Lecture 12-14

BT 636
Tissue Engineering and Regenerative Medicine
(3-0-0-6)

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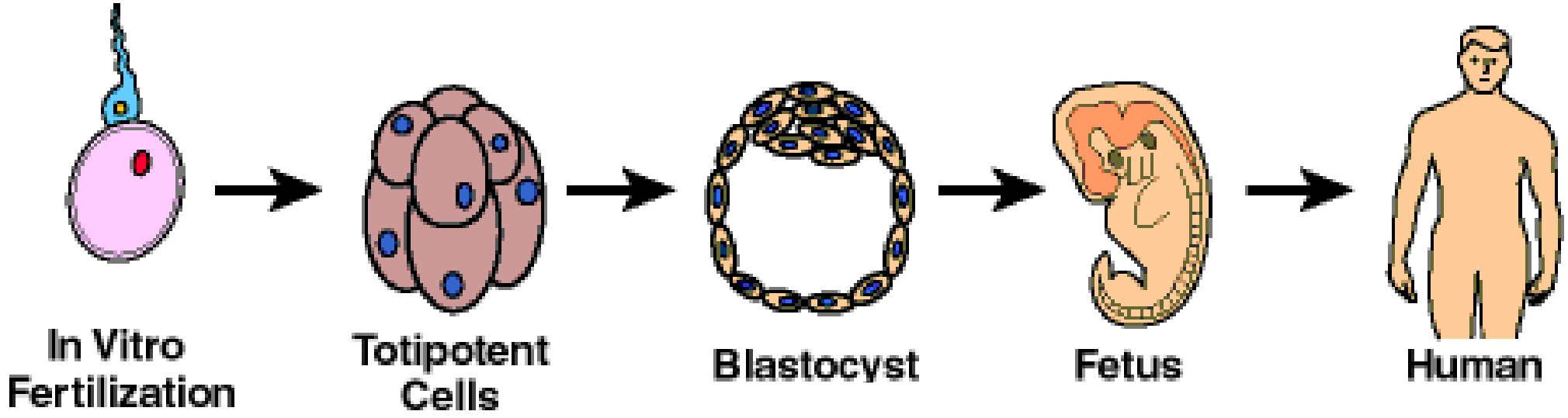
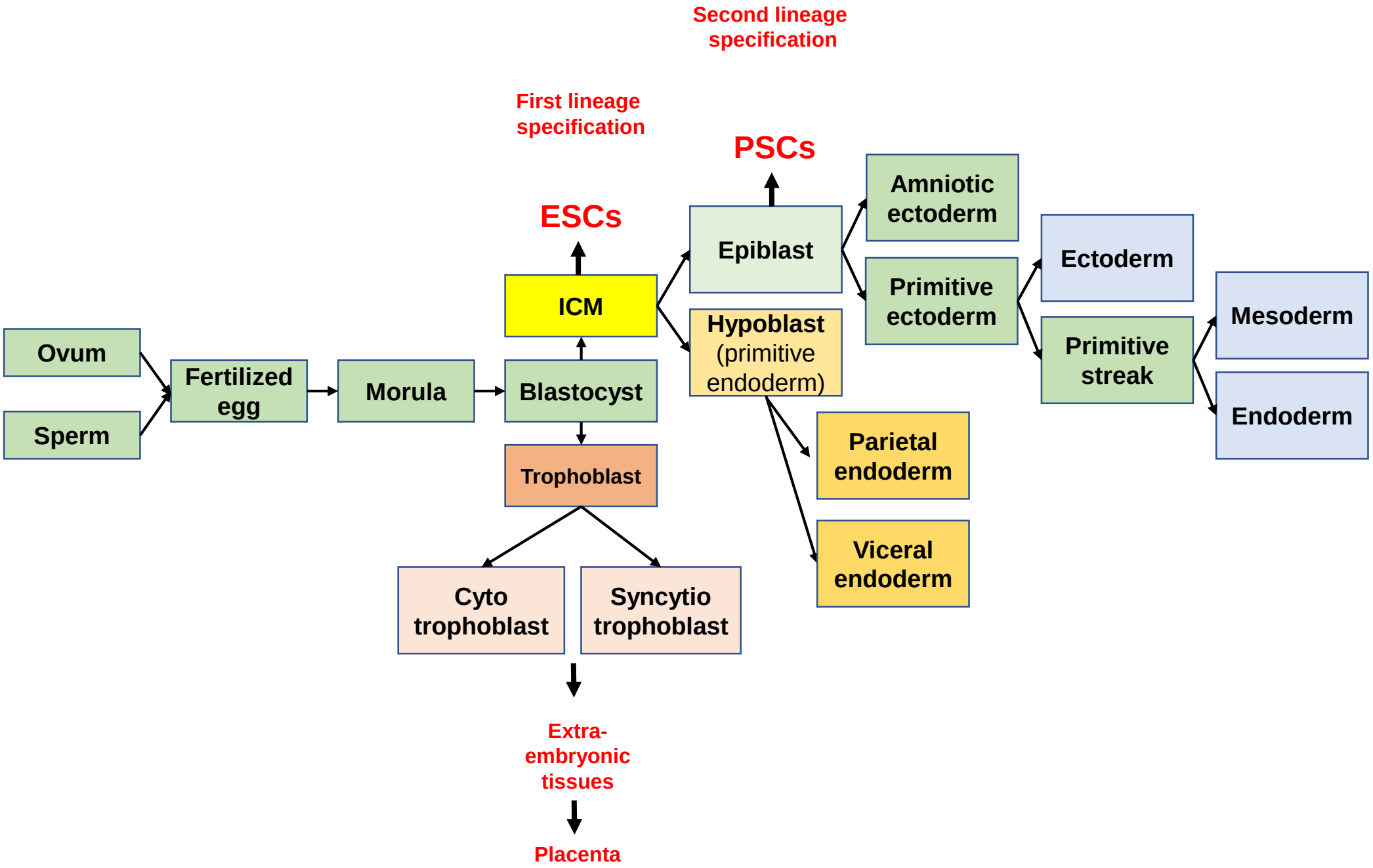
Human development



Embryonic development is a series of processes by which:

- **a fertilized egg divides (cleavage),**
- **forms a ball of cells (morula),**
- **develops a cavity (blastocyst stage),**
- **forms the three primary germ layers of cells that will ultimately give rise to all the cell types of the body (gastrula stage),**
- **and eventually generates all the specialized tissues and organs of a mature organism**


Second lineage specification
First lineage specification
PSCs
ESCs
Amniotic ectoderm
Epiblast
ICM
Ectoderm
Primitive ectoderm
Hypoblast (primitive endoderm)
Primitive streak
Mesoderm
Ovum
Fertilized egg
Morula
Blastocyst
Endoderm
Sperm
Parietal endoderm
Trophoblast
Viceral endoderm
Cyto trophoblast
Syncytio trophoblast
Extra-embryonic tissues
Placenta

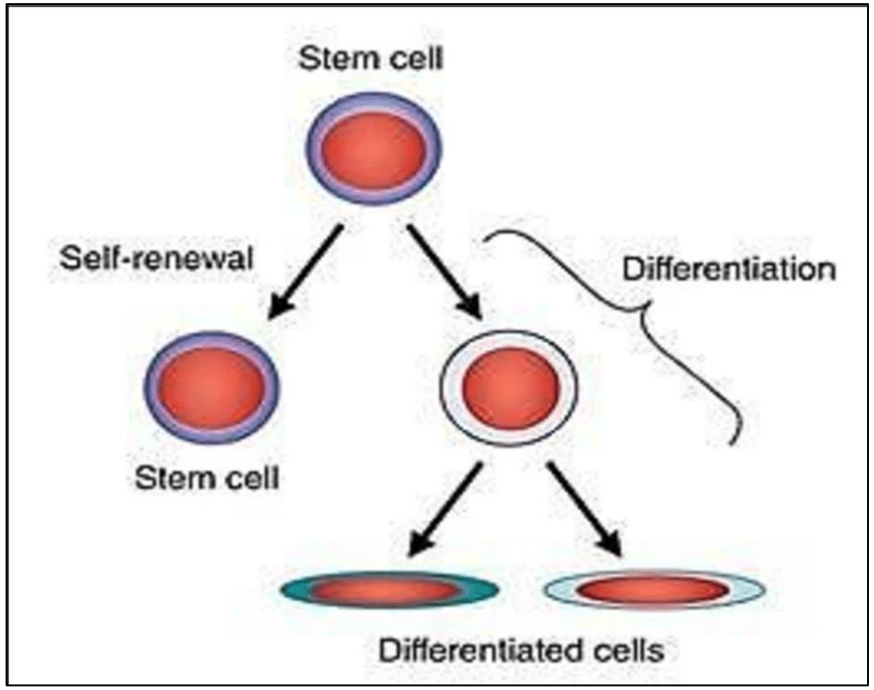
- After the blastocyst stage, once an embryo implanted in endometrium, the inner cell mass (ICM) of a fertilized embryo segregates into two layers: **hypoblast and epiblast**.

- The **epiblast** cells are the functional progenitors of somatic and germ cells. These cells later differentiate into 3 layers: definitive endoderm, mesoderm and ectoderm.

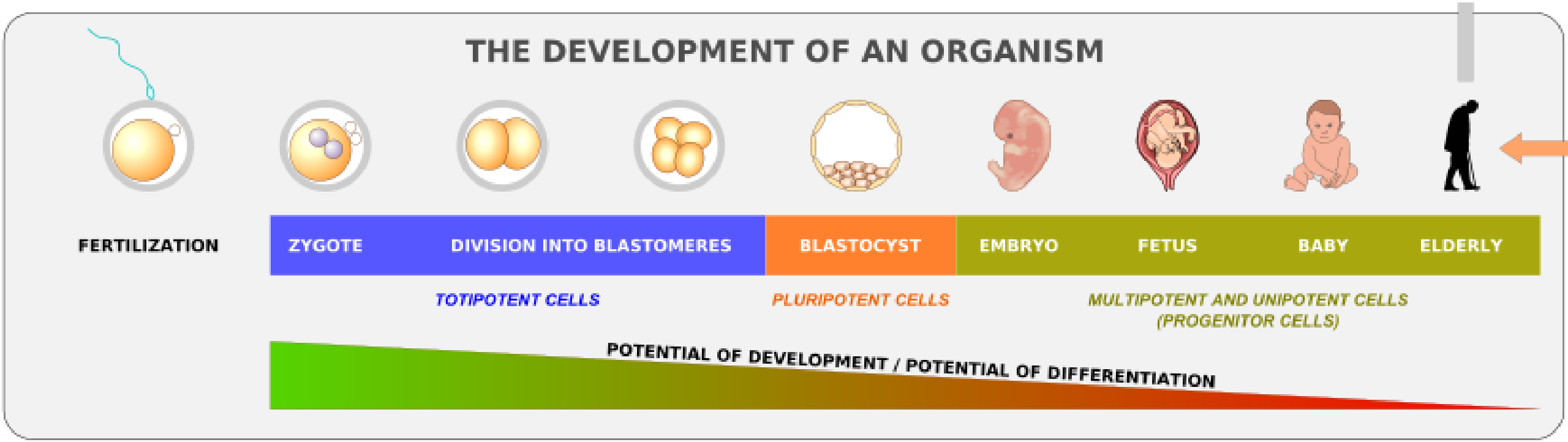
- Stem cells derived from **epiblast** are pluripotent. These cells are called epiblast-derived stem cells (EpiSC) and have several different cellular and molecular characteristics with Embryonic Stem Cells (ESC) (De-Miguel et al., 2009).

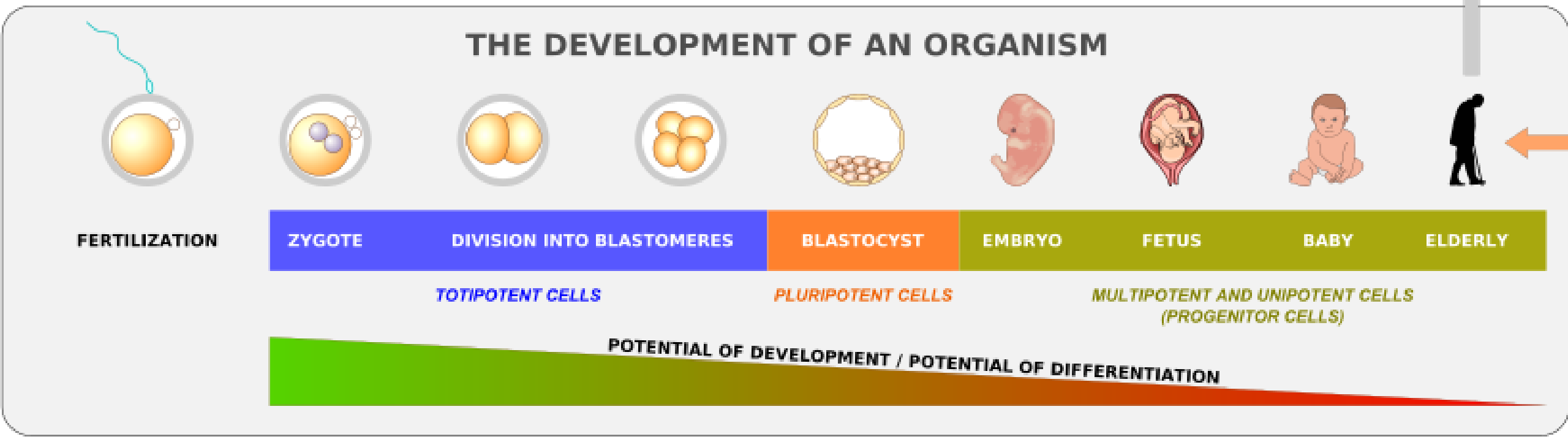
Stem cells and its special characteristics

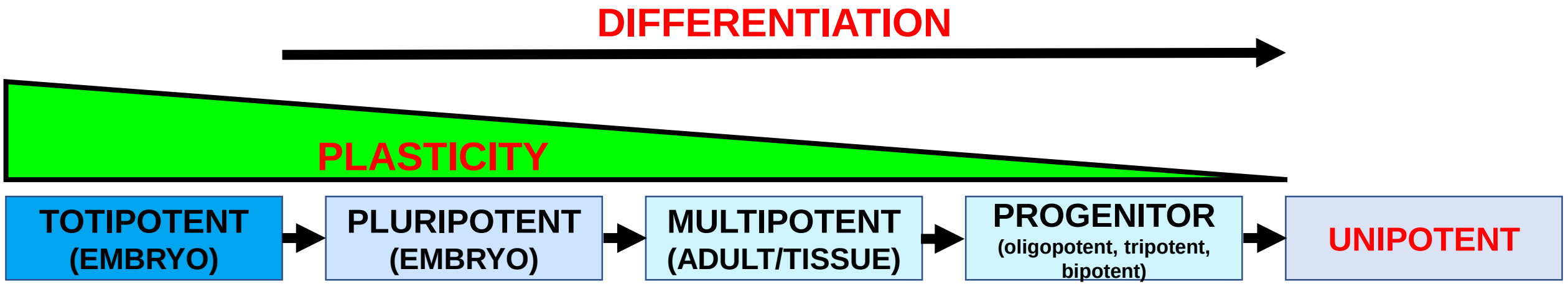
- **Undifferentiated** cells
- Ability to **self-renew** *in vitro*
- Ability to **differentiate** into multiple **OR** all cell types of the three germ layers
- Source for autologous (self) and allogenic (non-self) transplantation



Human development




THE DEVELOPMENT OF AN ORGANISM
FERTILIZATION
ZYGOTE
DIVISION INTO BLASTOMERES
BLASTOCYST
EMBRYO
FETUS
BABY
ELDERLY
TOTIPOTENT CELLS
PLURIPOTENT CELLS
MULTIPOTENT AND UNIPOTENT CELLS
(PROGENITOR CELLS)
POTENTIAL OF DEVELOPMENT / POTENTIAL OF DIFFERENTIATION


DIFFERENTIATION
PLASTICITY
TOTIPOTENT (EMBRYO)
PLURIPOTENT (EMBRYO)
MULTIPOTENT (ADULT/TISSUE)
PROGENITOR
(oligopotent, tripotent, bipotent)
UNIPOTENT

Types of Stem Cells

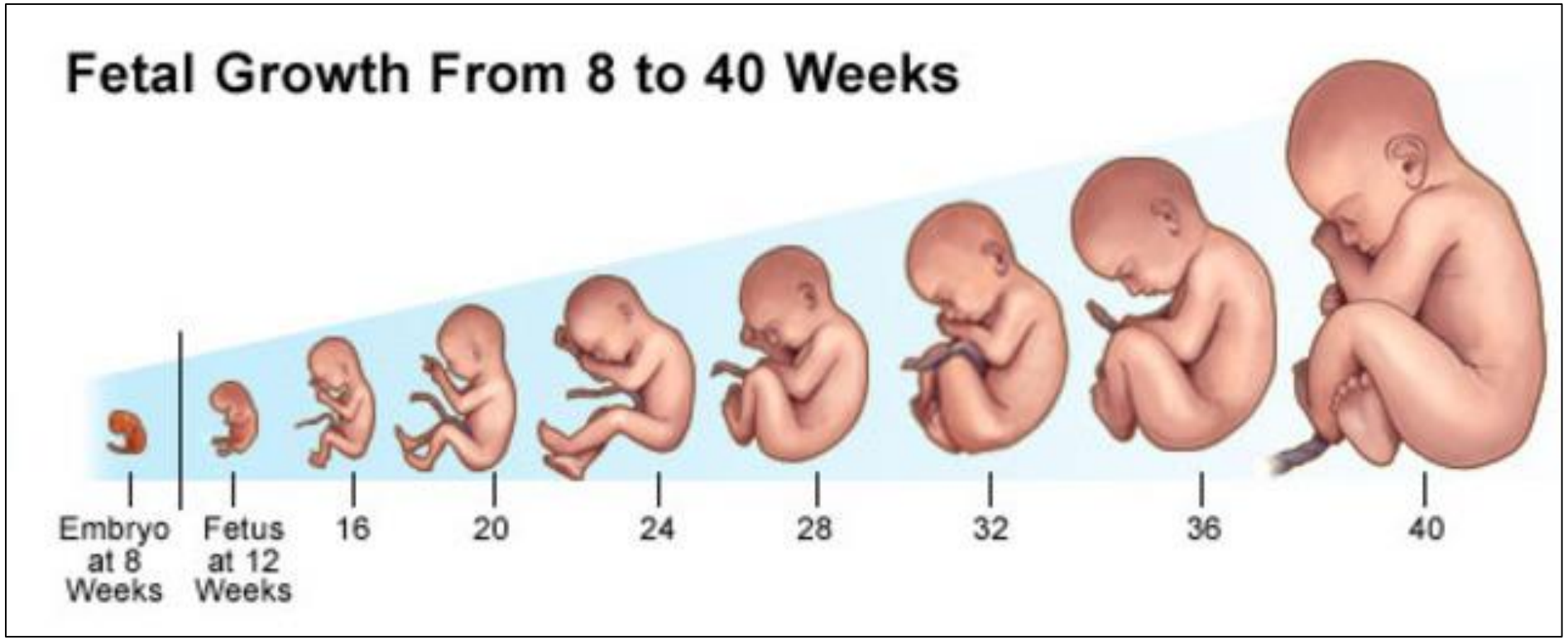
based on source

Embryo-derived stem cells	Fetal stem cells	Adult stem cells	Induced pluripotent stem cells
derived from an embryo (produced by IVF)	derived from fetus (aborted fetuses)	derived from adult (tissues of adults)	Generated artificially in a lab
E.g. Embryonic stem cells, Epiblast stem cells, Embryonic germ cells	E.g. Fetal blood and fetal tissues	E.g. HSCs, MSCs, NSCs, ADSCs, etc.	E.g. iPSCs

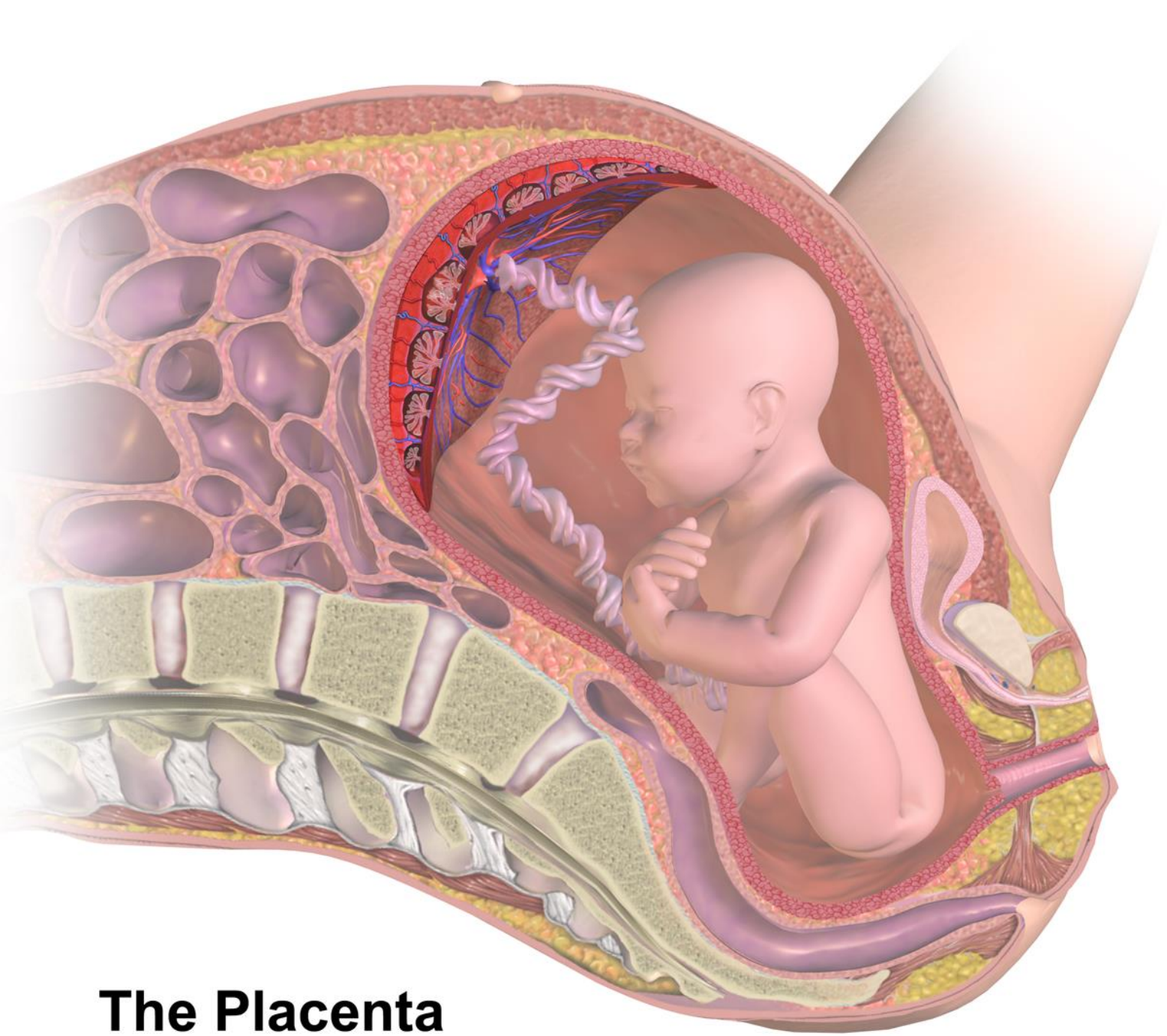
Umbilical cord blood stem cells ⟶ derived from umbilical cord

HSCs (cord blood), MSCs (Wharton's jelly)

Fetal development



A human fetus, attached to placenta, at around twelve weeks after fertilization.
Until around nine weeks after fertilization, this prenatal human would have been described as an embryo.



The Placenta

The **placenta** is a temporary organ that connects the developing fetus via the **umbilical cord** to the uterine wall to:

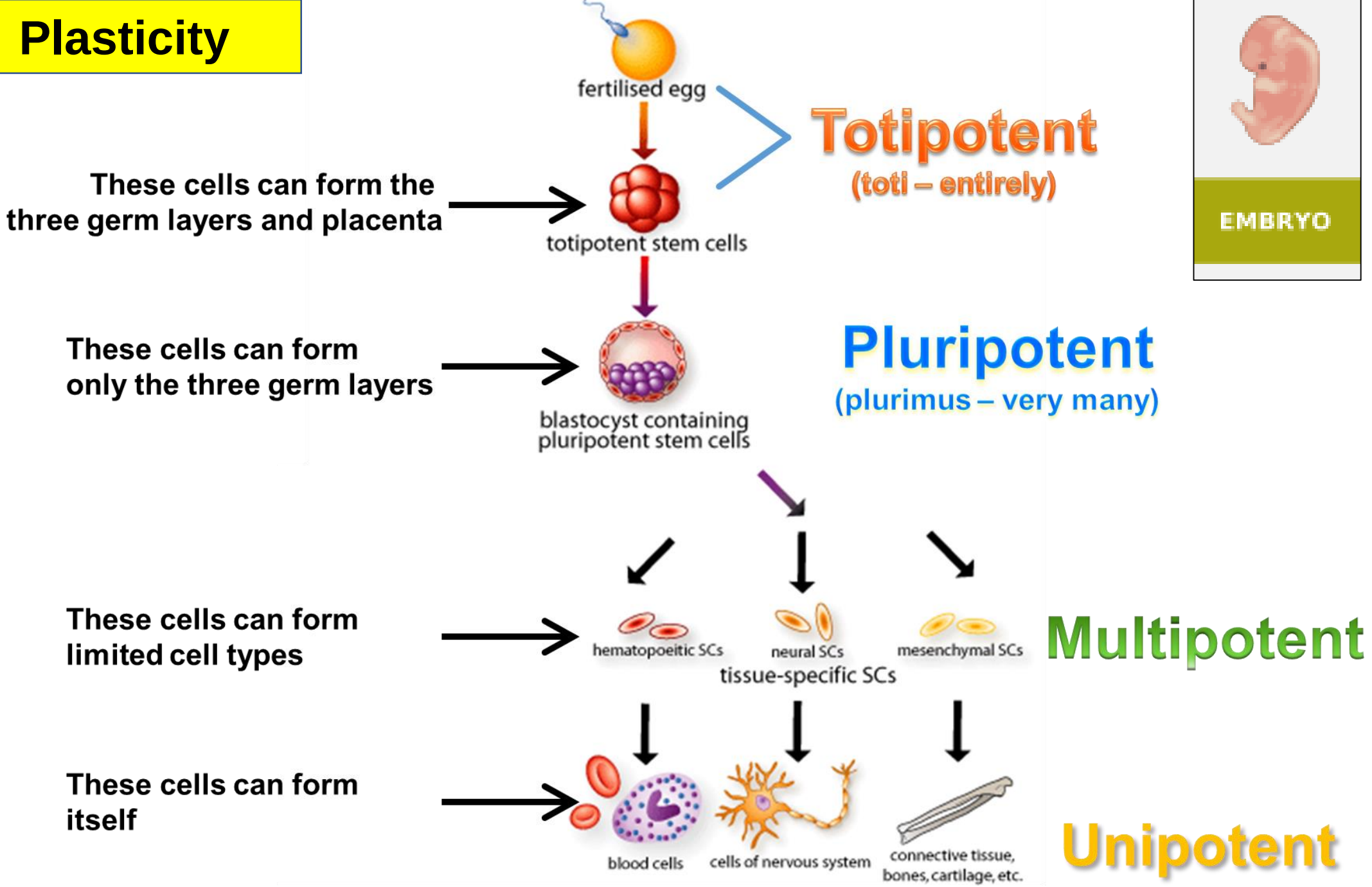
- Allow nutrient uptake
- Thermo-regulation
- Waste elimination
- Gas exchange via the mother's blood supply
- To fight against internal infection
- Produce hormones which support pregnancy.

Types of Stem Cells

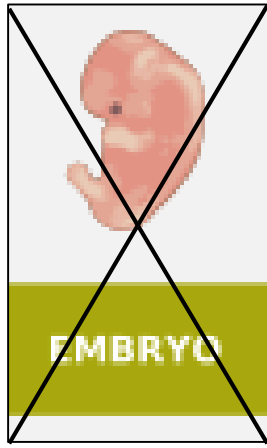
based on potency or differentiation ability

Totipotent cells	Pluripotent stem cells	Multipotent stem cells	Unipotent stem cells
present in embryo	derived from an embryo (produced by IVF)	derived from fetal, UC and adult tissues	present in adult tissues
E.g. Fertilized zygote Morula	E.g. Embryonic stem cells Epiblast stem cells Embryonic germ cells iPSCs	E.g. HSCs, MSCs, NSCs, ADSCs, UCBSCs, etc.	E.g. Skin stem cells, muscle stem cells, etc.

Plasticity


fertilised egg
Totipotent
(toti – entirely)
These cells can form the three germ layers and placenta
totipotent stem cells
These cells can form only the three germ layers
blastocyst containing pluripotent stem cells
Pluripotent
(plurimus – very many)
These cells can form limited cell types
hematopoeitic SCs
neural SCs
mesenchymal SCs
tissue-specific SCs
Multipotent
These cells can form itself
blood cells
cells of nervous system
connective tissue, bones, cartilage, etc.
Unipotent

EMBRYO


EMBRYO

Classification based on Potency of Cells

TOTIPOTENT

- Fertilized egg (or zygote)
- Morula

PLURIPOTENT

- Embryonic Stem cells (ESC)
- Epiblast Stem Cells (EpiSCs)
- Embryonic Germ Cells (EGCs)
- Embryonic Carcinoma Cells (ECCs)
- Induced Pluripotent Stem Cells (iPSCs)

MULTIPOTENT

PROGENITOR
(oligopotent, tripotent, bipotent)

ADULT STEM CELLS (ASCs) or TISSUE STEM CELLS

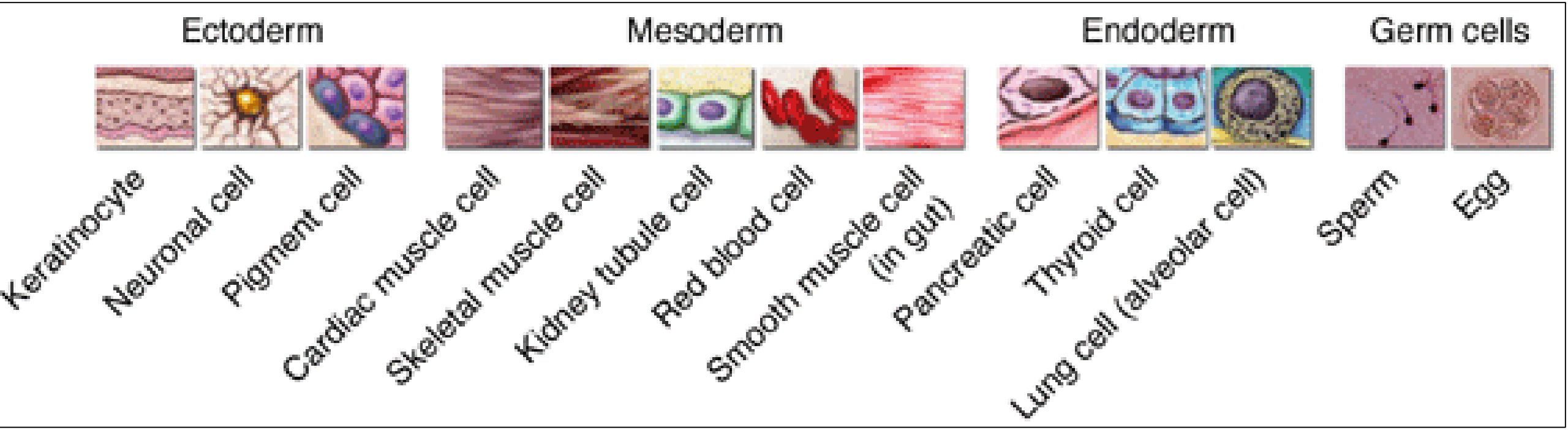
- Hematopoietic Stem Cells (HSCs) – from bone marrow and umbilical cord blood stem cells
- Mesenchymal Stem Cells (MSCs)
- Neural Stem Cells (NSCs)
- Intestinal Stem Cells
- Endothelial Stem Cells
- Spermatogonial Stem Cells
- etc. etc. etc.
- ASCs or Multipotent SCs give rise to progenitor cells, which are intermediate stem cells that lose potency. Progenitor stem cells are committed to differentiating along a particular cell developmental pathway.

UNIPOTENT

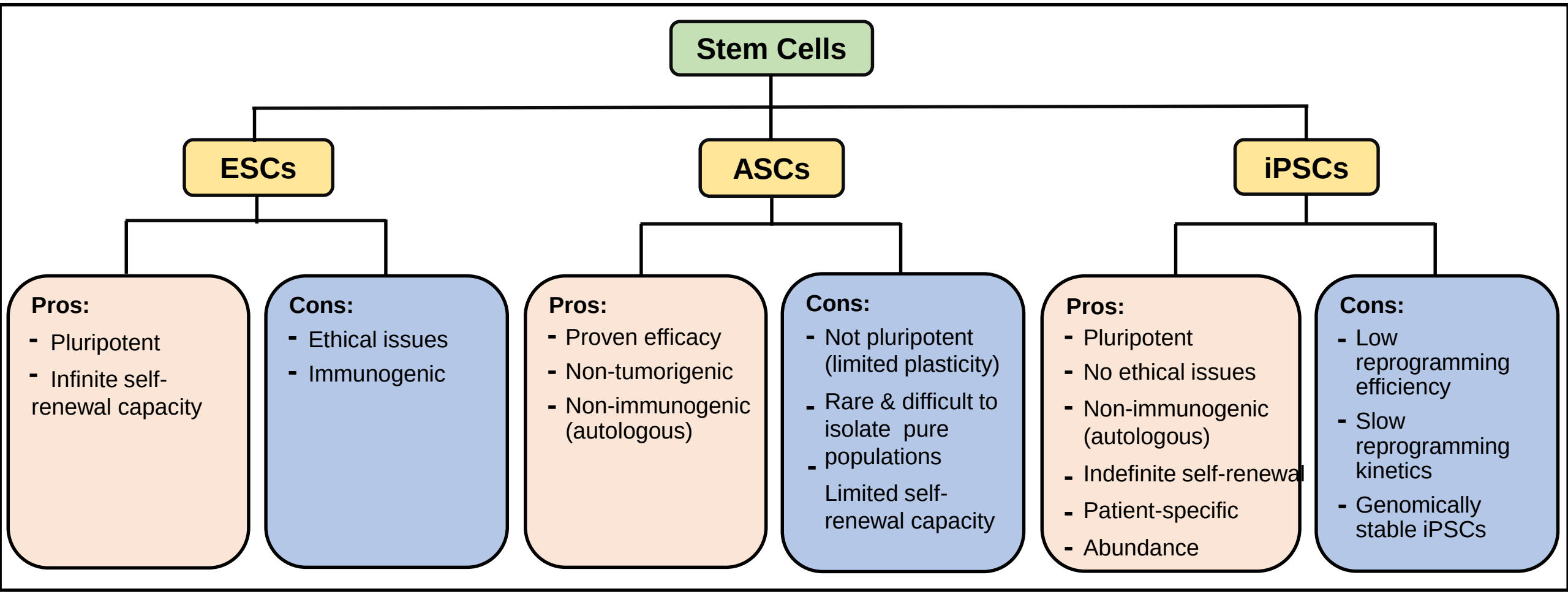
DIFFERENTIATED CELLS

- Fibroblasts
- Keratinocytes
- Cardiomyocytes
- Hepatocytes
- RBCs, WBCs
- β-cells
- etc. etc. etc.

Three germ layers

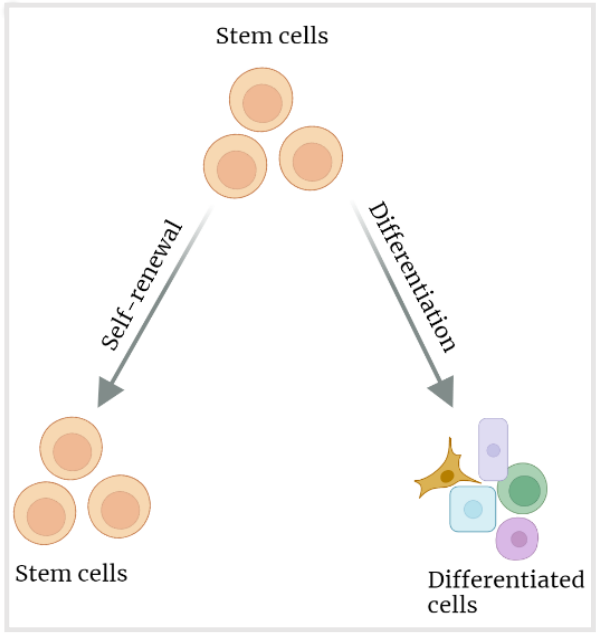


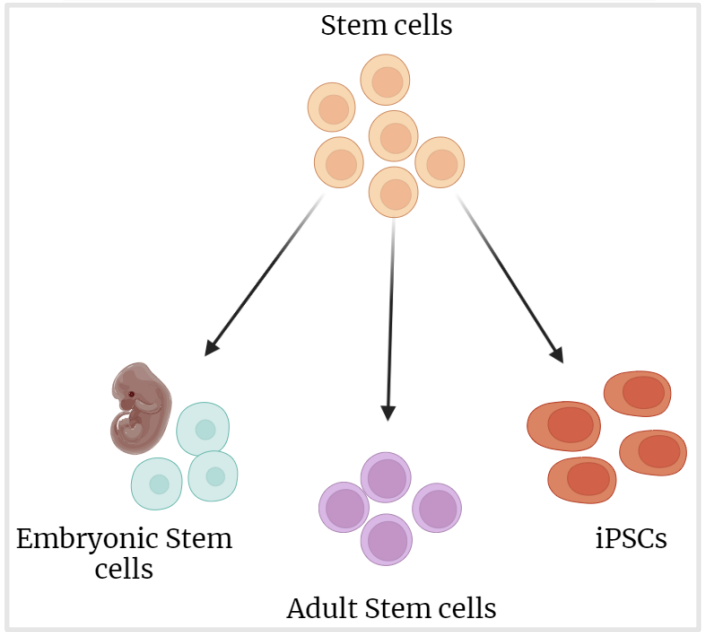
Types of stem cells



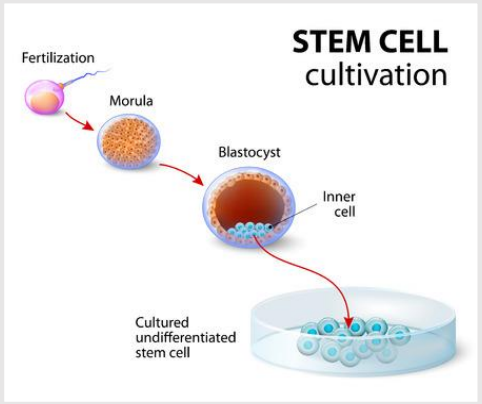
Saha B, …, **Thummer RP** (**2018**) Prospective applications of Induced Pluripotent Stem Cells in Military Medicine. Med J Armed Forces India. 74(4): 313-320.

Stem Cells





Embryonic stem cells



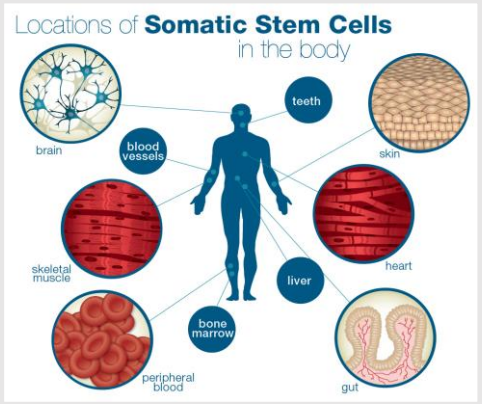
ADVANTAGES:

- Pluripotent
- Indefinite Self-renewal
- Development biology studies

DISADVANTAGES:

- Ethical consideration
- Possible immune rejection after implantation
- May lead to teratocarcinomas

Adult stem cells



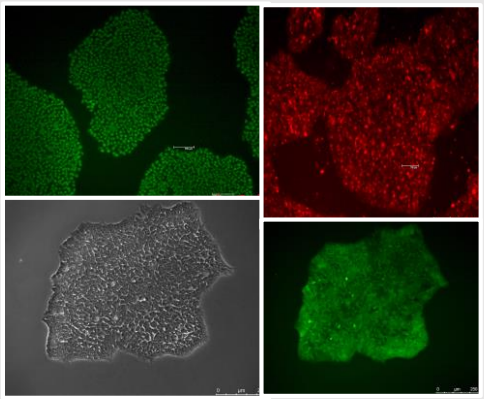
ADVANTAGES:

- Multi-potent
- Avoids ethical concerns
- Lower risk of immune rejection

DISADVANTAGES:

- Limited plasticity
- Isolation and acquisition difficulties
- Difficult to maintain in laboratory for long periods

induced Pluripotent stem cells



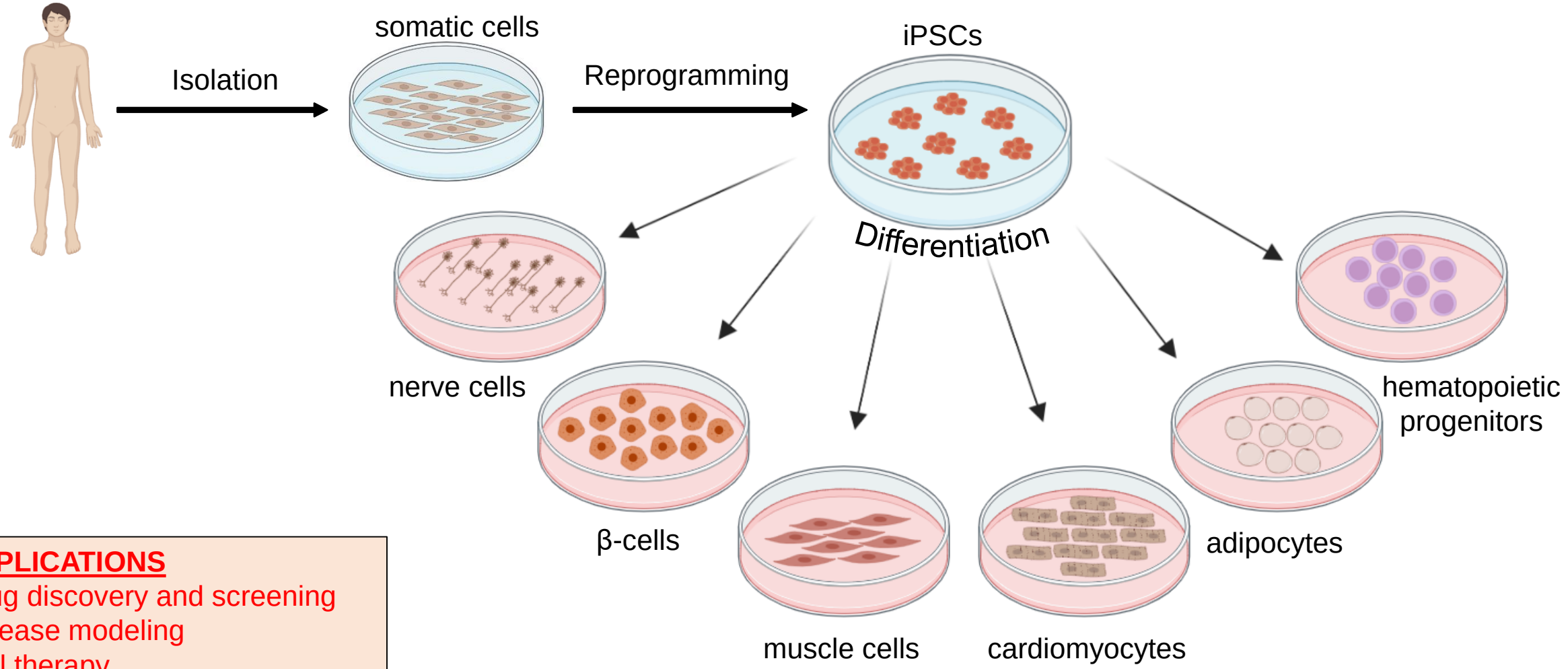
ADVANTAGES:

- Pluripotent
- No ethical consideration
- Indefinite self-renewal
- Patient-specific
- Abundance and Availability

DISADVANTAGES:

- Potential to form tumors when used integrative approaches
- Generation and maintenance is expensive and cumbersome

Induced Pluripotent Stem Cells (iPSCs)

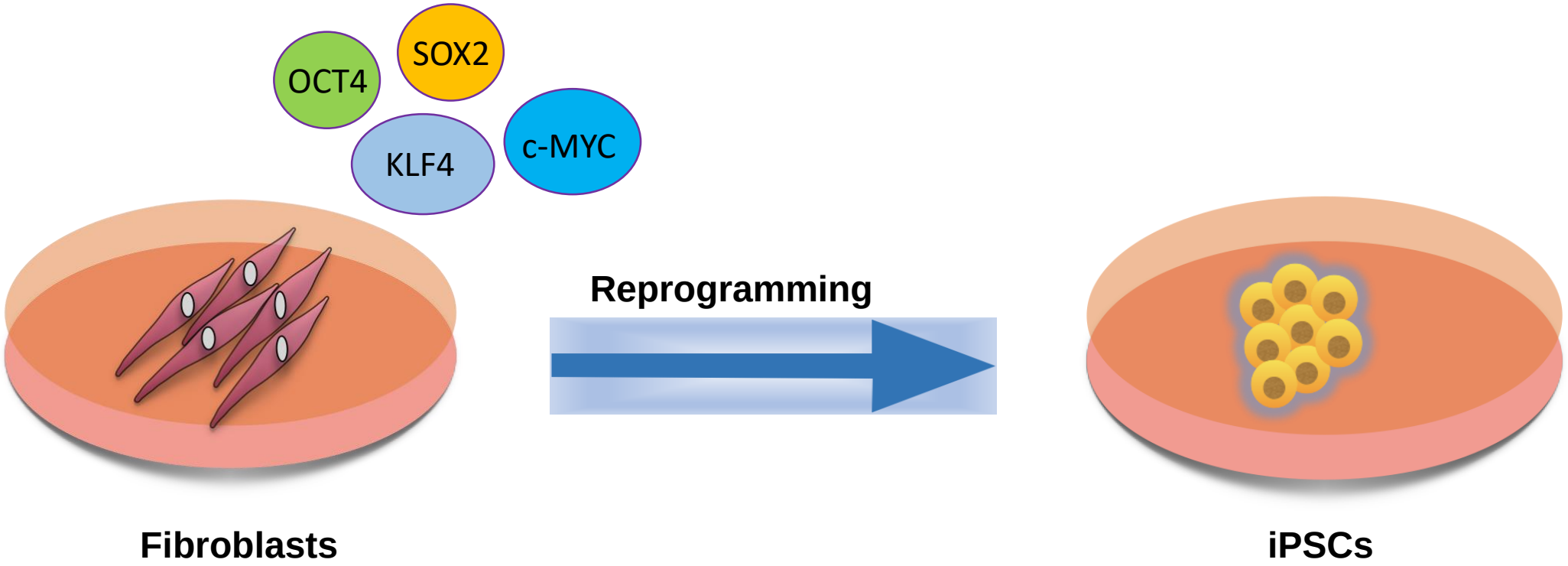


APPLICATIONS

- Drug discovery and screening
- Disease modeling
- Cell therapy
- Tissue regeneration
- Organoid production
- Genetic modification
- Basic research

Can differentiate into all 3 germ layers: Ectoderm, Endoderm and Mesoderm

Induced Pluripotent Stem Cells



24 genes

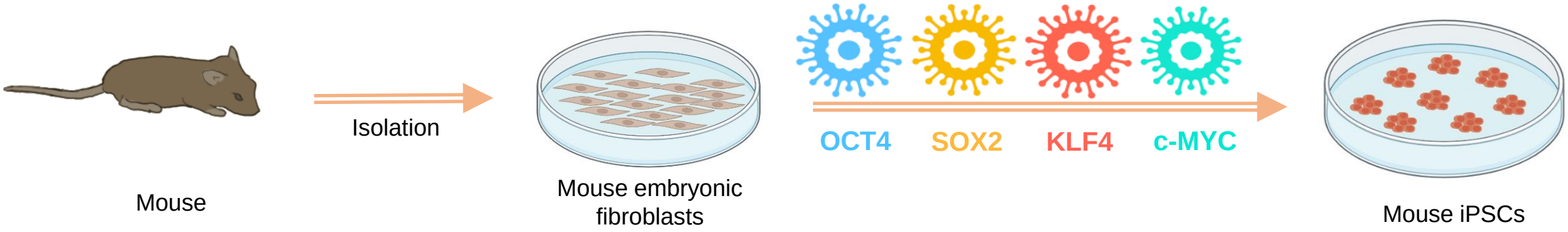
Induced Pluripotent Stem Cells (iPSCs)

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Cell

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

Prof. Shinya Yamanaka
(2006)
Nobel Prize (Medicine)
(2012)



Mouse

Mouse embryonic fibroblasts

Mouse iPSCs

- Derived by reprogramming somatic cells to pluripotent state

Induced Pluripotent Stem Cells (iPSCs)

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

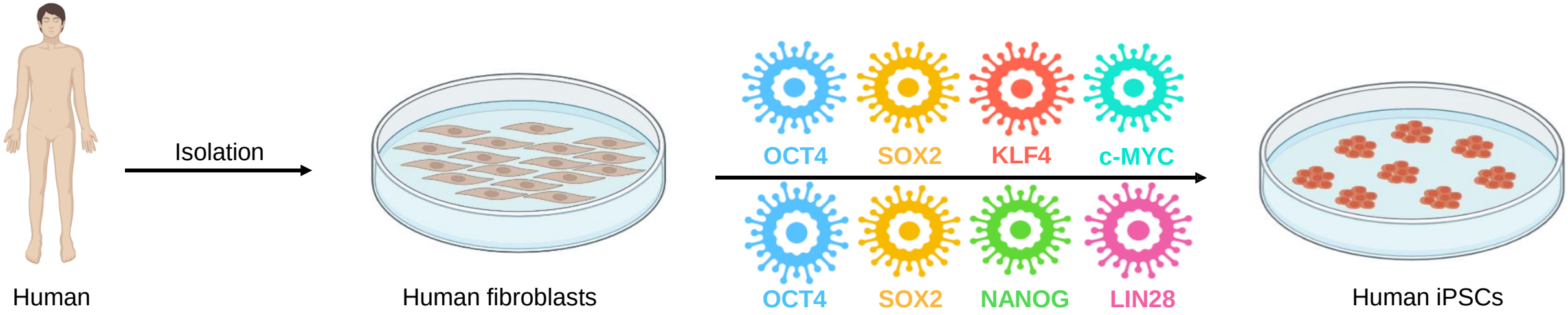
Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
[3]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
[4]Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

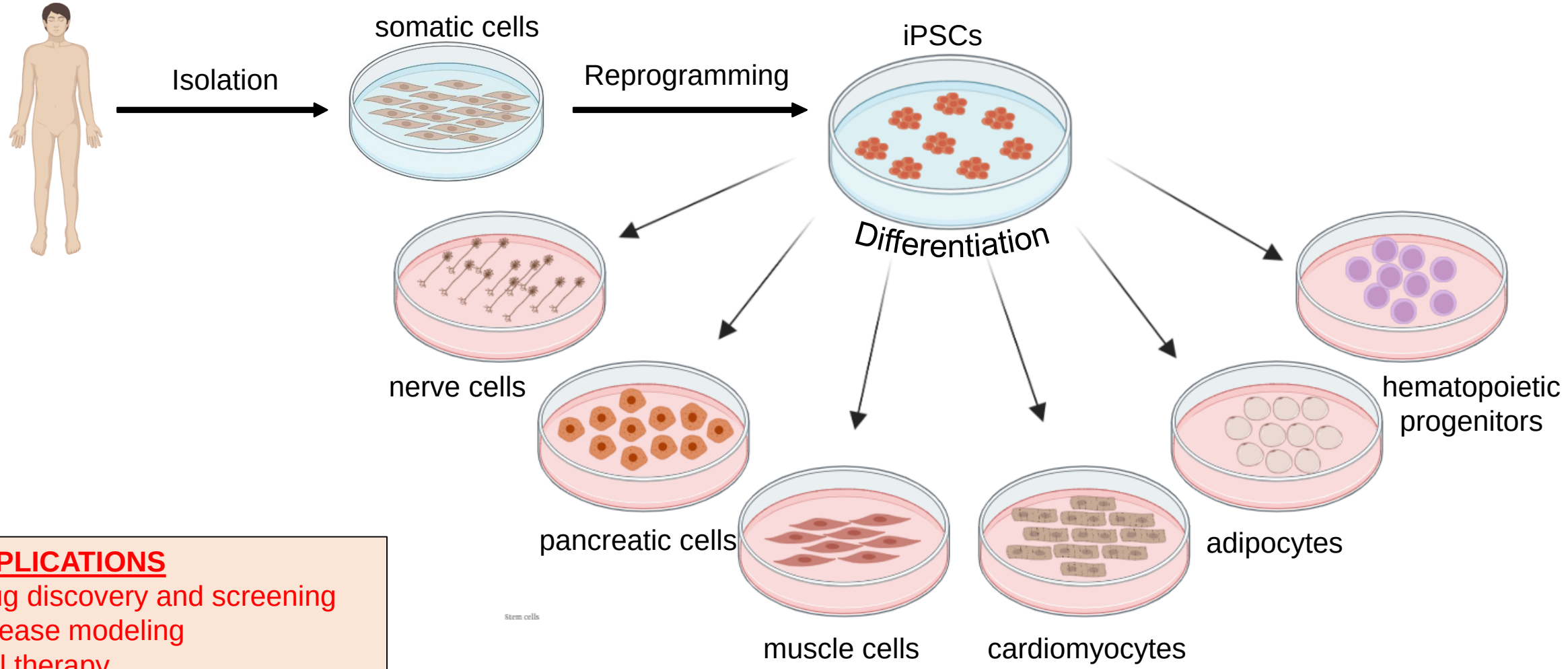
Junying Yu,[1,2*] Maxim A. Vodyanik,[2] Kim Smuga-Otto,[1,2] Jessica Antosiewicz-Bourget,[1,2] Jennifer L. Frane, Shulan Tian, Jeff Nie, Gudrun A. Jonsdottir, Victor Ruotti, Ron Stewart,[3] Igor I. Slukvin,[2,4] James A. Thomson[1,2,5*]

Yamanaka factors
OCT4, SOX2, KLF4, c-MYC

Thomson factors
OCT4, SOX2, NANOG, LIN28



Induced Pluripotent Stem Cells (iPSCs)



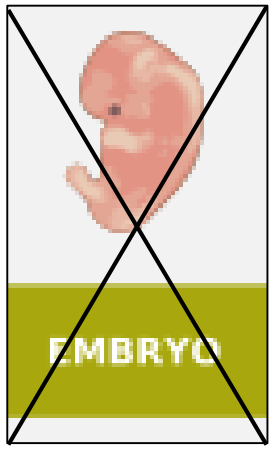
APPLICATIONS

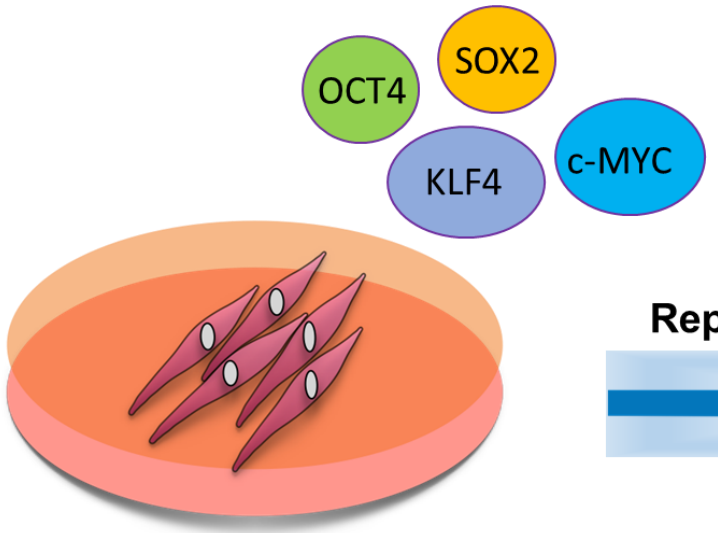
- Drug discovery and screening
- Disease modeling
- Cell therapy
- Tissue regeneration
- Organoid production
- Genetic modification
- Basic research

Can differentiate into all 3 germ layers: Ectoderm, Endoderm and Mesoderm

Advantages of iPSCs

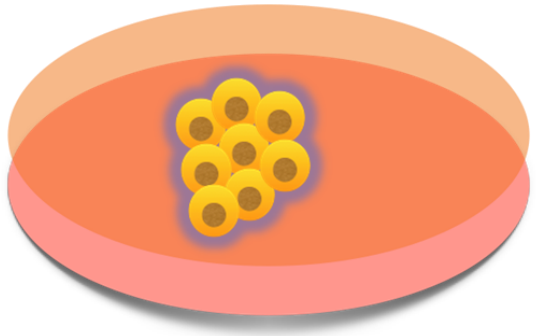
➢ **No destruction of embryo (non-invasive source)**





Fibroblasts

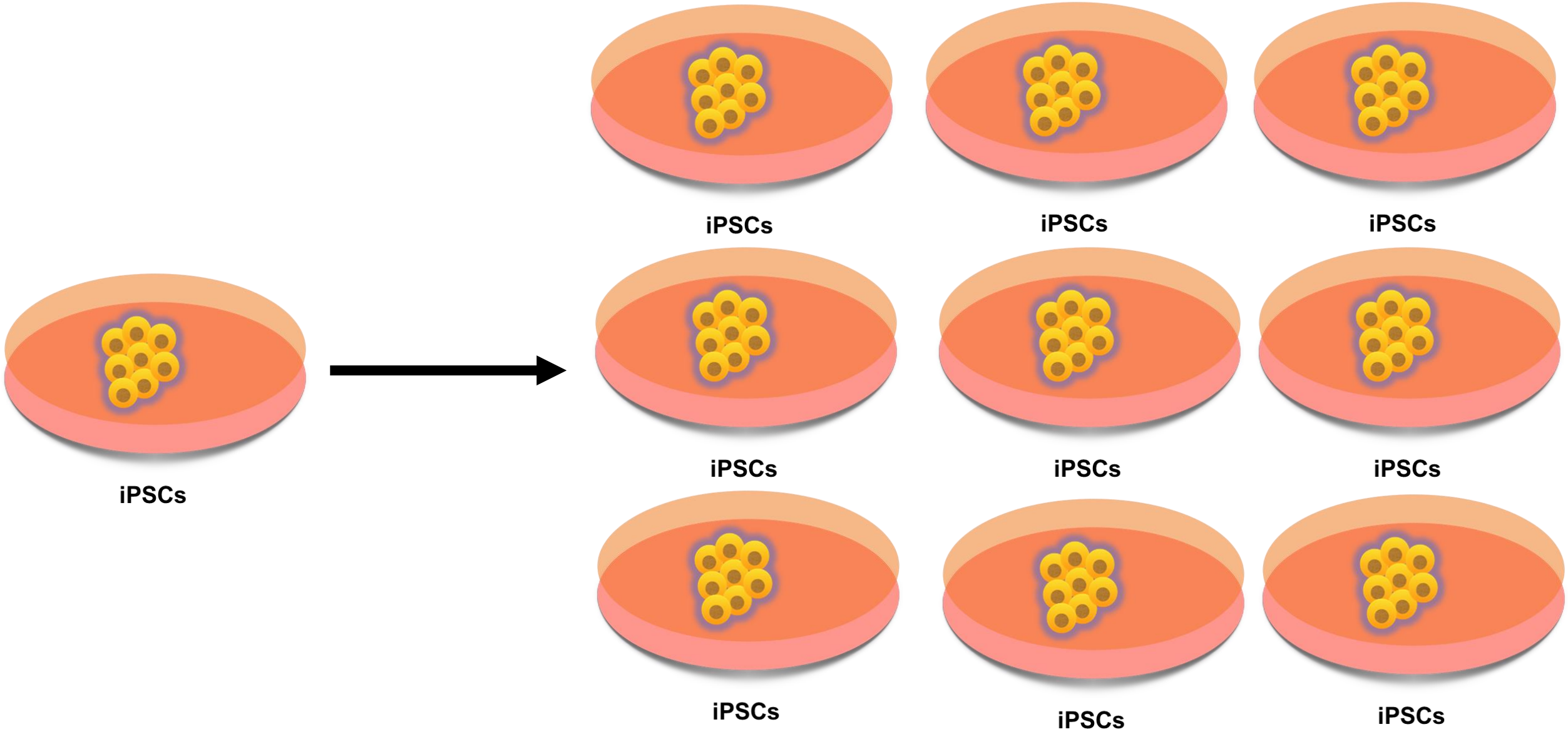
Reprogramming



iPSCs

Advantages of iPSCs

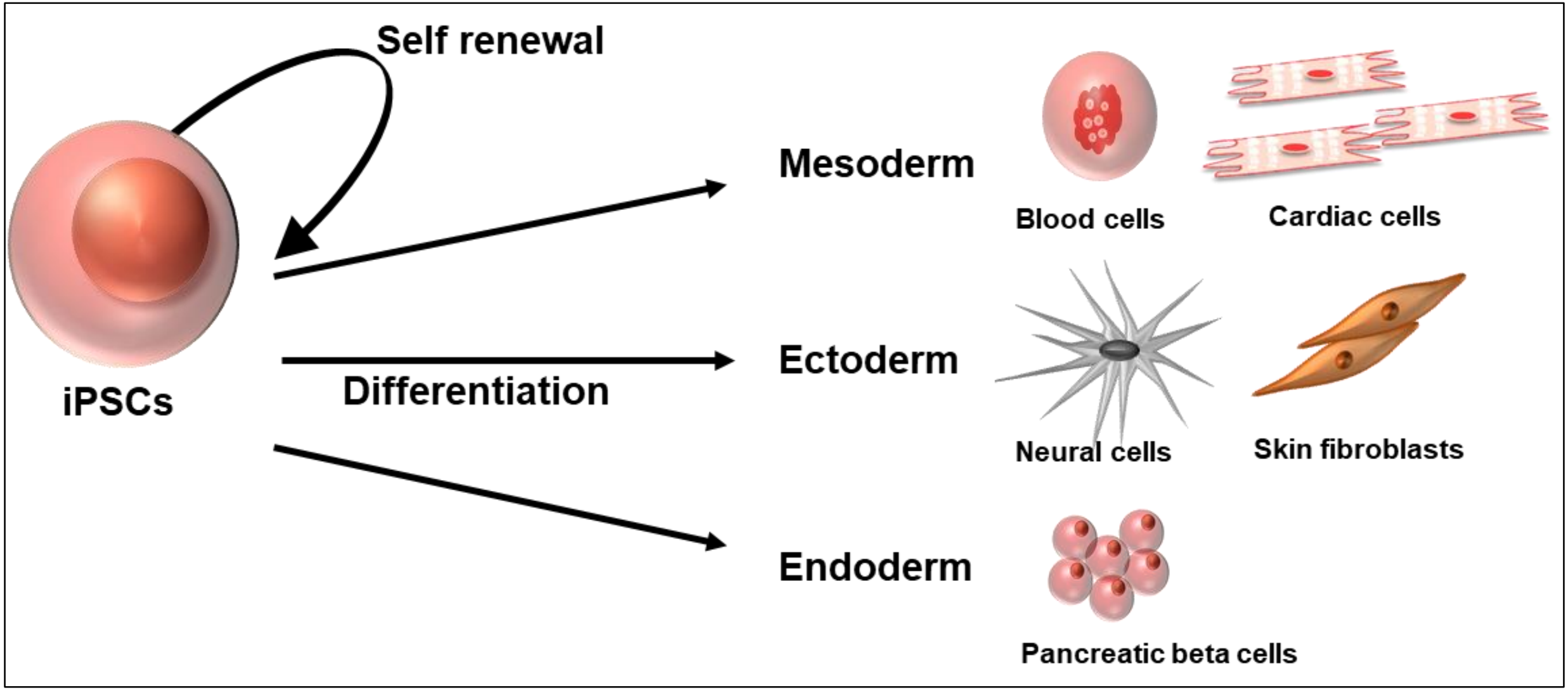
- **Unlimited self-renewal ability (maintaining normal karyotype)**



Advantages of iPSCs

➢ Source of pluripotent cells

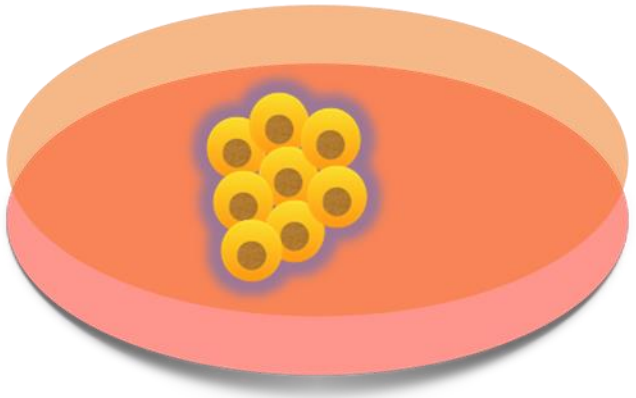
200-220 cell types



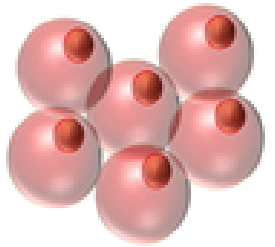
Advantages of iPSCs

- **Generates autologous cells (patient-specific iPSCs)**

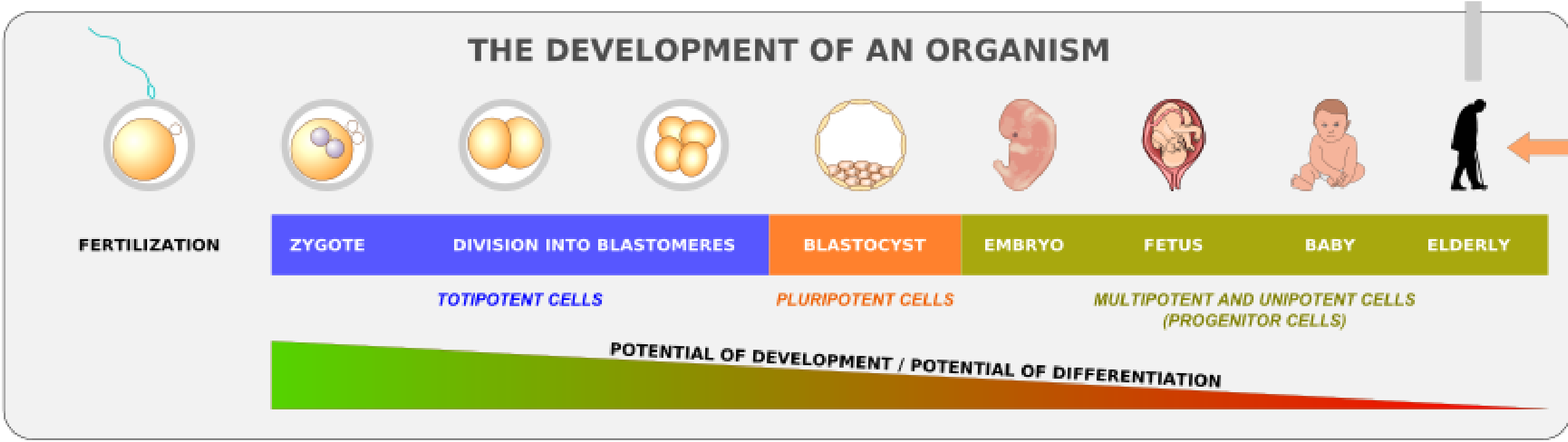
Type 1 DM

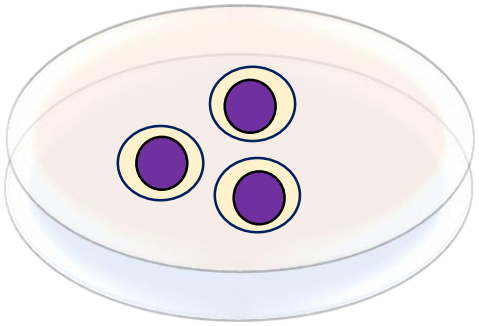


iPSCs



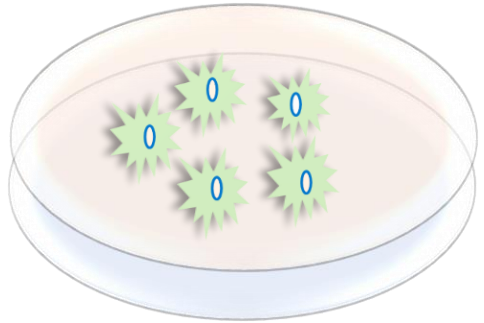
β-cells





iPSCs

Reprogramming

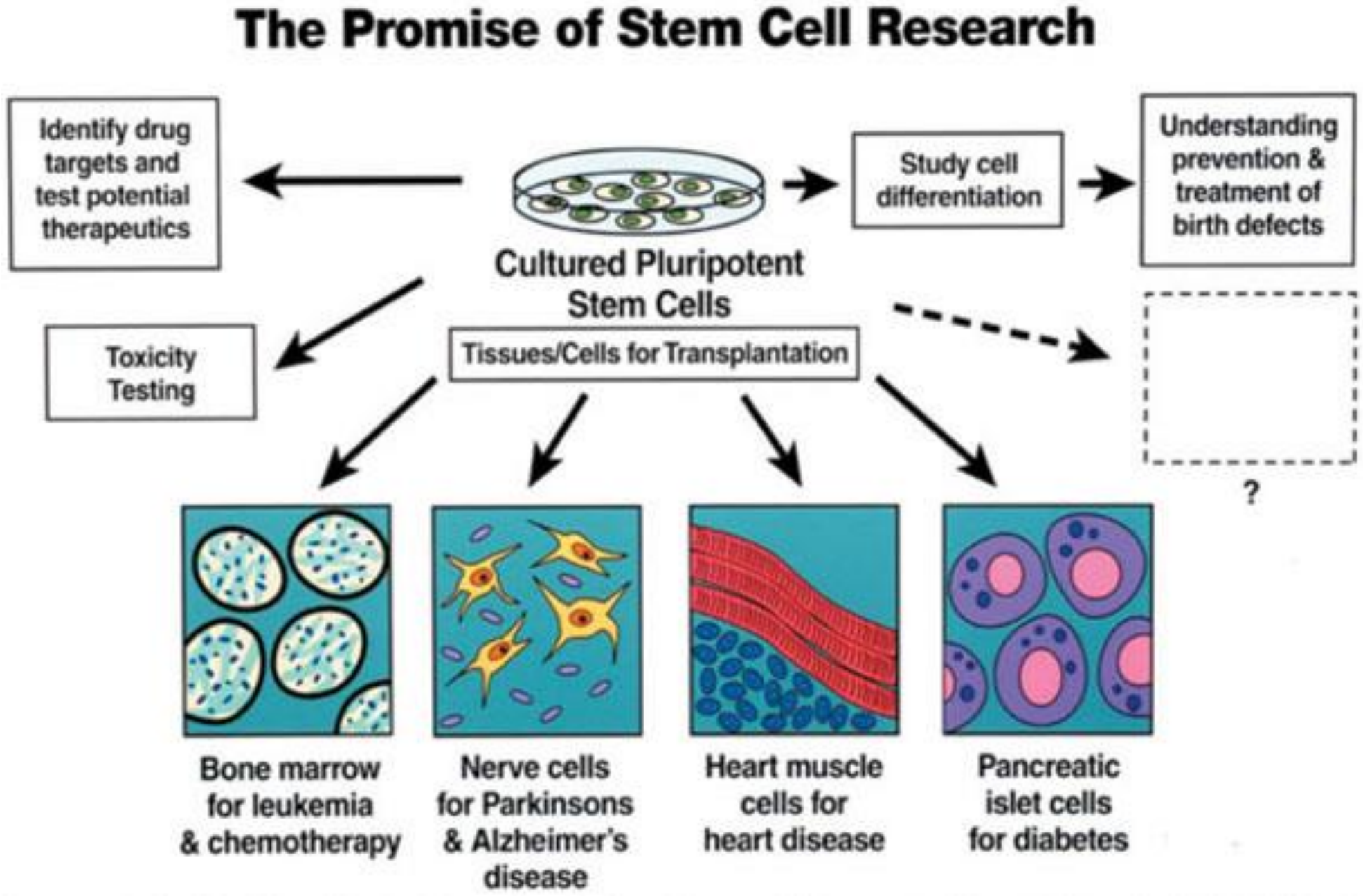


Somatic cells

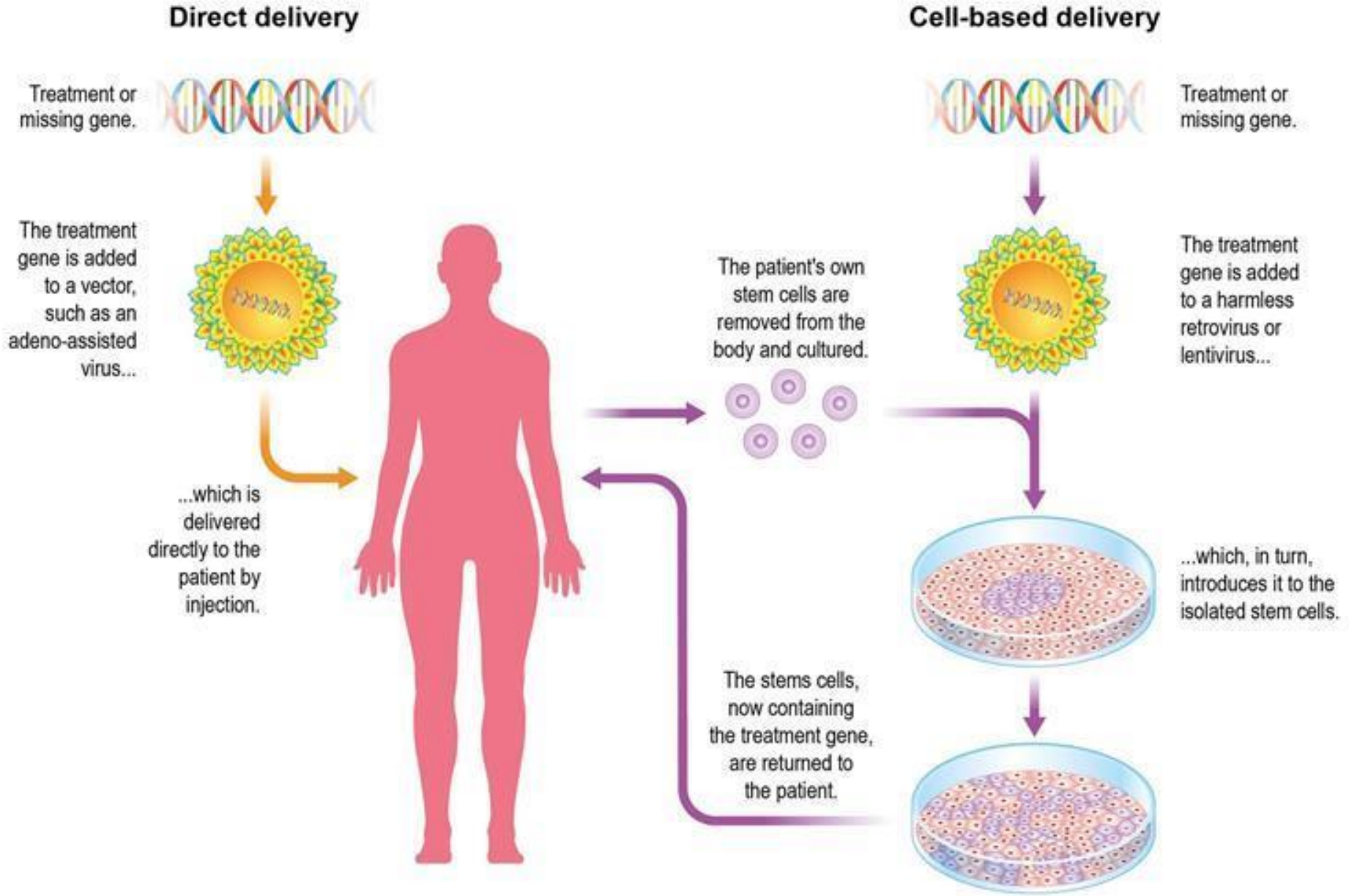
Advantages of iPSCs

- **No destruction of embryo (non-invasive source)**
- **Unlimited self-renewal ability (maintaining normal karyotype)**
- **Source of pluripotent cells (form desired cell type)**
- **Generates autologous cells (patient-specific iPSCs)**

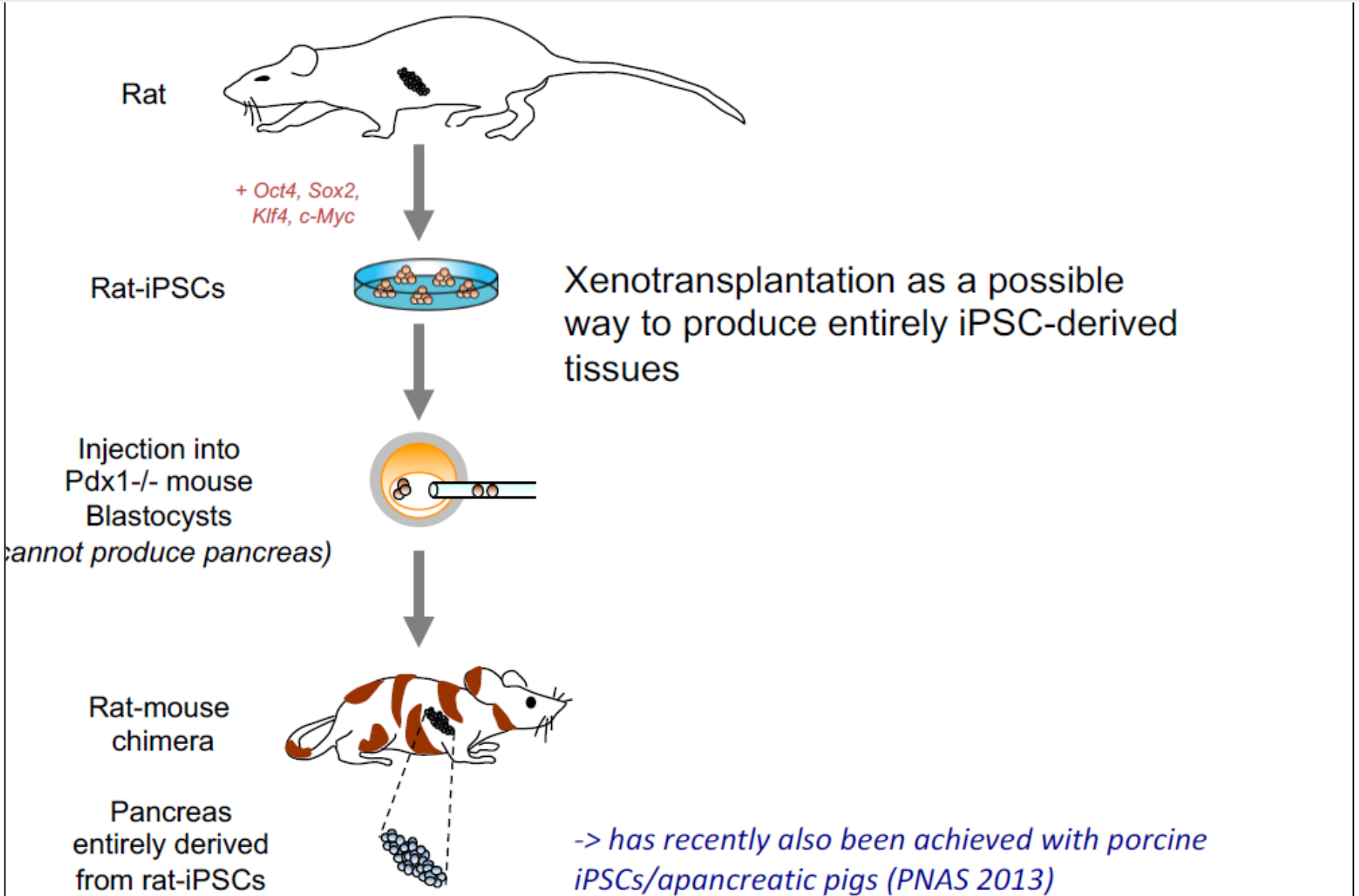
Applications of Stem Cells



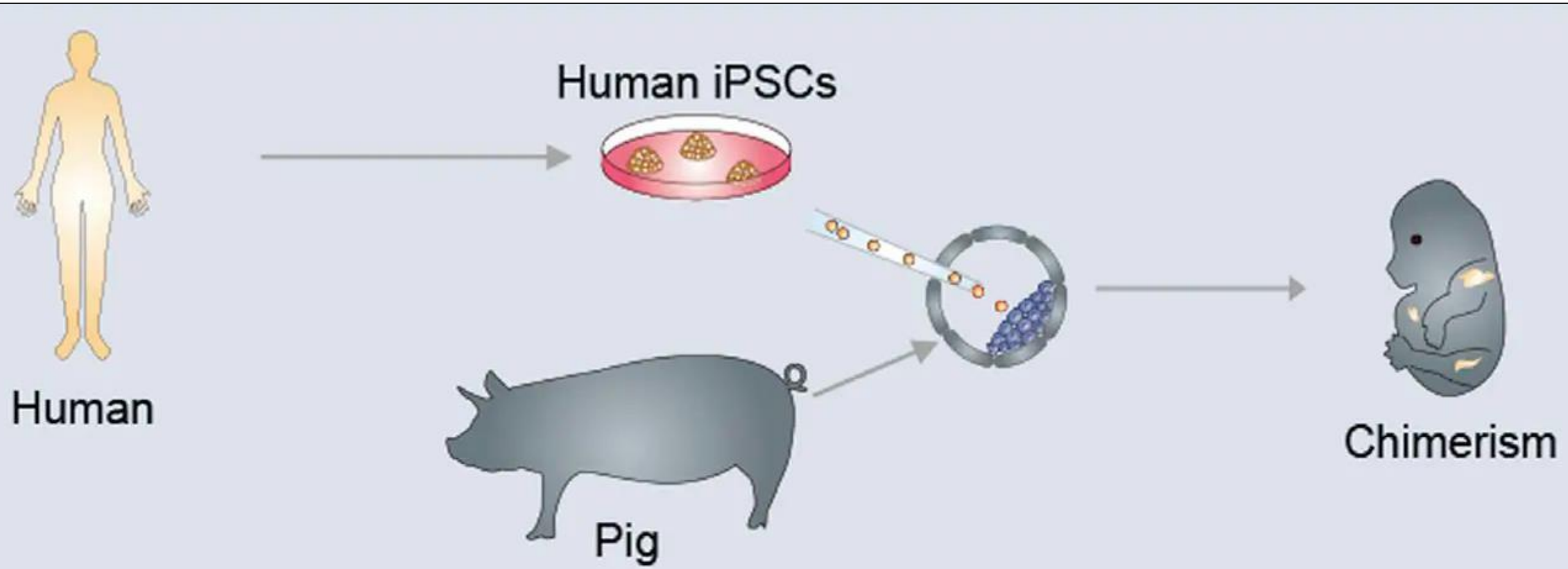
Applications of Stem Cells



iPSC as a tool to generate entire organs



Kobayashi et al., Cell (2010)


Human iPSCs
Human
Pig
Chimerism

Cells isolated from variety of species

Mammalian Cell Culture Lab

Laminar Flow Cabinet

Refrigerated Centrifuge

AC

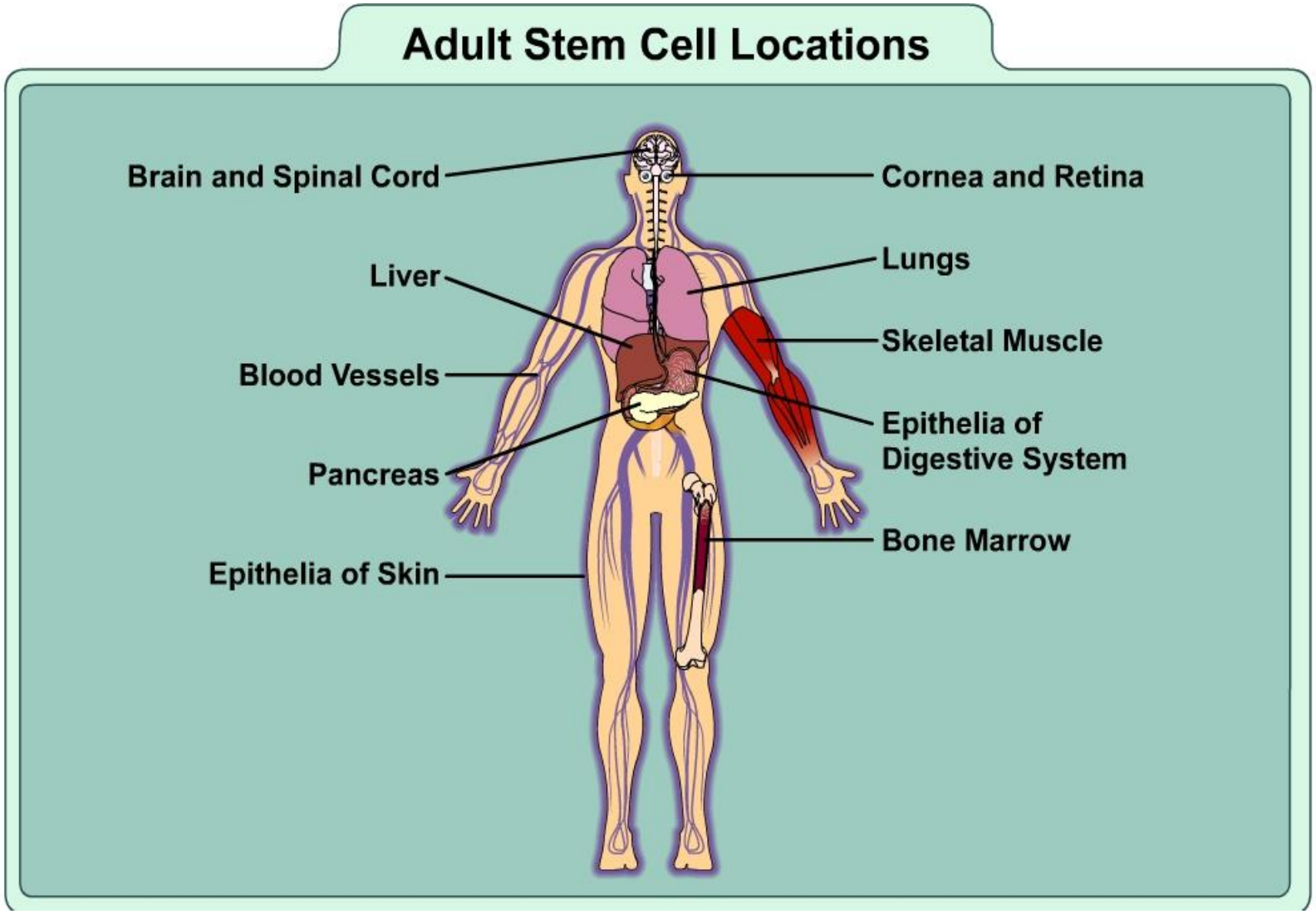
Cell Culture Plastics

Cell Incubator

Microscope

Adult stem cells

(most common)



Limitations of multipotent (adult/tissue) stem cells

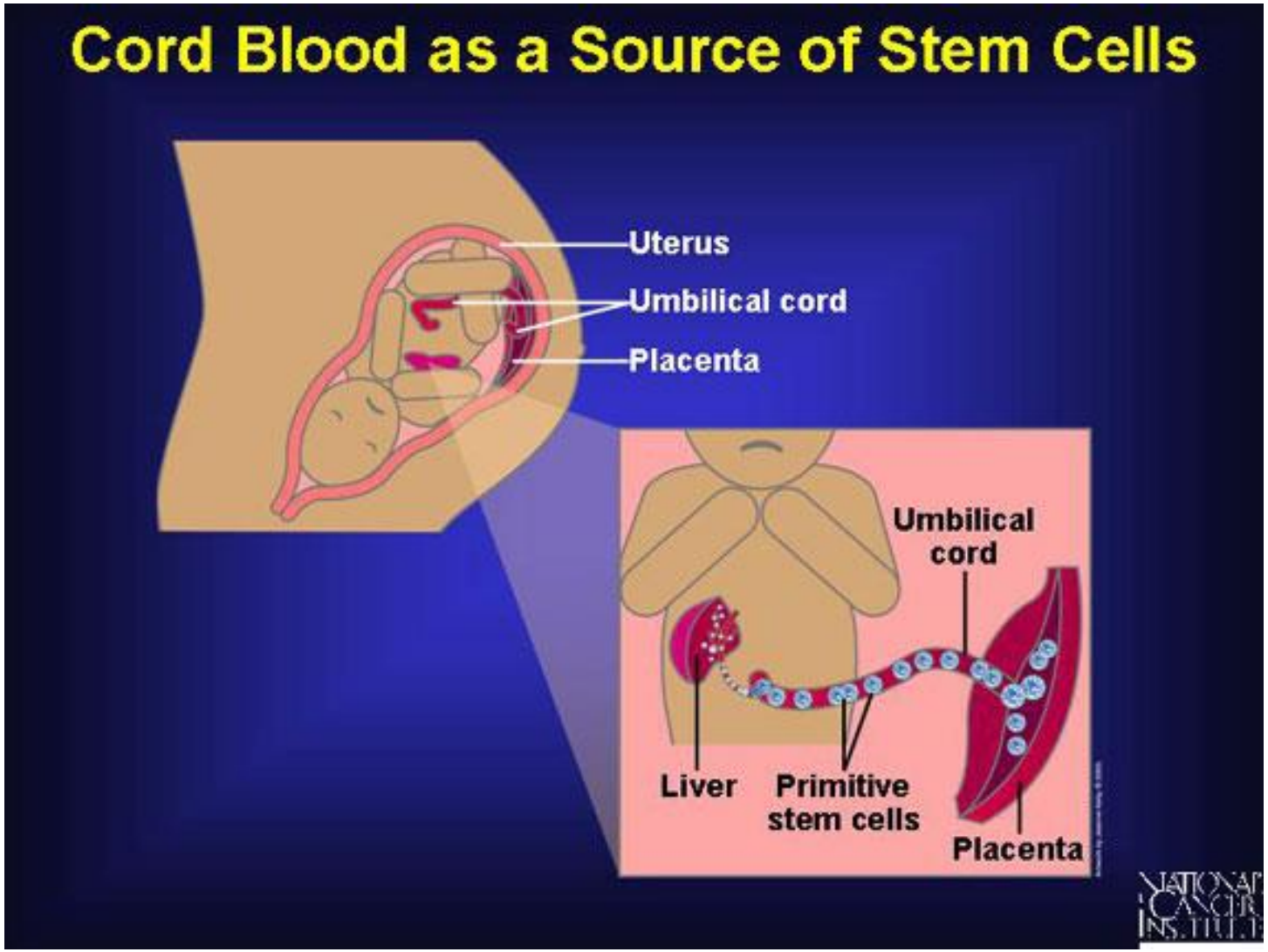
- No ethical issues (consent required)
- Immune rejection (only when transplanted from different patient)
- Multipotent
- Scarce
- Difficult to obtain pure populations
- Difficult to maintain these pure population of cells indefinitely in culture; limited life-span

iPS technology seems to be the answer but still a long way to go

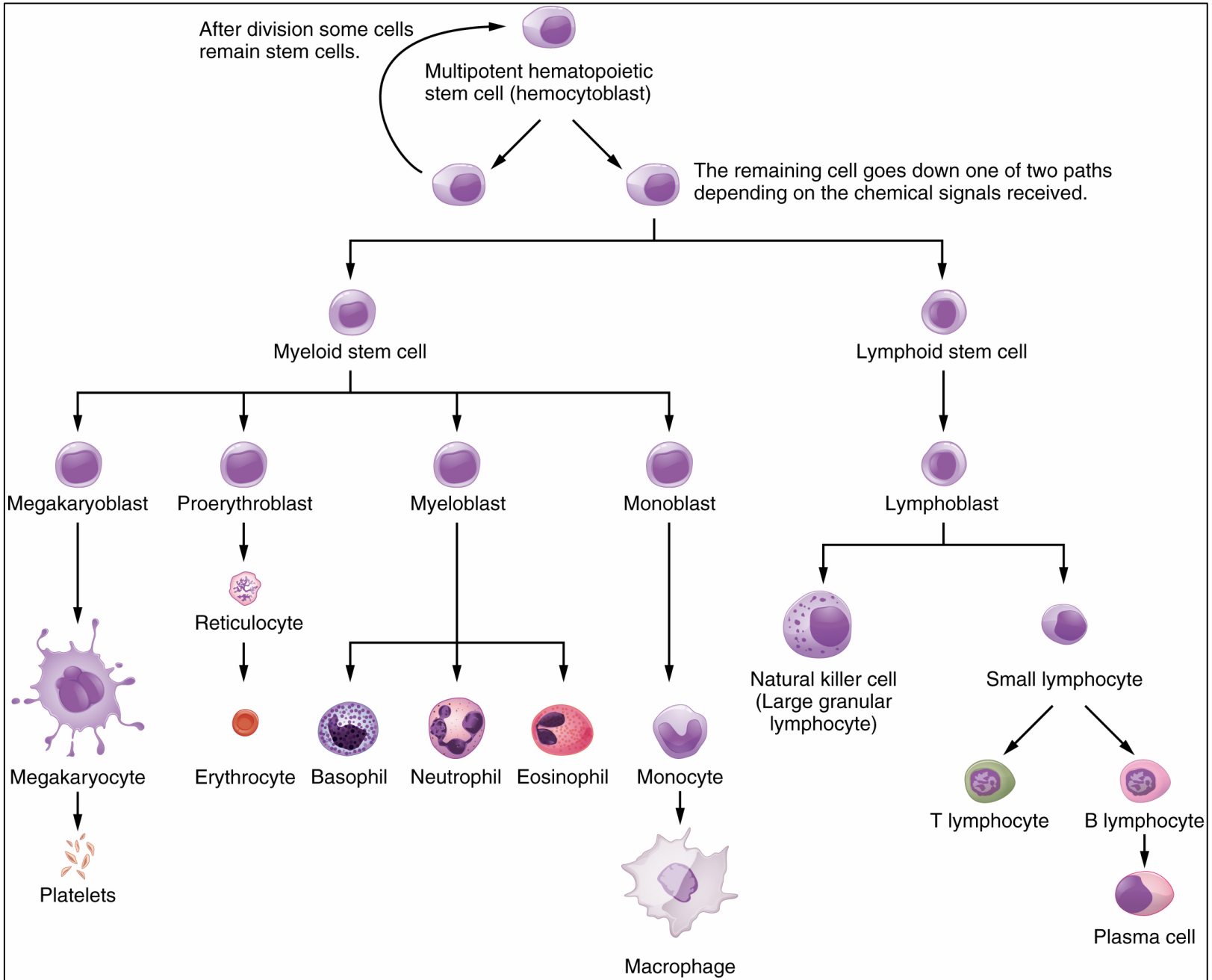
Why are adult stem cells preferable to ESCs?

- Naturally exist in our body, and they provide a natural repair mechanism for many tissues.
- Do not generate tumors on transplantation.
- Do not cause an immune reaction (in case of autologous; patient's own stem cells).
- Successfully used in human therapies for many years. Adequate clinical safety and efficacy data available.
- No therapies in humans (clinical trials are still ongoing) have ever been successfully carried out using ESCs.

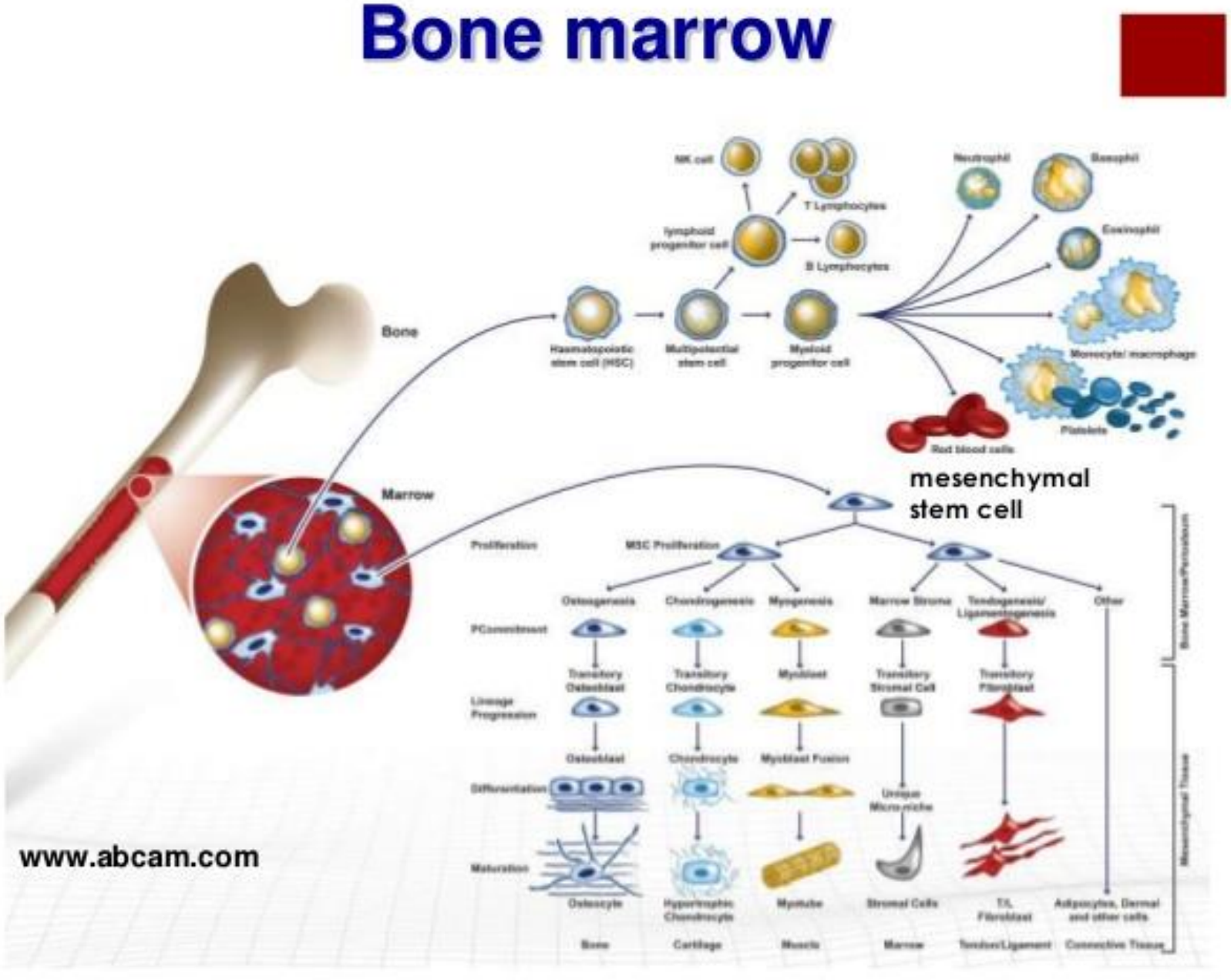
Isolation of adult stem cells



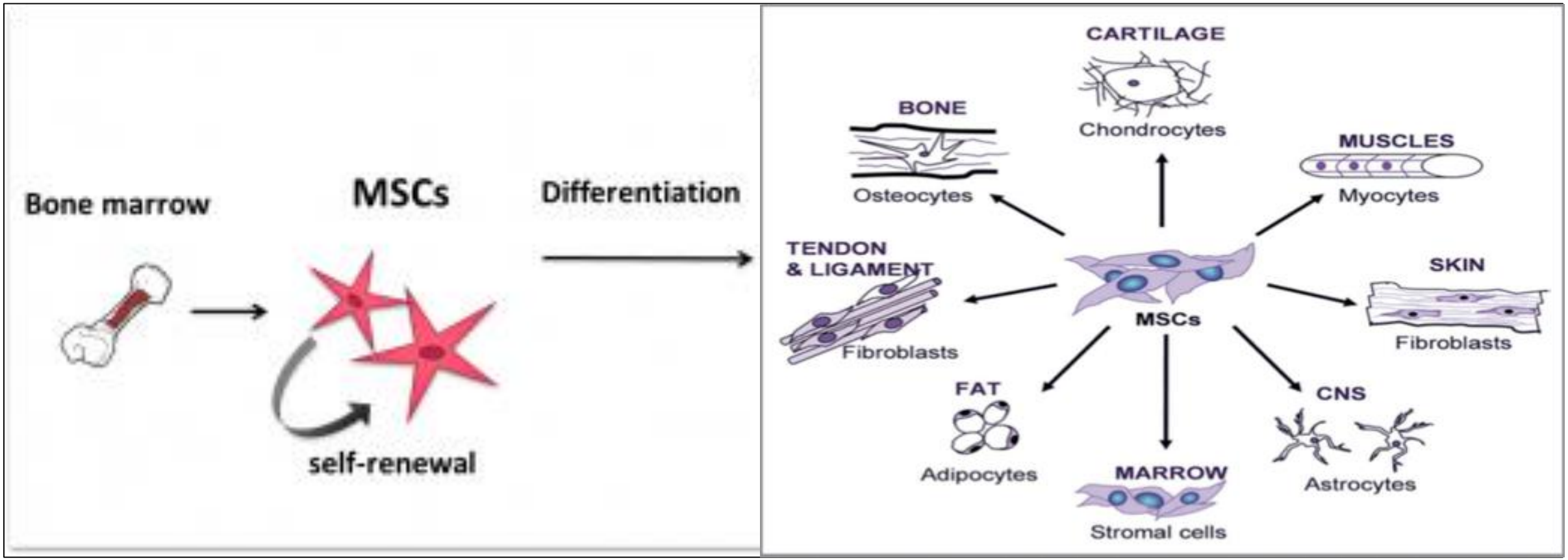
Isolation of adult stem cells



Isolation of adult stem cells

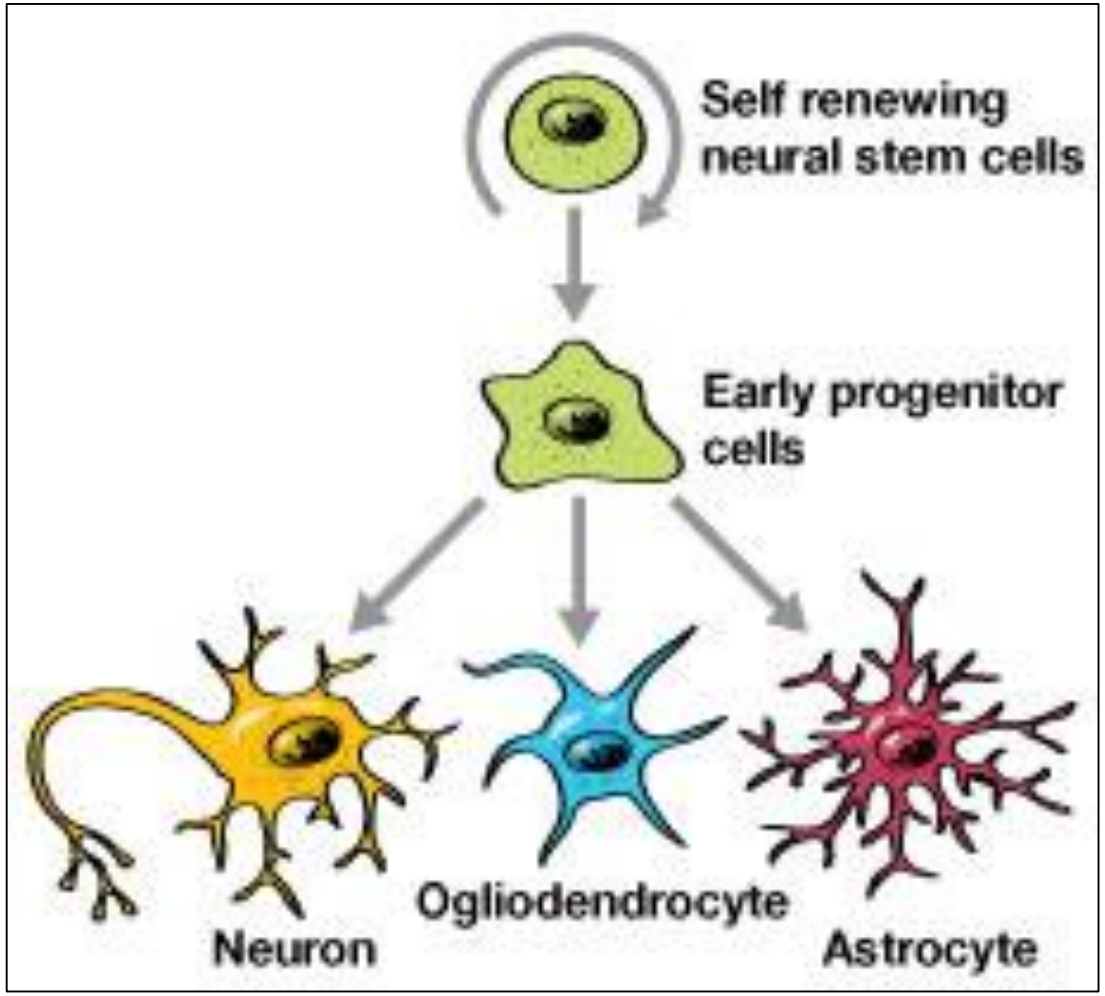


Isolation of adult stem cells

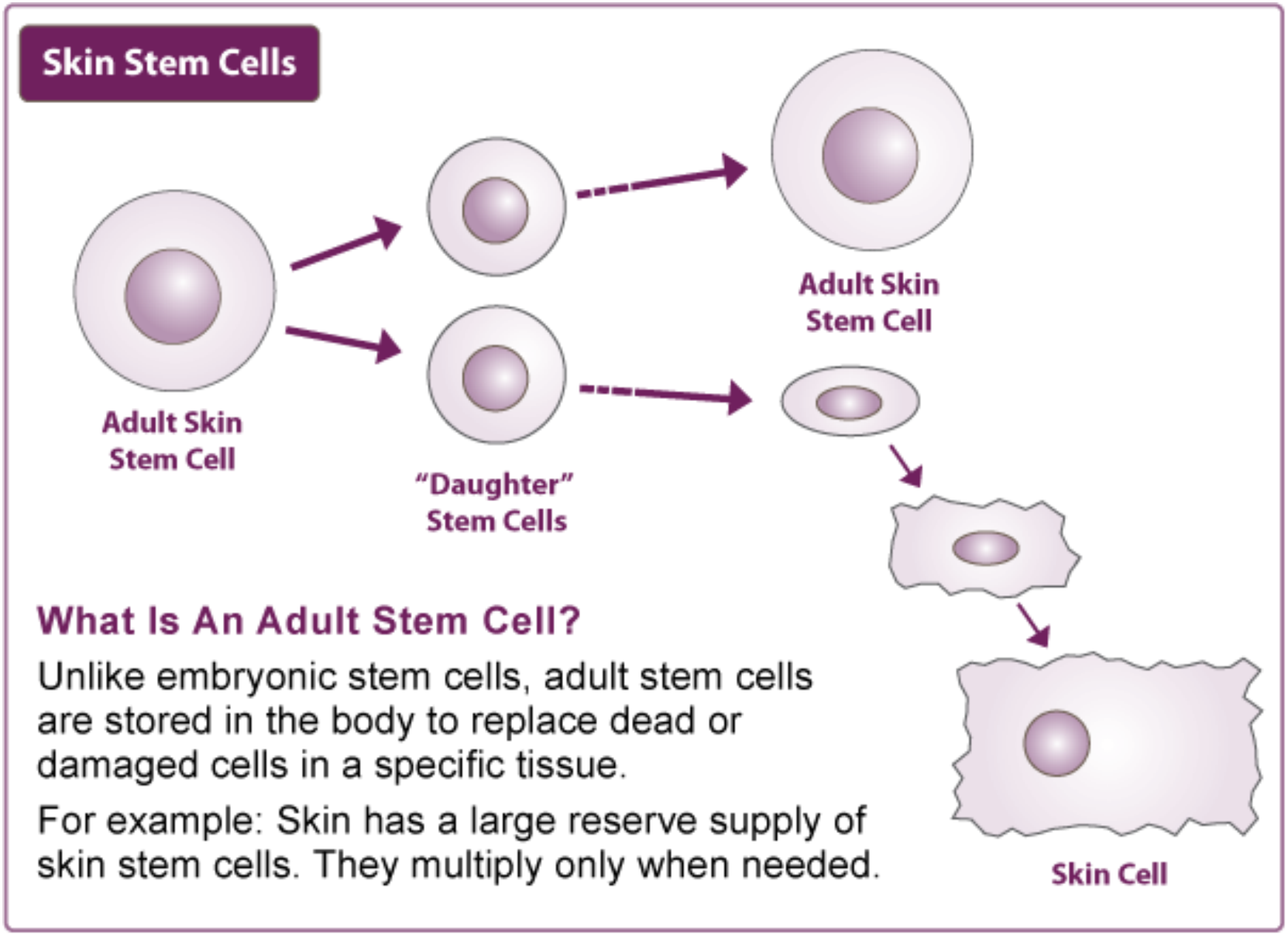


Isolation of adult stem cells

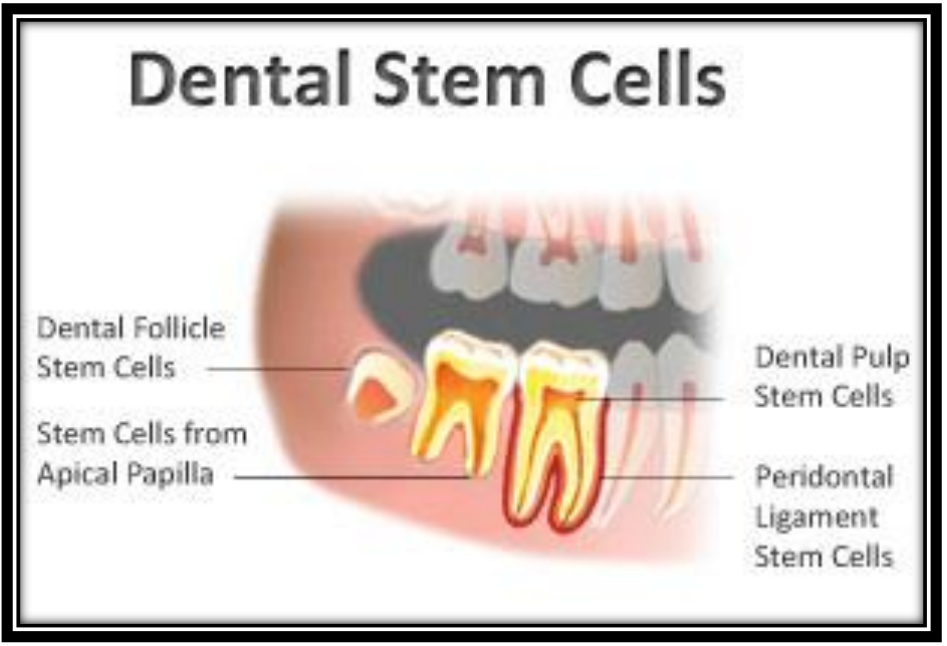
Neural stem cells persist in the adult vertebrate brain and continue to produce neurons throughout life.

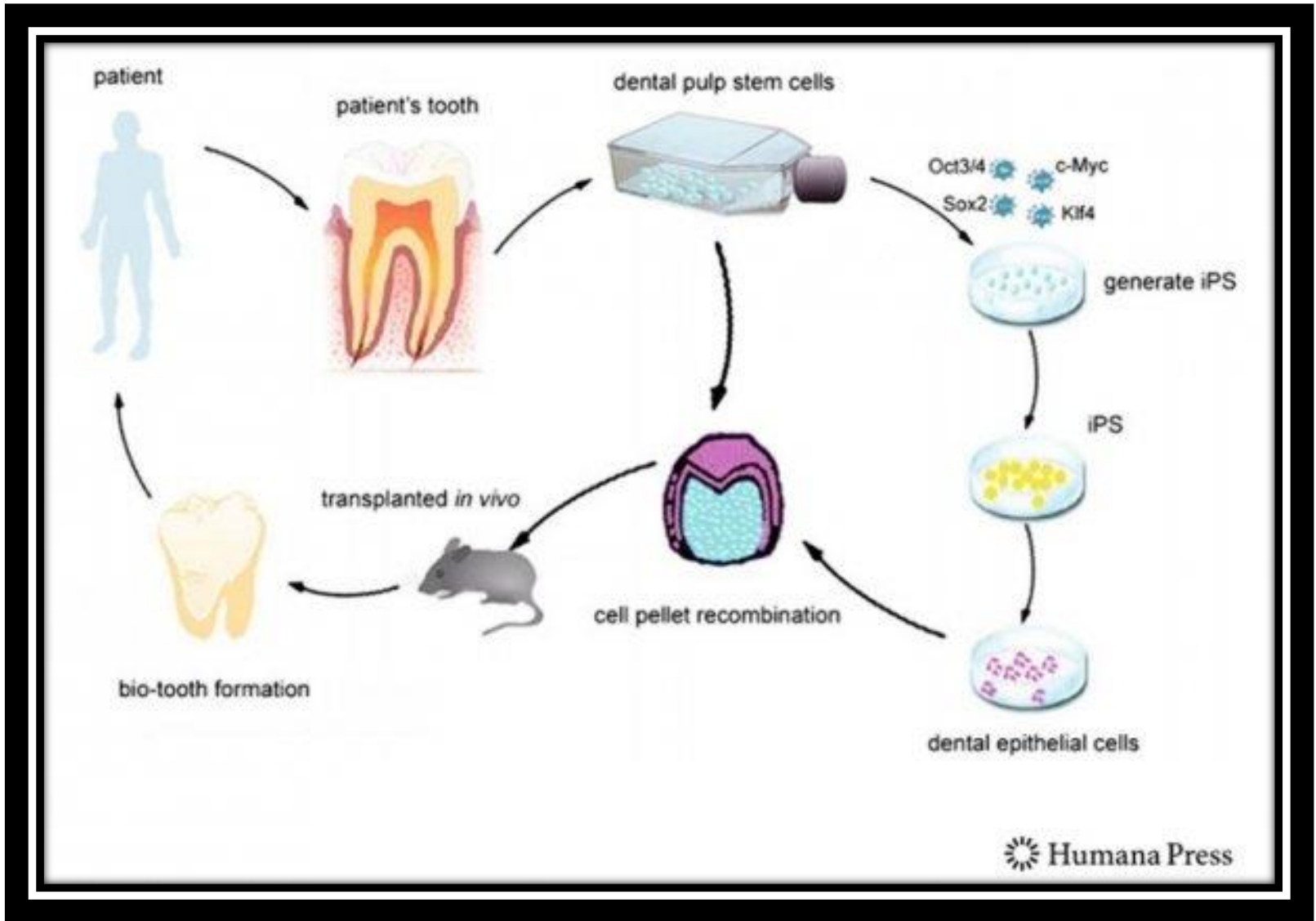


Isolation of adult stem cells

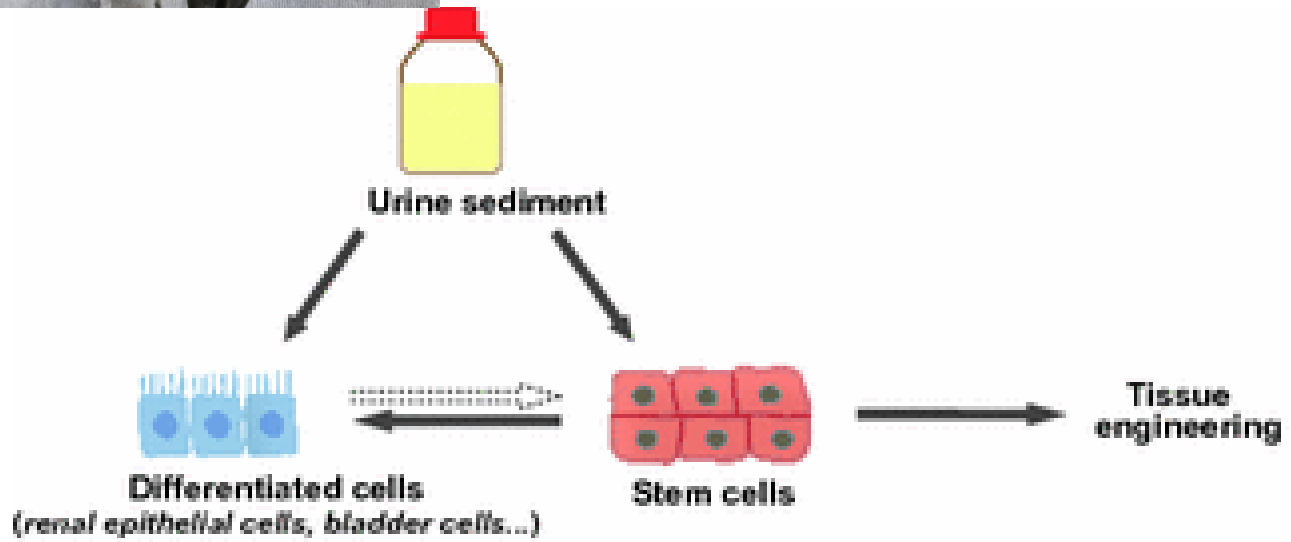


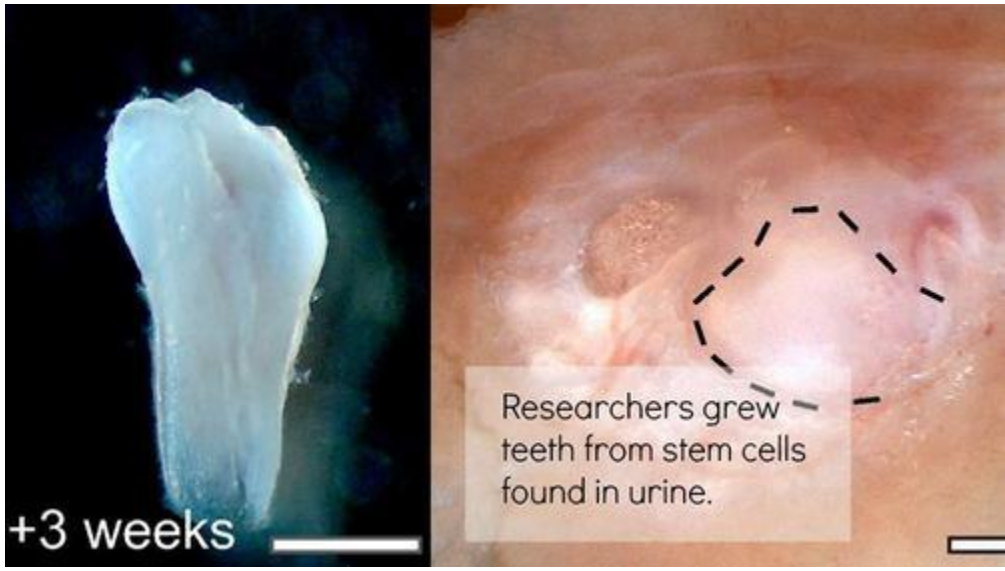
Isolation of adult stem cells





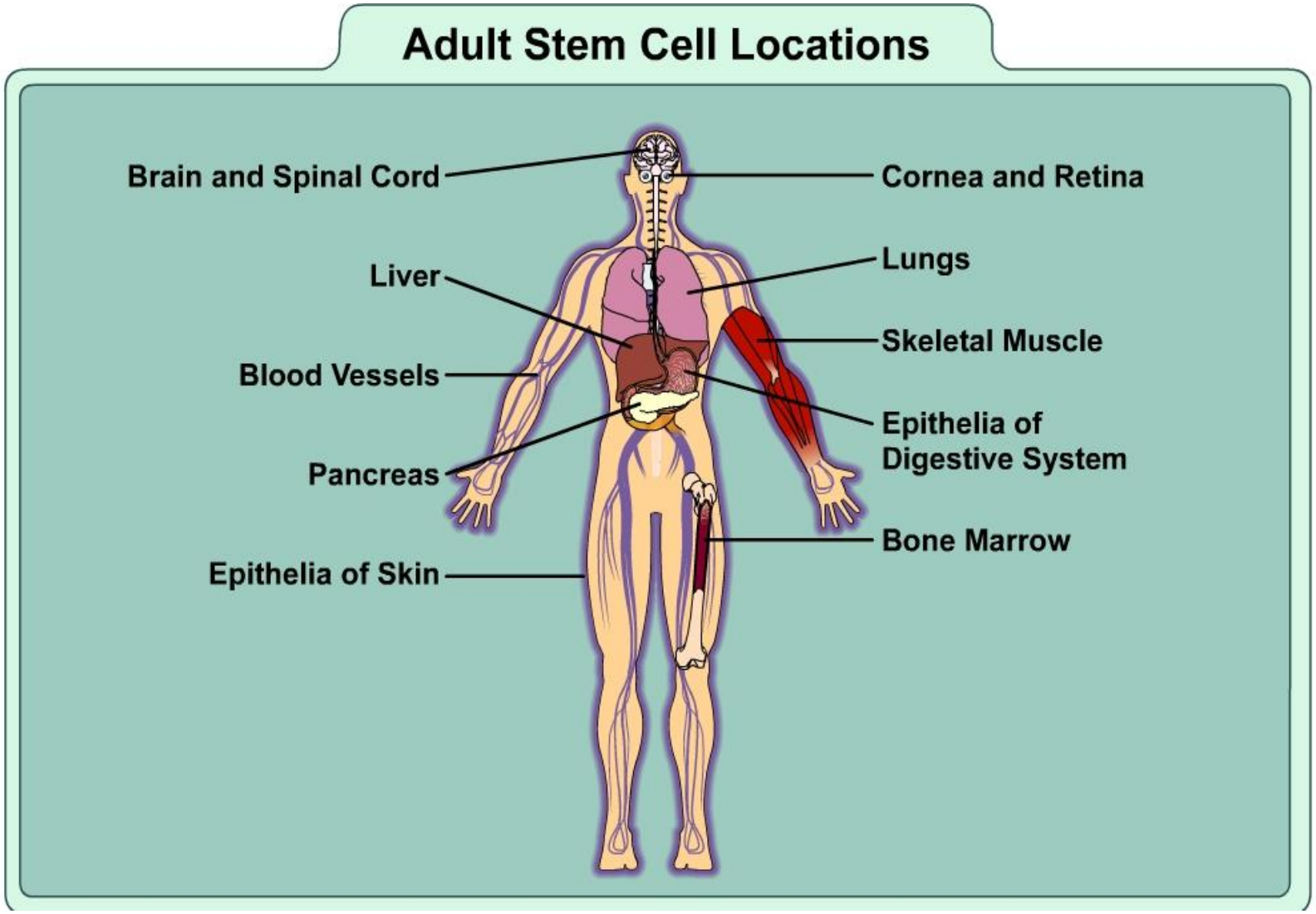
Isolation of adult stem cells





Isolation of adult stem cells

(most common)

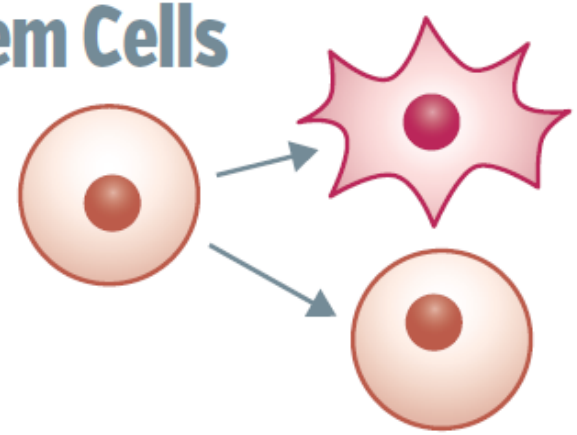


Pluripotent stem cell vs Multipotent Stem Cell

	Pluripotent Stem Cells (PSCs) Embryonic Stem Cell (ESC) Induced Pluritpotent Stem Cell (iPSC)	**Multipotent Stem Cells (MSC)** Adult Stem Cells (Hematopoietic Stem Cells; Mesenchymal Stem Cells, Neural Stem Cells, etc.
Potency	Pluripotent (differentiates into all cell types)	Multipotent (differentiates into limited number of cell types)
Biomedical Potential	Vast	Limited
Proliferation capacity	Unlimited (immortal)	Limited Life-span
Proliferation rate	Fast (symmetric division)	slow compared to PSC (asymmetric division)
Derived from	Embryo or Artificially reprogrammed	Adult human body
Availability	Grown relatively easily in culture … one requires large amount of cells for cell therapy	are scarce and difficult to obtain pure populations
Ethics …	ESCs are controversial as destruction of embryo is involved (but not iPS) …	non-controversial …
Cancerous	can be cancerous	are non-cancerous

Three Key Facts About Stem Cells

1. The defining characteristic of a stem cell is that it can self-renew or differentiate.
2. Stem cells enable the body to grow, repair and renew.
3. There are three types of stem cells:



Differentiation (Specializing)

Specialized cell
(e.g. muscle cell, nerve cell)

Self-Renewal (Copying)

Stem cell

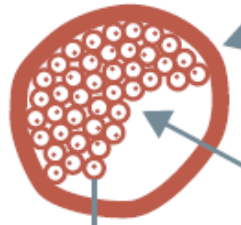
Tissue Stem Cells

In the fetus, baby and throughout life.

Found throughout the body, each type gives rise to at least one type of more specialized cell.

For example, blood stem cells are found in the bone marrow.

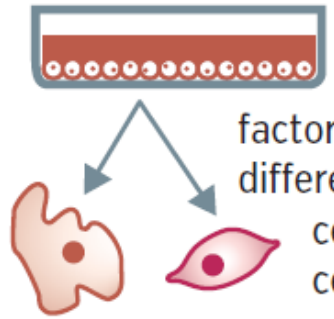
Embryonic Stem Cells



A *blastocyst*

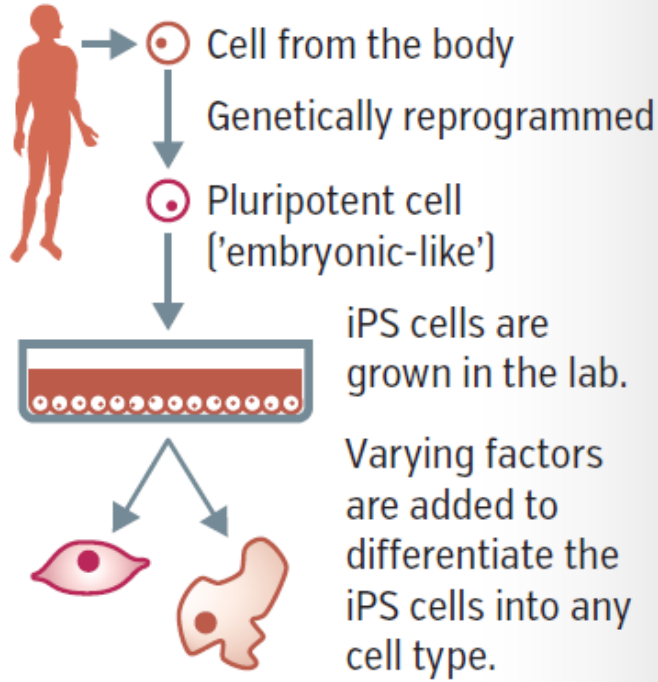
The cells inside are the *inner cell mass.*

These cells, then grown in the lab, are called *embryonic stem cells.*



Varying factors are added to differentiate the ES cells into any cell type.

Induced Pluripotent Stem Cells (iPS)



Cell from the body

Genetically reprogrammed

Pluripotent cell ('embryonic-like')

iPS cells are grown in the lab.

Varying factors are added to differentiate the iPS cells into any cell type.

Embryonic stem cells and iPS cells are *pluripotent;* they can generate all the specialized cells of the body.

Thank you for your attention