Stem Cells:Past-Present-Future

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Preview

- **Stem cells**
  - Set the stage
  - A little history
  - Definitions and clarifications
  - Explanation of stem cell biology
  - Hopes for regenerative medicine
- **Cloning**
  - Definitions and types
  - Therapeutic uses
  - Public perceptions
- **Final thoughts**

- **Throughout: Why the controversy?**

Obvious first slide

STEM CELLS

Can they be described in one sentence?

Stem Cells

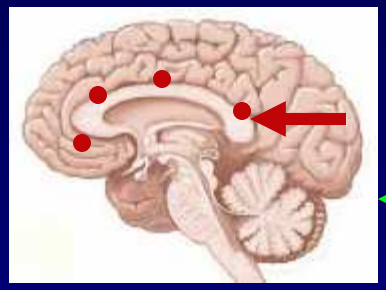
- Cells that can give rise to cells like themselves or other cell types.

- In You as you sit there…

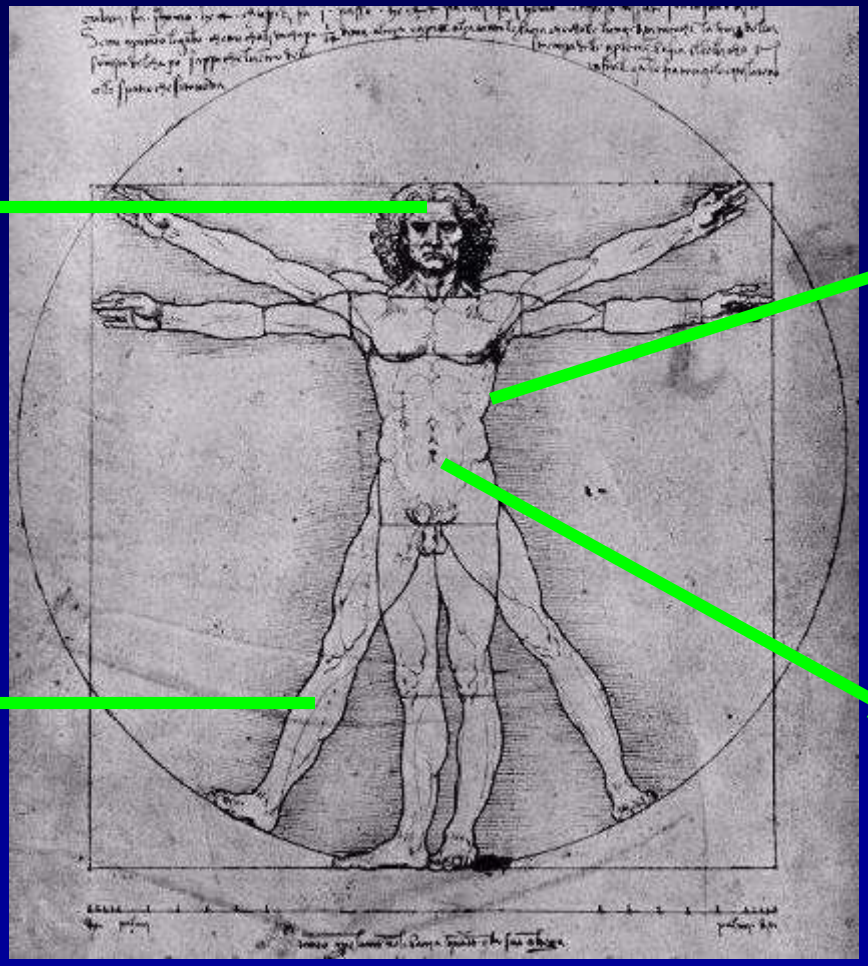
Present in constantly renewing tissues

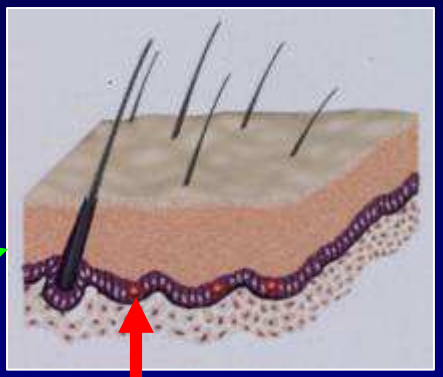
The Human Body

Contains more than 10 trillion cells, of more than 250 different types
Some tissues continually renew themselves from stem cells (adult stem cells)



Neuronal stem cell





Skin stem cell

Bone marrow stem cell

Intestinal stem cell

Embryonic vs. Adult Stem Cells

- **Embryonic stem cells**
  - Derived from early embryos
  - Depending on the stage at harvesting, capable of becoming a wide variety of adult cell types
  - (*Totipotent*-1-8 cell embryo; *Pluripotent*-embryonic stem cells)
- **Adult stem cells**
  - Rare: difficult to identify and isolate
  - Progenitor cells capable of becoming a limited number of cell types
  - (*multipotent*-can form a few cell types but not all)
- **Adult cells induced to become pluripotent**

Origin of the term Stem Cells

- **The words ‘stem cells’ were first used by Haeckel in 1868 to describe the origin of multicellular organisms from a unicellular organism.**

- **Regaud in 1901 used the term FIRST for self-renewal for spermatogonial stem cells in the testis. He recognized that for spermatogenesis to occur there must be a self-renewing ancestral cell.**

[1]Haeckel V. Natürliche Schöpfungsgeschichte (Berlin: Georg Reiner).

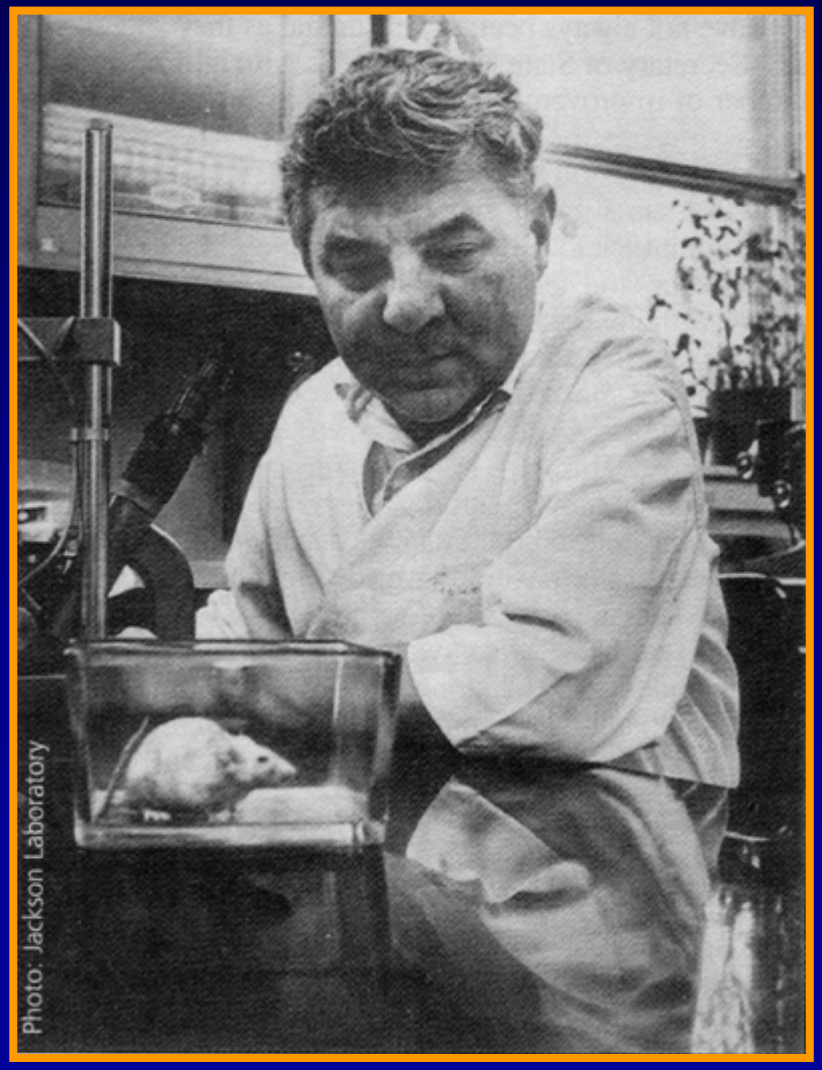
[2]Regaud C. Études sur la structure des tubes séminifères et sur la spermatogénèse chez les mammifères. Archives d'Anatomie Microscopiques et de Morphologie Expérimentale 1901; 4: 101-156; 231-380.

[3]Robey PC. Stem cells near the century mark. J Clin Invest 105:1489-1491, 2000.

[4]Ramalho-Santos M, Willenbring H. On the origin of the term "stem cell". Cell Stem Cell 1: 35-38. 2007.

Leroy Stevens: Stem Cell Pioneer

Jackson Laboratory, Bar Harbor, Late 1950s



A Brief History of Stem Cell Research

1958	**Leroy Stevens traces teratomas in fetal mice to primordial germ cells in genital ridge at gestational day 12 – these are pluripotent stem cells**
1975	Beatrice Mintz & Karl Illmensee (Philadelphia ICR) show ESCs can give rise to an organism
1986	Tom Doetschman (U Cincinnati) uses genetic modification of inner cell mass to create knockout technology.
1989	Mario R. Capecchi, Martin Evans, and Oliver Smithies **Knockout mouse technology**
1996	James Thomson (U Wisconsin) develops culture technology for ESCs of a variety of species
1998	**Thomson collects human ESCs from residual blastocysts after in vitro fertilizations**
1999	FDA clinical trial: Neural stem cells effective in a Parkinson's patient (adult stem cells)
2001	Multiple new ES cell lines derived and shared
2002	**House of Representatives bans "reproductive cloning"; President Bush limits stem cell lines to ~60 currently available lines**
2005	Harvard group develops method to make tailored SCs from existing cell lines
2006-2009	**iPS and Spermatogonial Stem Cells**
July 2009	**President signs order for new hESC guidlines**

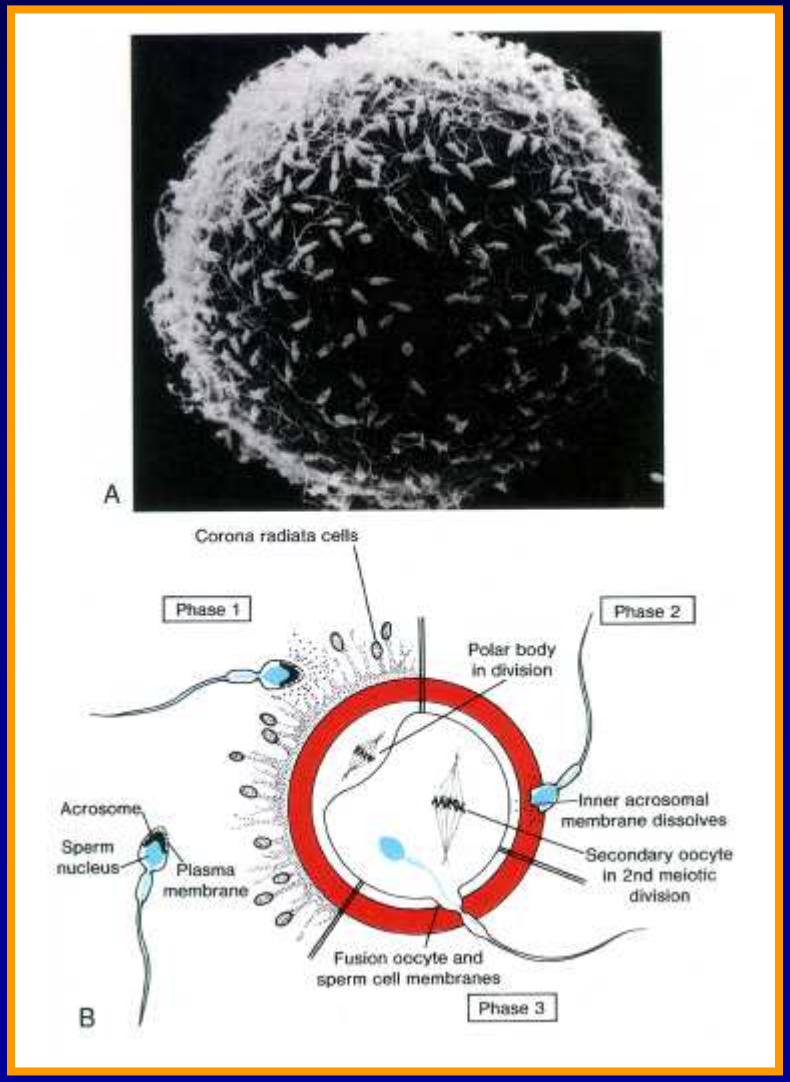
Biology Relevant to Embryonic Stem Cells

Stem Cells

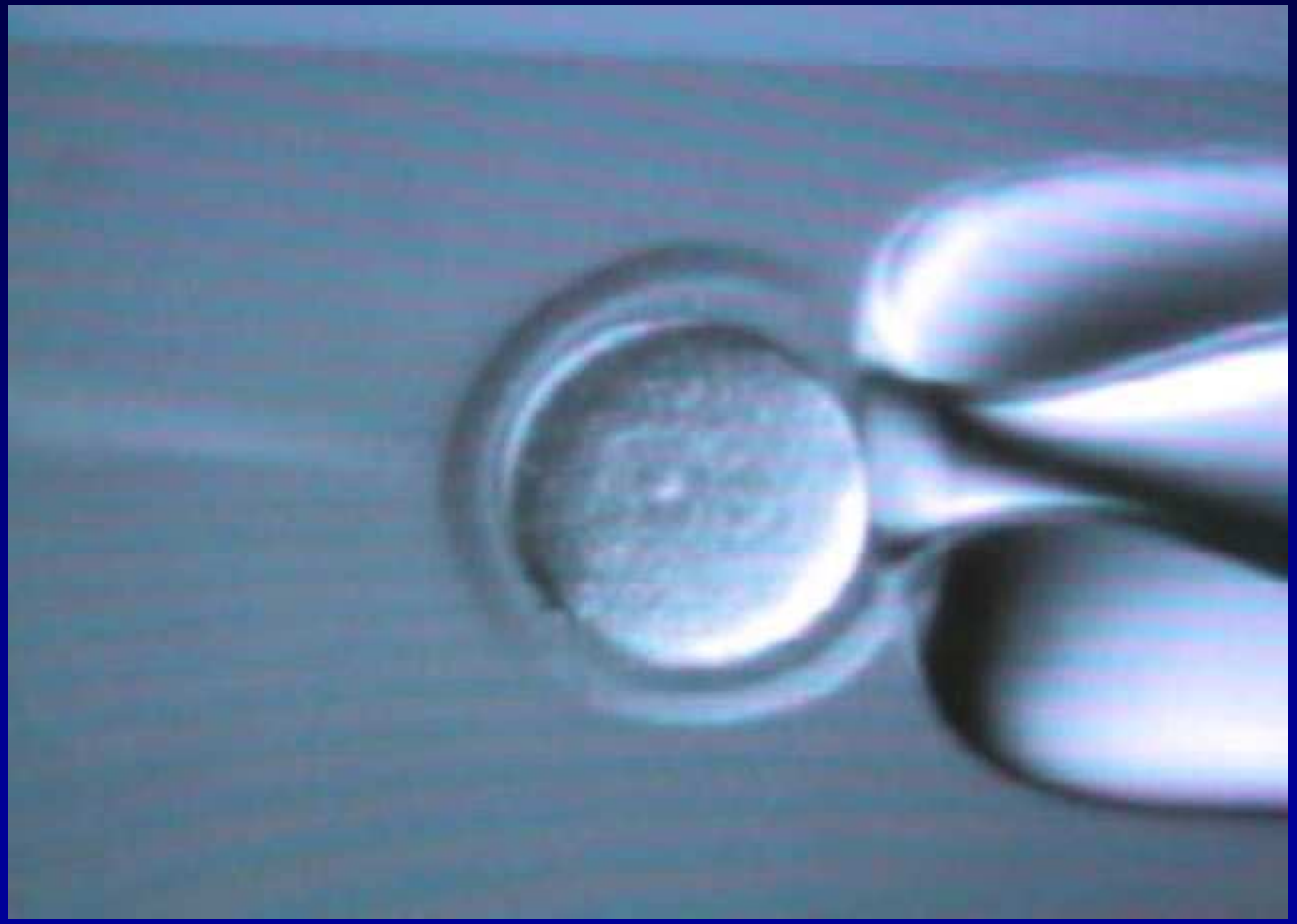
- **Ultimate stem cells**
  - The Single cell embryo
  - Inner Cell Mass of an embryo
    - These are considered Embryonic Stem cells

Start with the ultimate *totipotent* stem cell

A Single Sperm Fertilizes the Ovum



Holding pipette secures embryo

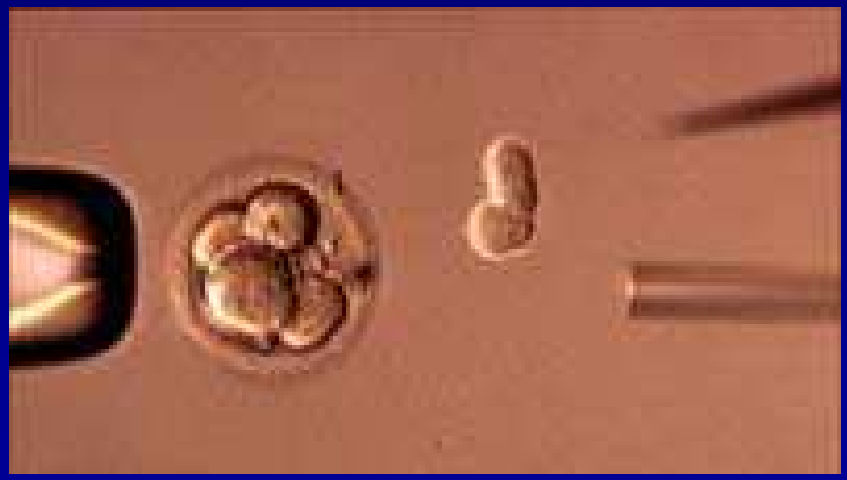


Injecting an embryo

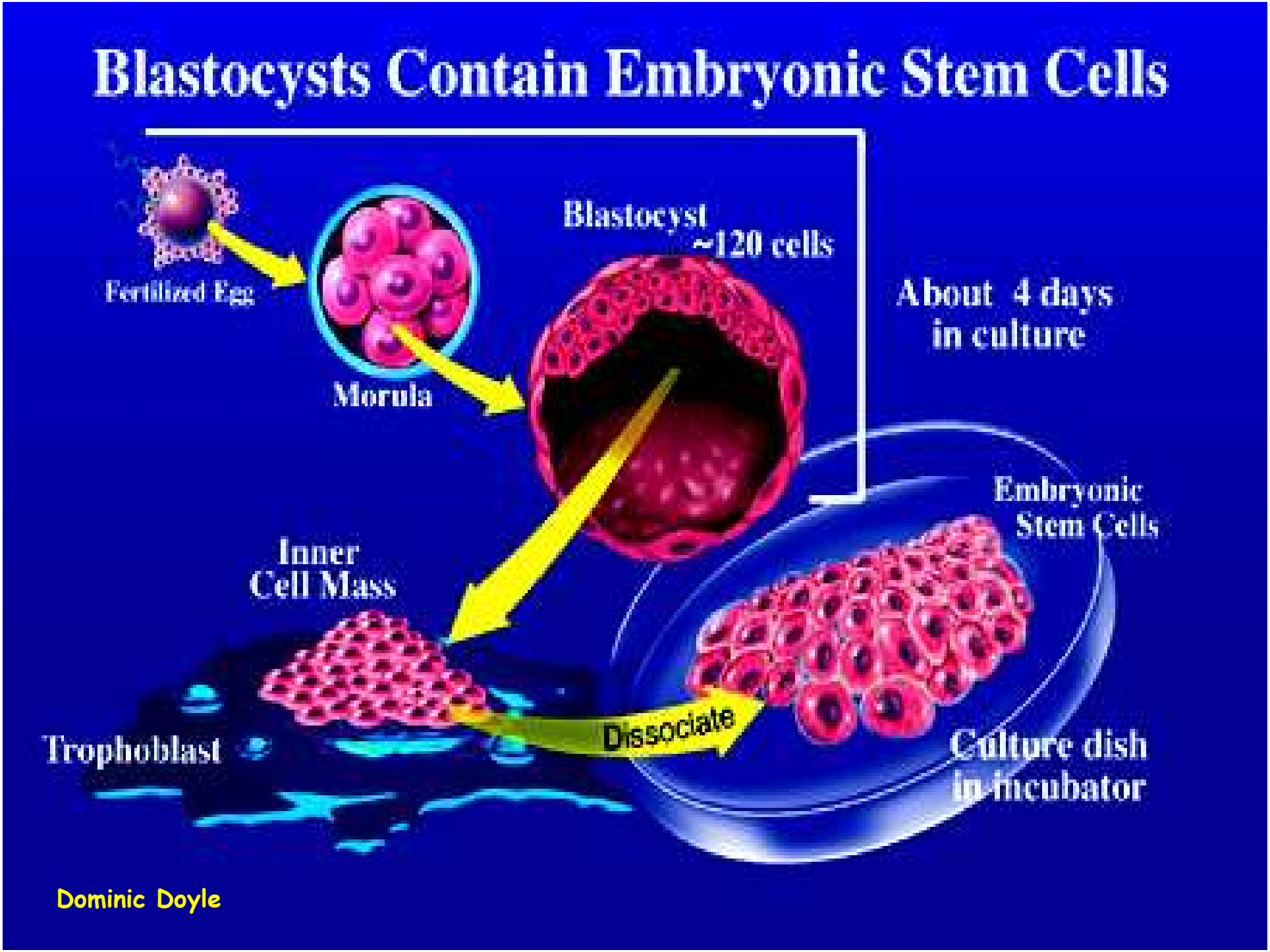
Actin-GFP transgenic mice

Alternative method of retrieving *totipotent* cells

-Based on a procedure used since the mid 1990's called **Preimplantation genetic diagnosis (PGD)**

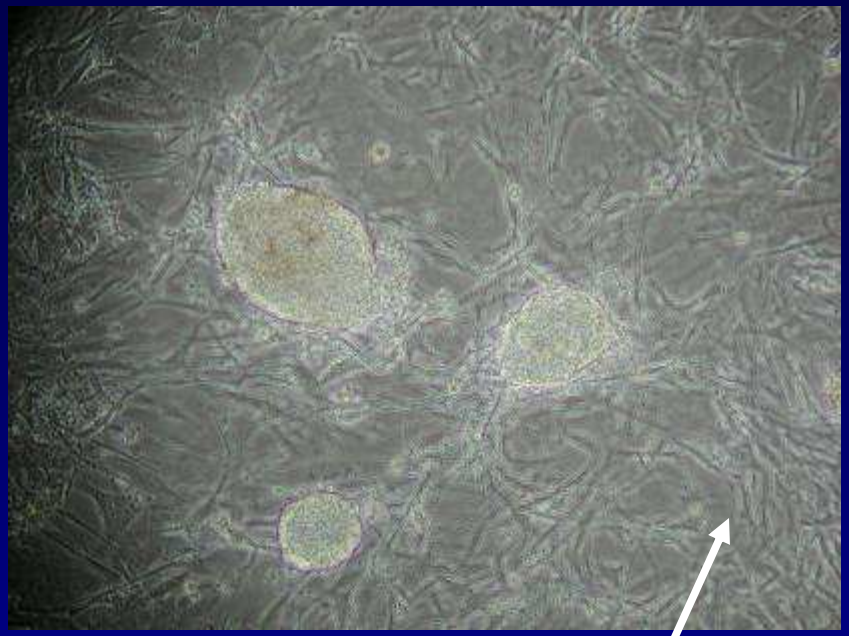


Blastocysts Contain Embryonic Stem Cells

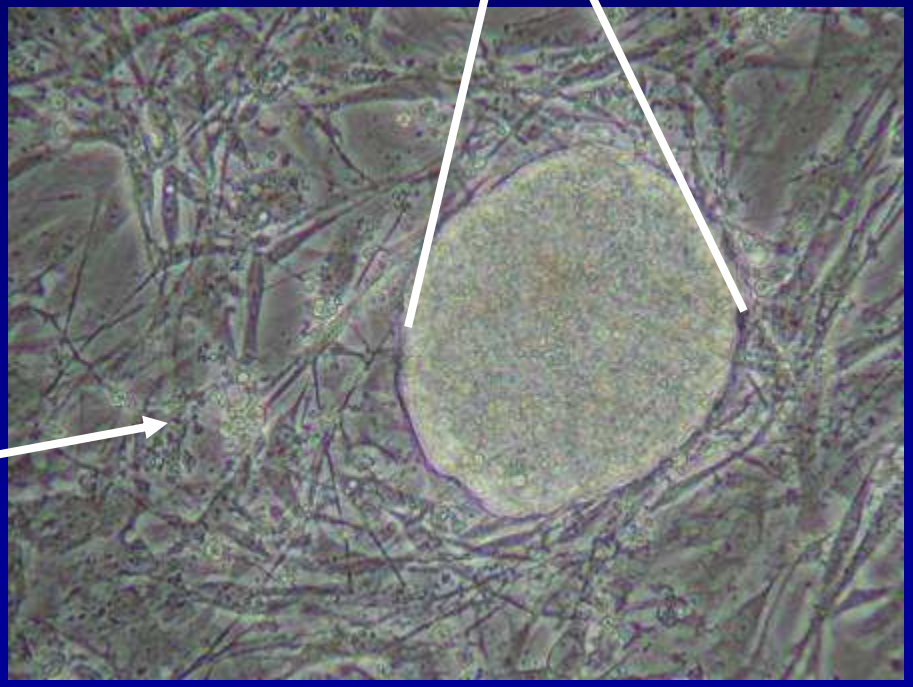


Dominic Doyle

ES colonies



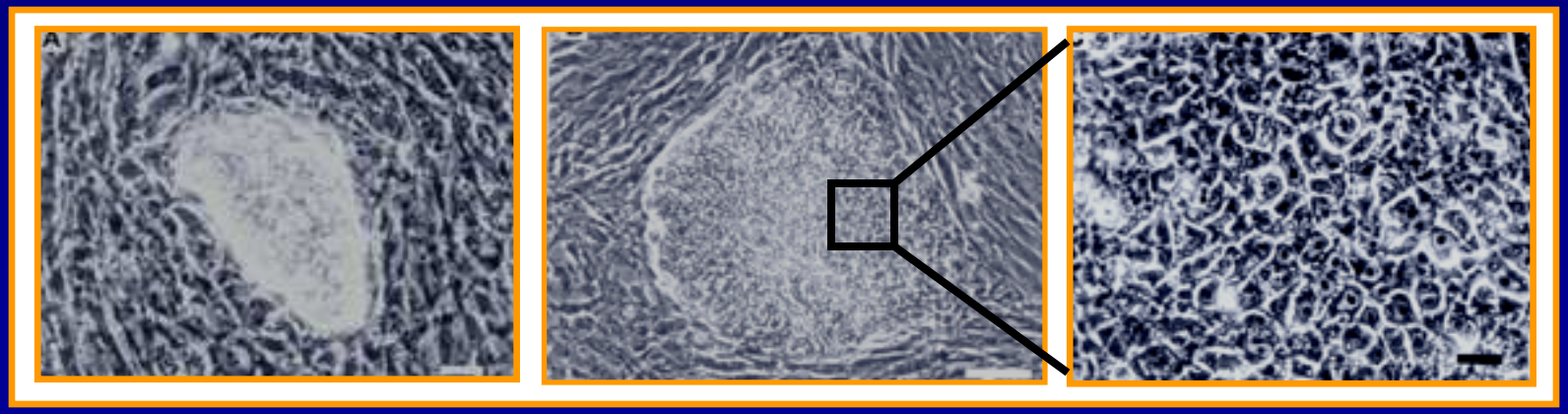
~1,000 ES cells



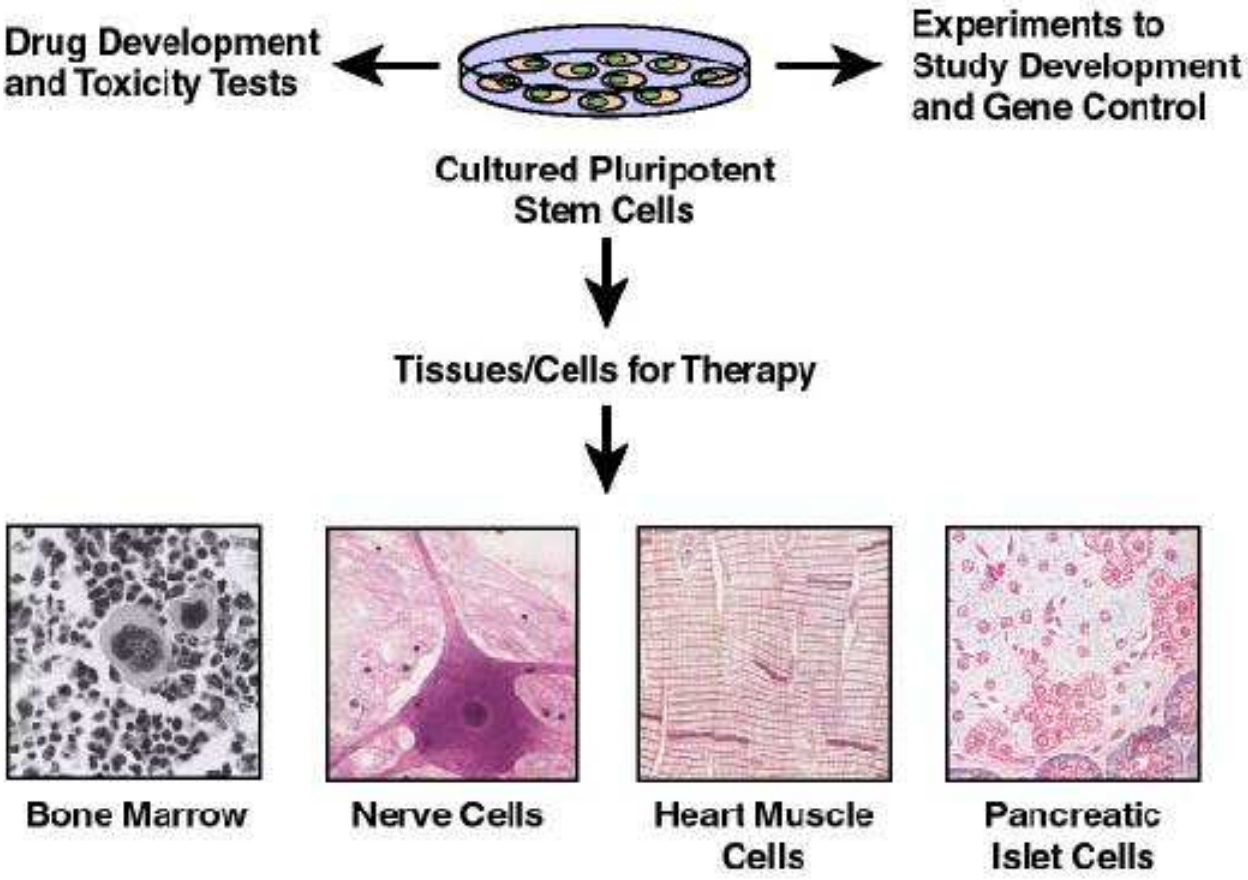
Single layer of firbroblast cells

Human Embryonic Stem Cells

First isolated in 1998
from spare blastocysts in
In Vitro Fertilization (IVF) program

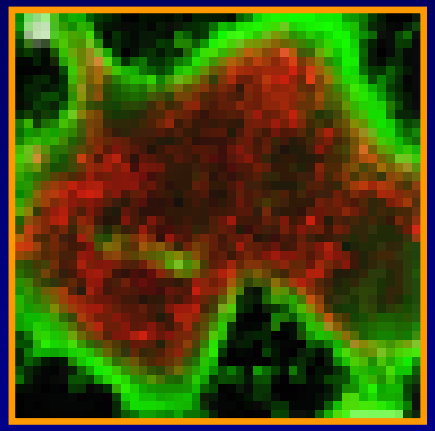


The Promise of Stem Cell Research

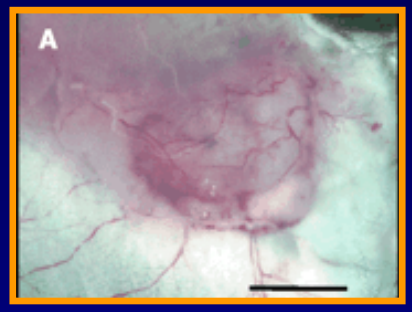


Source: NIH website "Stem cells: A Primer"

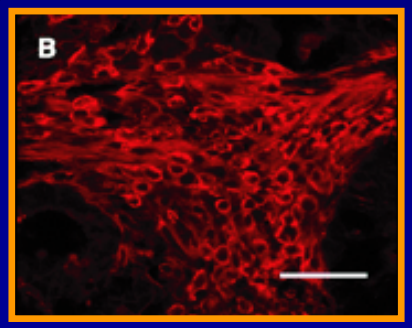
Mouse and Human Embryonic Stem Cells Can Give Rise to Insulin Secreting Cells



Cells secrete insulin

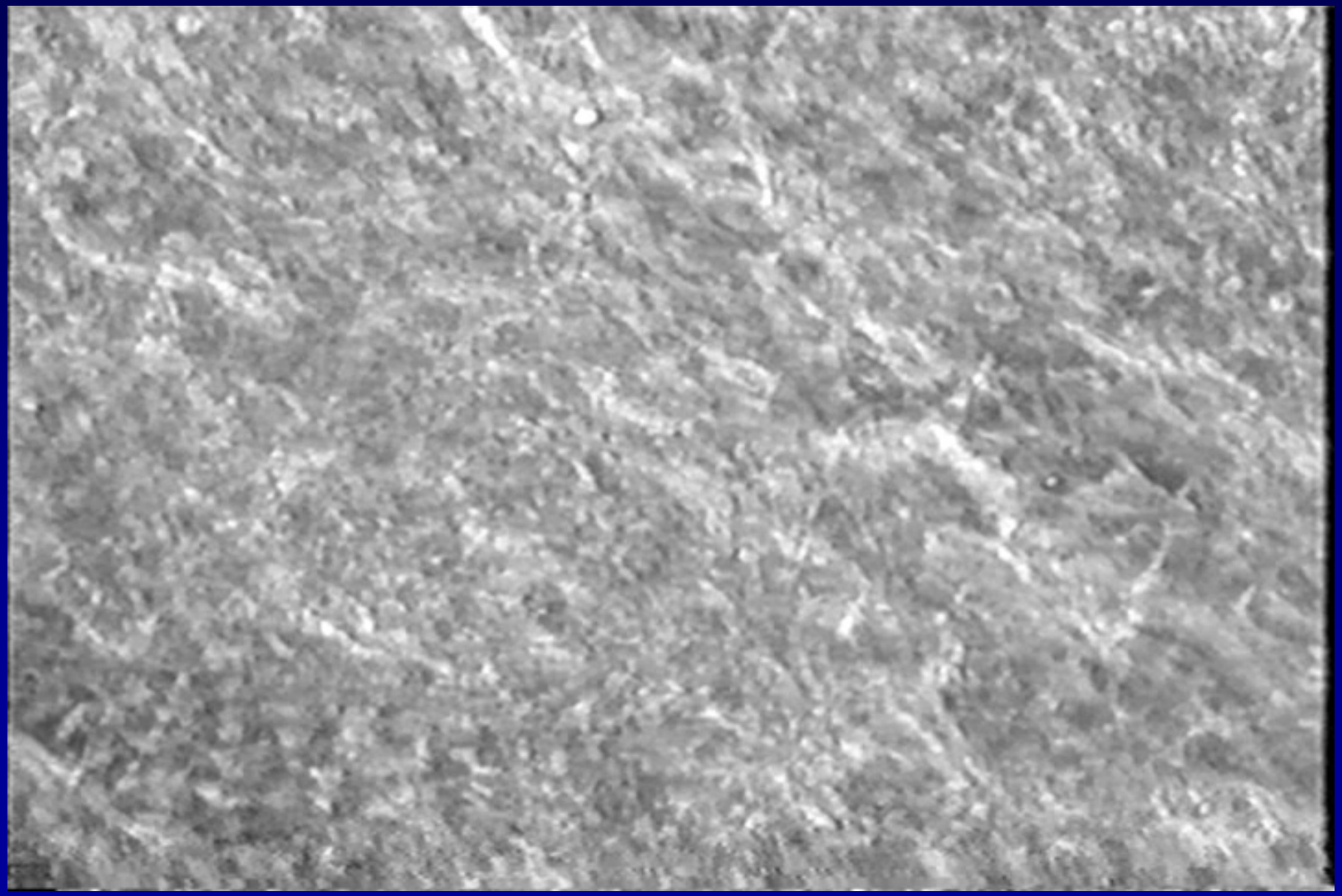


Transplanted into diabetic mouse



Stained for insulin

Pleuripotent non-beating ES cells that are allowed to form EBs begin to beat about 10 days after removal of LIF



Parkinson's Disease

articles

Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease

Jong-Hoon Kim*, Jonathan M. Auerbach*†, José A. Rodríguez-Gómez, Iván Velasco, Denise Gavin, Nadya Lumelsky, Sang-Hun Lee†, John Nguyen†, Rosario Sánchez-Pernaute†, Krys Bankiewicz† & Ron McKay

Laboratory of Molecular Biology, National Institute of Neurological Disorders and Stroke, National Institute of Health, Bethesda, Maryland 20892, USA
** These authors contributed equally to this work*

Parkinson's disease is a widespread condition caused by the loss of midbrain neurons that synthesize the neurotransmitter dopamine. Cells derived from the fetal midbrain can modify the course of the disease, but they are an inadequate source of dopamine-synthesizing neurons because their ability to generate these neurons is unstable. In contrast, embryonic stem (ES) cells proliferate extensively and can generate dopamine neurons. If ES cells are to become the basis for cell therapies, we must develop methods of enriching for the cell of interest and demonstrate that these cells show functions that will assist in treating the disease. Here we show that a highly enriched population of midbrain neural stem cells can be derived from mouse ES cells. The dopamine neurons generated by these stem cells show electrophysiological and behavioural properties expected of neurons from the midbrain. Our results encourage the use of ES cells in cell-replacement therapy for Parkinson's disease.

NATURE | VOL 418 | 4 JULY 2002 | www.nature.com/nature

A.L.S.

(LOU GEHRIG'S DISEASE)

Cell, Vol. 110, 385–397, August 9, 2002, Copyright ©2002 by Cell Press

Directed Differentiation of Embryonic Stem Cells into Motor Neurons

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Jeffery A. Porter,[2] and Thomas M. Jessell[1,3]
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Cambridge, Massachusetts 02138

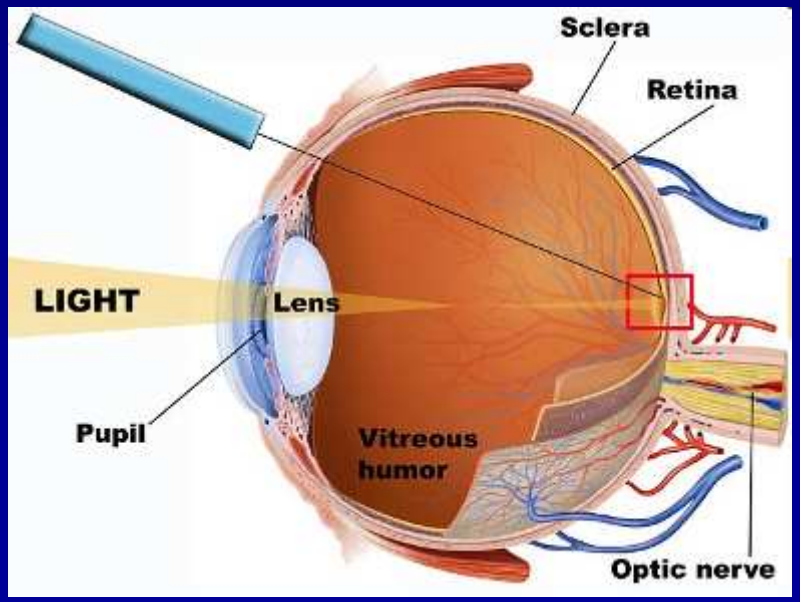
What diseases have been cured using embryonic stem cells?

- **None, yet.**

- **Geron corporation was the first to get FDA approval to test hESC safety in human spinal cords. Was to begin in August 2009**
  - Was asked by the FDA to halt the trial before it began because of safety concerns.

What diseases have been cured using embryonic stem cells?

- **Next up…Advanced Cell Technology Inc. to treat Stargardt's macular dystrophy.**
  - ES cells are pre-differentiated into retinal pigment epithelial cells, which are injected into the eye near the retina
  - In mouse and rat studies 99.9% success rate



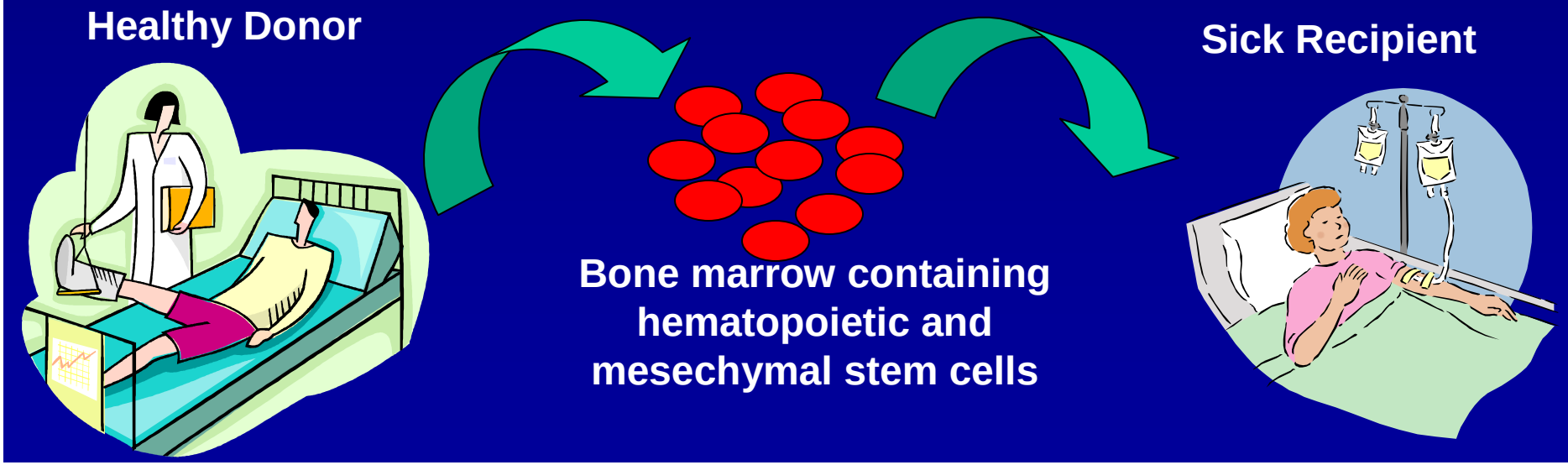
Problems to overcome with ES cells

- Teratoma
- Immunogenicity
- Ethical
- Full understanding of terminal differentiation (quality control)

Adult Stem Cells

Theories behind AS Cell Therapy

- Theory 1
  - **Direct use of AS cells derived from a healthy donor**
  - **Bone marrow transplants to cure leukemias and other blood disorders**

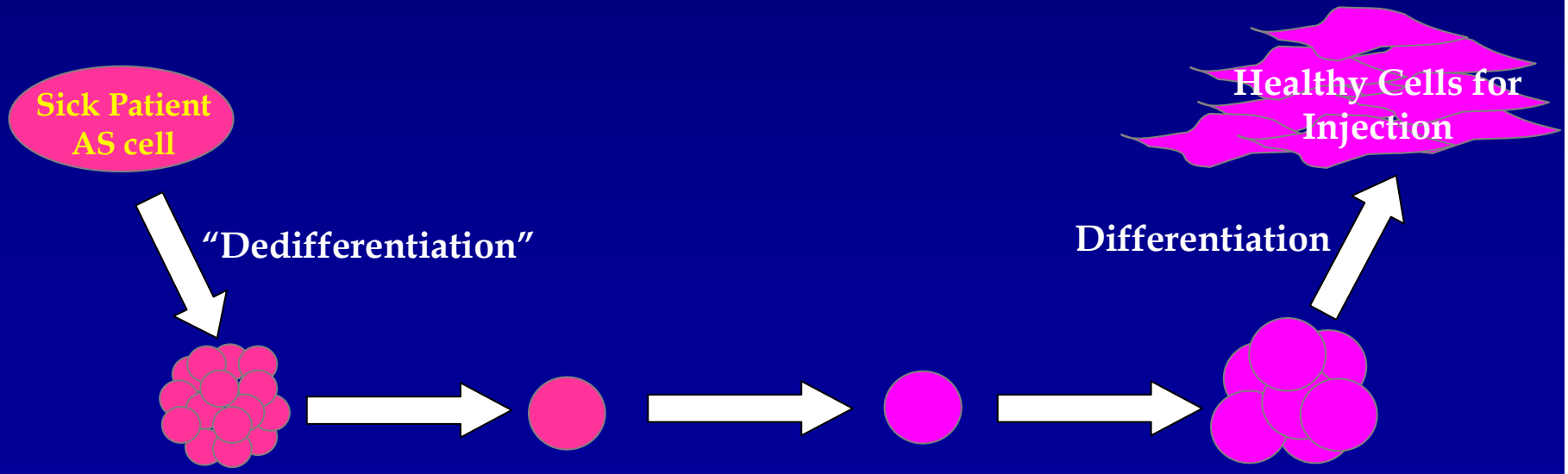


Problems and Benefits of Theory 1

- **Immunogenicity**
  - Because this method uses donor derived stem cells, the recipient must take immuno-suppressants to prevent transplant rejection
- **Donor Availability**
  - Bone marrow harvest is a painful procedure and donor matches are difficult to find
- We can actually do this today to help cure diseases

Theories behind AS Cell Therapy

- Theory 2
  - **AS cells are harvested from a sick patient**
  - **Undergo "dedifferentiation" to an ES cell-like state**
  - **Genetically modified to correct the disease**
  - **Differentiate to form the necessary cell**
  - **Cells are injected back in to the patient**



Problems and Benefits of Theory 2

- We haven't been able to do this yet in people with high success, although preliminary studies are underway (using spermatogonia, mesenchymal stem cells, etc.)

- **Immunogenicity**
  - Because this method uses patient derived cells, immunosuppressive therapy is not necessary

- **Availability**
  - In theory, a small number of cells from a biopsy of the testis or the skin should yield enough cells for this procedure

Stem Cells from Adult Tissues

- **Bone marrow stem cells**
  - Hematopoeitic (Celeste Simon)
  - Adipose Stem Cells (Jeffrey Gimble)
  - Mesenchymal,
  - Multipotent Adult Progenitor Cells (MAPCS) (Catherine Verfaillie)
- **Neural stem cells** (Larysa Pevney)
- **Cardiac stem cells** (Richard Lee)
- **Epidermal stem cells** (Elaine Fuchs)
- **Spermatogonial stem cells** (Martin Dym; Guan et al., 2006-mouse; Conrad et al., 2008-Human)
- **Umbilical cord, placenta,**
- **Stem cells from Human Exfoliated Deciduous teeth (SHED)** (Miura et al., 2003)
- **Amniotic fluid derived stem cells (AFS)** (Anthony Atala)

Reviewed by John Gearhart

Questions Concerning Adult Stem Cells

- **What is the best source for treating/curing disease of interest?**
- **Why do they not differentiate like the surrounding cells?**
  - MSC don't differentiate well when injected into heart
- **What conditions or signals cause them to proliferate and differentiate?**

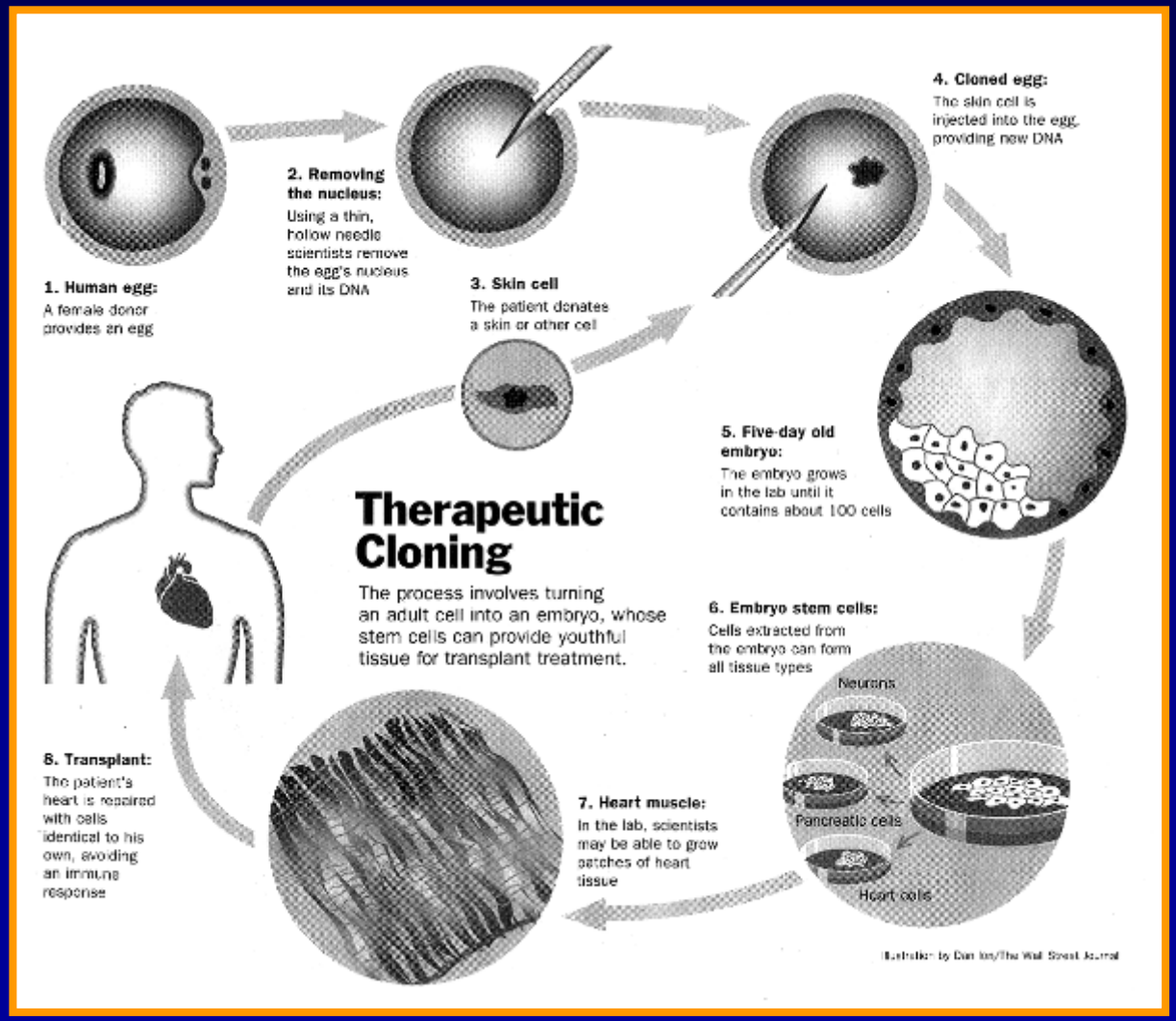
Ongoing Clinical Trials Employing Mesenchymal Stem Cell therapy in Cardiovascular Disease

Trial ID	Start Date	End Date	Condition	Study Type	Phase	Number Enrolled	Delivery Method
NCT00260338[a]	Dec-2005	Nov-2009	Myocardial Infarction, Coronary Heart Disease	Safety/Efficacy	I/II	40	Intramyocardial Injections
NCT00418418[a]	Oct-2006	Dec-2010	Heart Failure, Myocardial Infarction, Coronary Artery Disease	Efficacy	II	60	Intramyocardial Injections
NCT00587990[a]	Nov-2007	Jun-2011	Left Ventricular Dysfunction, Stem Cell Transplantation	Safety/Efficacy	I/II	45	
NCT00555828[b]	Mar-2008	Dec-2013	Myocardial Infarction	Safety/Efficacy	I/II	25	Transendocardial Injections
NCT00768066[a]	Aug-2008	N/A	Left Ventricular Dysfunction, Stem Cell Transplantation	Safety/Efficacy	I/II	60	Transendocardial Injections
NCT00721045[b]	Aug-2008	Jul-2011	Heart Failure	Safety/Efficacy	II	60	Transendocardial Injections
NCT00644410[a]	Sep-2008	Sep-2012	Congestive Heart Failure	Safety/Efficacy	I/II	60	Intramyocardial Injections
NCT00810238[a]	Dec-2008	Dec-2010	Chronic Heart Failure (Class II/III)	Safety/Efficacy	I/II	240	Intraventricular Injections
NCT00883727[a]	Apr-2009	Dec-2011	Myocardial Infarction	Safety/Efficacy	I/II	20	Intravenous Injections
NCT00877903[a]	Mar-2009	Mar-2012	Myocardial Infarction	Safety/Efficacy	II	220	Intravenous Injections
NCT00927784[b]	Jun-2009	Sep-2010	Congestive Heart Failure	Safety/Efficacy	II	80	Intramyocardial Injections

The table summarizes the ongoing clinical trials on mesenchymal cells. Trial ID corresponds to each study as listed on **http://clinicaltrials.gov/**.
[a]Clinical trials use mesenchymal stem cells.
[b]Clinical trials use mesenchymal precursor cells

Adult/ES-like cells

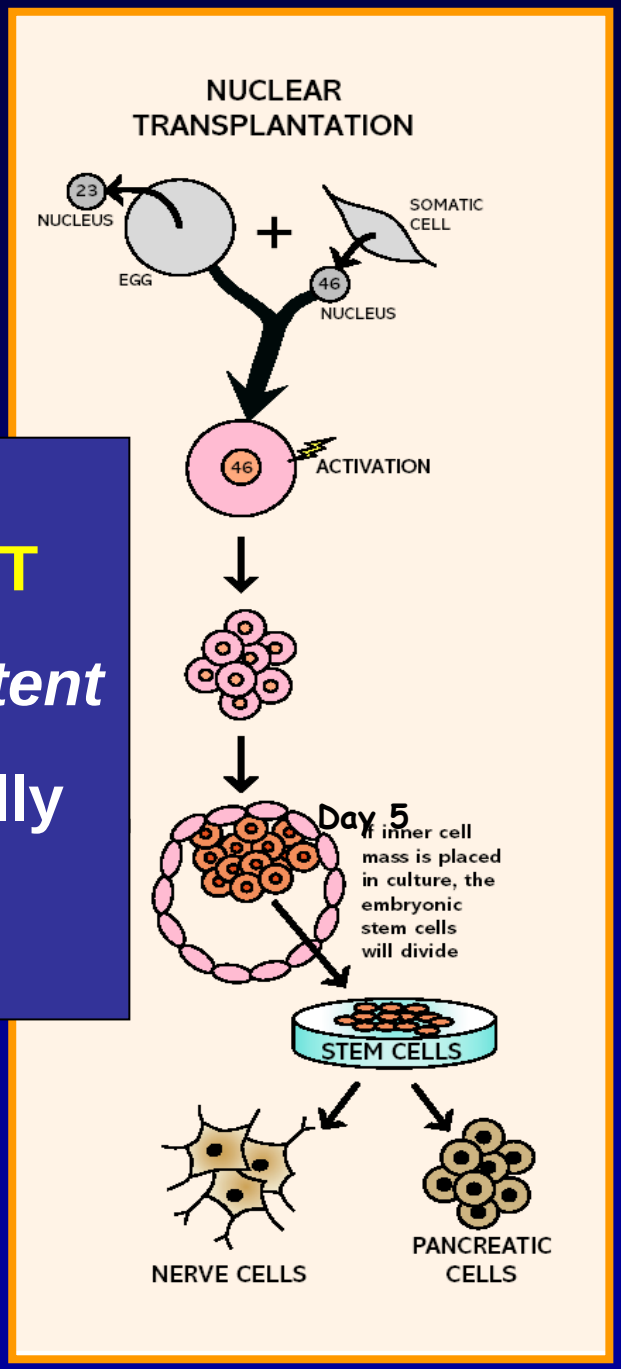
Somatic Cell Nuclear Transfer (SCNT)



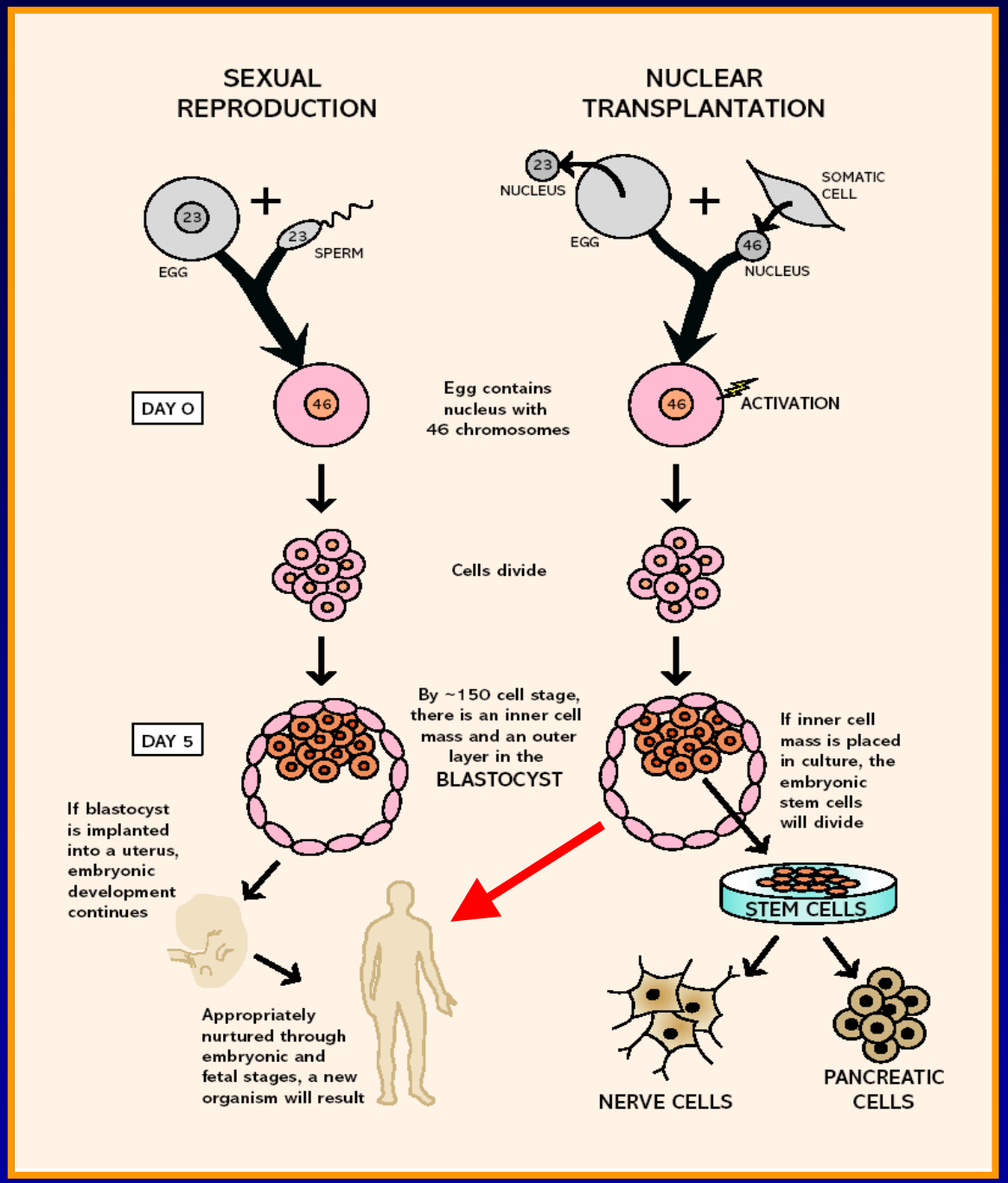
“Therapeutic Cloning”

Potential Advantage of SCNT

The Ability to Produce *Pluripotent* Stem Cells that are Genetically Identical to the Patient



The Moral Crossroads



iPS

Kazutoshi Takahashi and Shinya Yamanaka

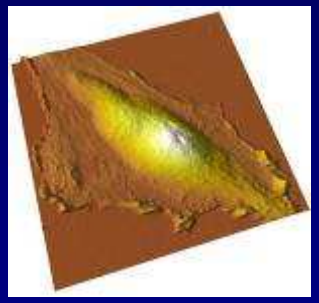
- **Virally transfect 4 genes into fibroblasts and they reprogram into ES-like cells**

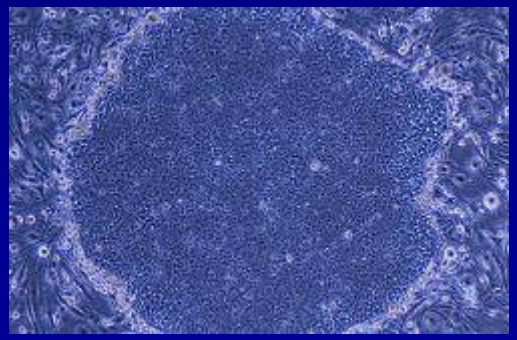
cMyc-may now be dispensable –Lin 28

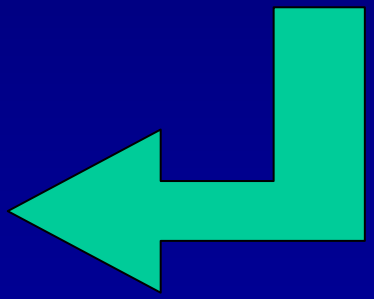
Klf4

Sox2

Oct4







Problems being resolved:

-Retroviruses or adenoviruses are needed

-iPS form teratomas

-Genes are not controlled

-Genes may insert into genome replacing a tumor suppressor

(Fixed using transposon technology? A. Nagy)

Pluripotency of Spermatogonial Stem Cells (gPS cells)

Spermatogonial Stem Cell → Embryonic Stem Cell-like → Different cell lineages

- **Golestaneh et al., 2009 Stem Cells and Development 2009 Oct;18(8):1115-26**

- Guan *et al*. Pluripotency of spermatogonial stem cells from adult mouse testis. Nature 440:1199-1203, 2006.

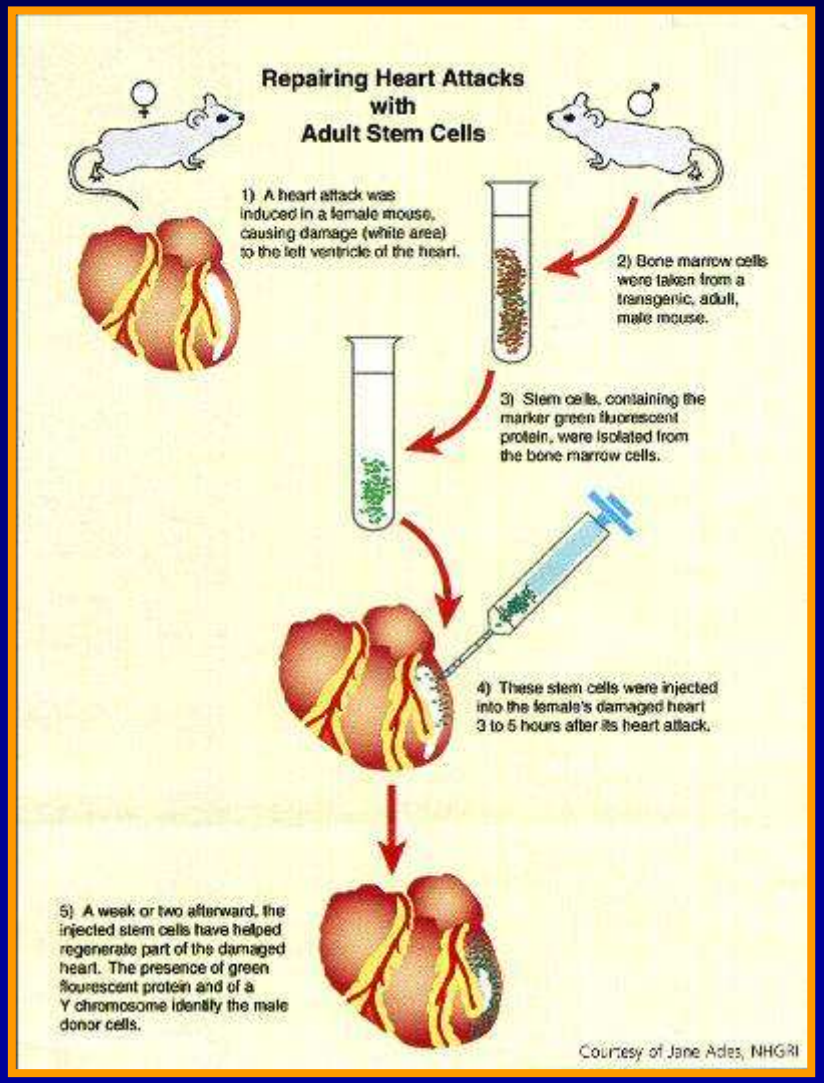
- Shinohara *et al*. The germ of pluripotency. Spermatogonial stem cells in adult testis may be more versatile than embryonic stem cells. Nature Biotechnology 24, June 6; 663-664, 2006

- Baba *et al*. Generation of Cardiac and Endothelial Cells from Neonatal Mouse Testis-derived Multipotent Germline Stem Cells. Stem Cells. 2007 Feb 22; [Epub ahead of print] .

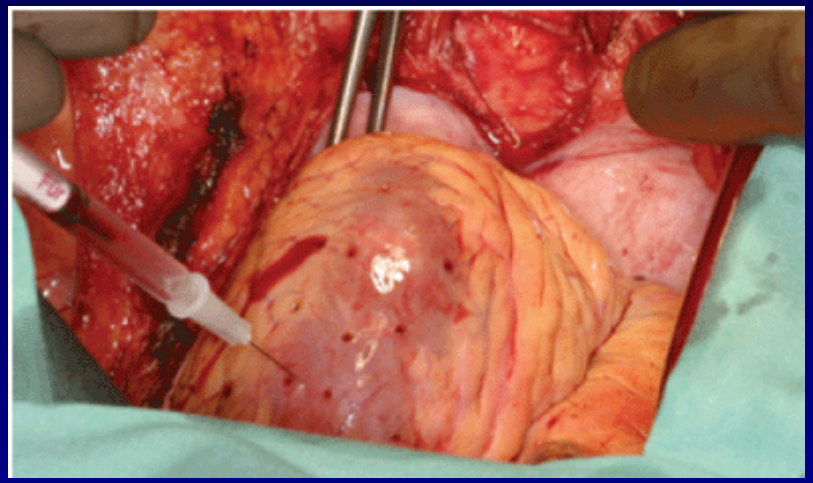
Human

- **Conrad S, et al. (2008). Generation of pluripotent stem cells from adult human testis. Nature.**

A Therapeutic Regimen Using Adult Stem Cells/iPS/SCNT



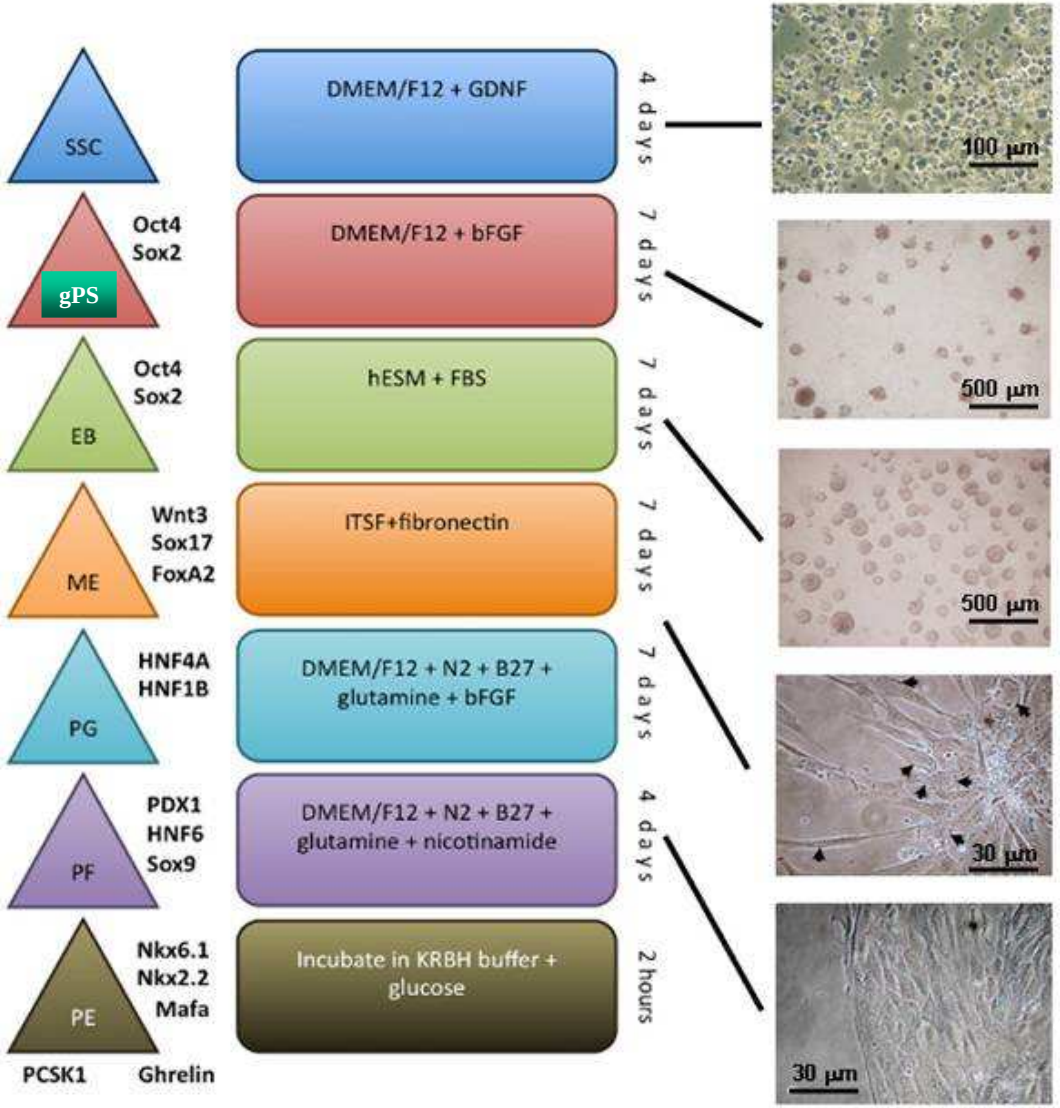
Adapted from Orlic *et al.*, *Nature* 410:701-705 (2001)

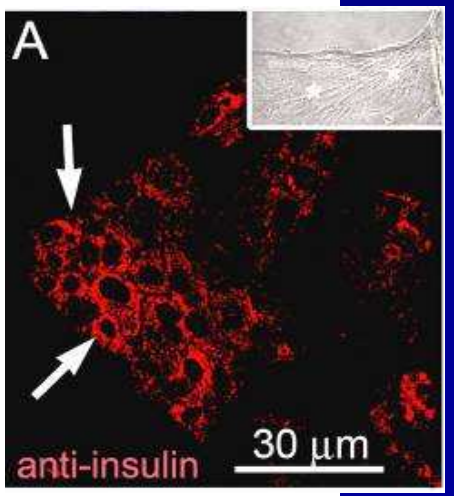


Transmyocardial Injection

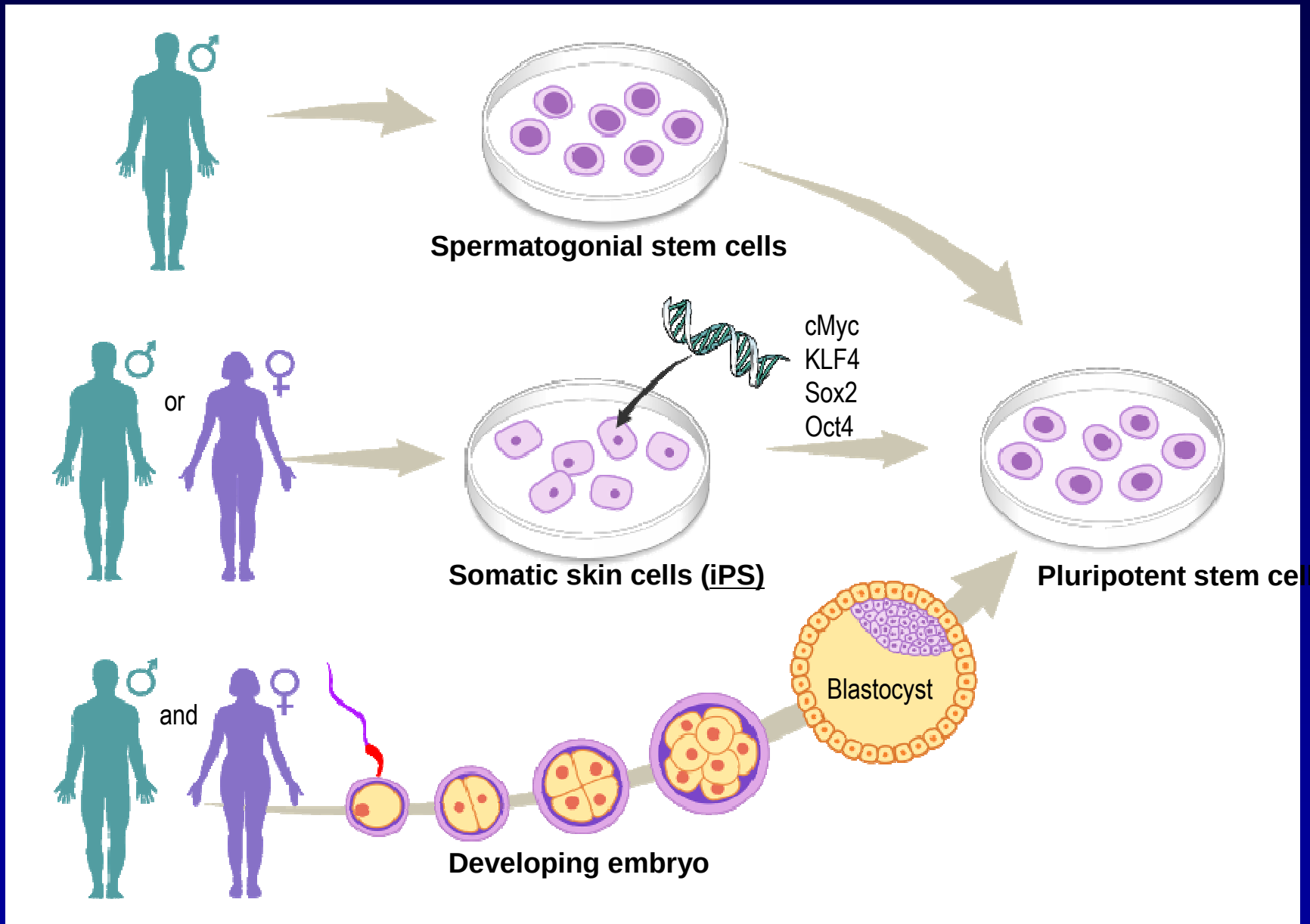
Aka Direct epicardial Injection

Can pancreatic cells generated from human gPS cells secrete insulin? Protocol for generating insulin-secreting cells?





An "Ethical" Stem Cell is Born?



Stem Cell Funding

- Federal funds can only support human ES cell research using approved cell lines (Pres. Bush)
  - **However, most educational institutions rely on government funding from the NIH**
  - **NEW Guidelines (Pres. Obama)-Federal funds can be used to generate new hESC lines from frozen IVF embryos. MUST HAVE PERMISION FROM PARENTS.**
- Some State governments are providing additional funding for ES cell research
  - **i.e. California Institute for Regenerative Medicine**
  - **New Jersey Commission on Stem Cell Biology**
  - **New York Commission on Stem Cell Biology (NYSTEM)**
- There are currently no funding restrictions on the use of adult stem cells

ES Cell Lines

- In the USA, until July 2009, no new human ES cell lines could be made
- **http://grants.nih.gov/stem_cells/registry/current.htm**
  - **22 old lines are in limbo/no approval was obtained from parents**
  - **There are 44 new, approved cell lines as of ~1/10**
  - **114 lines are pending**
  - **Many of the old lines are not genetically normal or undifferentiated due to length of use (bad hands using them)**
  - **Many of the new lines are too young to determine genetic stability**
  - **The new guidelines allow new hESC lines to be produced, but will take over a year to generate and test.**

What about IVF embryos?

- Now that federal restrictions have been lifted, frozen IVF embryos can be used to generate ES cell lines
- However…
  - **This is a limited resource**
  - **Many of the leftover embryos were not used for implantation due to genetic or developmental abnormalities**
  - **Good for studying genetic diseases, but may not be therapeutically viable**

Scientific and Philosophical Juncture

- **Should we continue to lead the world in asking important scientific questions ?**
- **Do we allow ourselves to become a backwater in an area of research destined to have profound therapeutic and economic implications?**
- **Embryonic stem cell research holds similar promise as well as a heavy burden of social and moral responsibility.**
- **We faced this type challenge previously when DNA technology was in its infancy**
- **Louise Brown**

  Born-July 25, 1978