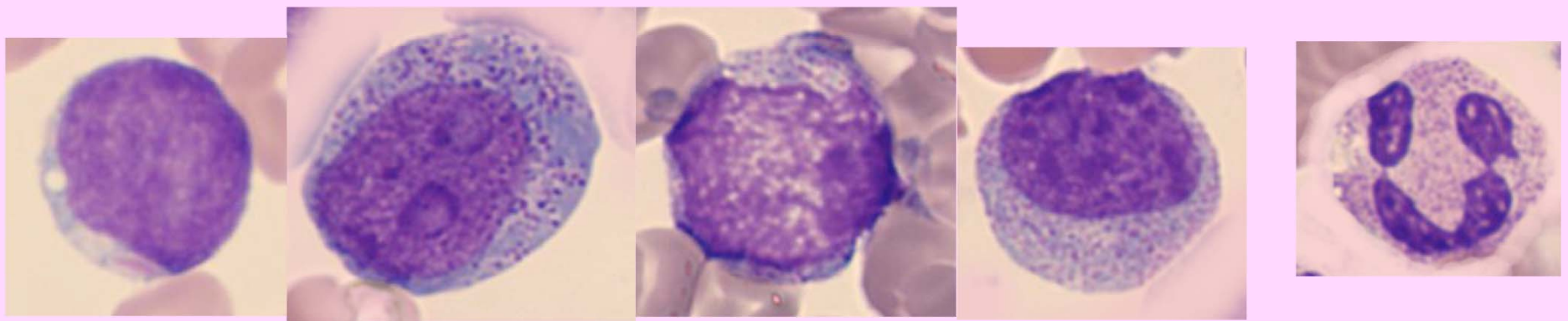
MIELOBLASTO

PROMIELOCITA

MIELOCITA

METAMIELOCITA

GRANULOCITA



CD13

CD16

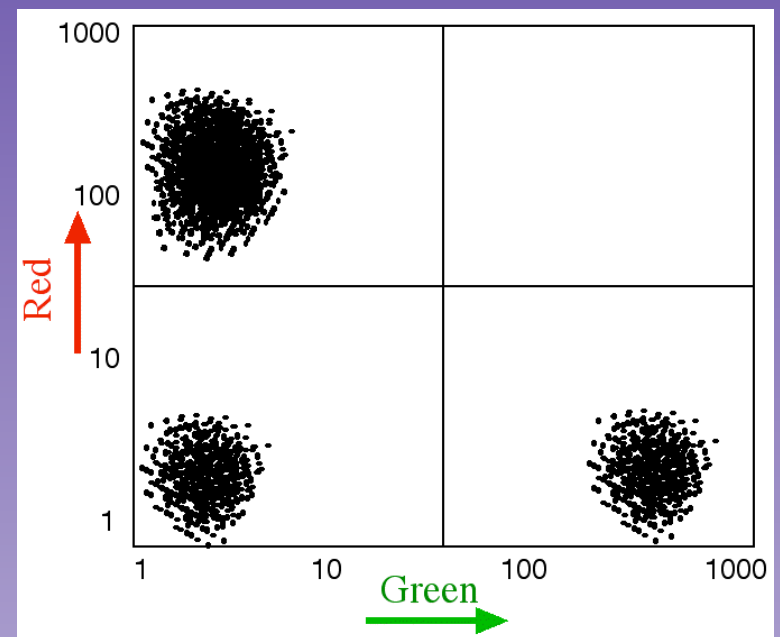
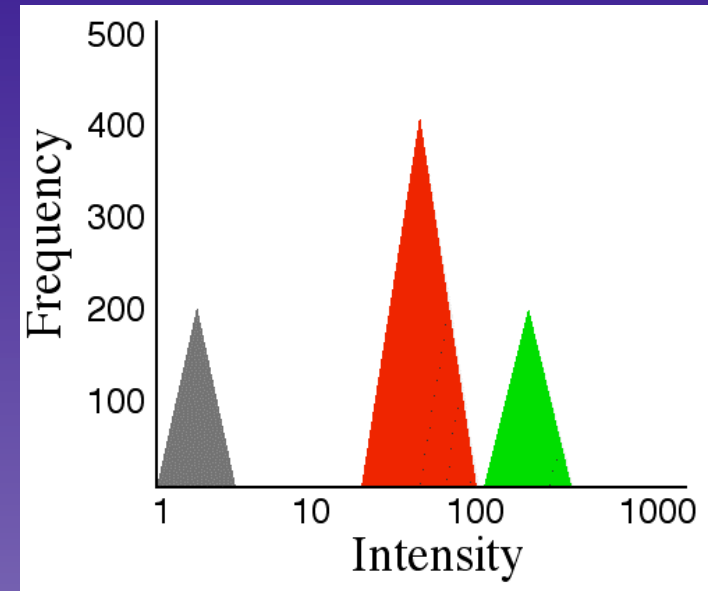
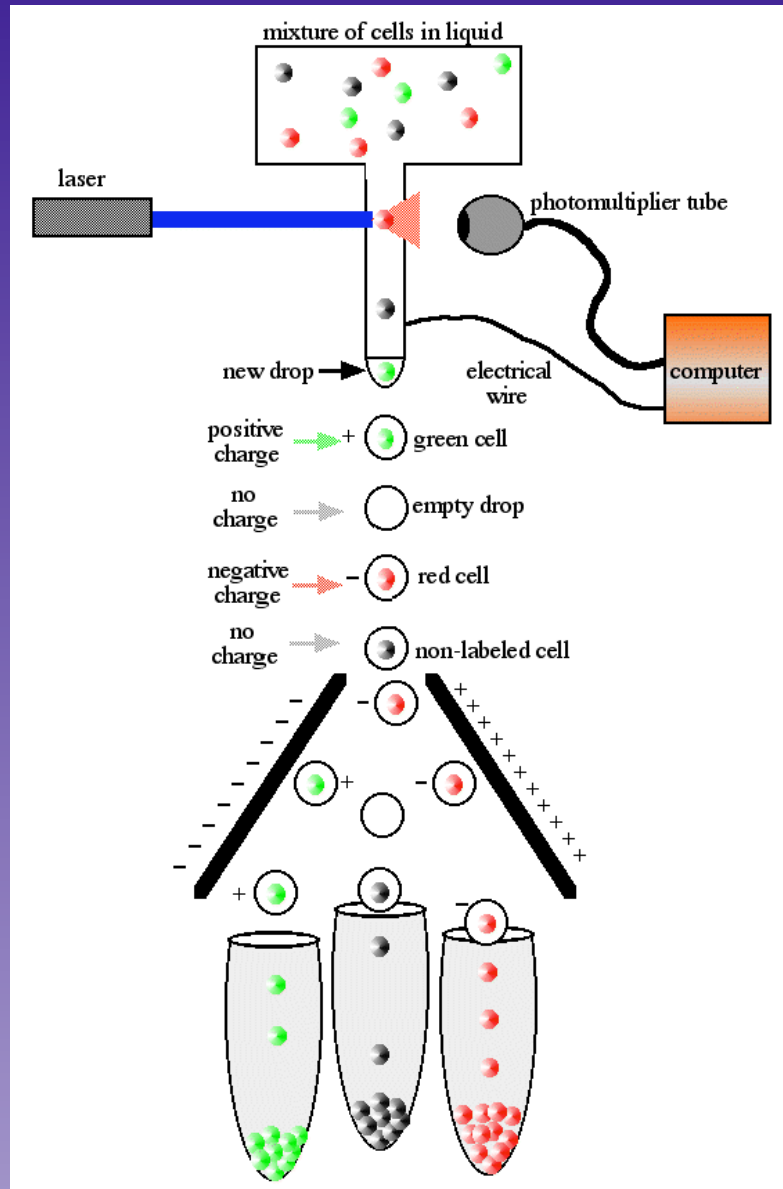
CD64

CD66c

CD34/DR

CD10

Fluorescence Activated Cell Sorting (FACS)



Midollo Osseo Normale

CD45

Linfociti

Linfoblasti

Ematogoni

Eritroidi

Monociti

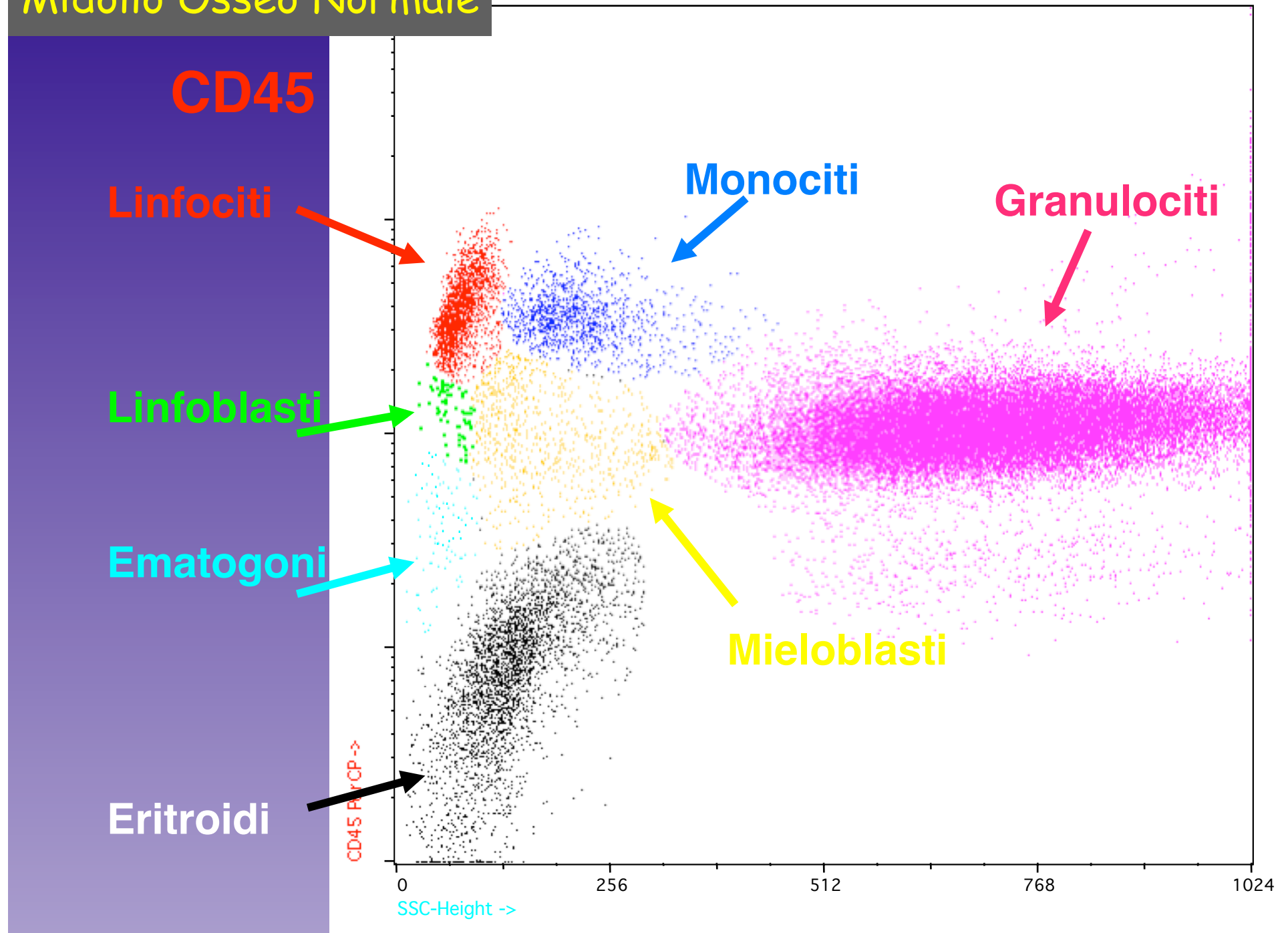
Granulociti

Mieloblasti

CD45 PE/CP ->

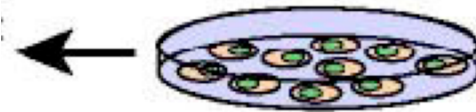
SSC-Height ->

0 256 512 768 1024



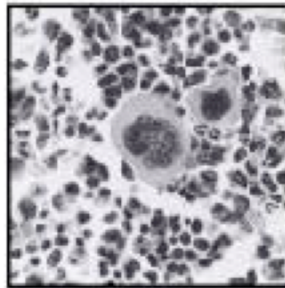
Le prospettive per le cellule staminali

Sviluppo di
farmaci e test
di tossicità



Esperimenti per
studiare sviluppo e
regolazione genica

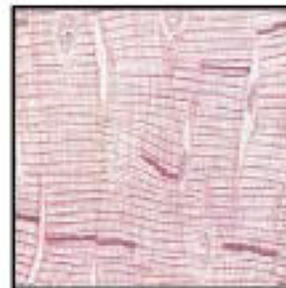
Tessuti e cellule per la terapia



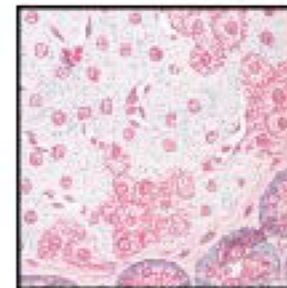
Midollo
Osseo



Cellule
nervose

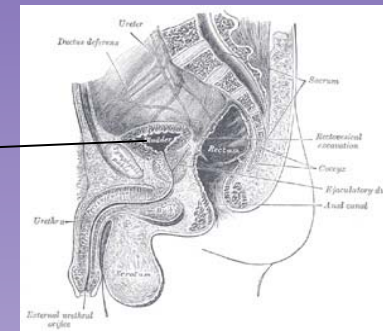
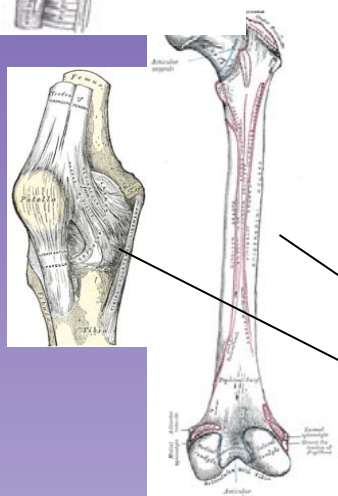
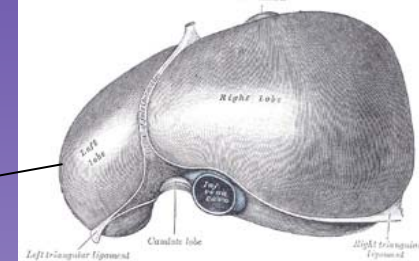
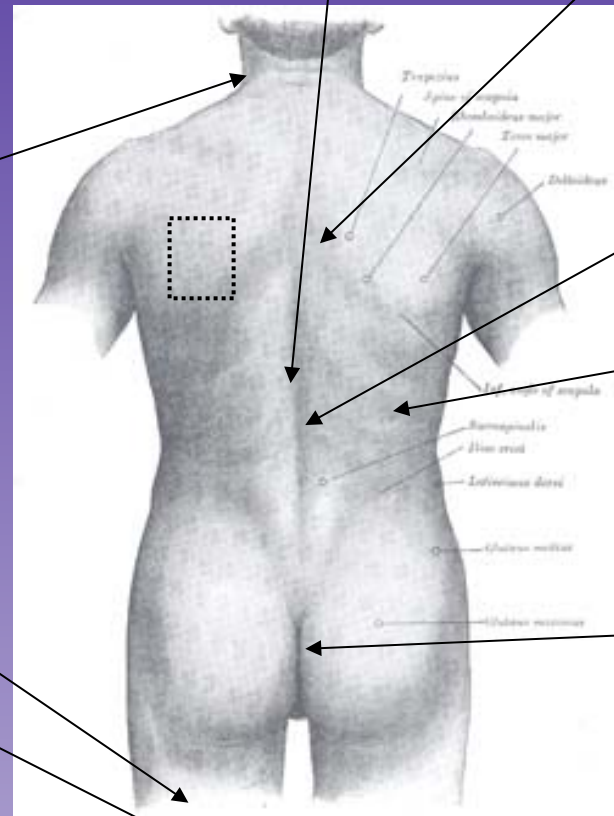
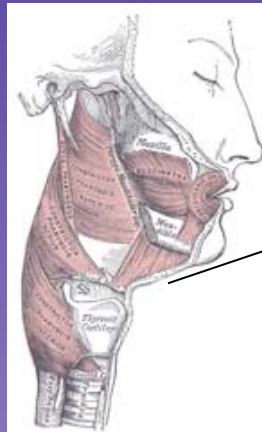
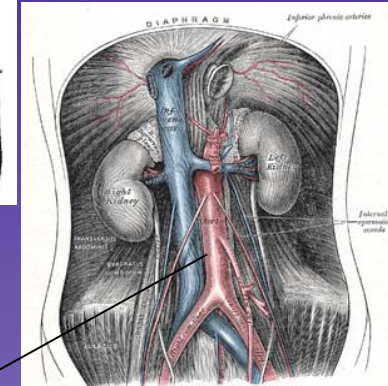
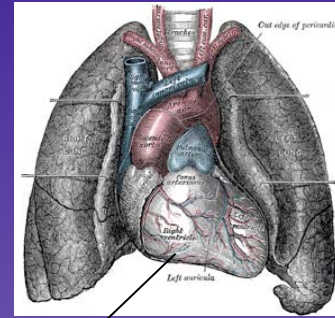
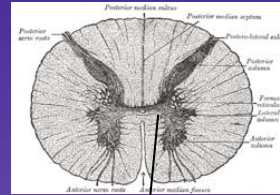
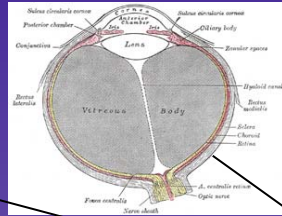
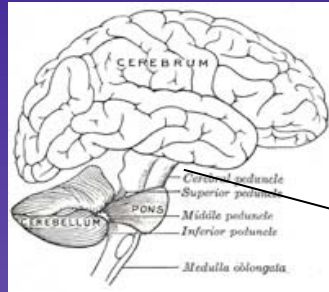


Cellule
cardiache



Isole del
Pancreas

Organi che oggi possiamo o domani vorremmo poter ricostruire



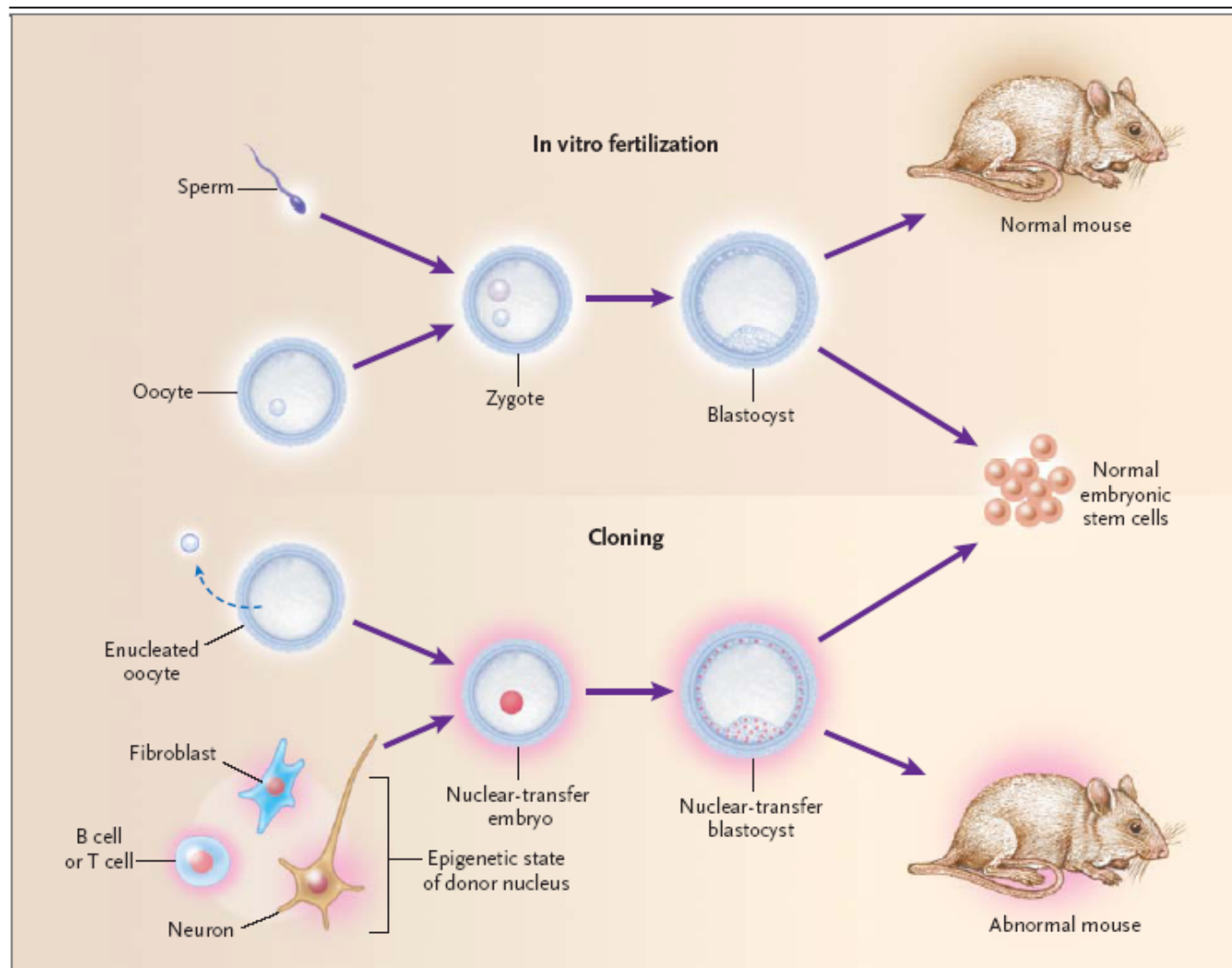


Figure 3. Derivation of Embryonic Stem Cells from a Blastocyst, Resulting in Selection for Functional Cells.

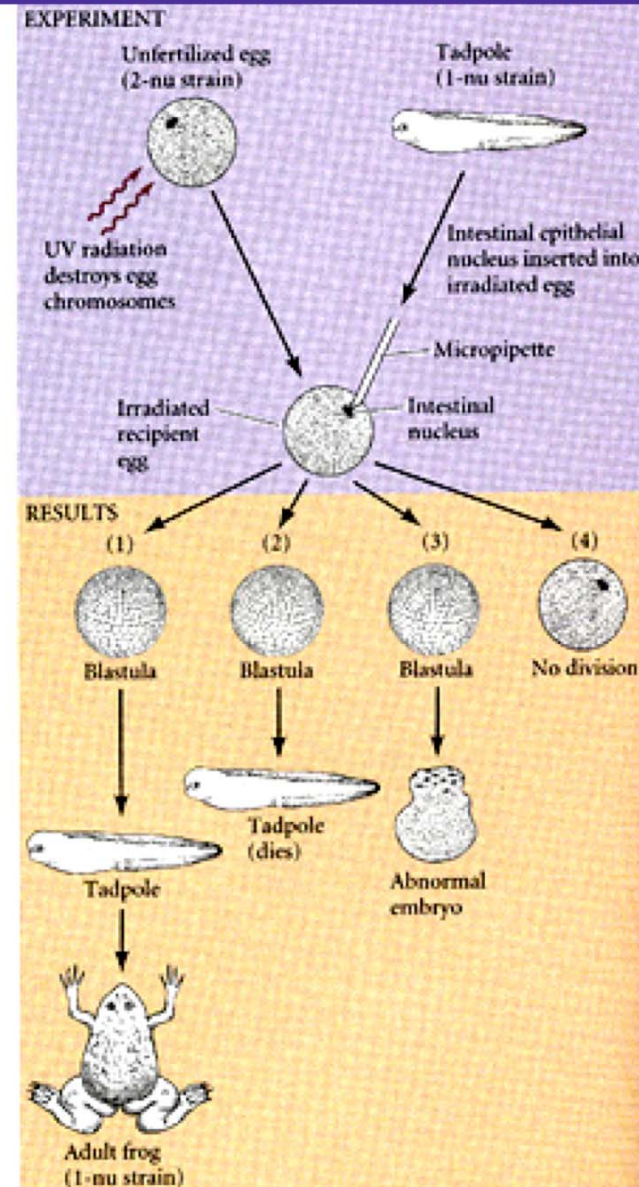
Only a few of the cells of a blastocyst derived from a fertilized zygote have the potential to produce an embryonic stem-cell line, and these cells seem to be selected for by culture conditions (top panel). Mice derived from fertilized zygotes are normal because the sperm and oocyte genomes have undergone proper reprogramming during gametogenesis (top panel). However, cloned blastocysts and the resultant mice seem to maintain a memory of the epigenetic state of the donor nucleus they were derived from (bottom panel). This is probably due to faulty reprogramming of the somatic donor nucleus after nuclear transfer and results in abnormal phenotypes and aberrant patterns of gene expression. These epigenetic abnormalities are represented by the pink halo surrounding the cloned embryo and mouse. In contrast, the derivation of embryonic stem cells from cloned blastocysts appears to select for fully reprogrammed, functional cells that have lost this epigenetic memory.

Nel 1963 John Gurdon dimostrò che era possibile generare delle rane a partire dal nucleo di una cellula somatica dell'intestino di un girino

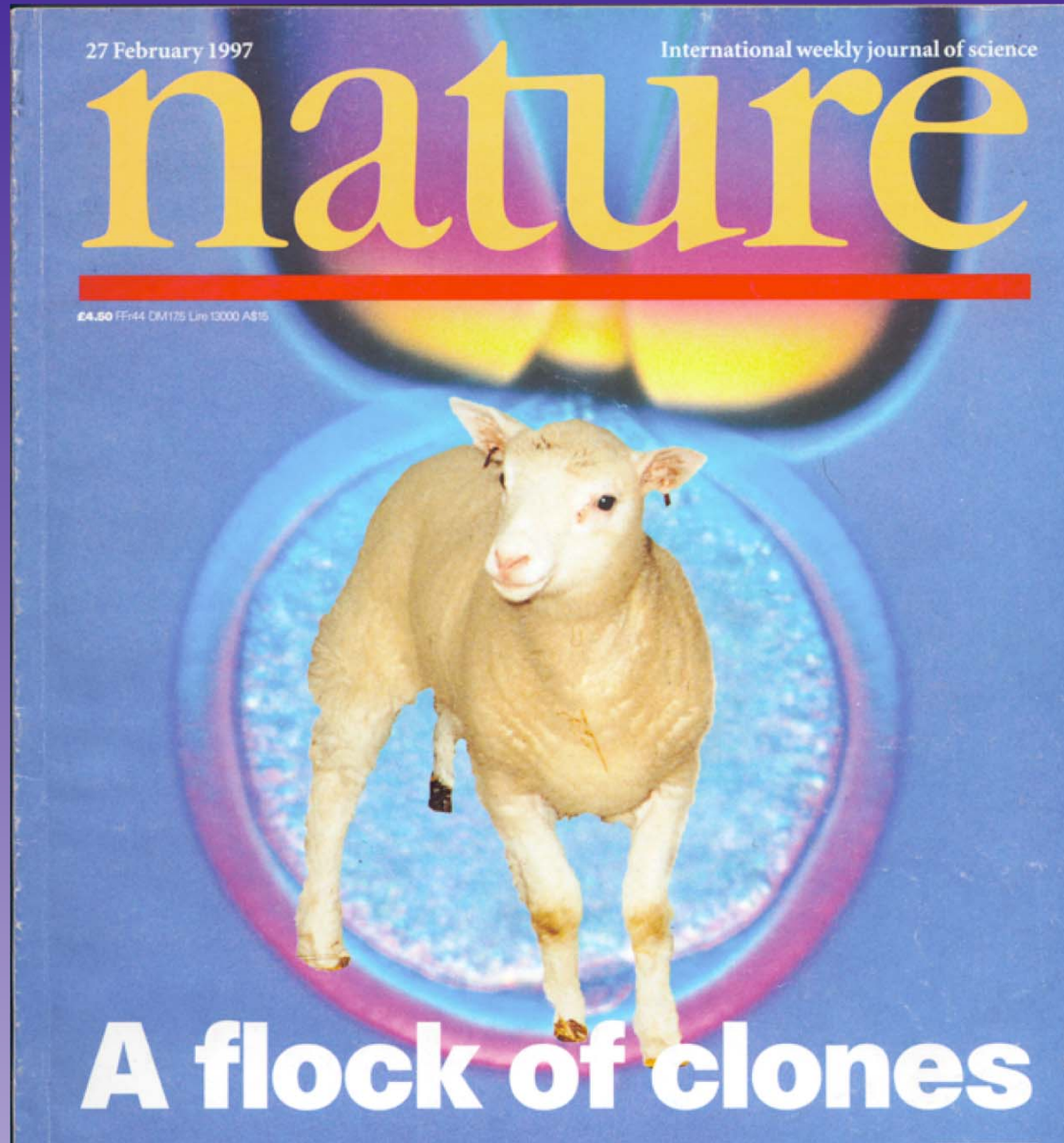


Wild-type female donor
of enucleated eggs

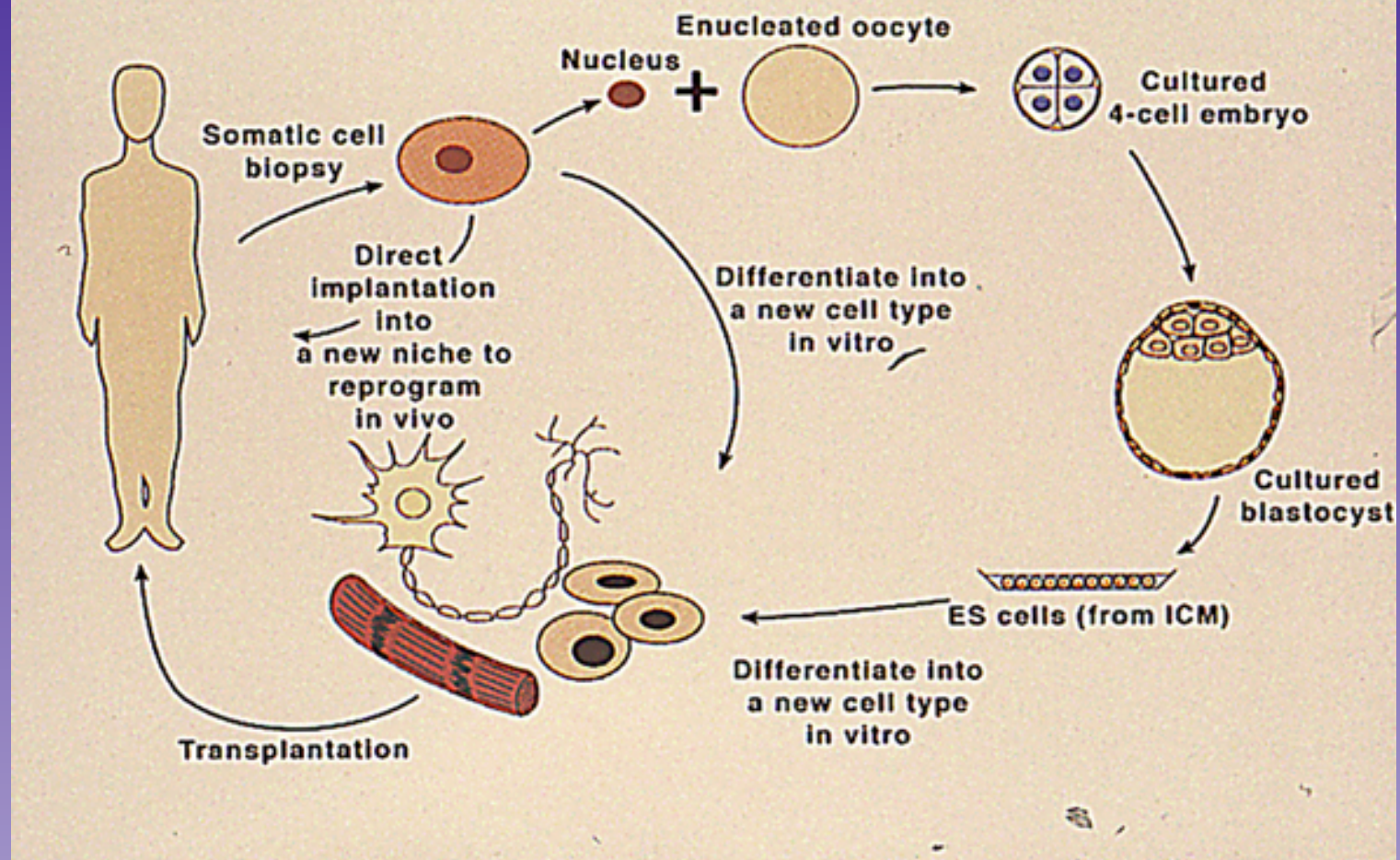
Albino parents
of nucleus donor



Nel 1997 Wilmuth e i suoi colleghi dimostrarono lo stesso fenomeno in un mammifero



Therapeutic cloning



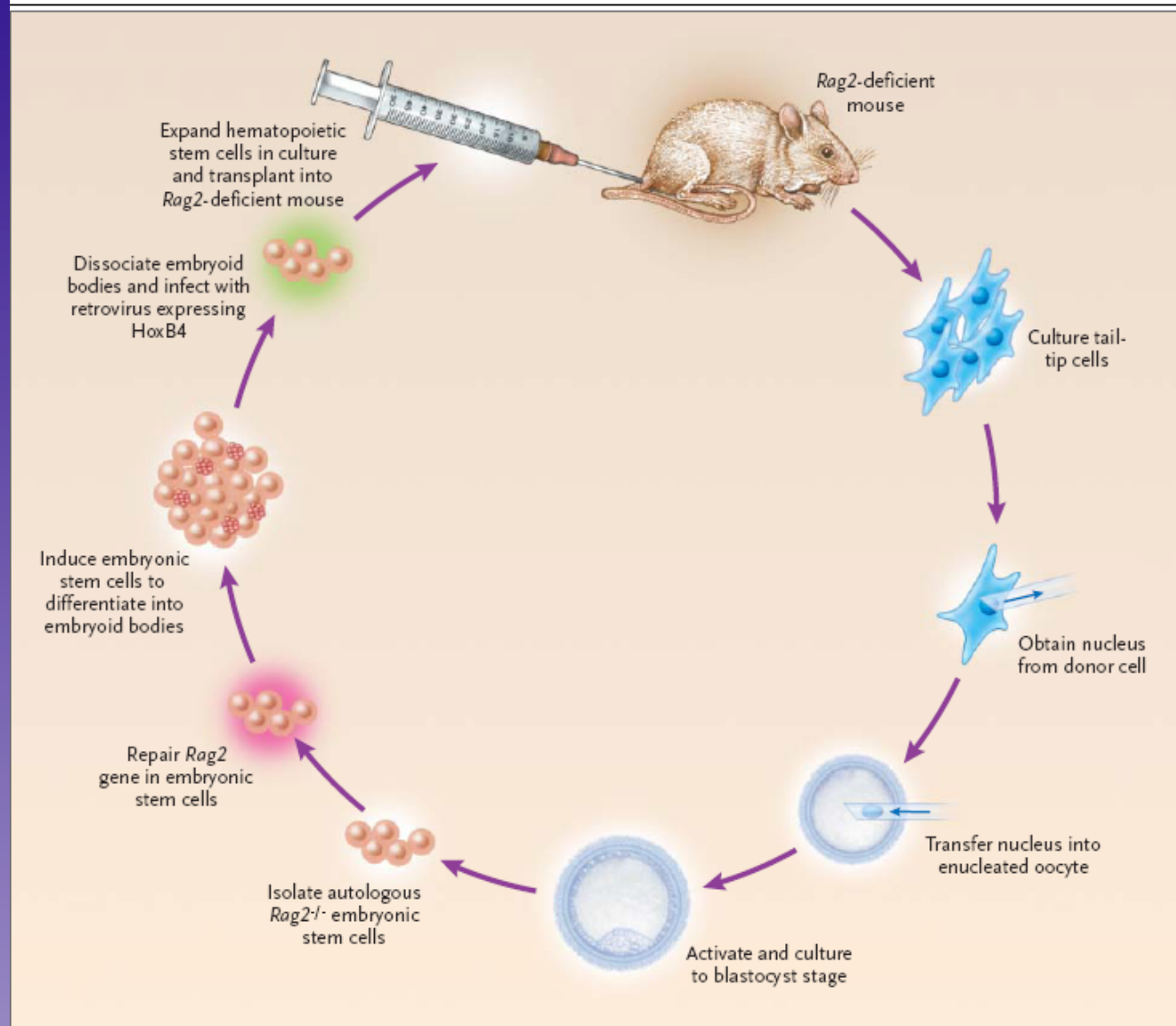


Figure 2. Mouse Model of Therapeutic Cloning.

Tail-tip cells were obtained from mice with a deficiency of recombination-activating gene 2 (*Rag2*) and cultured, and the nuclei were transferred into enucleated oocytes. The cloned embryos were cultured to the blastocyst stage to derive autologous embryonic stem cells. After one of the mutant *Rag2* alleles was repaired by homologous recombination, embryonic stem cells were induced to differentiate into embryoid bodies (embryo-like structures that contain various types of somatic cells) and infected with a retrovirus expressing the homeobox protein HoxB4. The resultant hematopoietic stem cells were clonally expanded and injected intravenously into irradiated *Rag2*-deficient animals to reconstitute their immune system. Adapted from Rideout et al.,⁵⁶ with the permission of the publisher.

Possible alternative ways to generate Embryonic Stem cells

1. From oocytes (H. Scholer, Nature 2003, not confirmed)
2. From neonatal spermatogonia
3. From adult spermatogonia
4. By expressing 4 genes (Oct4, Sox2, Klf4, c-Myc) into embryonic and adult mouse fibroblasts (T. Yamanaka, Keystone on Stem Cells)

Induced Pluripotent Stem Cells mouse neonatal cardiomyocytes

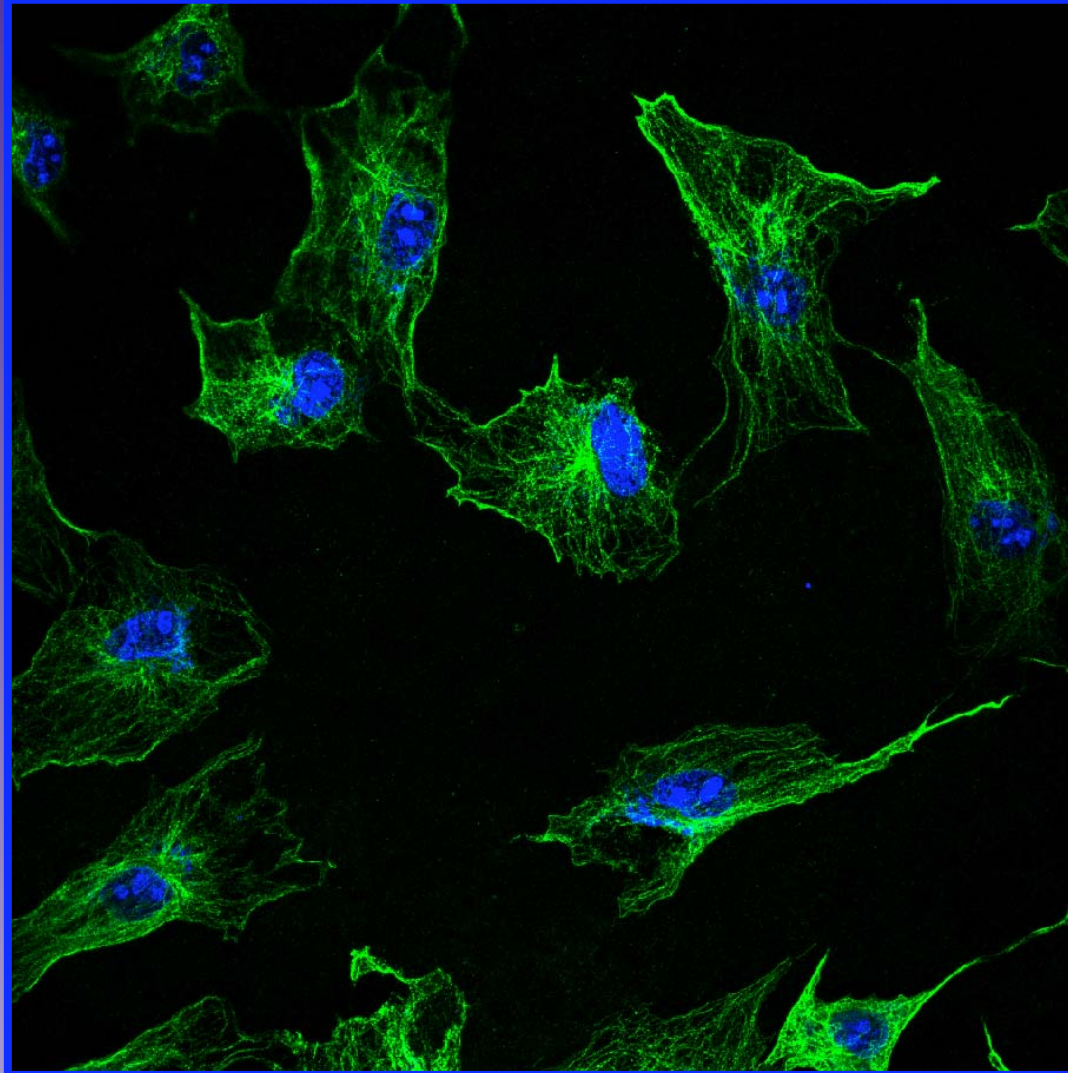
by defined factors

Oct 4, Sox 2, Klf ,c-mic

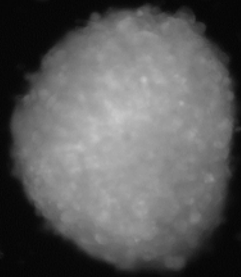
Oct 4, Sox 2, Nanog, lin 28

Oct 4, sox 2, valproic acid (VPA)

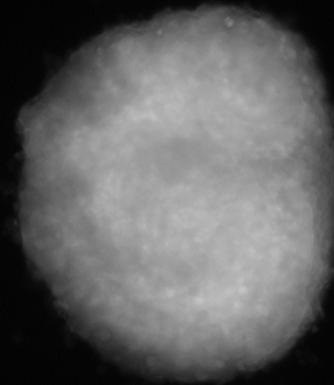
Neonatal cardiomyocyteis



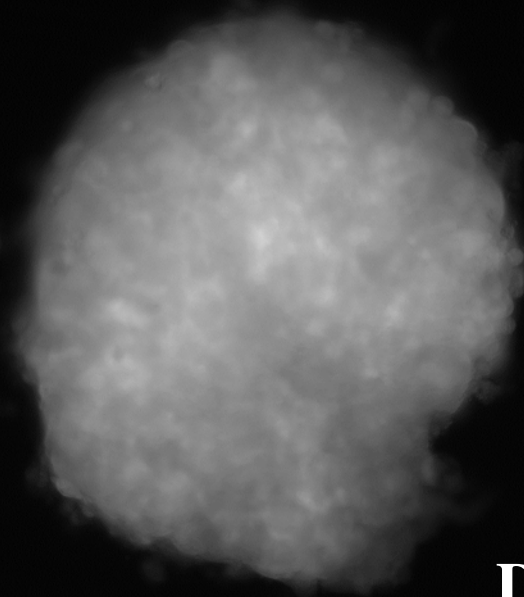
Embryos body formation



Day 7



Day 10



Day 15

