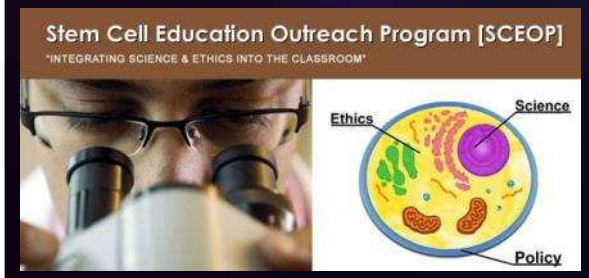
Stem Cells and Regenerative Medicine



Presented by
Yung-Jen Chuang 莊永仁
Dept. of Medical Science
National Tsing Hua University

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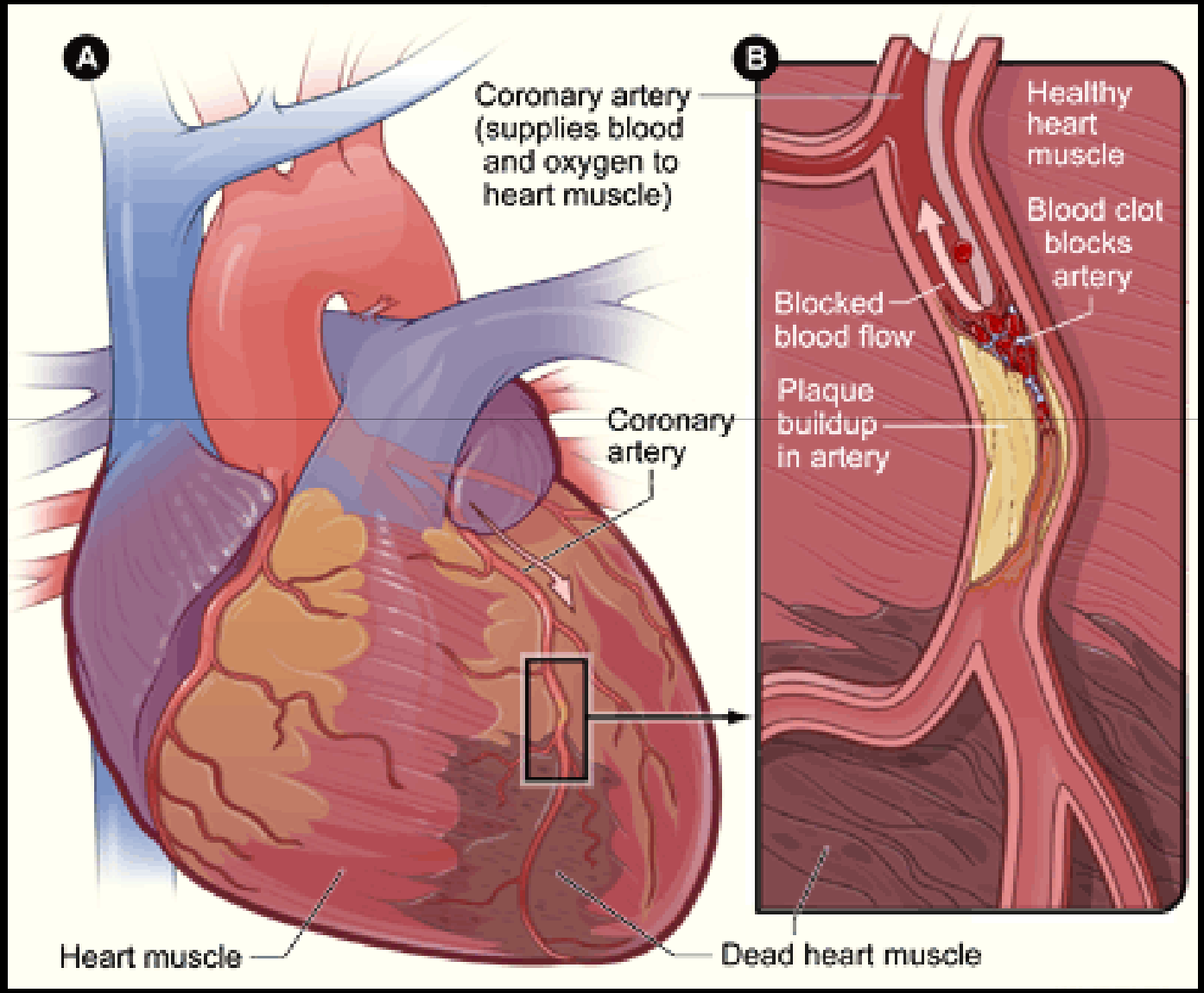
This presentation was modified from online course of

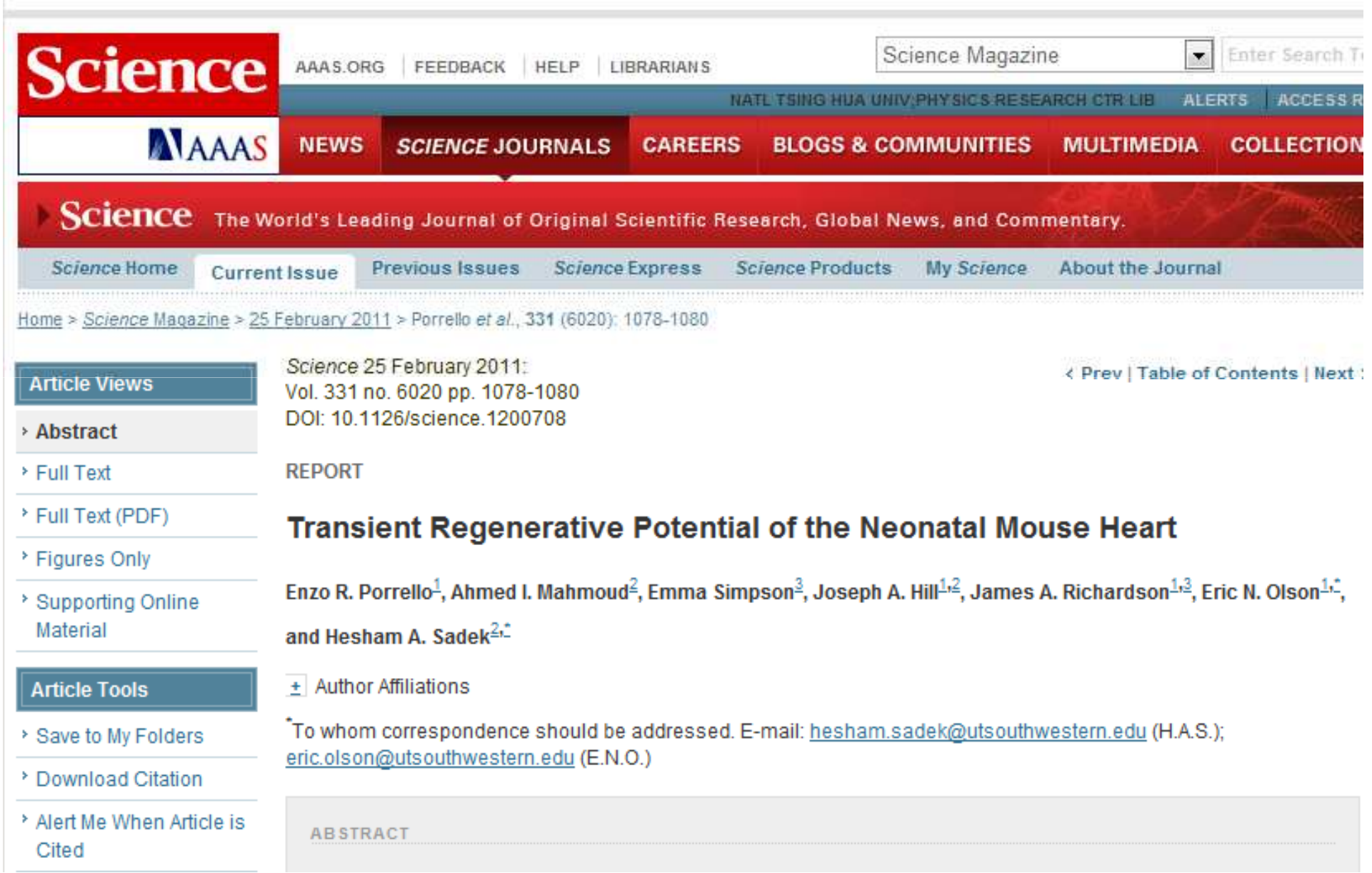
California Institute for Regenerative Medicine

http://www.cirm.ca.gov/

Heart Attack



New Born mouse can fully regenerate the damaged heart



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Science 25 February 2011:
Vol. 331 no. 6020 pp. 1078-1080
DOI: 10.1126/science.1200708

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REPORT

Transient Regenerative Potential of the Neonatal Mouse Heart

Enzo R. Porrello[1], Ahmed I. Mahmoud[2], Emma Simpson[3], Joseph A. Hill[1,2], James A. Richardson[1,3], Eric N. Olson[1,*], and Hesham A. Sadek[2,*]

± Author Affiliations

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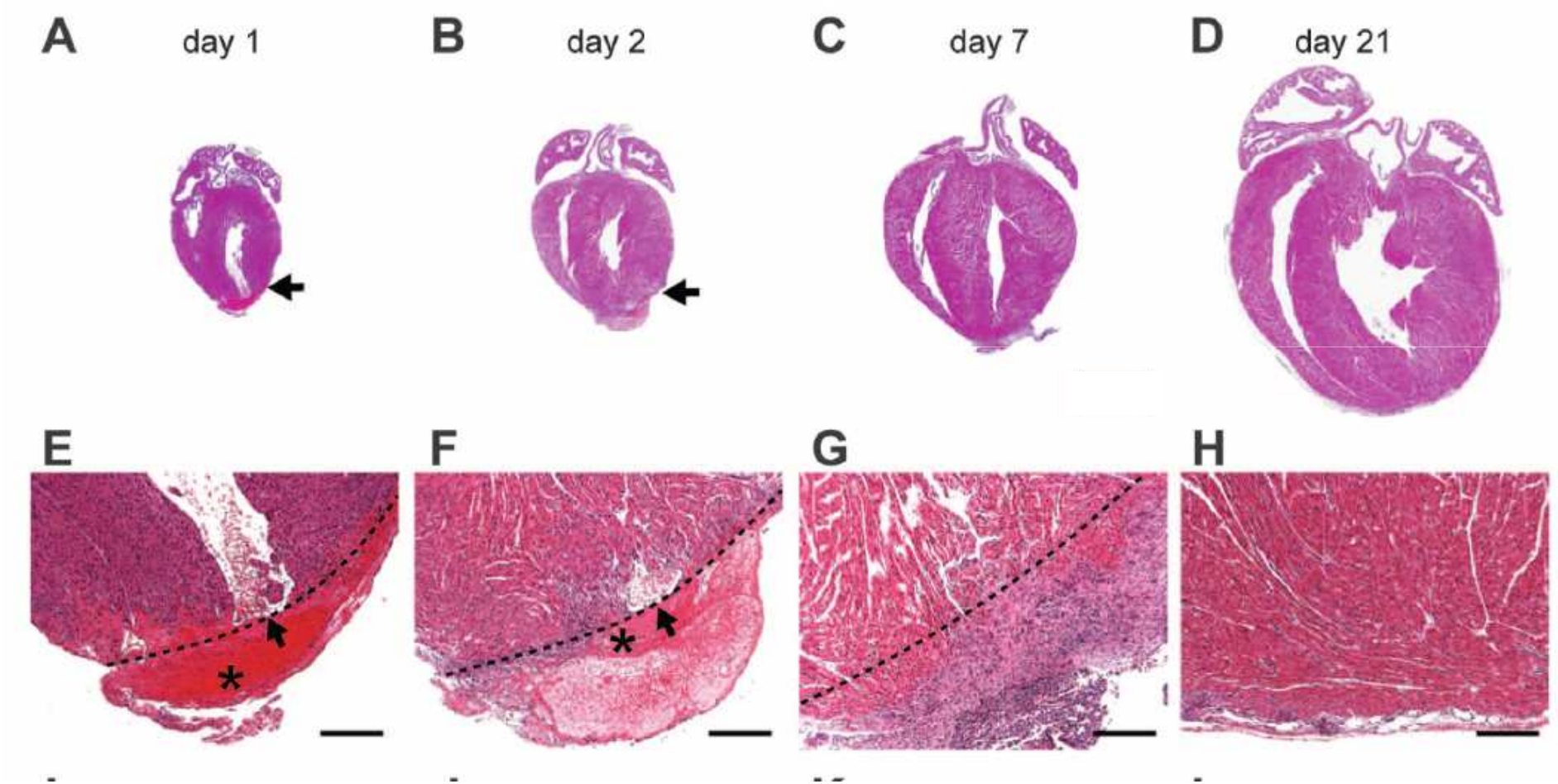
ABSTRACT

But the regeneration ability **will lost by 7 days after birth**



So, some mechanism is switched off!

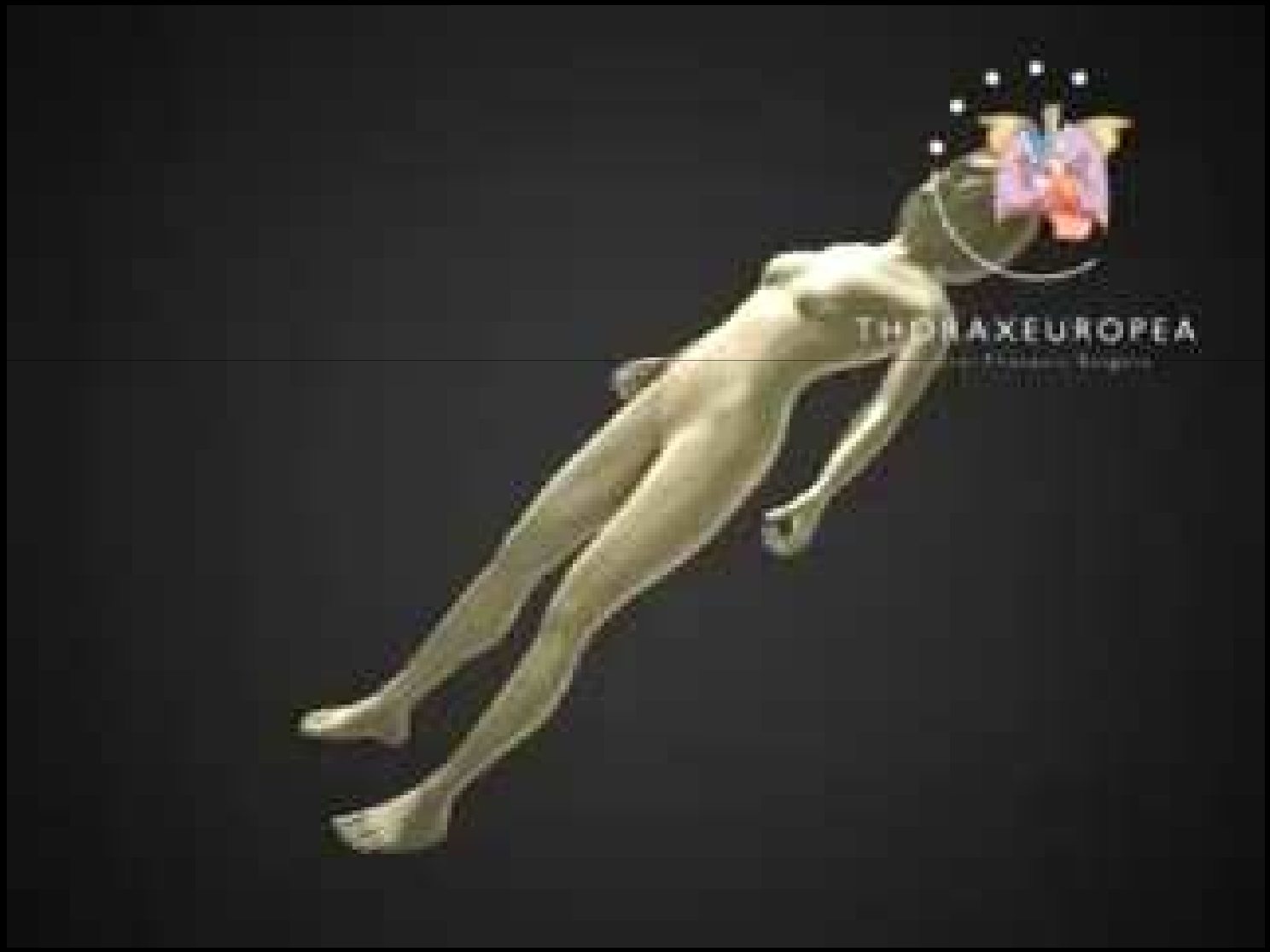
“Glow-in-the-dark” dogs - Ruppy!

What is stem cell research?

- Prevent/treat diseases and injuries
  - Cell-based therapies (細胞療法)
  - Pharmaceutical development (藥物研發)
    - Includes drug discovery, testing and delivery, etc.
- To learn about basic biological science such as development, aging, and evolution.
  - Experimental model systems (i.e. 模式生物)

Trachea transplantation:

Example of adult stem cell-based tissue regeneration



Source of Stem Cells
如何獲得幹細胞?

Conception in a dish

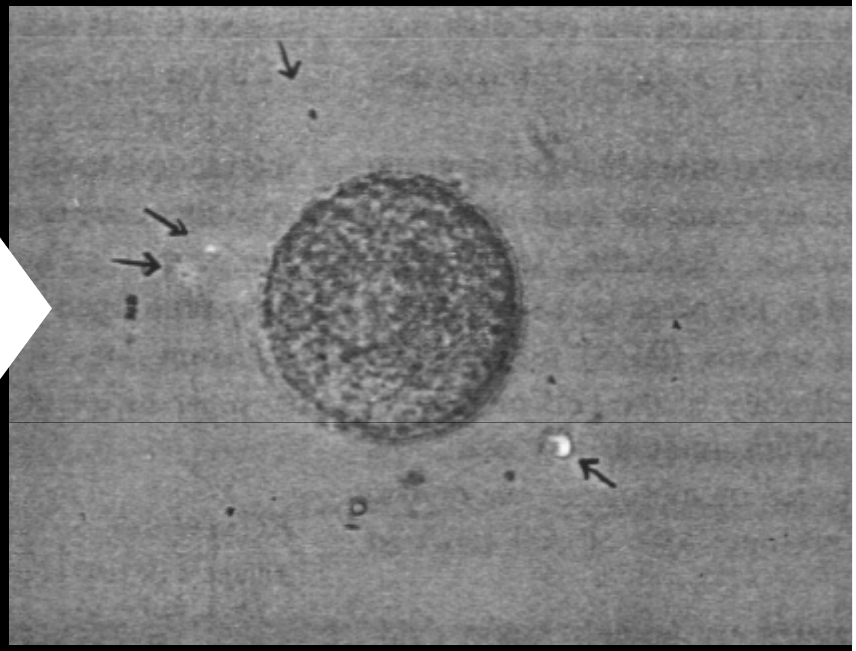
Day 1

Reducing the time of sperm–oocyte interaction in human in-vitro fertilization improves the implantation rate

Luca Gianaroli[1,3], M.Cristina Magli[1], Anna Pia Ferraretti[1], Agnese Fiorentino[1], Elisabetta Tosti[2], Sergio Panzella[1] and Brian Dale[2]

[1]S.I.S.ME.R., Medicine Reproductive Unit, V. Mazzini, 12, 40137

possessing fertilizing capacity is reduced (Holde
Trounson, 1991), and where up to 500000/ml motil
tozoa per oocyte are used (Fiorentino *et al.*, 199
1994).



In the ***In-vitro*** **fertilization** (**IVF)** procedure, sperm and eggs “interact” in a dish leading to insemination.
They literally swim up to the egg and burrow toward the nucleus.
The first one to get there wins, and all others are blocked out.

竹科男 精液品質差

邱俊吉 / 專題報導

3對夫妻1對不孕 男性因素佔4成

疑與高齡結婚、工作壓力及環境污染有關民眾提到「竹科男」，會認為他們多金社經地位高，但他們有他們的辛苦，尤其是生育後代。醫師指出，不孕症的男性因素通常佔3成，但竹科人不孕症的男性因素超過4成，其精液平均品質較一般人差。

國泰醫院新竹分院的生殖醫學中心，成立雖不久，院內人員卻常以「爆滿」二字來形容其盛況，粗估近2年已至少有1000對夫妻前往治療。該中心主任林正凱說，國內平均6至7對夫妻中，就有一對曾面臨不孕的問題，但在園區，平均每3對便有一對為不孕所困，無子煩惱比一般人更嚴重。

不孕普遍，林正凱認為有幾個原因，可能是高齡婚姻，或是工作量太大，或是環境污染，都可能使精、卵子的品質下降，但就該中心所見，竹科不孕症夫妻的男性因素，也就是精液品質，似乎比一般不孕症夫妻，佔了更大比率。

Male fertility issue:

Sometimes sperm cannot latch onto and penetrate the egg. They may choose to have Intra-Cytoplasmic Sperm Injection (ICSI)

Intra-Cytoplasmic Sperm Injection

Intra-Cytoplasmic Sperm Injection

Day 1

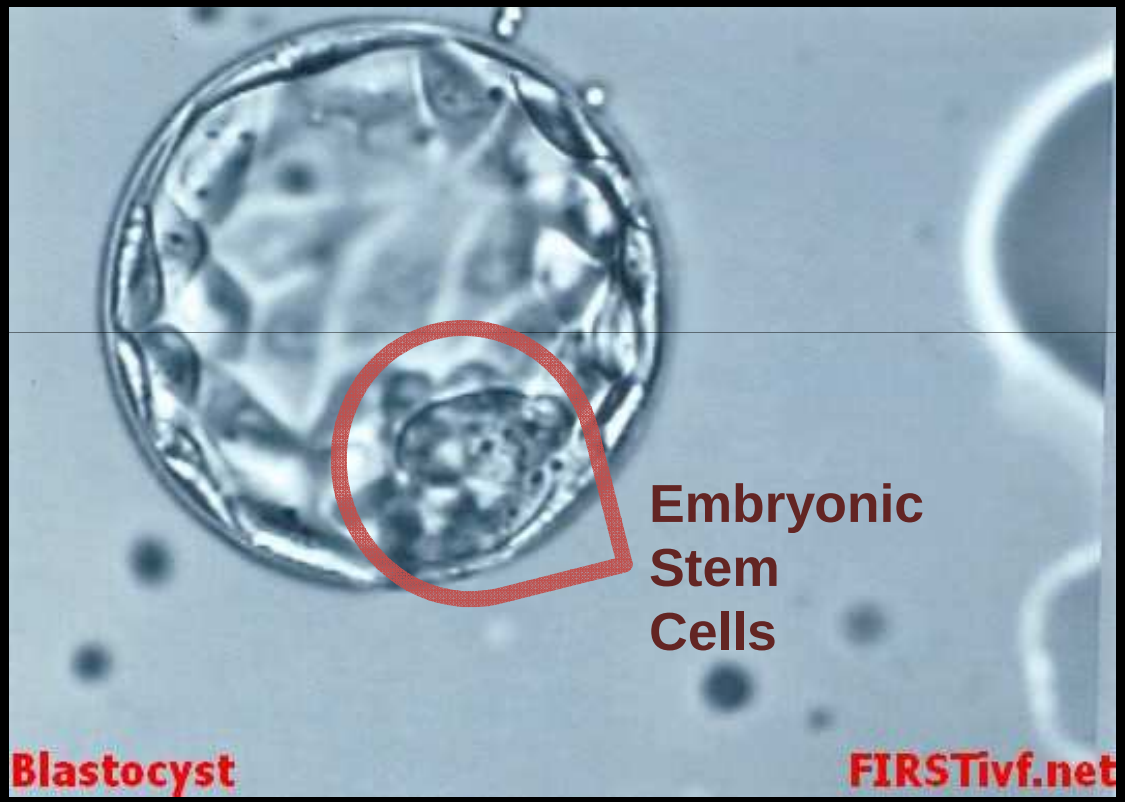
Day 2

Day 2

Day 3

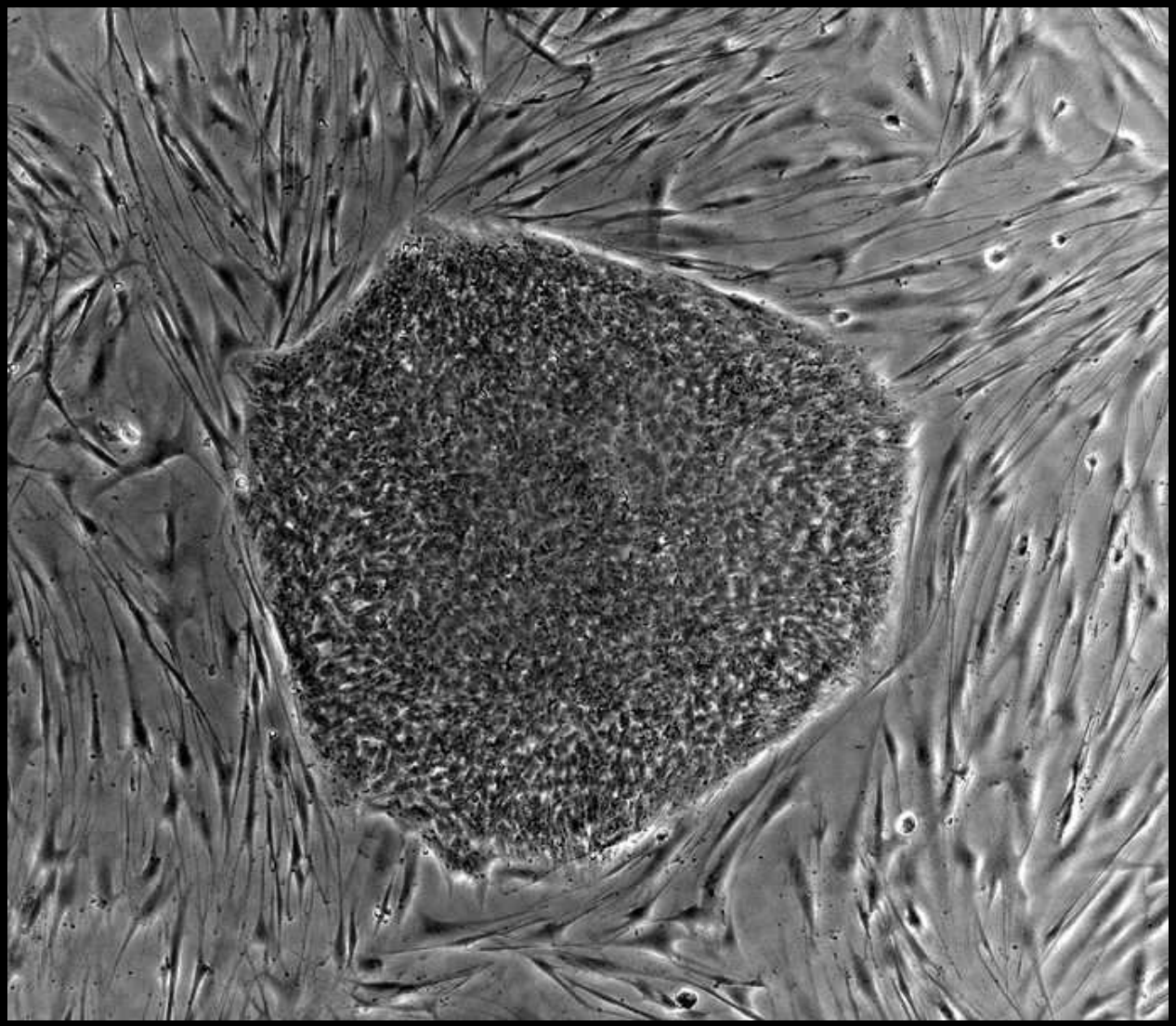
Day 4

Day 5

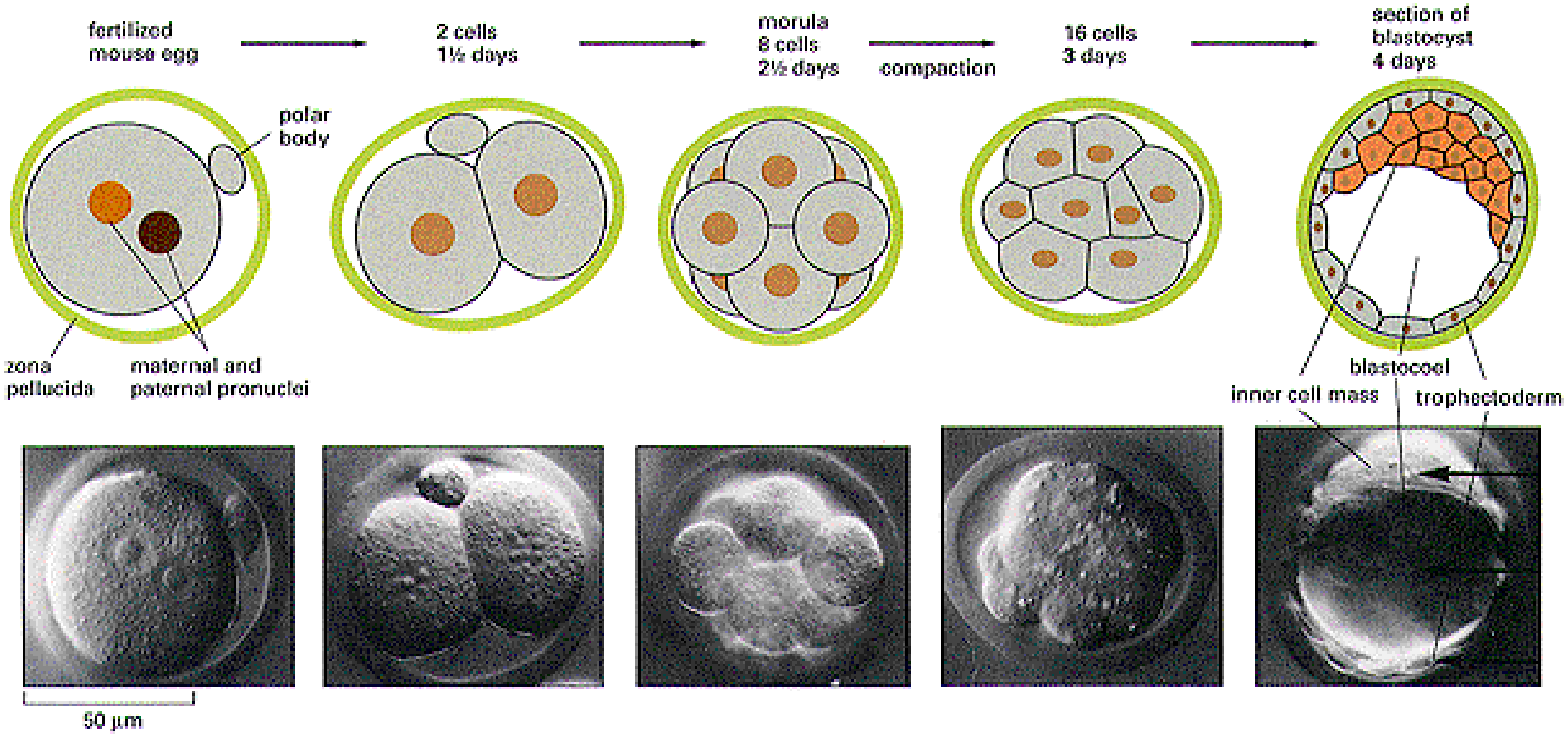


Embryonic stem cells in the dish:

What do cultured ES cells look like?



Human embryo, the first 4 days

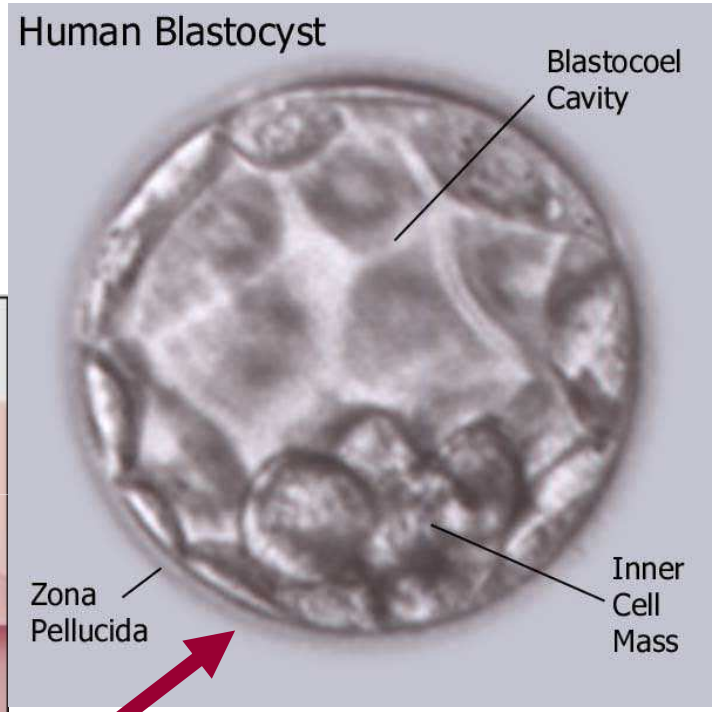


From The Art of MBoC³ © 1995 Garland Publishing, Inc.

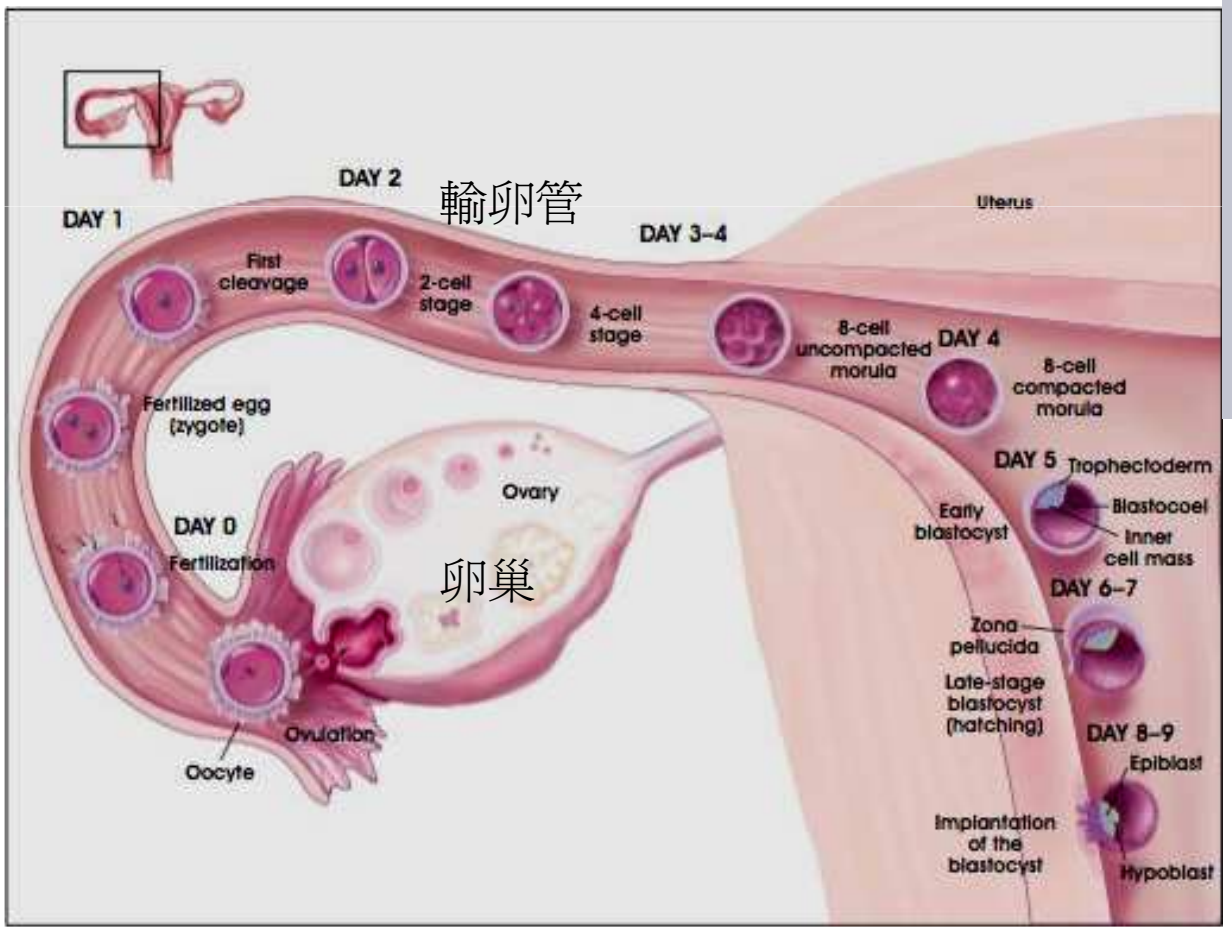
Development & Differentiation
發育 & 分化

Real-time Video Clip of Blastocyst Formation

FF ~2000x



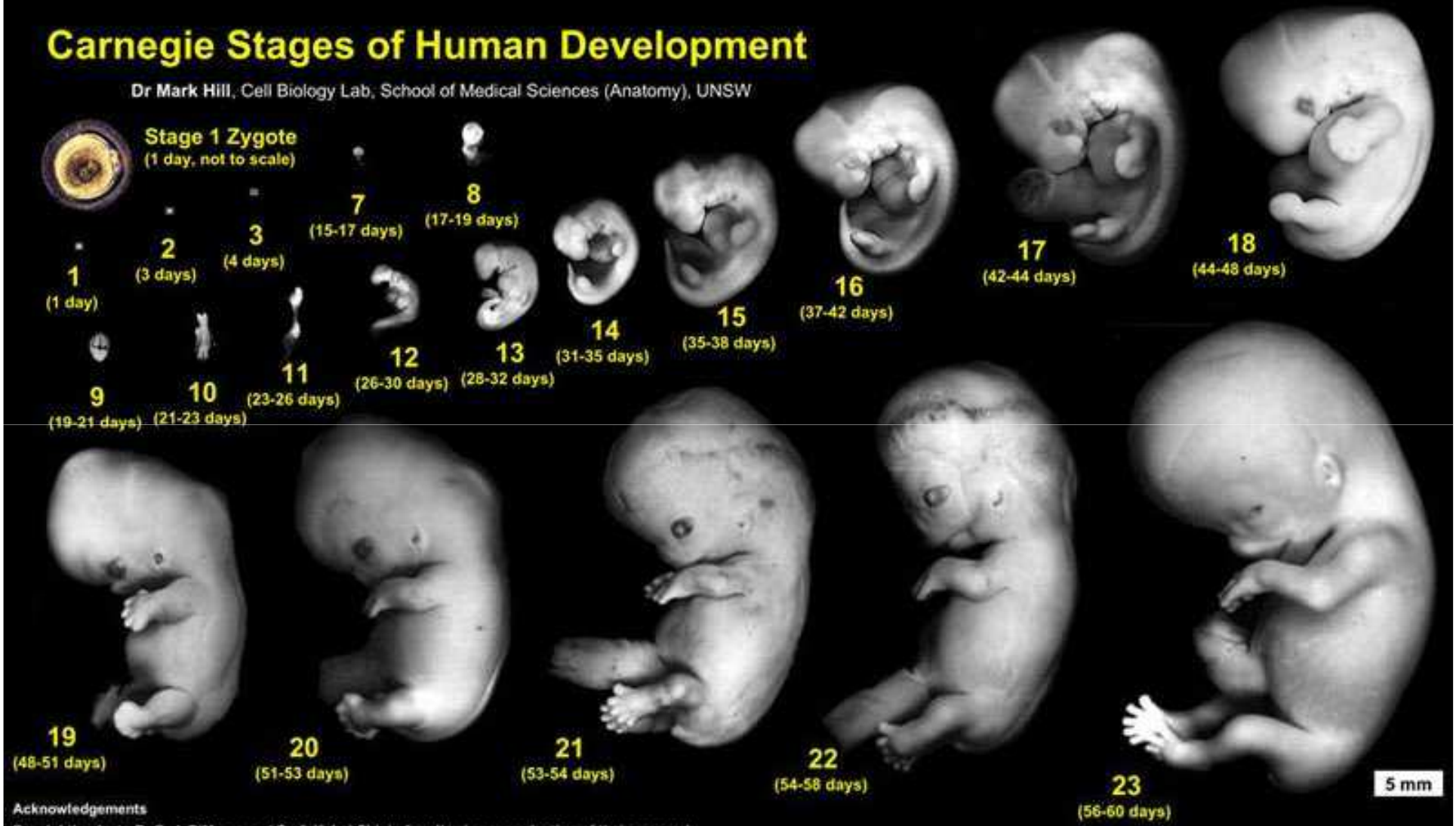
ESCs
胚胎幹細胞



子宮

Carnegie Stages of Human Development

Dr Mark Hill, Cell Biology Lab, School of Medical Sciences (Anatomy), UNSW



Acknowledgements

Special thanks to Dr S. J. DiMarzo and Prof. Kohei Shiota for allowing reproduction of their research images and material from the Kyoto Collection and Ms B. Hill for image preparation.

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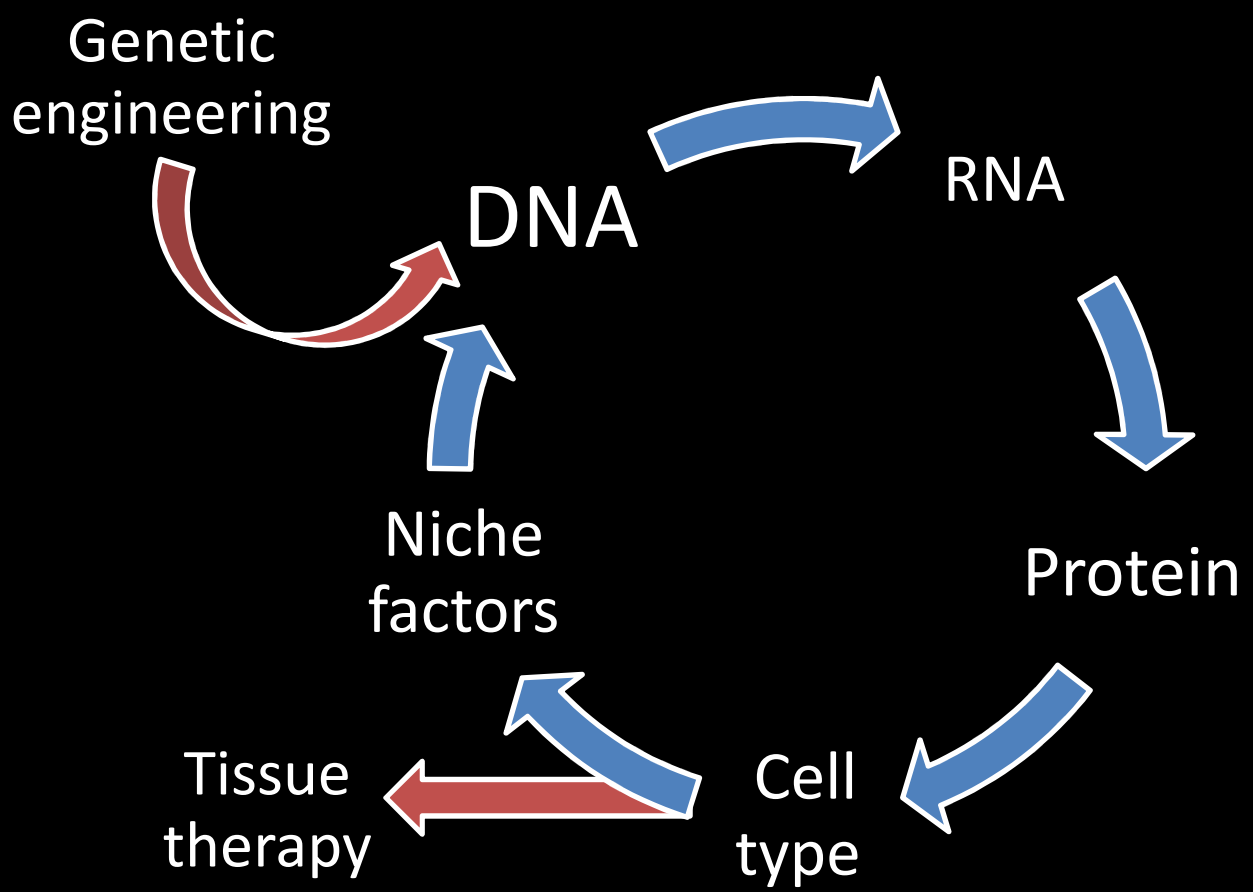
How do cells know what to become?

All cells in a person have the same DNA

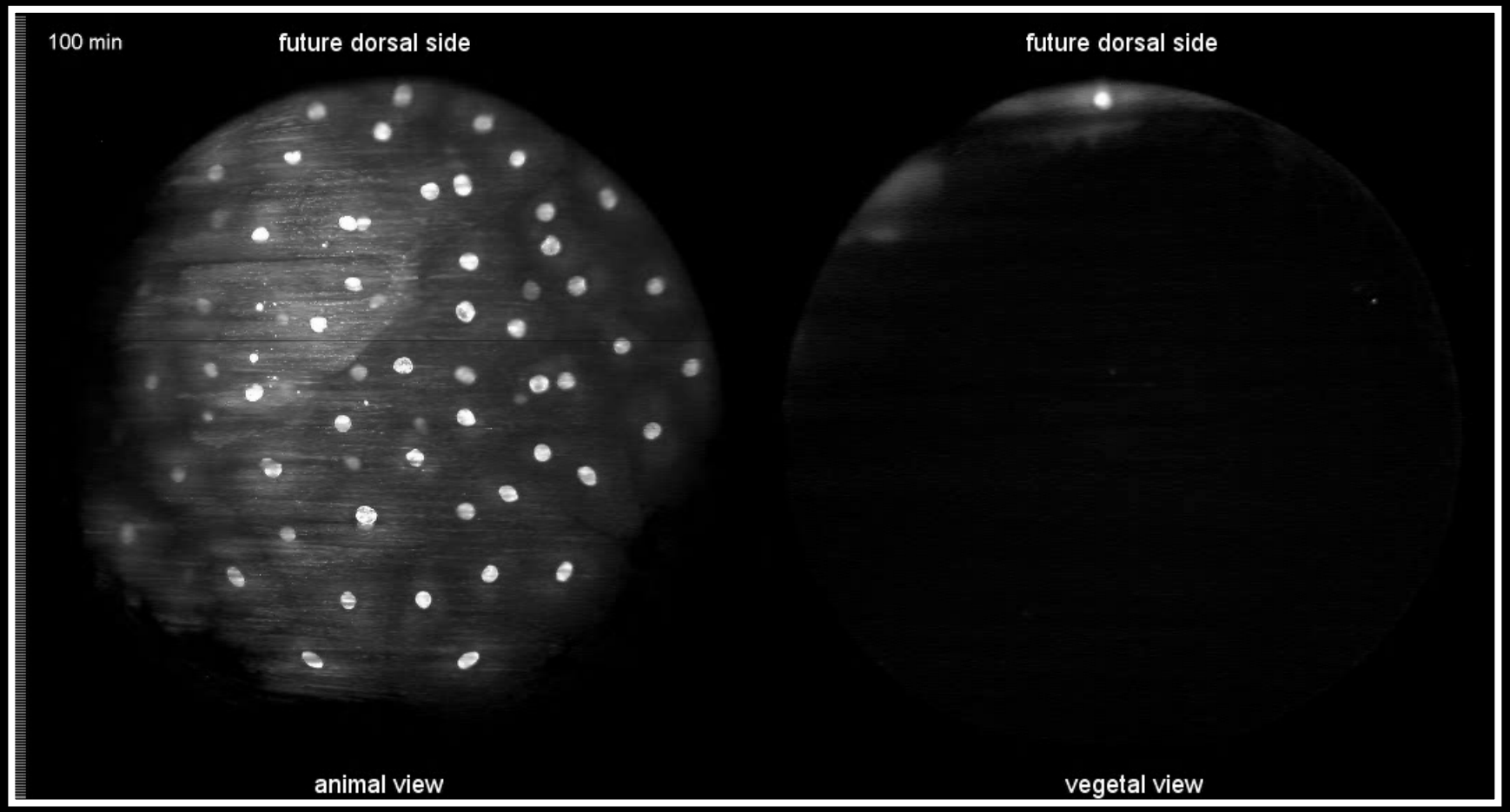
Yet eye cells differ from nose cells

Central dogma of biology



Embryonic Development

Zebrafish embryo



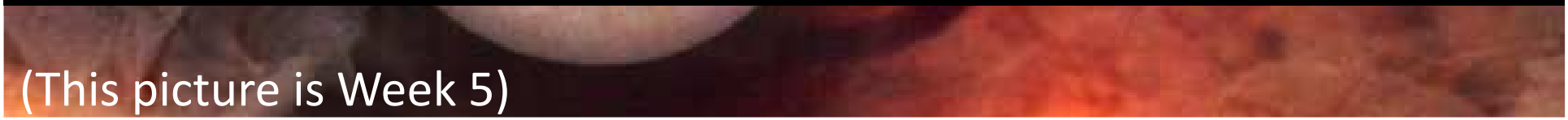
Keller et al. 2008

Supporting video

At what point is this a fetus?

(受精多久後才發育成胎兒?)

- Days 7-14: Uterine implantation
- Day 14: Three distinct layers begin to form (no more pluripotent stem cells)
- Days 14-21: Beginning of future nervous system
- Days 21-24: Beginning of future face, neck, mouth, and nose
- Weeks 3-8: Beginning of organ formation
- Week 5-8+: Now it's generally called a fetus (no consensus on exact time point)



(This picture is Week 5)

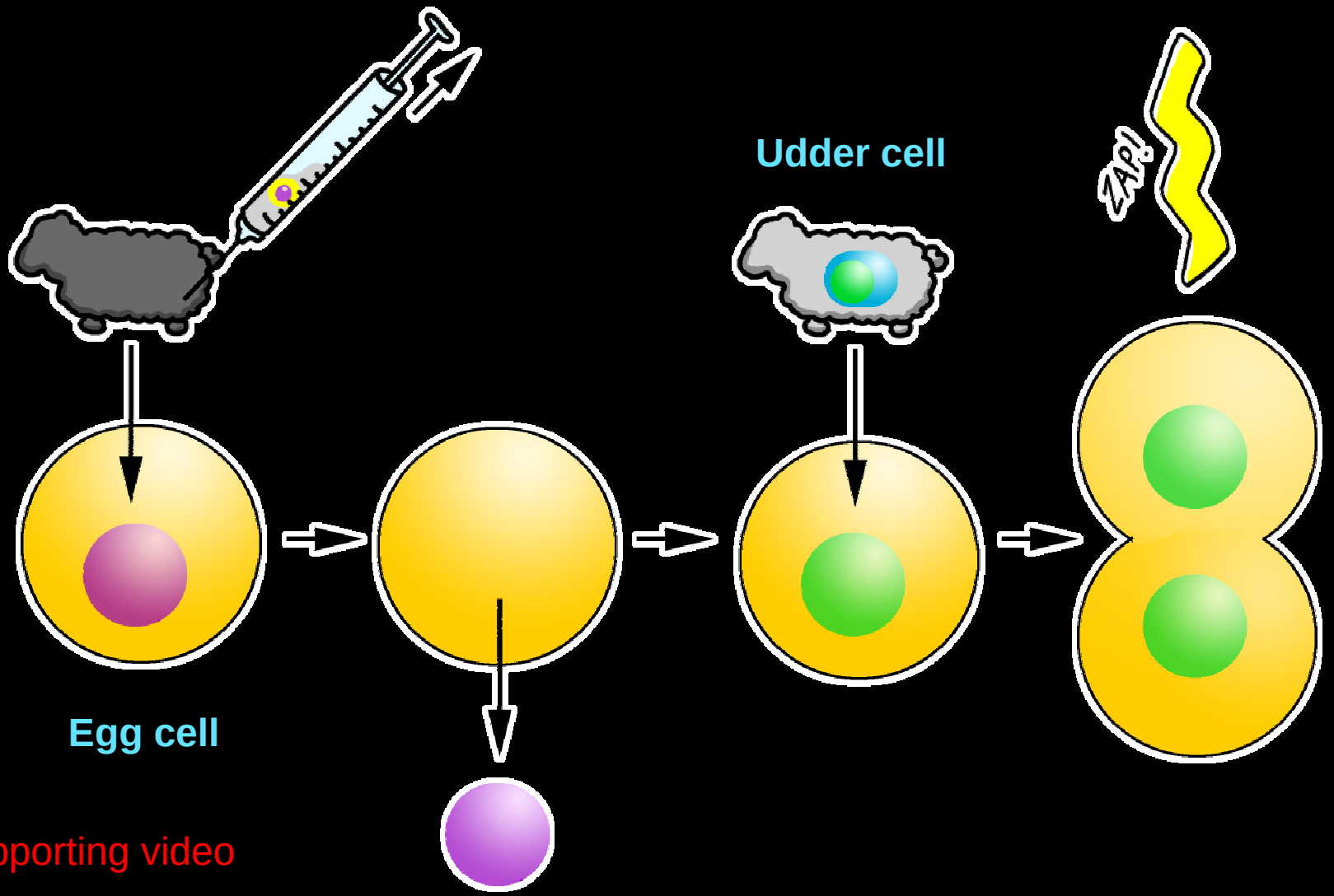
Divergent Religious View on Research and Clinical Use of ESC

Religions	Embryonic Stem Cell (ESC) Research
Catholic 天主教徒	**Prohibited** (life begins at conception)
Muslim 伊斯蘭教信徒	**Acceptable** (fetus has moral existence only at the end of the 4th month)
Jewish 猶太人	**Acceptable** (embryo has no moral status until 40 days)
Buddhist 佛教徒	**Prohibited** (life begins at conception)

Cloning
人工複製動物

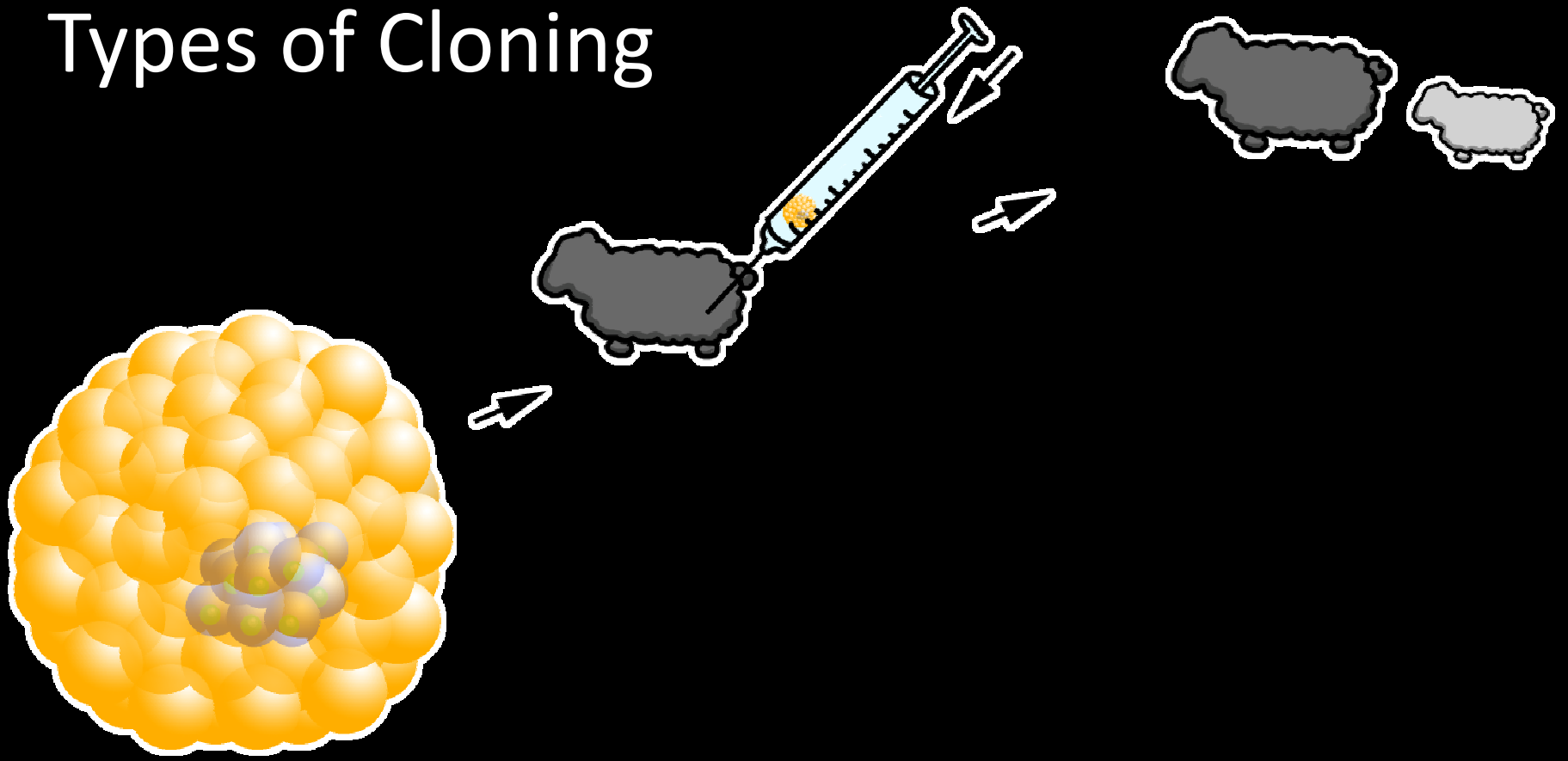
Somatic Cell Nuclear Transfer (SCNT)

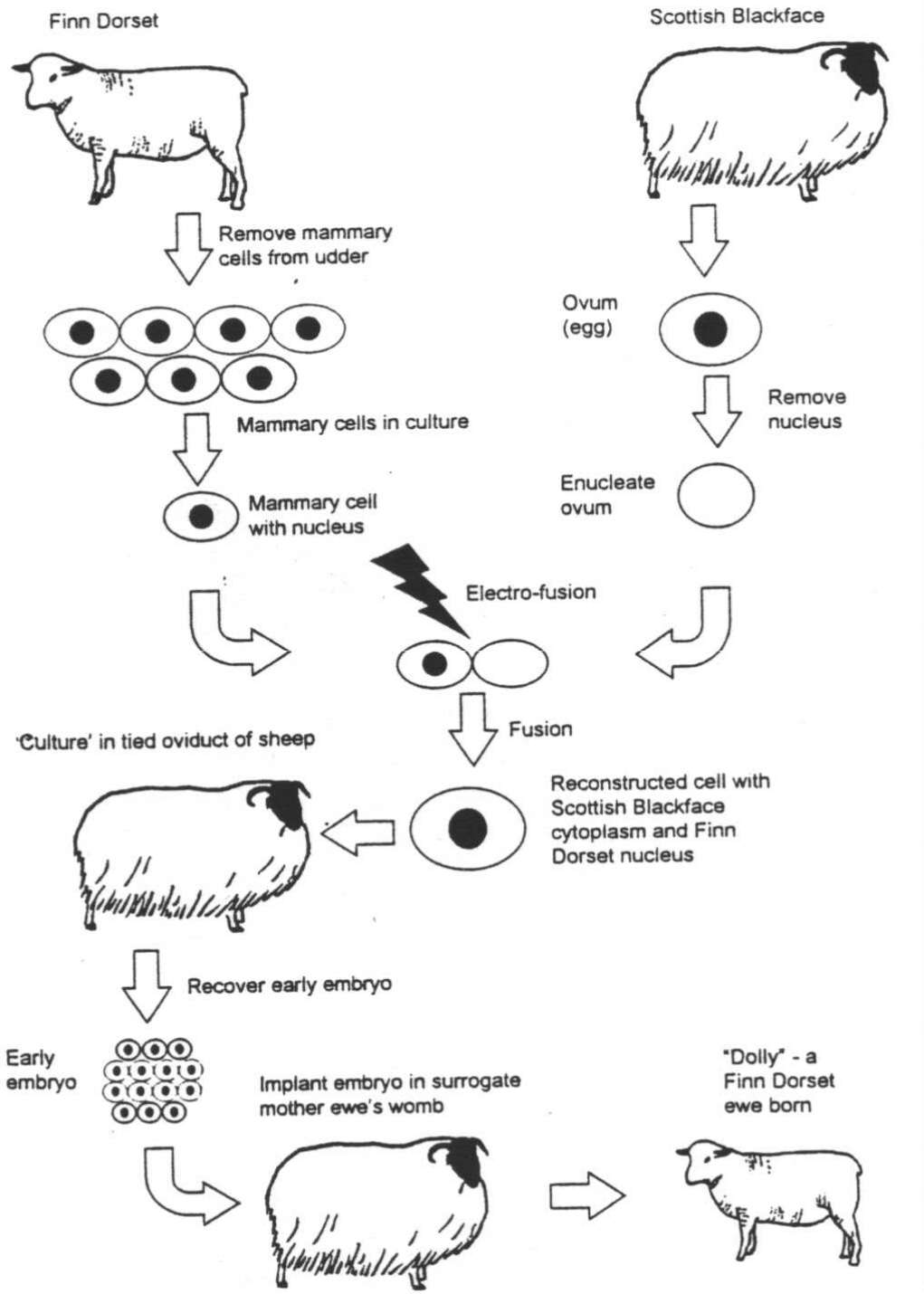
Cloning of embryonic stem cells



Supporting video

Types of Cloning





Cloning Dolly
桃莉羊

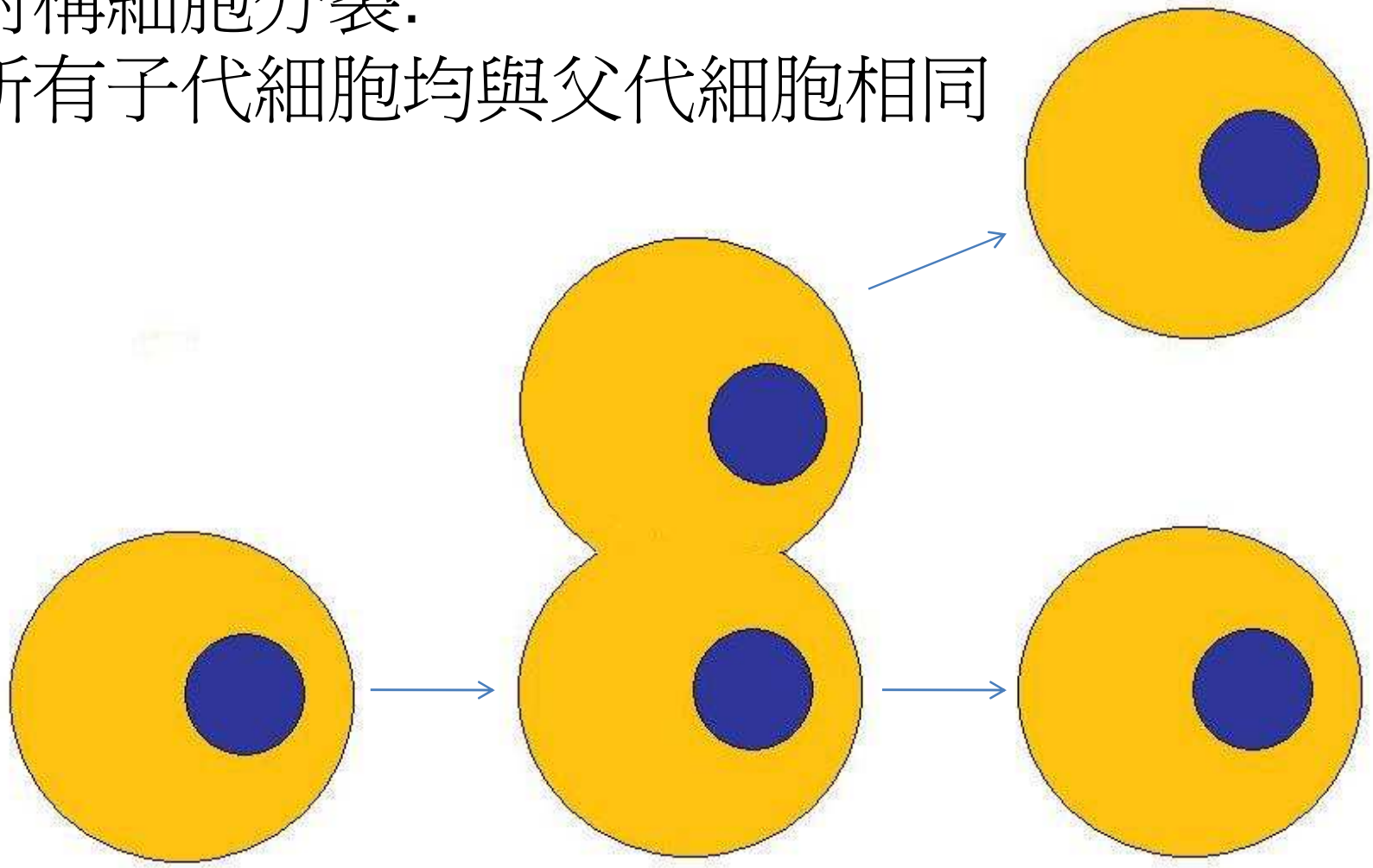
Complications
Observed in Reproductive Cloned Animals

- Extremely low reproductive efficiency（低繁殖率）
- High late fetal losses（高死胎率）
- Placental abnormalities（胎盘异常）
- Increased birth size（巨婴症）
  - Large offspring syndrome
    - Risk to mother and offspring
- High early neonatal death rates（高夭折率）
  - Respiratory deaths

What makes stem cells unique
幹細胞特性

Symmetric cell division

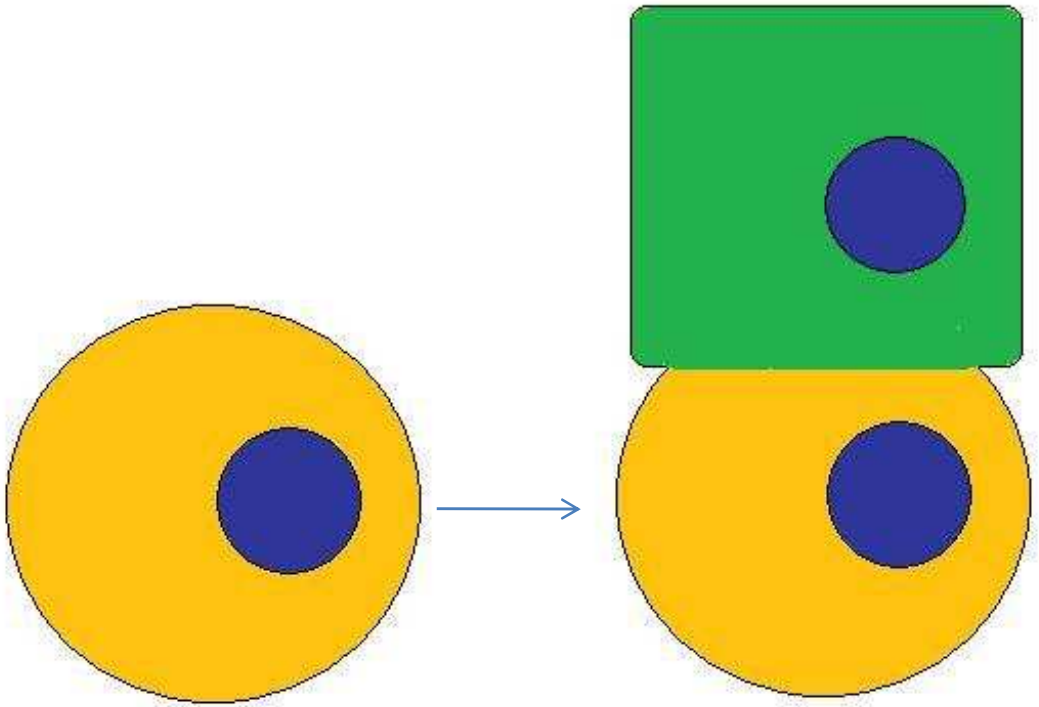
對稱細胞分裂:
所有子代細胞均與父代細胞相同

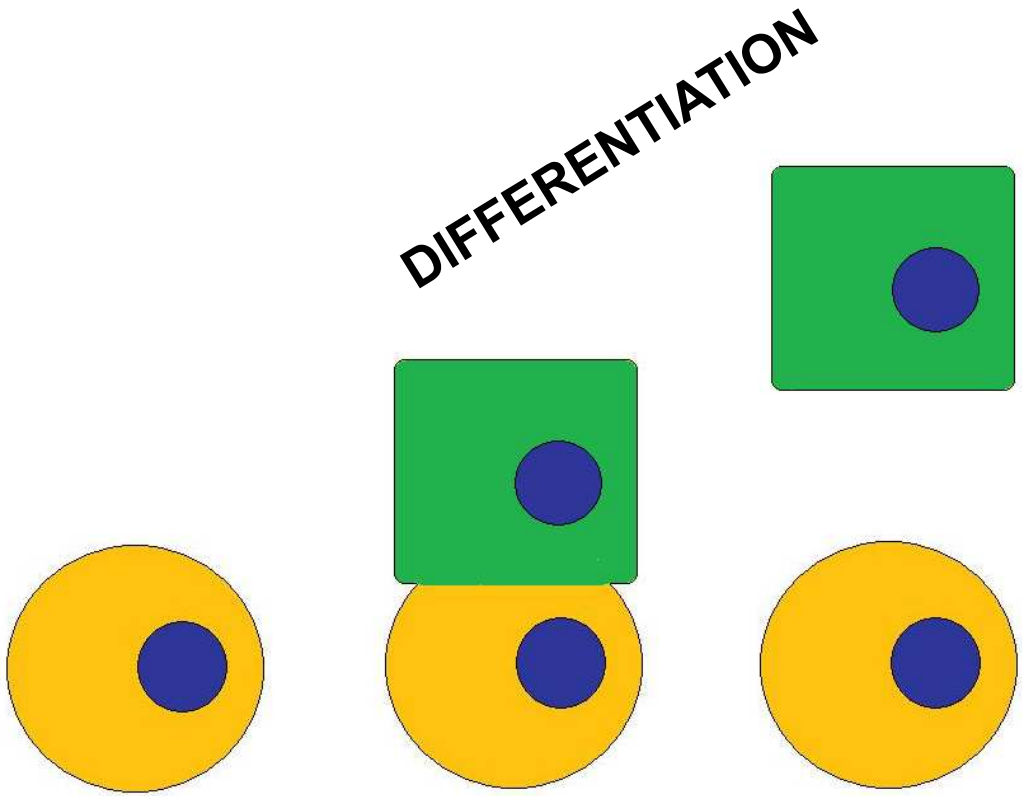


Asymmetric cell division

非對稱細胞分裂

1. Self-renews (自我更新)
2. Differentiates (分化)




DIFFERENTIATION
→ SELF – RENEWAL →

Stem Cell - Definition

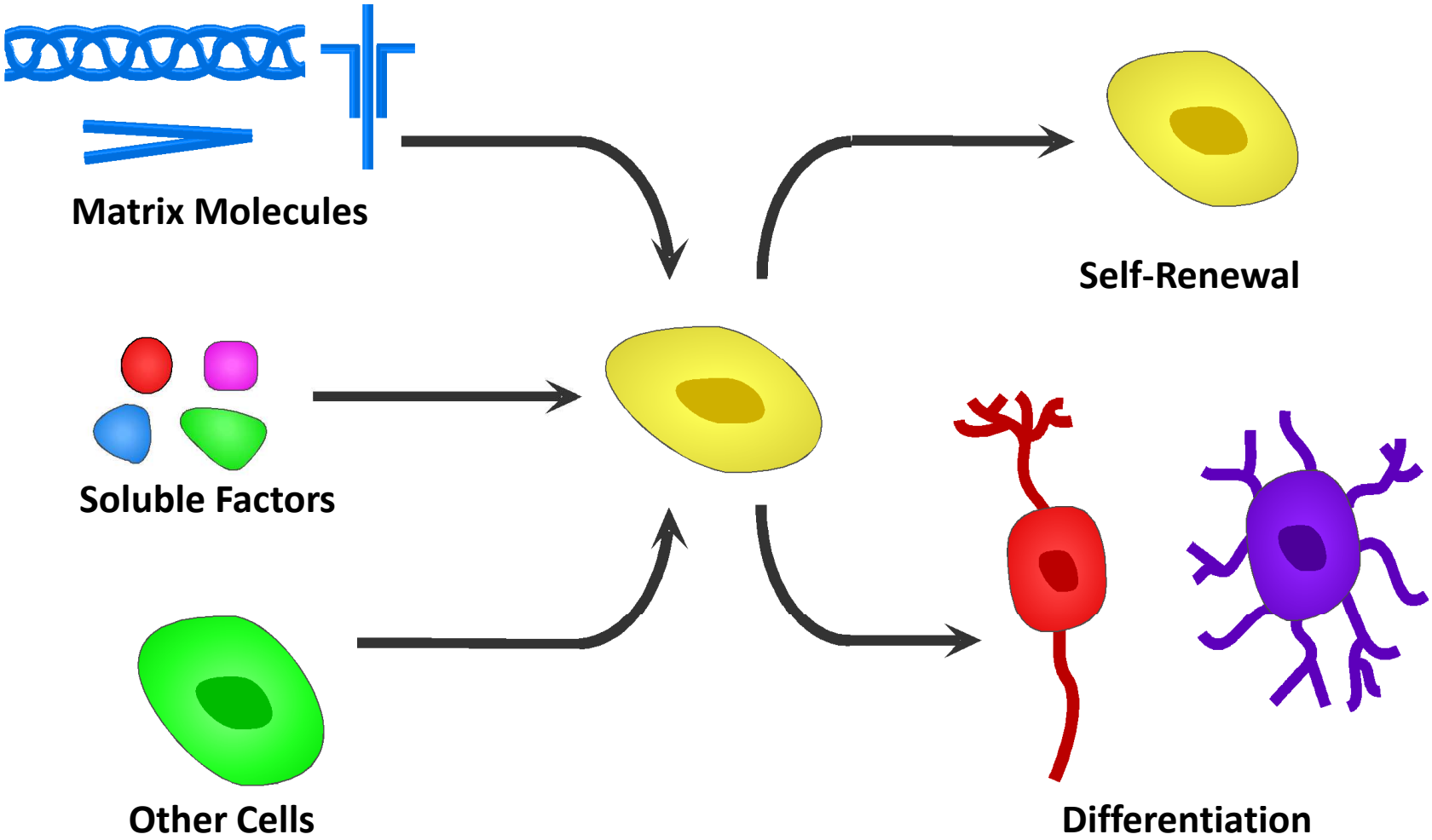
- **Self-Renewal**
  - It can **divide without limit**

    (可無限分裂**);** at least for the lifetime of animal
  - It is **not itself terminally differentiated**

    (未終止分化**) ;** Not at the end of differentiation pathway
- **Potency in differentiation**
  - When it divides each daughter cell **has a cell fate choice**

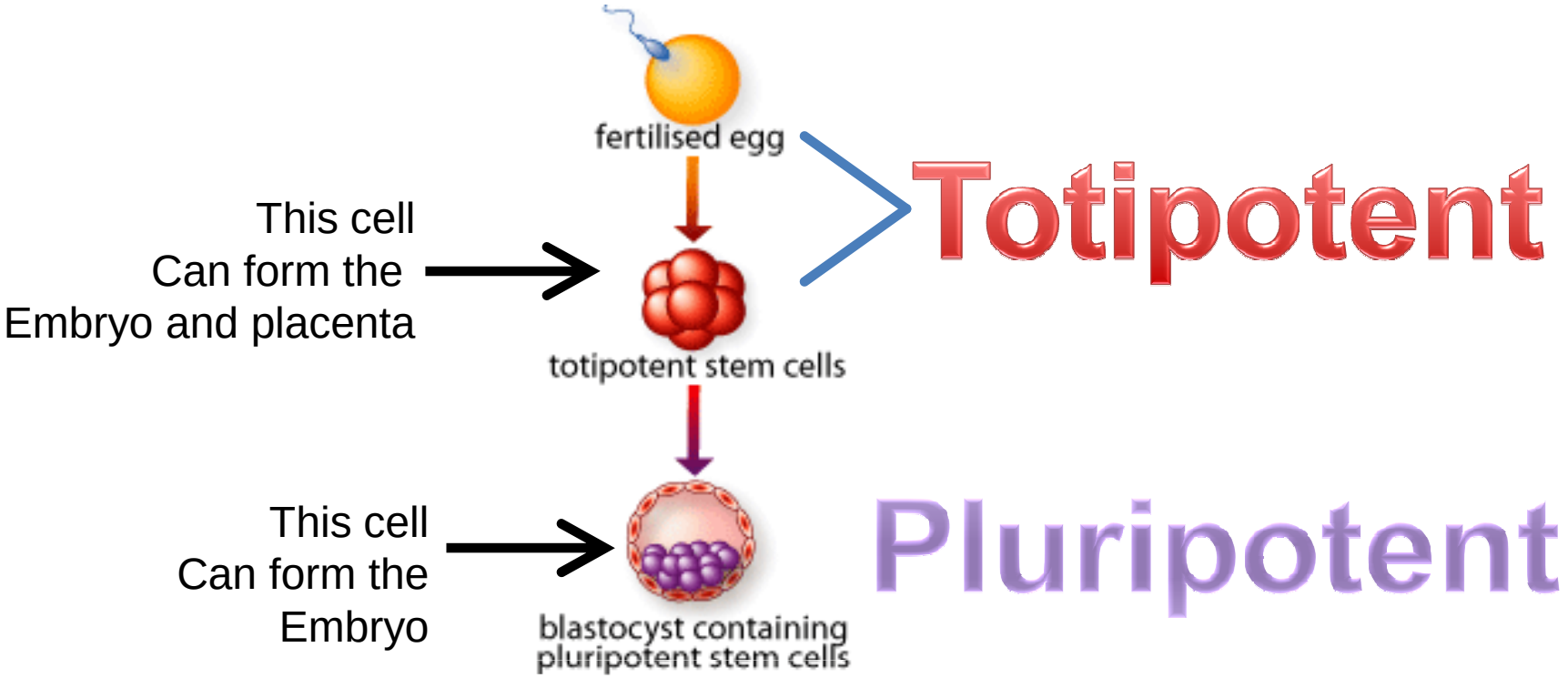
    (子代細胞擇定分化途徑); it can remain a stem cell, or it can embark on a course leading to terminal differentiation

Signals to Stem Cells



Little, et al. *Chemical Reviews* (2008).

Different types of stem cells
幹細胞種類


fertilised egg
This cell
Can form the
Embryo and placenta
totipotent stem cells
Totipotent
This cell
Can form the
Embryo
blastocyst containing
pluripotent stem cells
Pluripotent

Key Definitions:

Totipotent: toti-, from Latin *totus*, means whole
全能 = whole potent can form every cell type, including germ cells

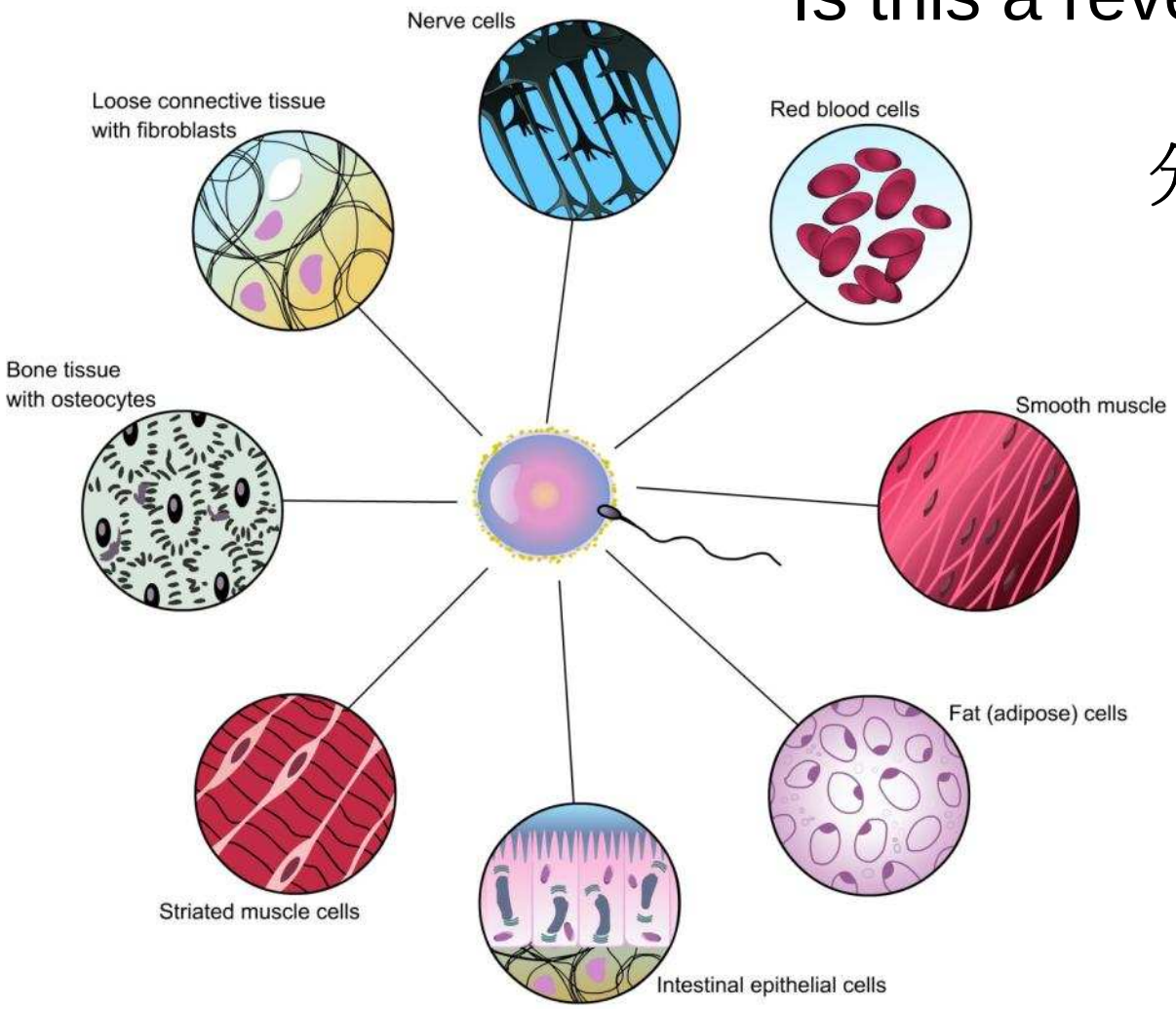
Pluripotent: pluri-, from Latin *plur*-, means more, several, more than one can form many different derivatives, but not germ cells
多高能

Multipotent: from Latin *multus*- means much or many. can form a few different cell types
多能

toti > pluri > multi

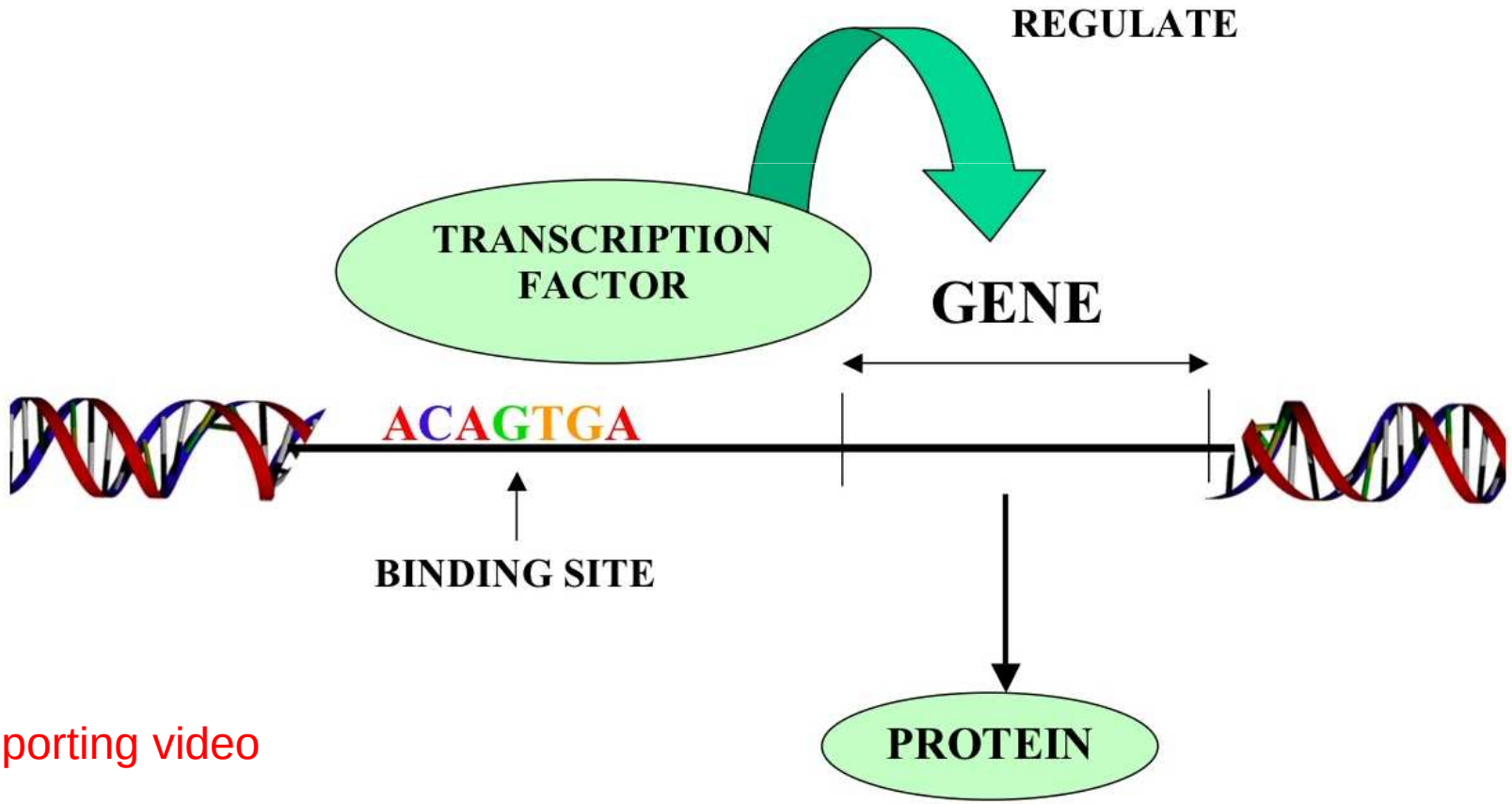
Cell Differentiation:
Is this a reversible process?

分化過程可逆嗎?



Transcription factor (轉錄因子) is a protein that binds to specific DNA sequences and thereby controls the transfer (or transcription) of genetic information from DNA to mRNA

Legend: A transcription factor molecule binds to the DNA at its binding site, and thereby regulates the production of a protein from a gene.



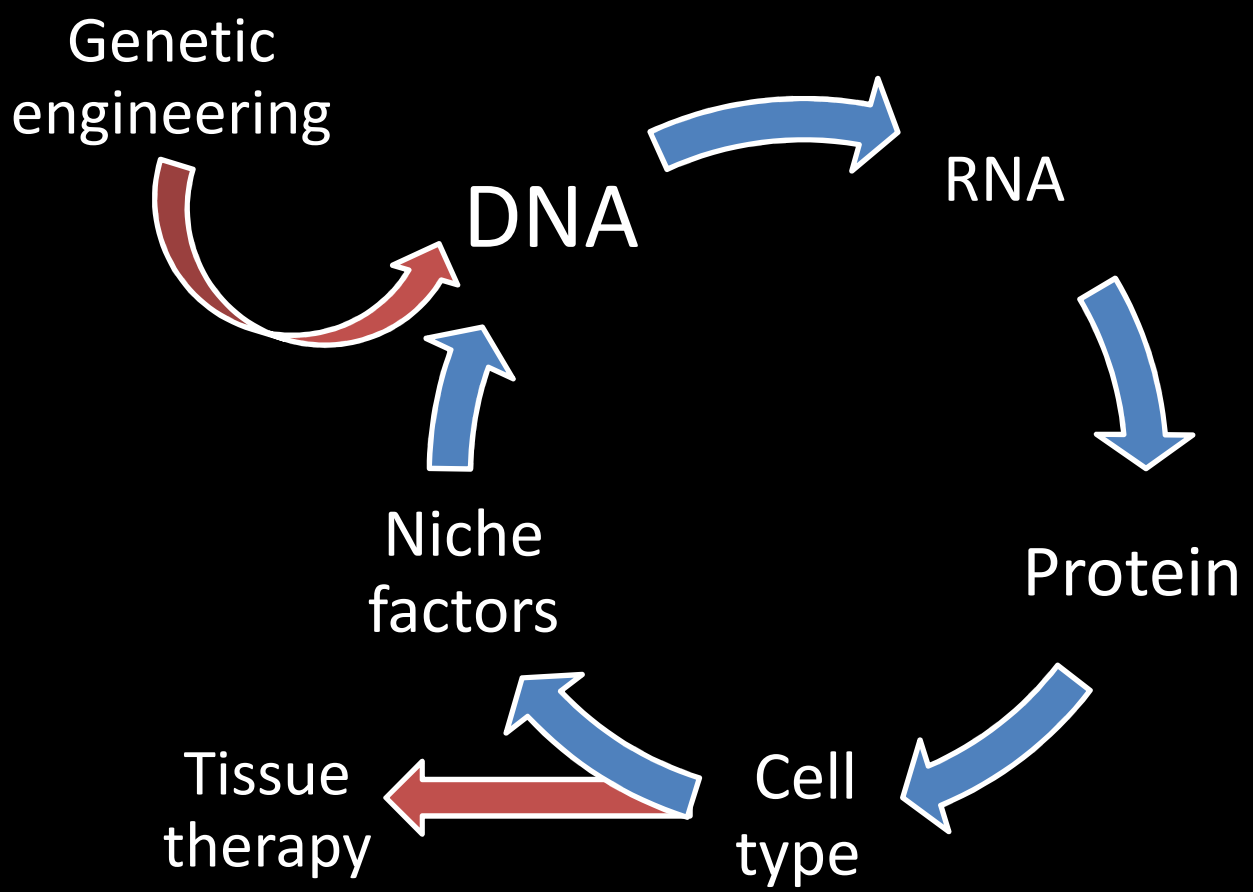
2 supporting video

How do cells know what to become?

All cells in a person have the same DNA

Yet eye cells differ from nose cells

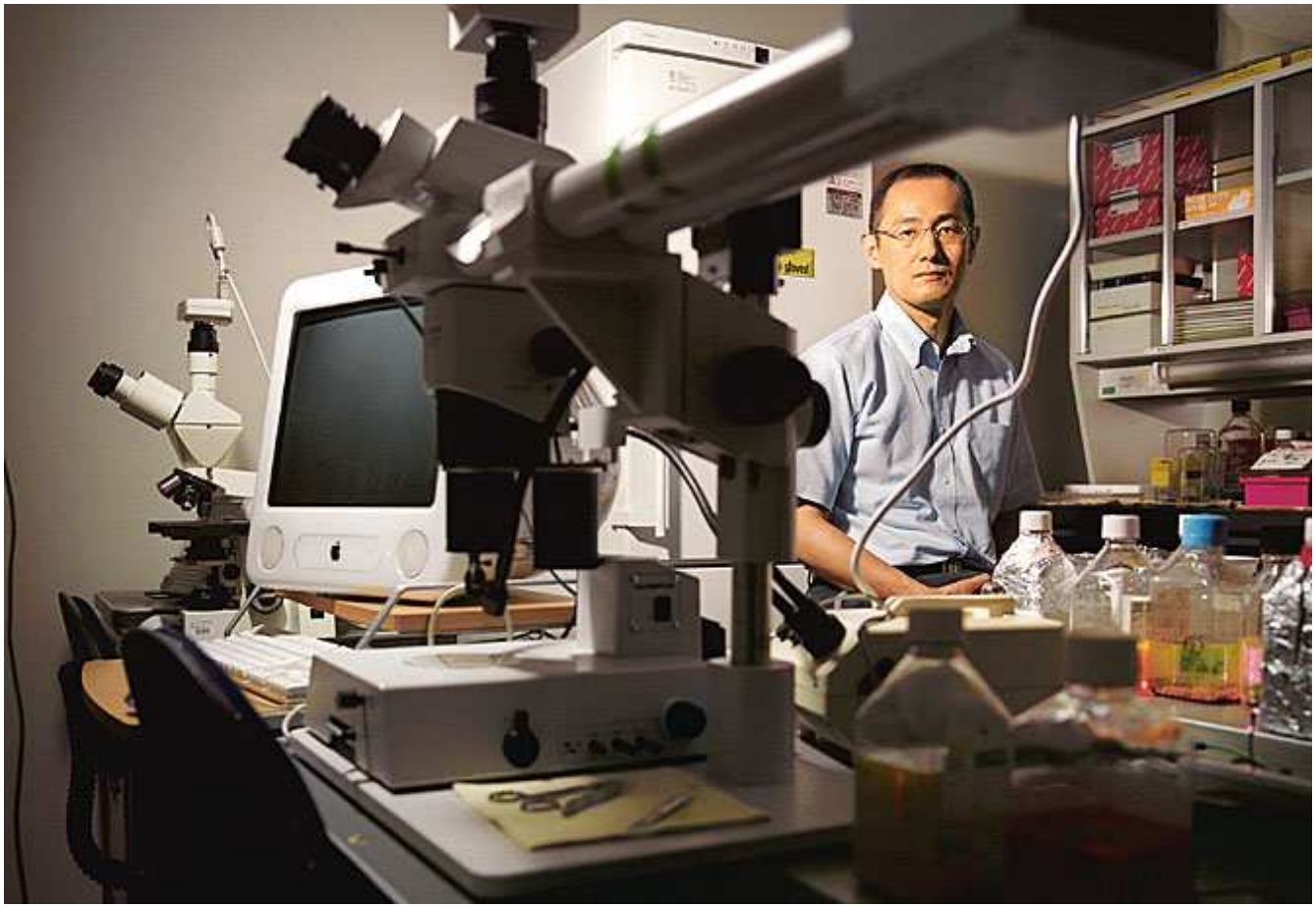
Central dogma of biology



Time Magazine : http://www.time.com/time/

Stem Cells: Japan Gets Ahead of the Curve

Thursday, Jun. 14, 2007 By BRYAN WALSH



LEADING LIGHT: Yamanaka, a former orthopedic surgeon, knows how to knit research together with practical applications

A breakthrough research!

Cell

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

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

SUMMARY

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we demonstrate induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions. Unexpectedly, Nanog was dispensable. These cells, which we designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Following injection into blastocysts, iPS cells contributed to mouse embryonic development. These data demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors.

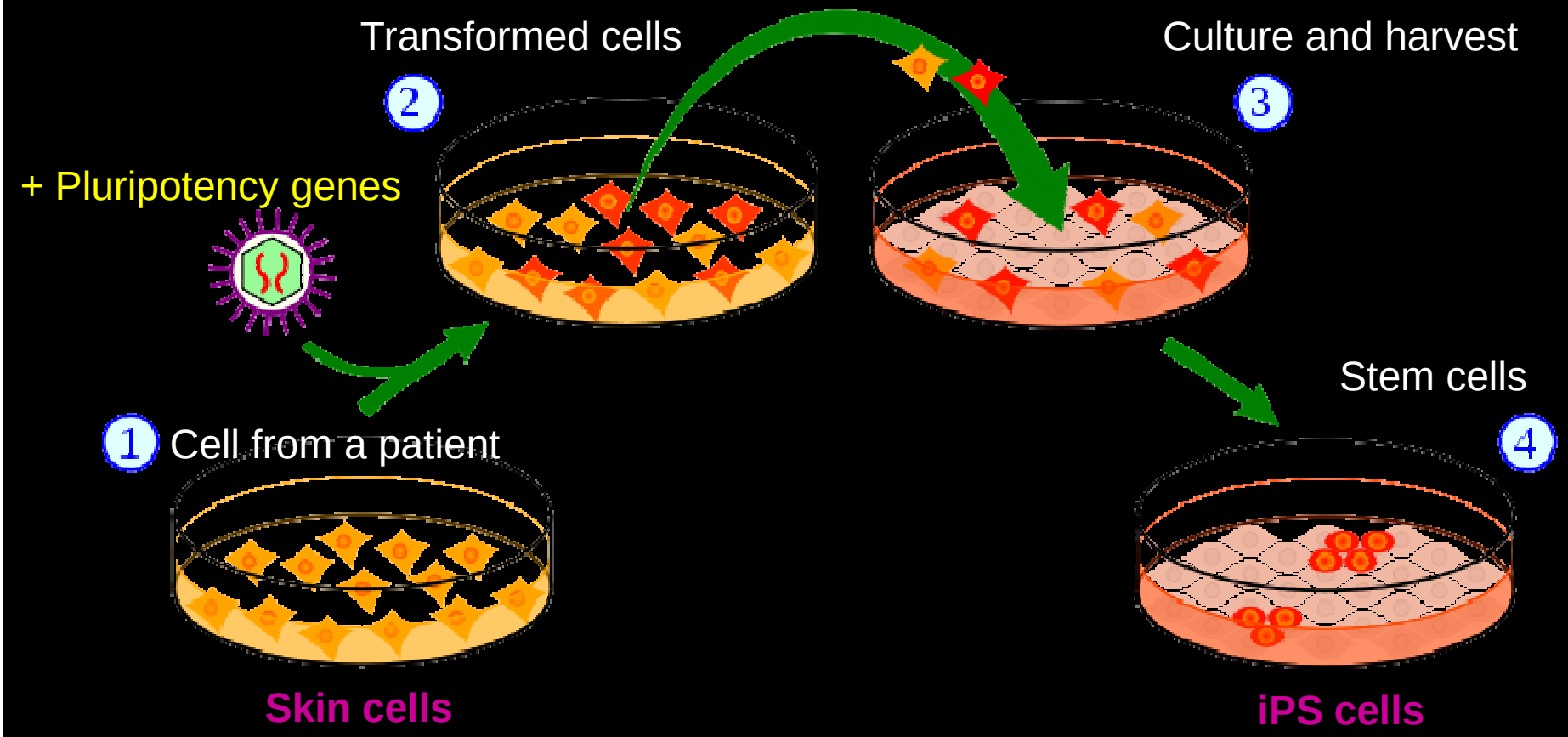
or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.

Several transcription factors, including Oct3/4 (Nichols et al., 1998; Niwa et al., 2000), Sox2 (Avilion et al., 2003), and Nanog (Chambers et al., 2003; Mitsui et al., 2003), function in the maintenance of pluripotency in both early embryos and ES cells. Several genes that are frequently upregulated in tumors, such as *Stat3* (Matsuda et al., 1999; Niwa et al., 1998), *E-Ras* (Takahashi et al., 2003), c-*myc* (Cartwright et al., 2005), *Klf4* (Li et al., 2005), and β-*catenin* (Kielman et al., 2002; Sato et al., 2004), have been shown to contribute to the long-term maintenance of the ES cell phenotype and the rapid proliferation of ES cells in culture. In addition, we have identified several other genes that are specifically expressed in ES cells (Maruyama et al., 2005; Mitsui et al., 2003).

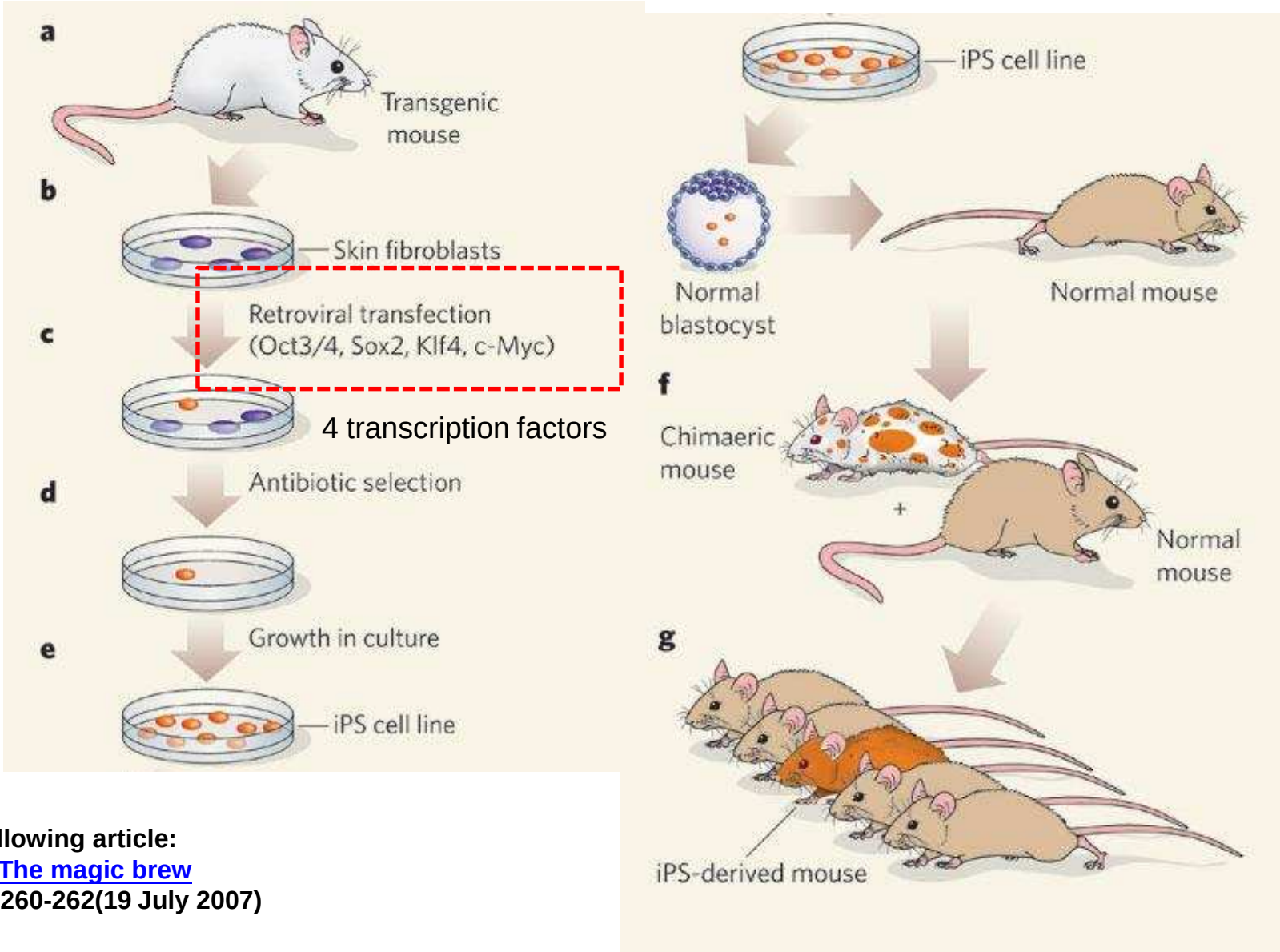
In this study, we examined whether these factors could induce pluripotency in somatic cells. By combining four selected factors, we were able to generate pluripotent cells, which we call induced pluripotent stem (iPS) cells, directly from mouse embryonic or adult fibroblast cultures.

Induced Pluripotent Stem (iPS) Cells

Genetically engineering new stem cells

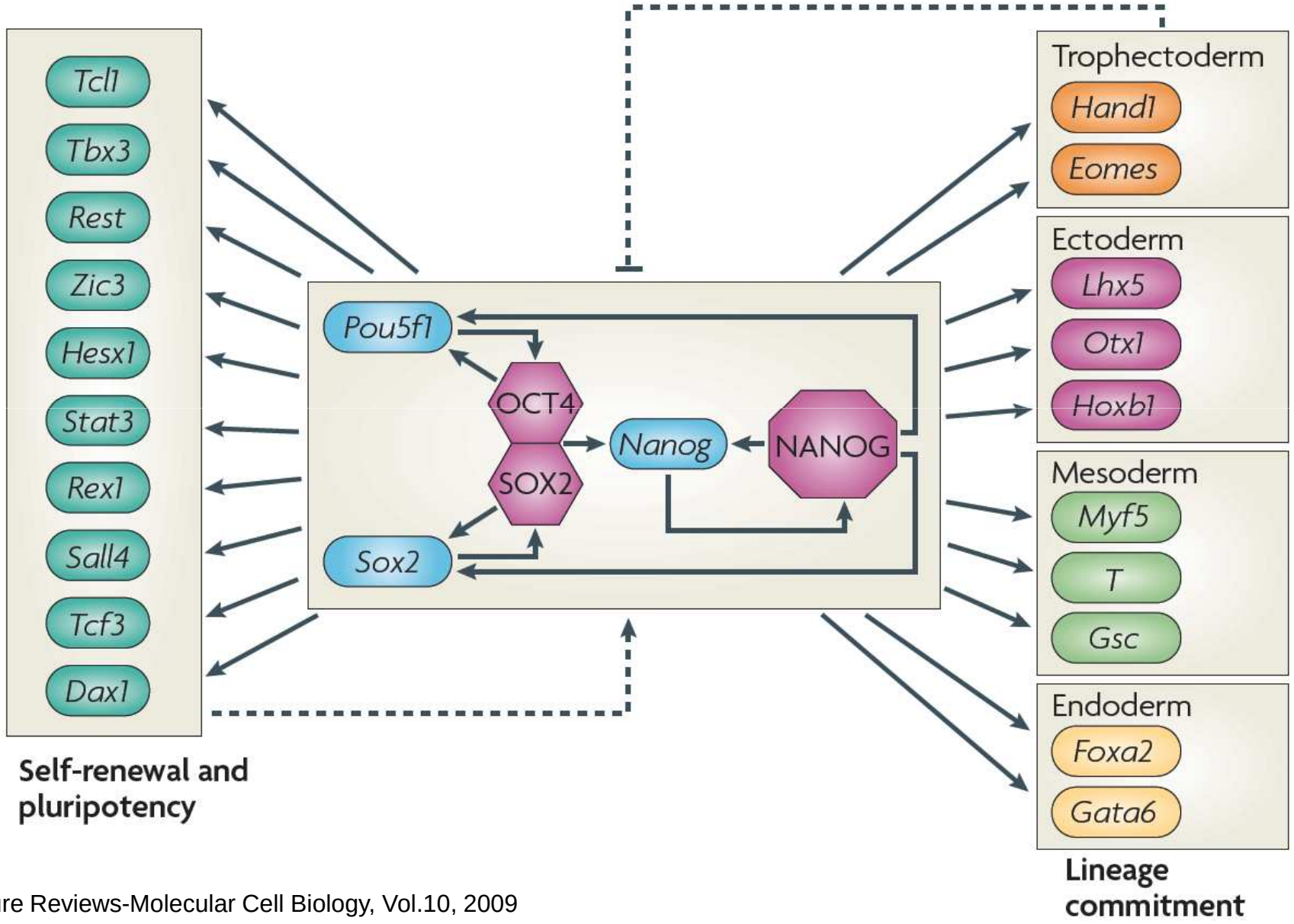


Turning adult skin fibroblasts into embryonic stem cells



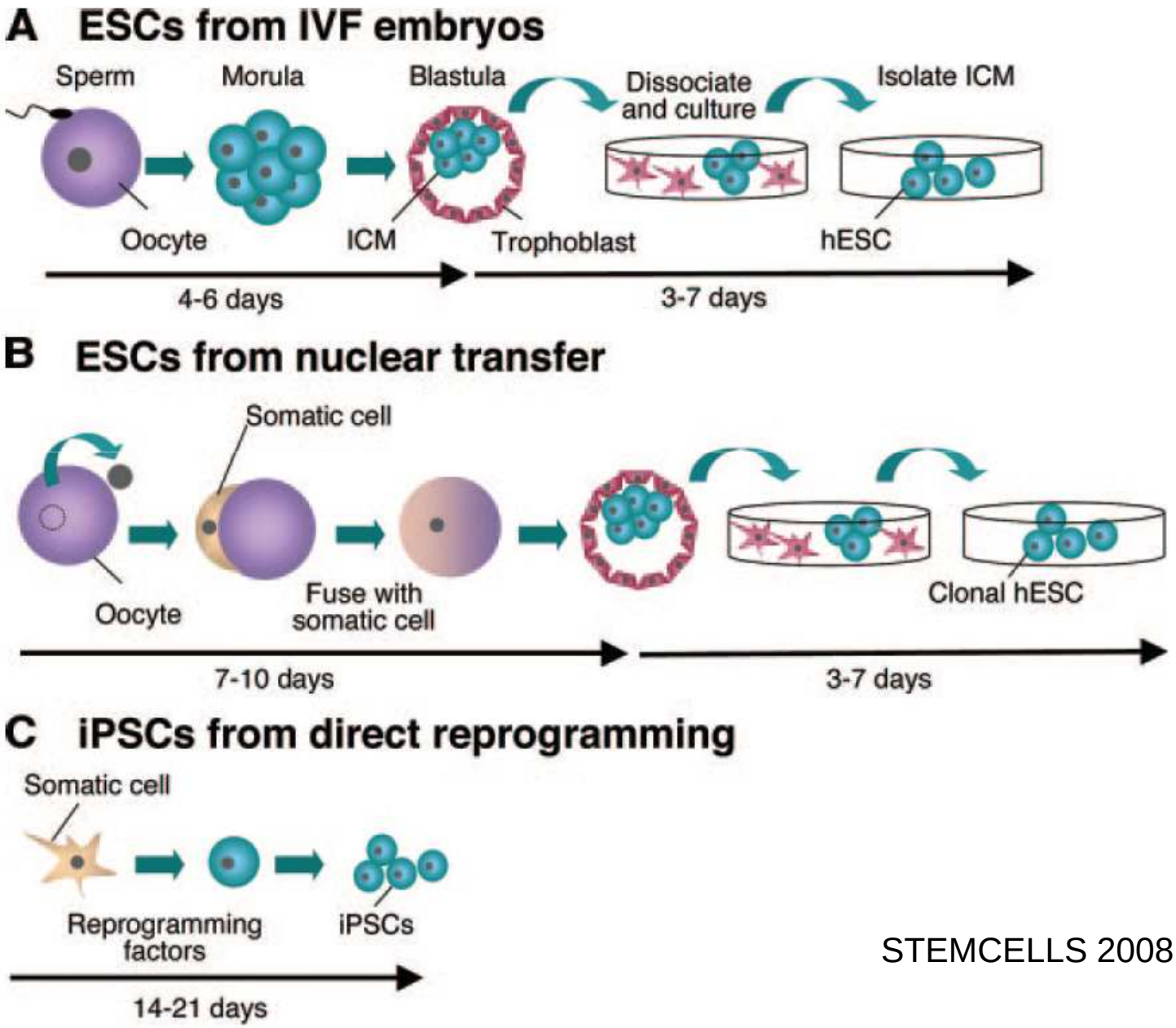
From the following article:
Stem cells: The magic brew
Nature 448, 260-262(19 July 2007)

Box 1 | **The core embryonic stem cell transcriptional circuit**



Nature Reviews-Molecular Cell Biology, Vol.10, 2009

Comparison of different methods to derive pluripotent stem cells



STEMCELLS 2008;26:2753–2758

Pros and Cons to iPS cell technology

- Pros:
  - Cells would be genetically identical to patient or donor of skin cells (no immune rejection!)
  - Do not need to use an embryo
- Cons:
  - Cells would still have genetic defects
  - One of the pluripotency genes is a cancer gene
  - Viruses might insert genes in places we don't want them (causing mutations)

Tedious Approach

- Yamanaka first selected 24 transcription factors from literature research (~1500 TFs in human)

- His student Takahashi then tried various possible combinations
  - One TF: 24 choices
  - Two TFs: 552 combinations
  - Three TFs: 12,144 combinations
  - Four TFs: 255,024 combinations

高橋和利

http://www.frontier.kyoto-u.ac.jp/

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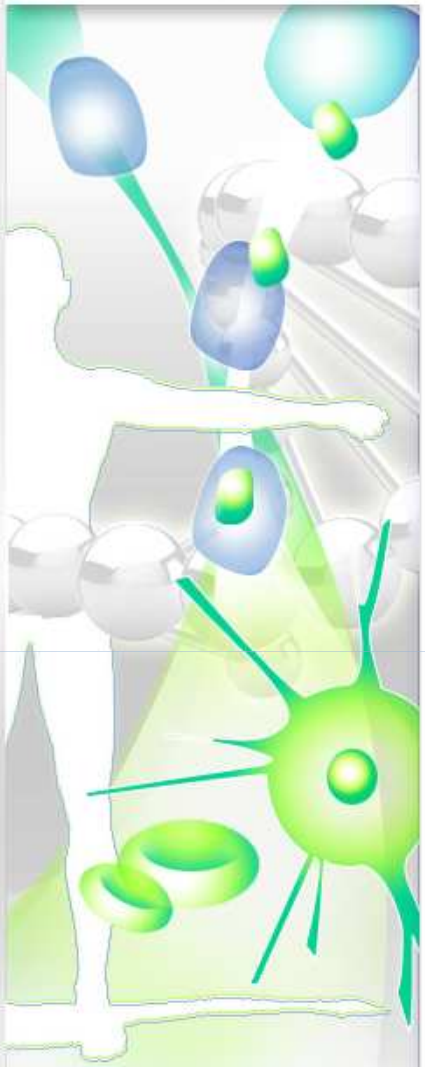


Welcome to
Institute for Frontier Medical Sciences,
Kyoto University

研究室紹介

◆ 生体機能学研究部門
- 細胞機能調節学分野
- 生体微細構造学分野
- 生体機能調節学分野
- 生体システム制御学分野
- 生体再建学分野

◆ 生体組織工学研究部門
- 生体分子設計学分野
- 生体材料学分野
- 組織修復材料学分野
- 生体物性学分野

◆ 再生統御学研究部門
- 発生分化研究分野
- 再生誘導研究分野
- 再生増殖制御学分野
- 再生免疫学分野

◆ 再生医学応用研究部門
- 生体修復応用分野
- 組織再生応用分野
- 器官形成応用分
- 臓器再建応用分野
- 再生医学応用流動分野

◆ 附属施設
■ 附属再生実験動物施設

■ 附属幹細胞医学研究センター
- 霊長類胚性幹細胞研究領域
- 幹細胞分化制御研究領域
- 幹細胞加工研究領域
- 細胞プロセシング研究領域
- 再プログラム化研究領域

■ 附属ナノ再生医工学研究センター
- ナノバイオプロセス研究領域
- シミュレーション医工学研究領域
- ナノバイオメカニズム研究領域
- 再生医工学研究領域

情報公開ページ
- ヒトES細胞プロジェクト
- 教員任期制に関する情報公開
- 政府の競争的資金一覧
- 文部科学省の競争的資金一覧
- 外部評価を受けて

教員公募案内

お知らせ

“

京都大学附置研究所・センター品
川セミナー のお知らせ

京都大学再生医科学研究所
第5回公開講演会のご案内

共同利用・共同研究について

＜その他＞
more

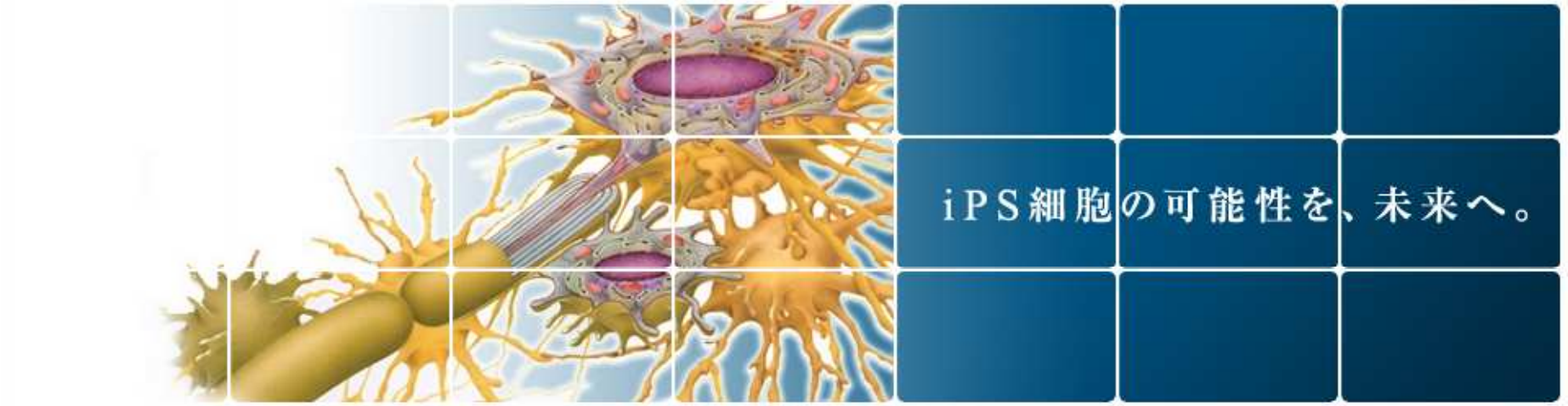
リンク
- 京都大学
- 国立大学附置研究所・センター長会議
- ナノメディシン融合教育ユニット

English | 交通アクセス | お問い合わせ | サイトマップ

検索 文字サイズ 小 中 大

京都大学
iPS細胞研究所
CiRA（サイラ）

- CiRA（サイラ）について About CiRA
- 研究活動 Research Activities
- よくある質問 Resources
- ニュースルーム Newsroom
- 採用情報 recruit



News ニュース

一覧 RSS

2010.04.28 イベント・セミナー
平成22年度iPS細胞樹立・維持培養講習会および実技トレーニングのご案内

2010.04.22 その他

Highlights ハイライト

2010.3.11
山本研究室　ポスドク研究員・技術員　募集

PDF ポスドク研究員・技術員募集要項

Support

iPS細胞研究基金
ご支援のお願い

Sources of Stem Cells

Stem cell type	**Description**
Embryonic 胚胎	Cells from human blastocysts
Fetal stem cells 胎兒	Cells from gonads of aborted fetuses
Umbilical cord blood stem cells 臍帶	Cells from the umbilical cord blood of newborns
Placenta derived stem cells 胎盤	Cells from the placenta of newborns
Adult stem cells 成人	Cells from adult tissues, such as bone marrow
Induced pluripotent stem cells (iPSCs) 誘導式多能性幹細胞	Adult cells that have been genetically reprogrammed to an embryonic stem cell–like state

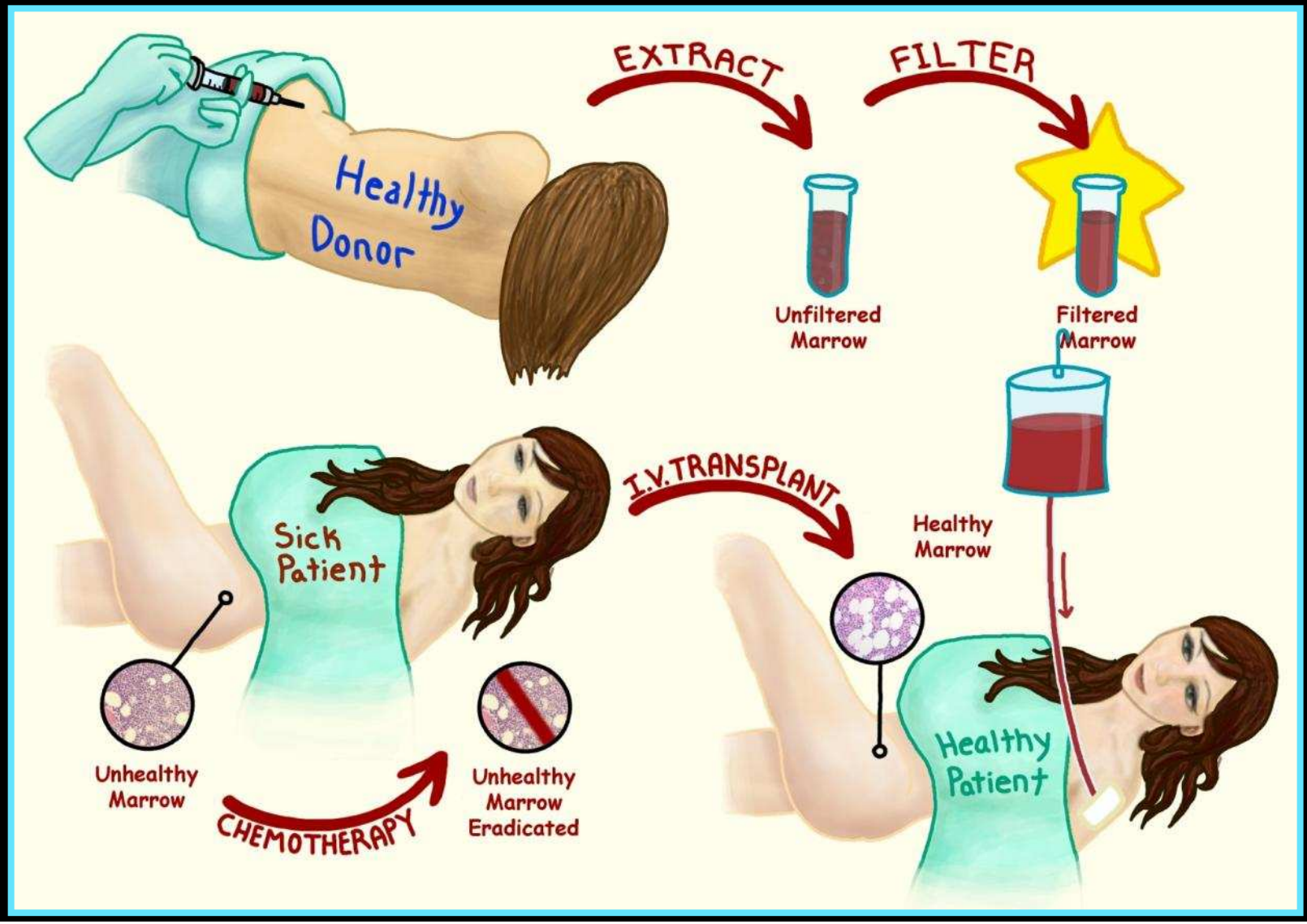
Stem cell therapy and technology
幹細胞療法與技術

How to collect bone marrow and cord blood

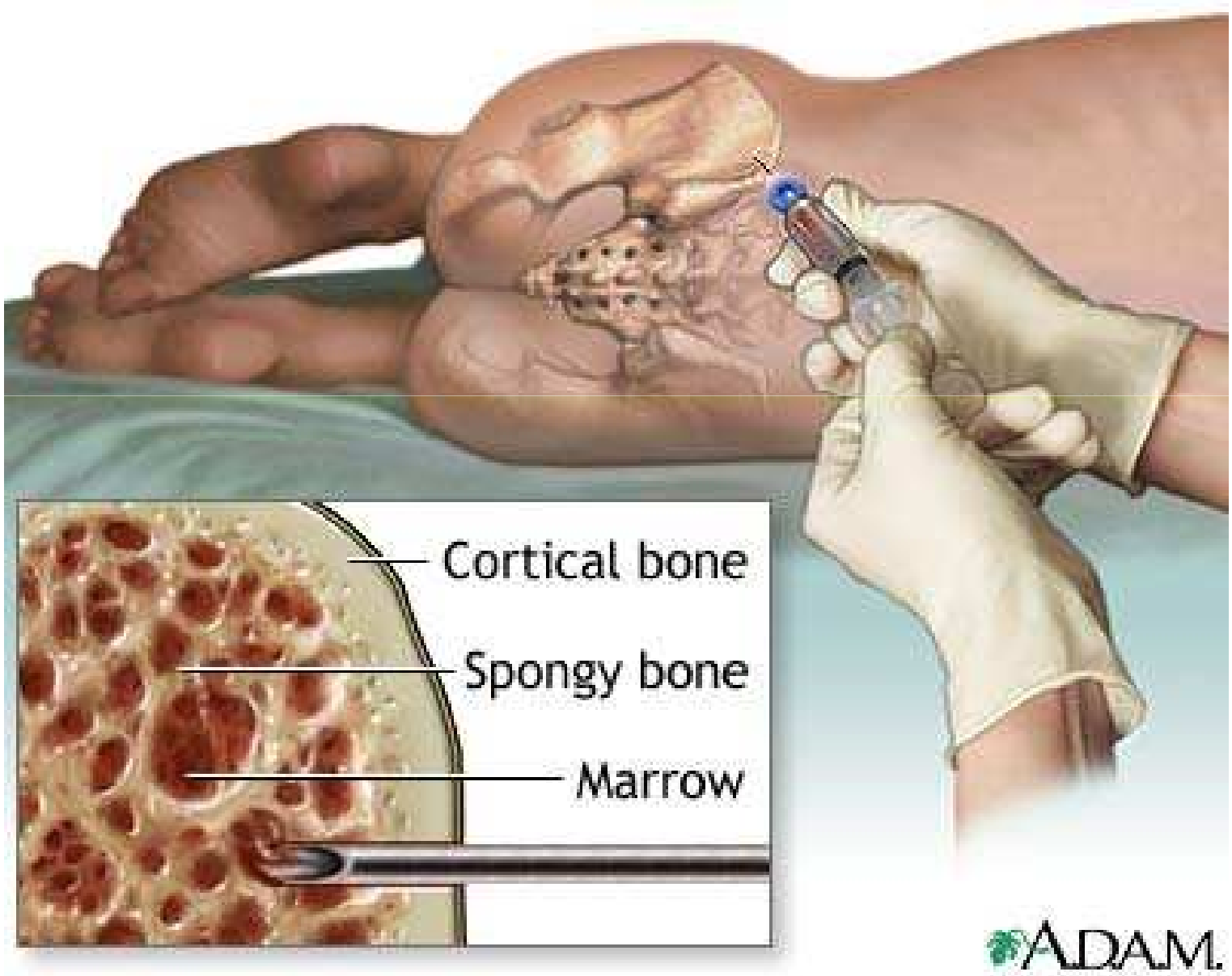
如何收集骨髓與臍帶血?

Bone marrow transplant:

Example of adult stem cell-based therapy



Adult Stem Cell:
Bone Marrow Extraction



Bone marrow aspiration needles

Maslak, P. ASH Image Bank 2002;2002:100529

Copyright ©2002 American Society of Hematology. Copyright restrictions may apply.

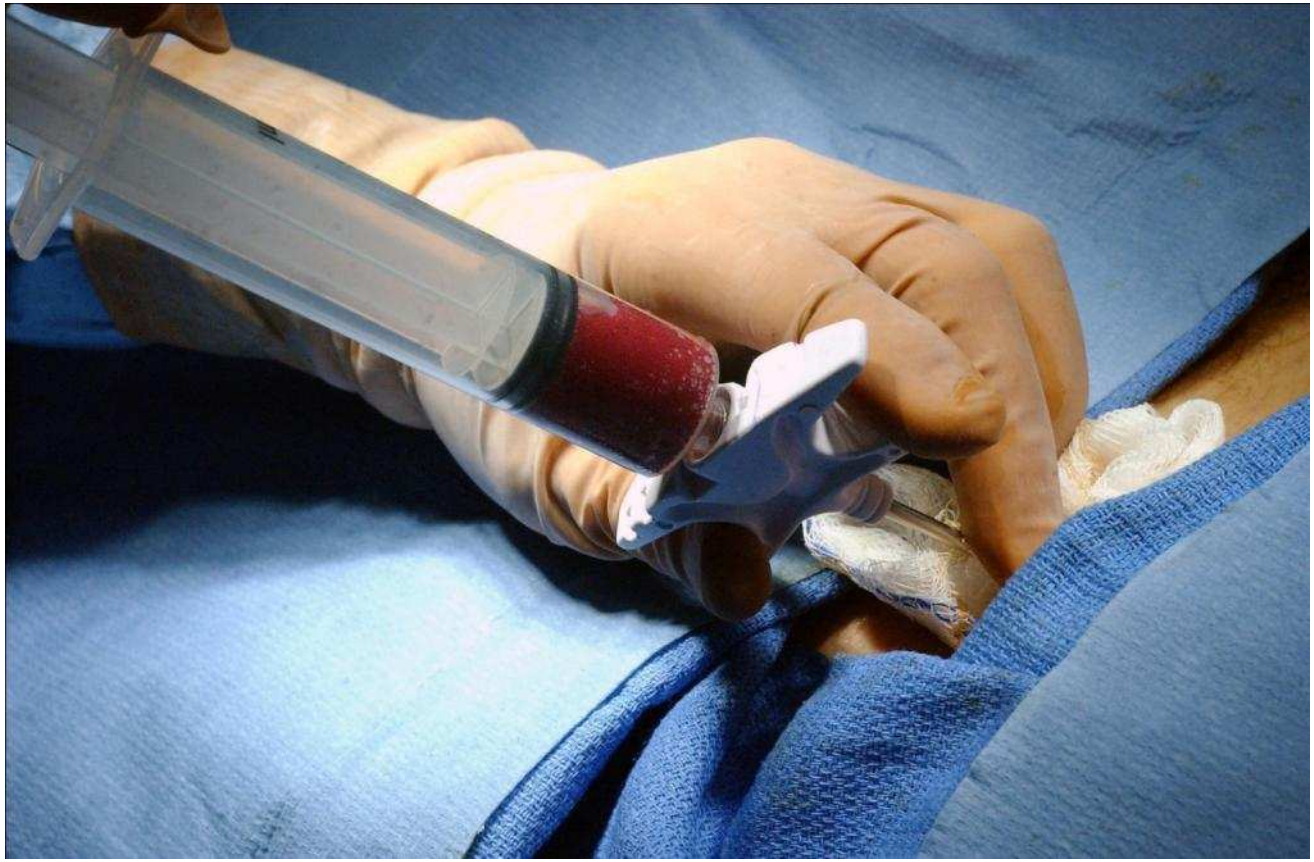
Bone marrow extraction – a bedside view






http://www.emedicine.com/med/topic2971.htm#target1

The amount of bone marrow harvested



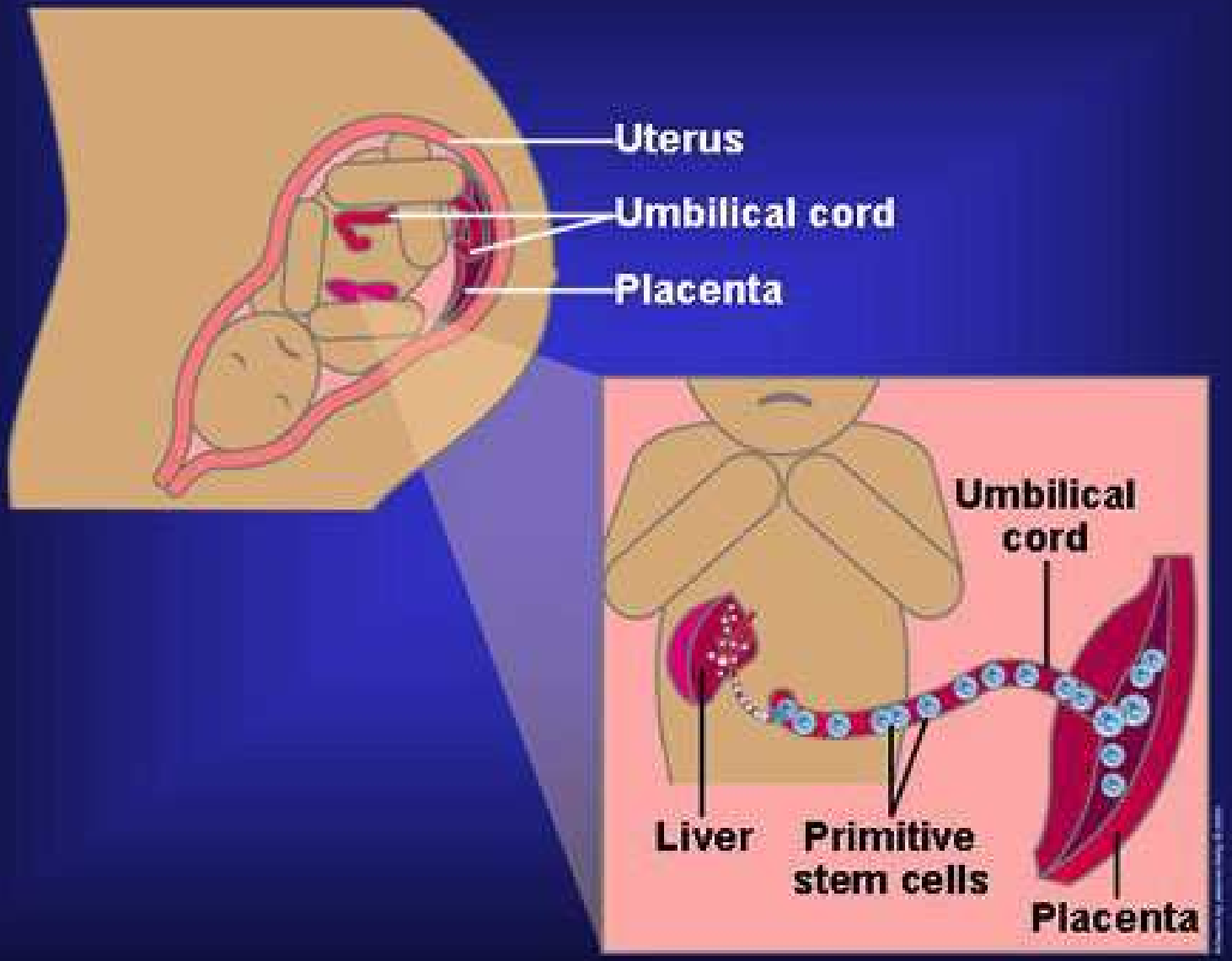
• It depends on the size of the patient and the concentration of bone marrow cells in the donor's blood.

• Usually **10 to 15 cc of bone marrow/kg of recipient weight** of marrow and blood are harvested, which represents about **2% of a person's bone marrow**, which the body replaces in four weeks.

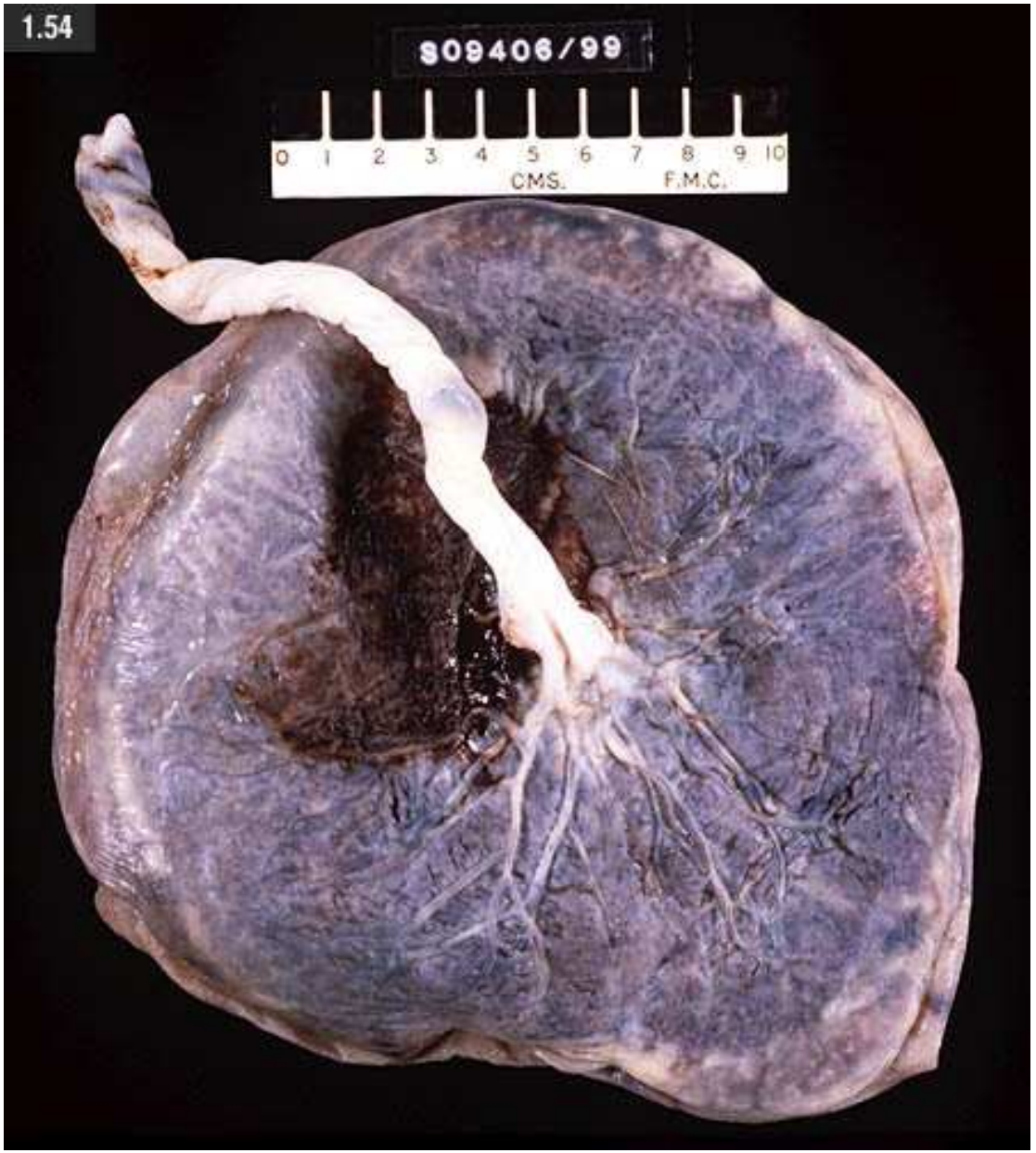
Alternative choice other than Bone Marrow

骨髓替代物

Cord Blood as a Source of Stem Cells



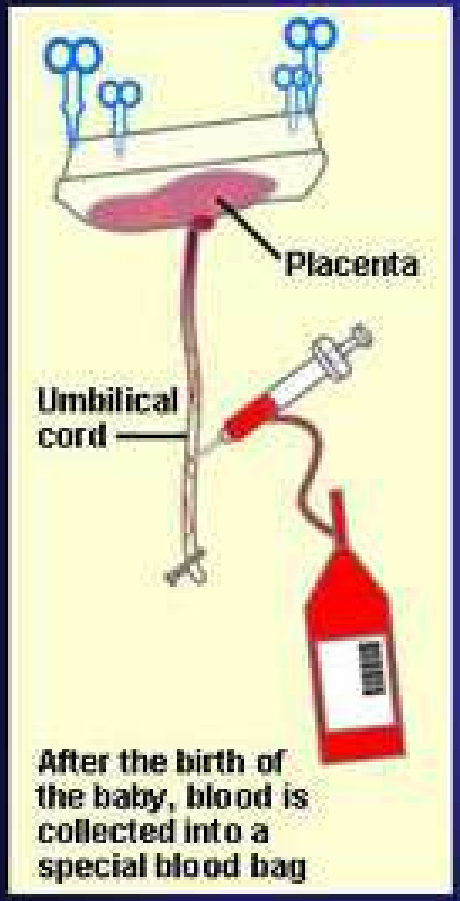
Human Placenta and Umbilical Cord

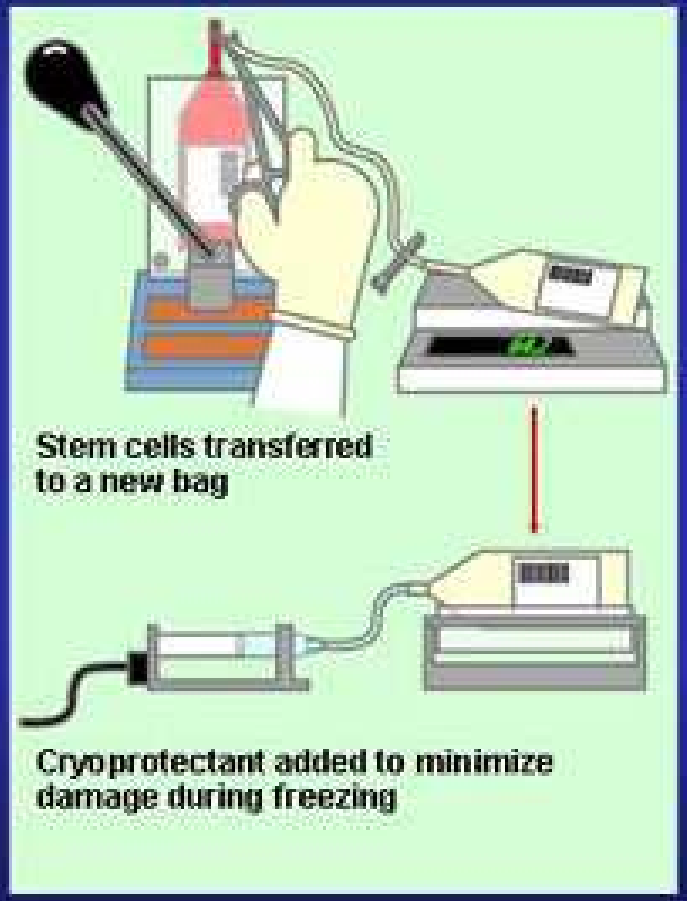


Collecting Cord Blood

After or before
placenta detach from Uterine

Placental and Cord-Blood Stem Cell Transplants





NATIONAL CANCER INSTITUTE

你要保存臍帶血嗎？

Cost-Benefit Analysis

臍帶血保存費用

- **一次付清**
  - 以目前台灣臍帶血銀行一般的價格，保存20~25年，約需要**6~8**萬元。

- **年繳方式**
  - 如果採用年繳方式，除了一開始採集與分離技術的費用外，每年需付保存費 **5000**元，**20**年累計下來，至少需要**10**萬元。

- 科學人, 2004

XX臍帶血銀行 Pricing information

專案價格標準 · · ·

SERVICE

◎ 專案一、「兩地存捐」方案

適合注重對品質要求及風險分散觀念的完美主義者。

方案	繳費方式	定價
兩地存捐	預繳20年全額費用	NT$140,500 (特惠價實施中，歡迎來電洽詢)

◎ 專案二、「單地儲存」方案

適合100%爲家人付出，將寶寶臍帶血預留給家人使用的家庭守護者。

方案	繳費方式	定價
單地儲存	預繳20年全額費用	NT$73,000

◎ 專案三、「單地抗凍袋儲存」方案

家族短期內有臍帶血移植需求者。

方案	繳費方式	定價
單地抗凍袋儲存	預繳20年全額費用	(特惠價實施中，歡迎來電洽詢)

Heart Disease	1 in 5
cancer	1 in 7
Stroke	1 in 24
Motor vehicle accident	1 in 84
Suicide	1 in 119
Falling	1 in 218
Firearm assault	1 in 314
Pedestrian accident	1 in 626
Drowning	1 in 1,008
Motorcycle accident	**1 in 1,020**
Fire or smoke	1 in 1,113
Bicycling accident	**1 in 4,919**
Air/space accident	1 in 5,051
Accidental electrocution	1 in 9,968
Alcohol poisoning	1 in 10,048
Hot weather	1 in 13,729
Hornet, wasp or bee sting	1 in 56,789
Legal Execution	1 in 62,468
Lighting	1 in 79,746
Earthquake	1 in 117,127
Flood	1 in 144,156
Firework discharge	1 in 340,773

Causes of Death

What are the odds?

http://www.nsc.org/lrs/statinfo/odds.htm

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What are the odds?

← **1 in 20,000 for Autologous transplant**

Royal College of Obstetricians and Gynaecologists

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What are the odds?

1in 2,700 for Pre-arranged usage

1 in 20,000 for Autologous transplant

Royal College of Obstetricians and Gynaecologists

要花錢保存臍帶血嗎？

保存臍帶血是[花錢買希望、花錢買保險]，畢竟並非每個人都需要做這項投資，如果家庭經濟寬裕，當然可以為寶寶保存臍帶血，也算是一種投資。

到目前為止，許多被宣稱的幹細胞的功用，都還沒有大規模的臨床應用，但科技進步得很快，以後的發展難以預料，但應有合理的期待，才不至於失望。

公益的臍帶血庫 vs 私人的臍帶血銀行

- 公益臍帶血庫的目的是提供需要骨髓移植的病人更多搜尋和配對的機會，藉以彌補骨髓資料庫配對不易的困擾。

- 私人的臍帶血銀行則是以自費保存臍帶血，是爲了小孩子和家人儲存，除非自己同意否則他人不得使用，不用擔心被別人用掉。

Stem cell Therapy

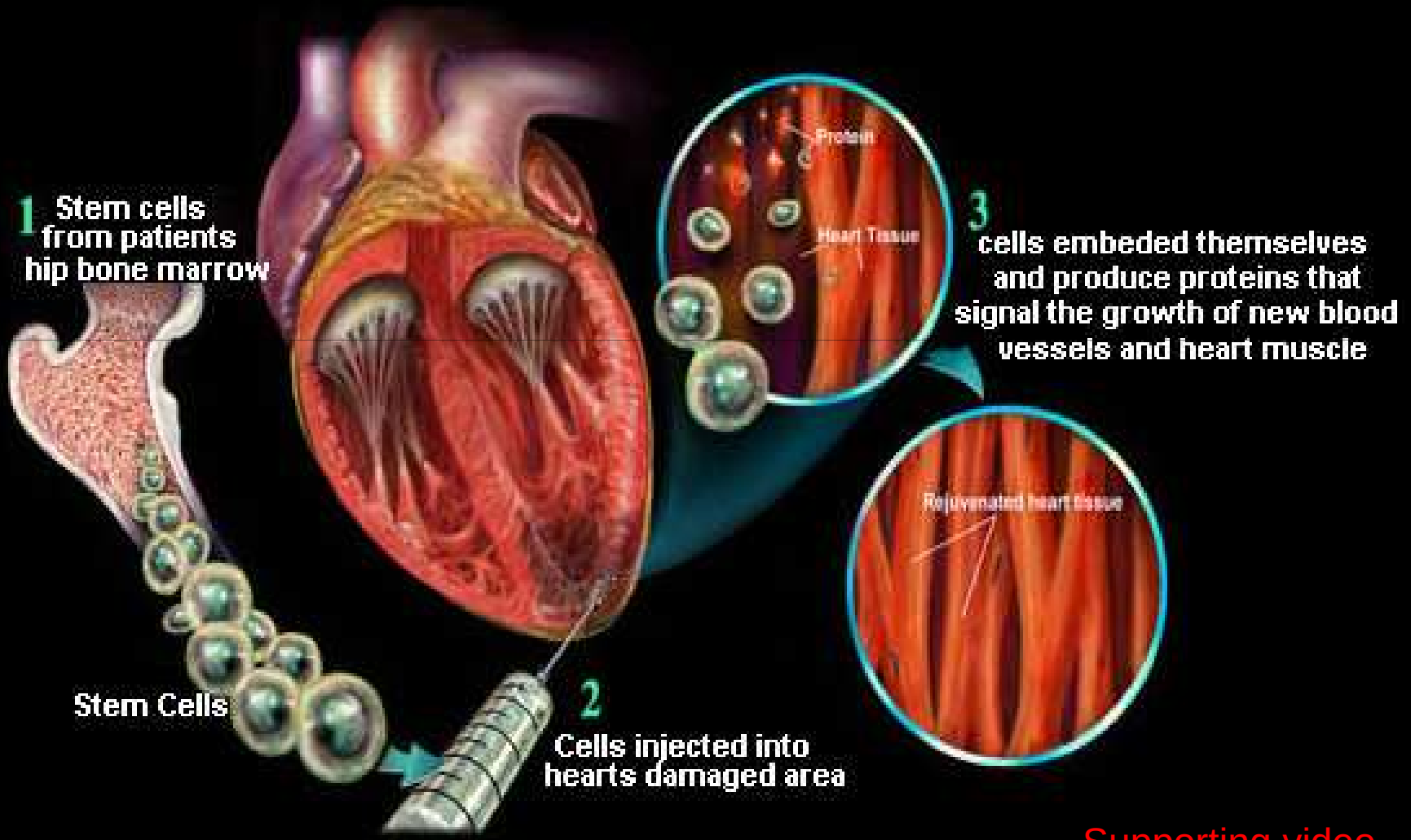
可以用幹細胞療法治療的疾病

Experimental model system

Heart muscle cells beating in a petri dish!

Videos by The Exploratorium

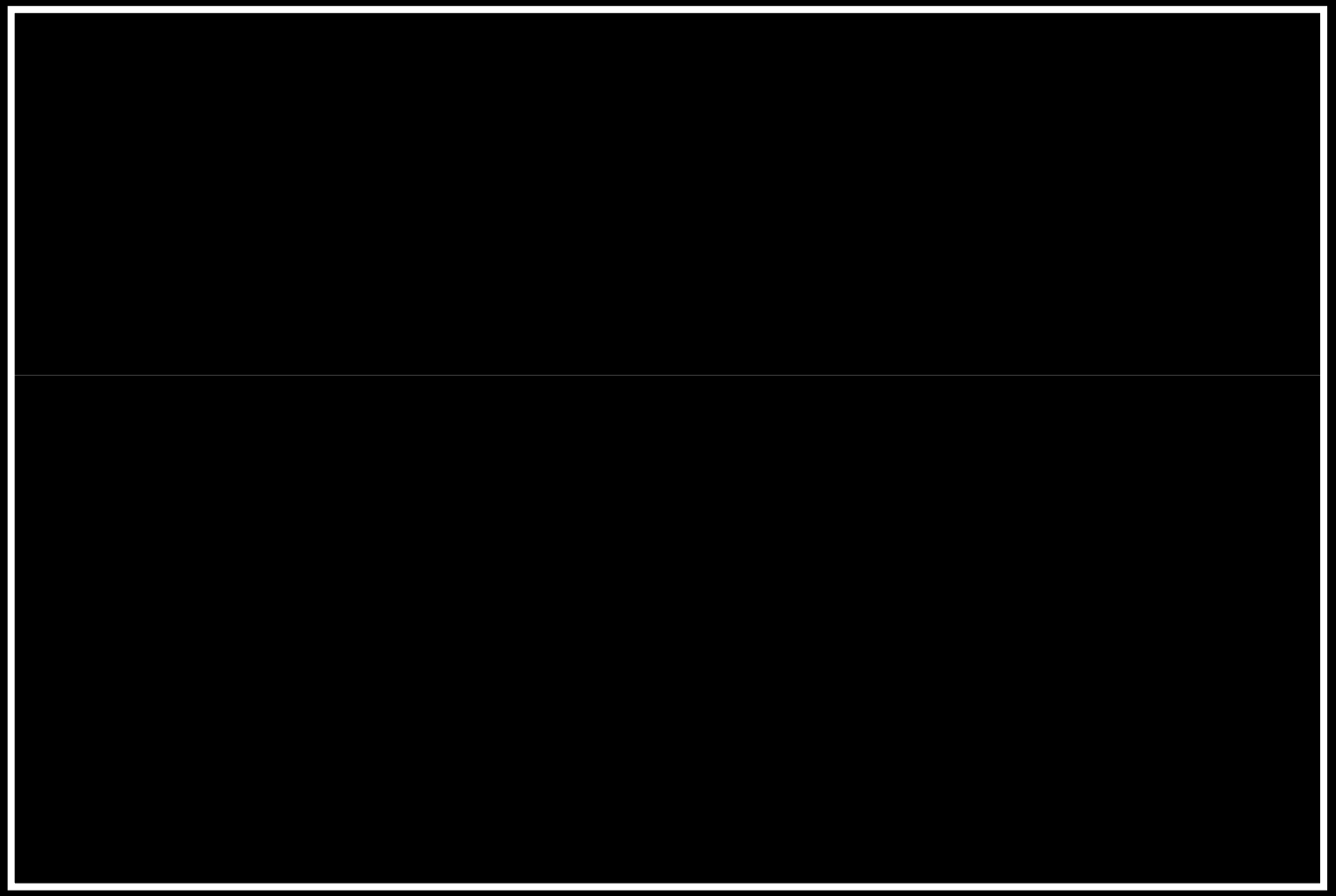
Treating cardiovascular diseases



Supporting video

Spinal cord injury:

Example of embryonic stem cell-based therapy



Geron video: http://www.geron.com/grnopc1clearance/

Why do researchers want to use embryonic stem cells along with other technologies?

- Pluripotent
  - Expanded developmental potential allows them to be used in ways that adult stem cells cannot
- Can proliferate indefinitely in culture
- Easier to obtain than adult stem cells

Science is discovering the unknown

- Stem cell field is still in its infancy
- Human embryonic stem cell research is a decade old, adult stem cell research has 30-year head start
- Holds hope for curing or improving treatments for 70+ diseases

How can you help to shape the direction of this field?

digitalblasphemy.com

Discussion

Or Quiz?

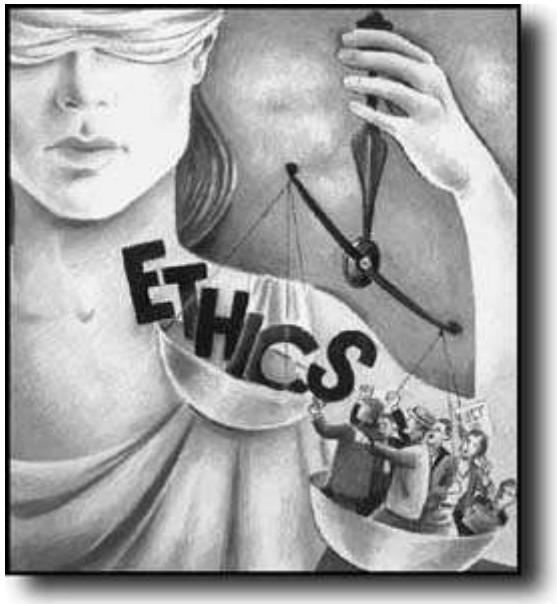
Discussion Topics

- Health insurance issue
- Ethics on stem cell therapy
- Bone marrow/cord blood donor
- Research funding support


健保財務收支
資料來源：健保局
保險支出
保險收入
保費收入
億元
4500
4300
4100
3900
3500
3300
3100
2900
2700
2500
3233
3371
3527
3674
3822
4011
4159
4349
3015
3270
3436
3554
3672
3699
3842
3853
3076
3368
3522
3611
3819
3874
4020
4027
(年)2002 2003 2004 2005 2006 2007 2008 2009

What is “ethics”?

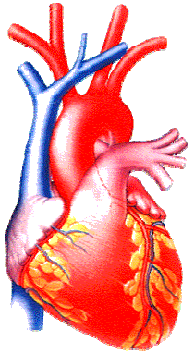
- **Ethics:** “the **rules of conduct** recognized in respect to a **particular class of human actions** or a particular group, culture”



What is “Bioethics”?

– **Bioethics:** “a field of study concerned with the **ethics** and **philosophical implications** of certain **biological** and **medical procedures, technologies, and treatments**, such as organ transplants, genetic engineering, and care of the terminally ill”

A classic bioethical decision



- One heart available → who should get it?

17-year old girl

40-year-old school principal

70-year-old woman

What diseases do we do stem cell research on first?

Muscular dystrophy

likely to die by age 20

VS.

Spinal cord injuries

paralyzed, but likely to live longer

What diseases do we do stem cell research on first?

- *Independent Citizen's Oversight Committee (ICOC)* includes:

– Spinal cord injuries	about 35,000 cases in CA
– Alzheimer's disease	about 470,000 cases in CA
– Type II (adult) diabetes	10% of adults (20 and up) have it
– Multiple sclerosis	
– Type I (juvenile) diabetes	1 in every 400-600 children/adolescents
– Heart disease	
– Cancer	
– Parkinson's disease	
– Mental illness	
– HIV/AIDS	

Common concerns in funding decisions

- Number of people with the disease.
- The groups that suffer from the disease.
- Severity of the disease.
- Disease mortality.
- Average age at death.
- Already available therapies or treatments.