Stem cells – state of the ART

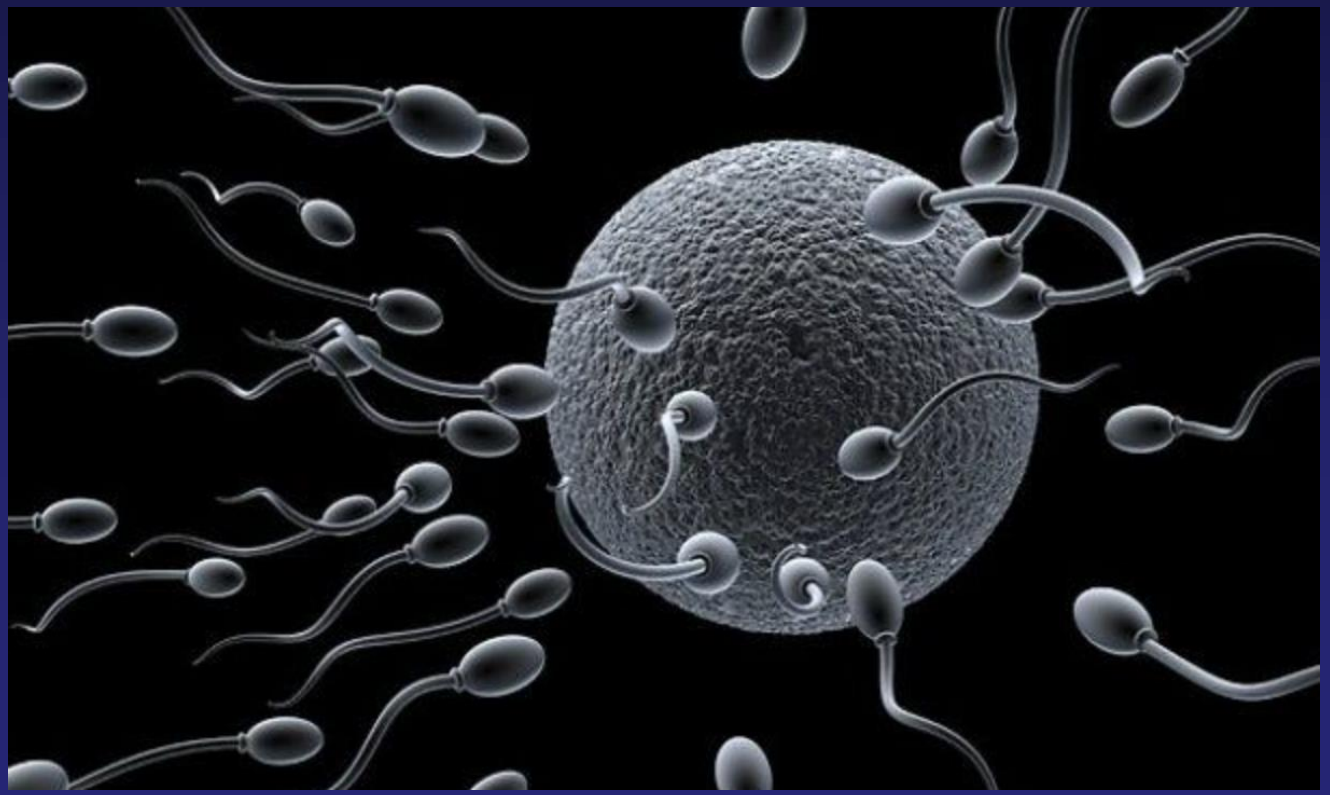
Pluripotent stem cells in ART – how far are we?

Peter Kragh

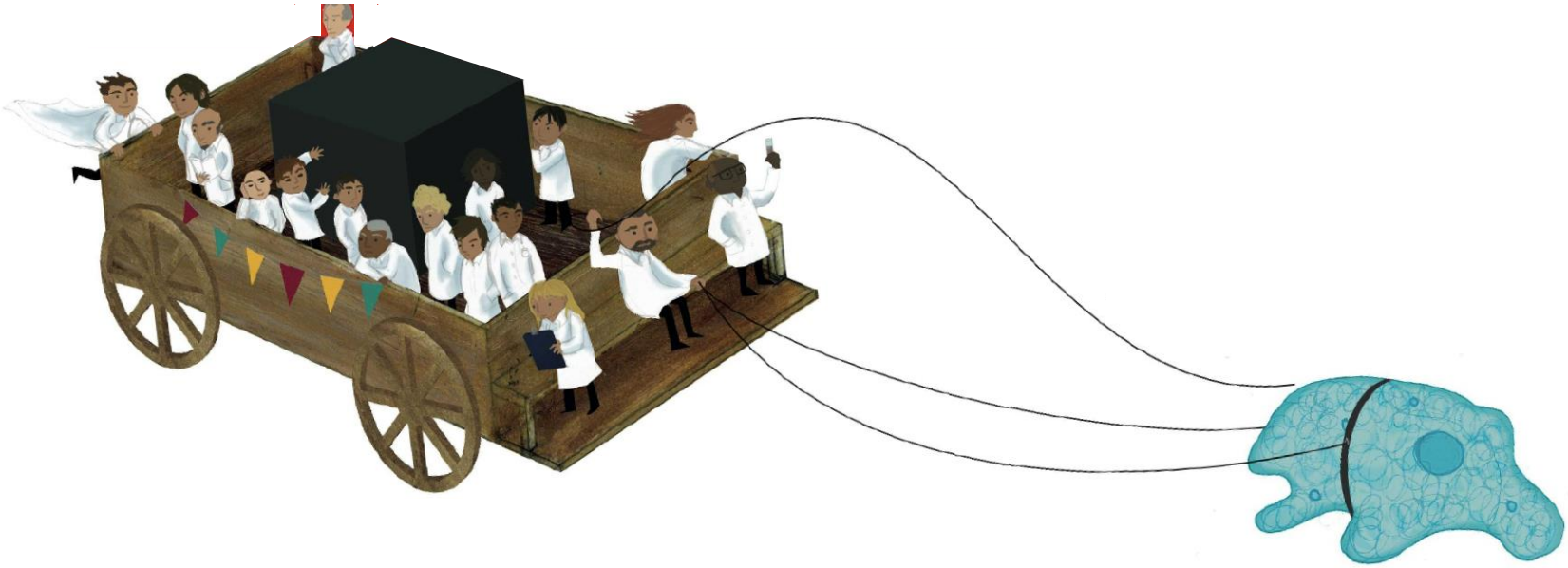
SPIREN FERTILITETSKLINIKK
&
FERTILITETSSEKSJONEN, ST OLAVS HOSPITAL

Today:

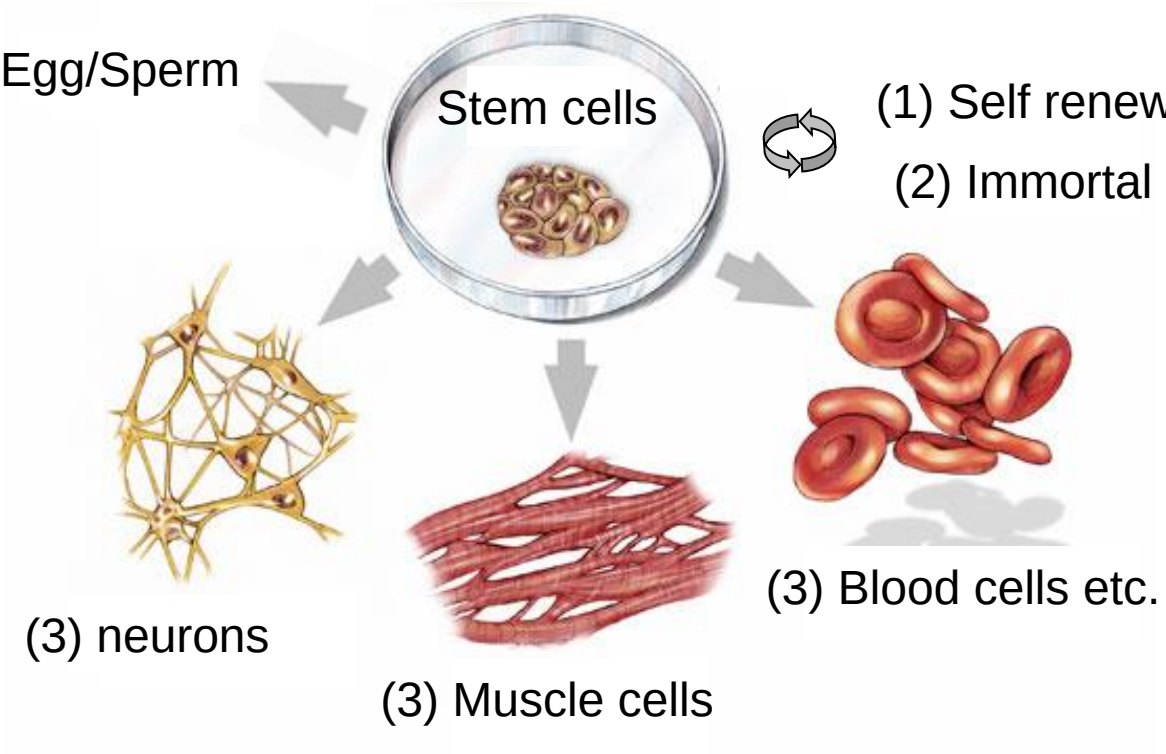
- Stem cells: origin, development
    - Embryonic stem cells
    - Induced pluripotent stem cells
- Cloning
    - Therapeutic cloning (human)
    - Reproductive cloning (animals)
- Generation of gametes ”in vitro”



The stem cell - Regenerative medicin



The three characteristics of stem cells



Stem cells have many similarities with cancer cells

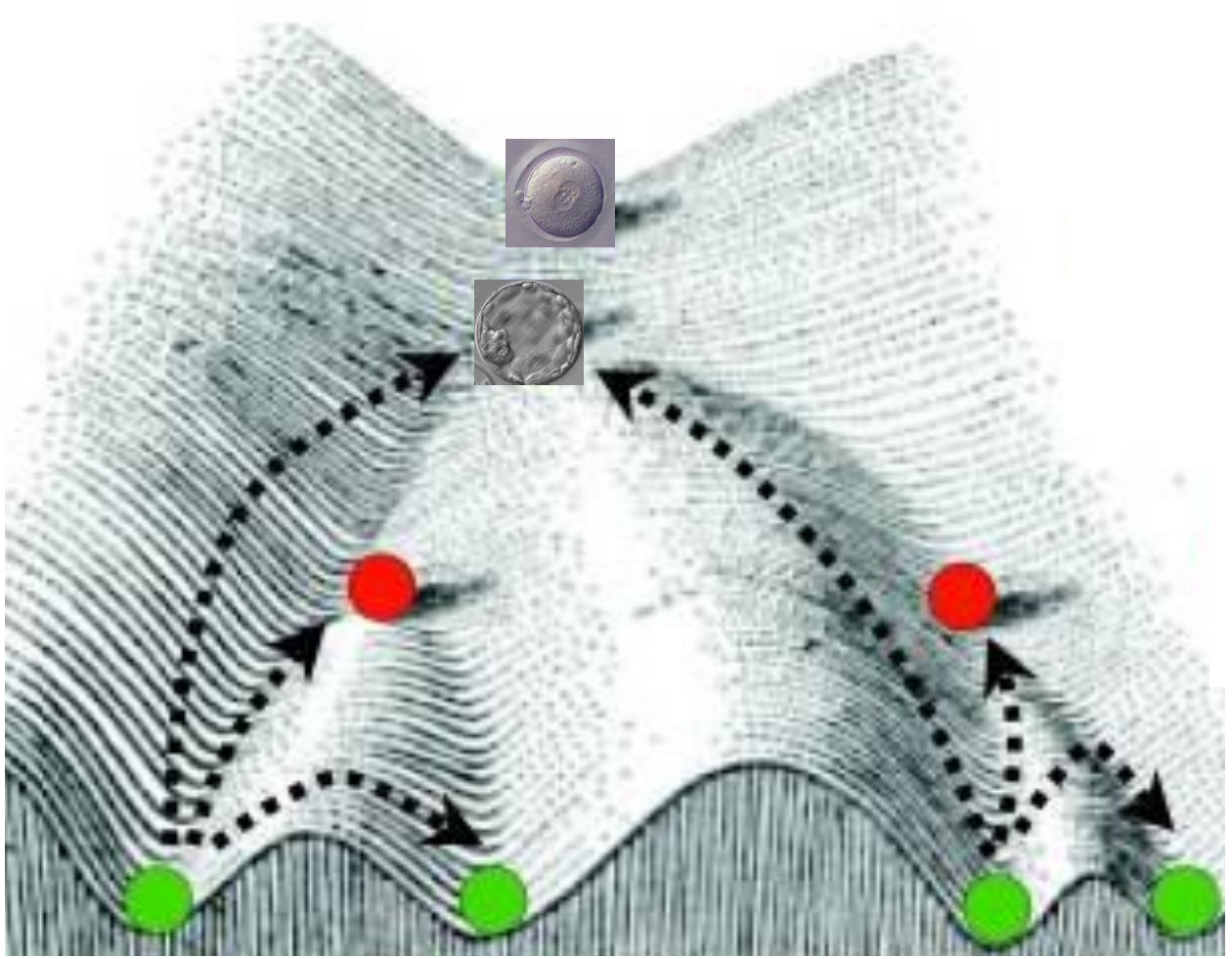
“Ski-map” for cell differentiation

TOTIPOTENT
zygote

PLURIPOTENT
ES / (iPS) cells

MULTIPOTENT
blood / mesenchymal stem cell

UNIPOTENT
differentiated cell

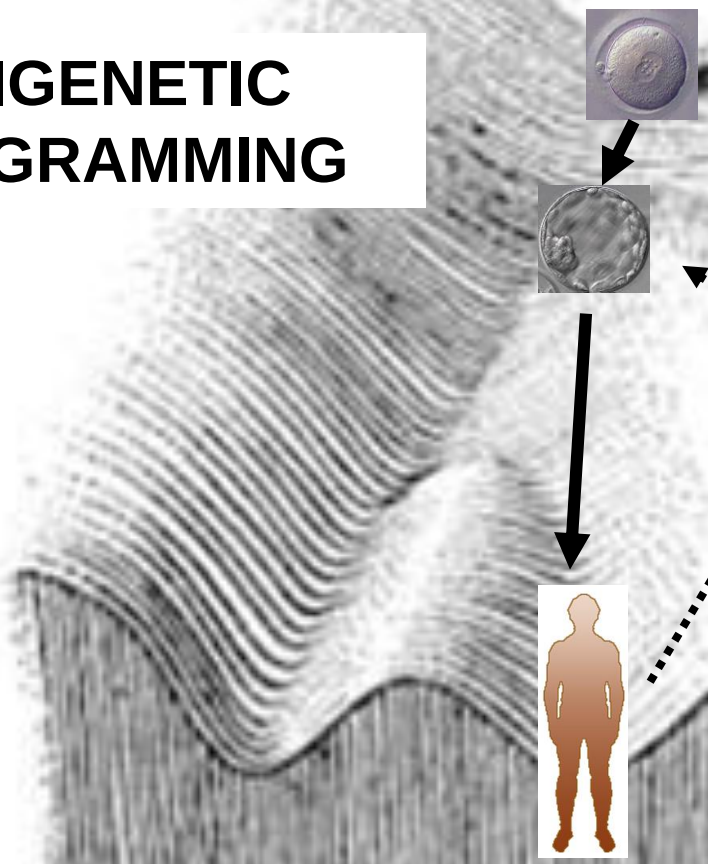


B-cell Monocyte Fibroblast Muscle cell

Stem cells and somatic cells:

SAME GENOME

EPIGENETIC PROGRAMMING



EPIGENETIC RE-PROGRAMMING:

-Gametogenesis

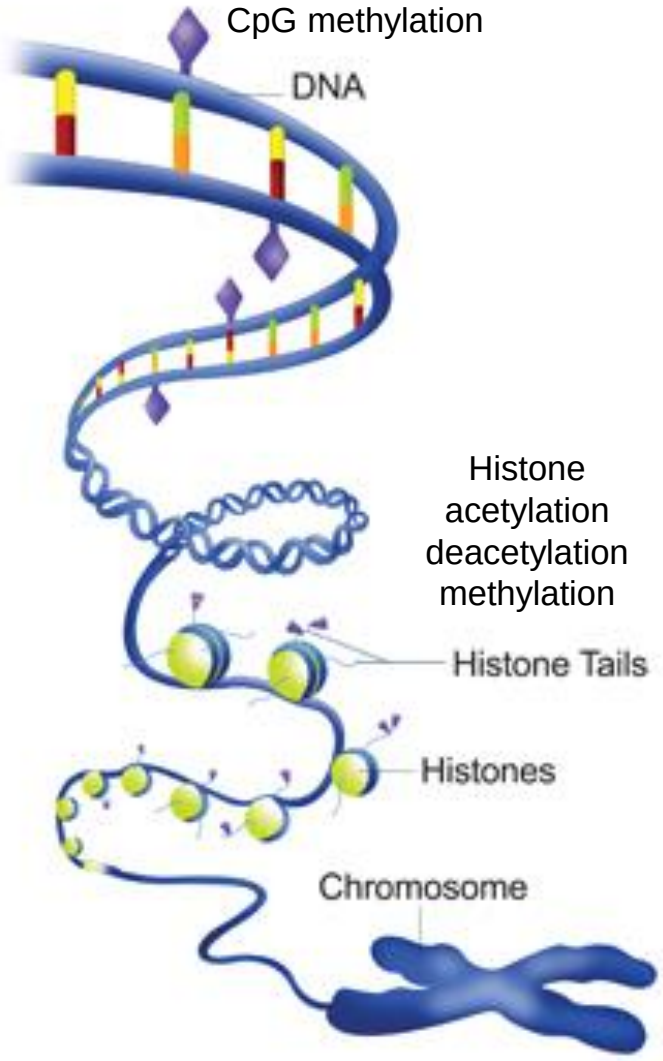
-Cloning

-Stem cell production

Stem cells and somatic cells:

Same genome (DNA sequence) – different epigenome

Epigenetics: turn genes on/off



Stem cell therapy regenerative medicine:

Stem cells replace diseased tissue:

Hematopoietic diseases:
Bone marrow transplantation – leukaemia

Pluripotent stem cells – differentiate into all cell types

How do we get hold of them….

Three ways to pluripotency:

Embryonic stem (ES) cells:

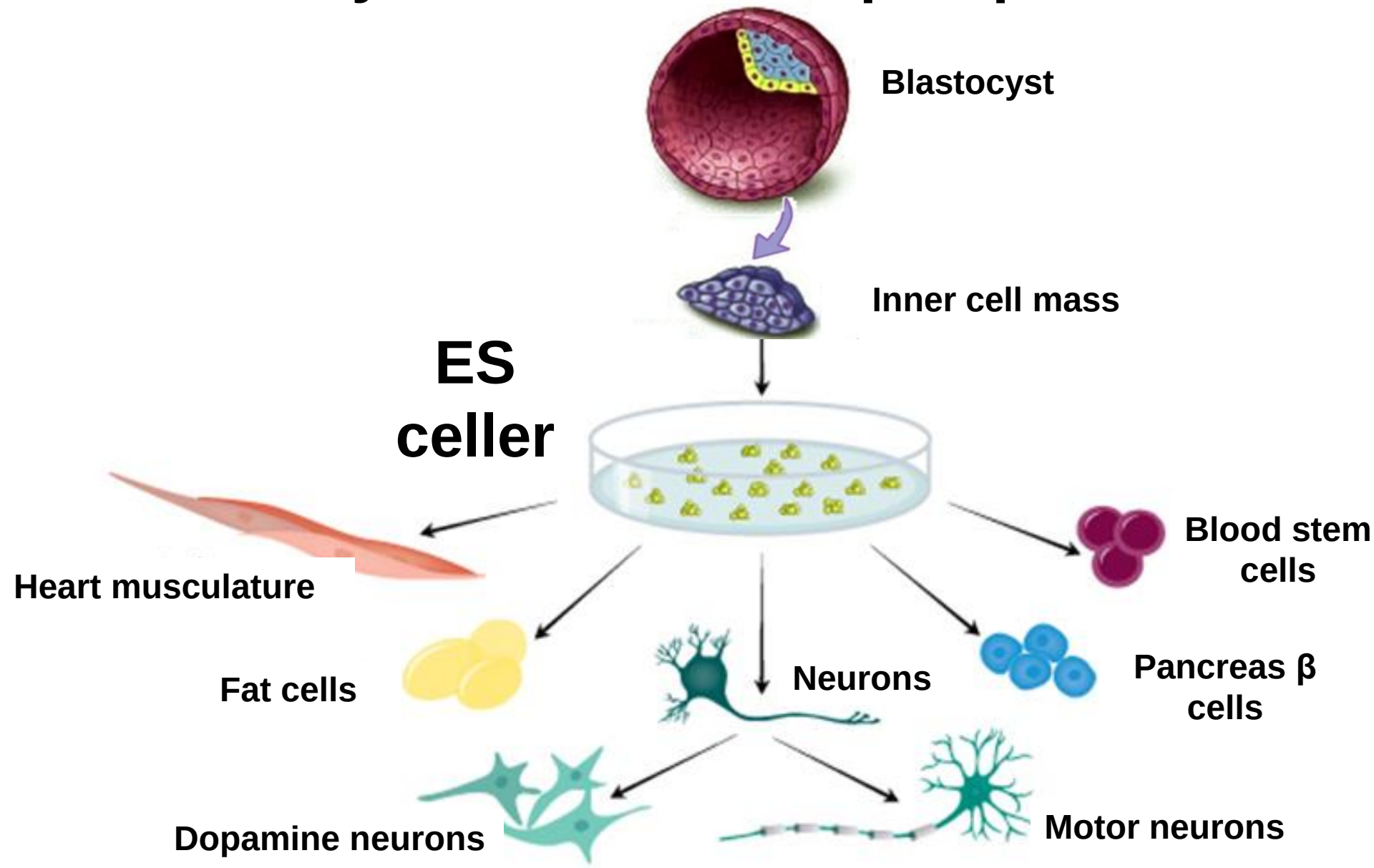
1. Obtained directly from inner cell mass of the blastocyst

Reprogrammed stem cells / Nuclear reprogramming:

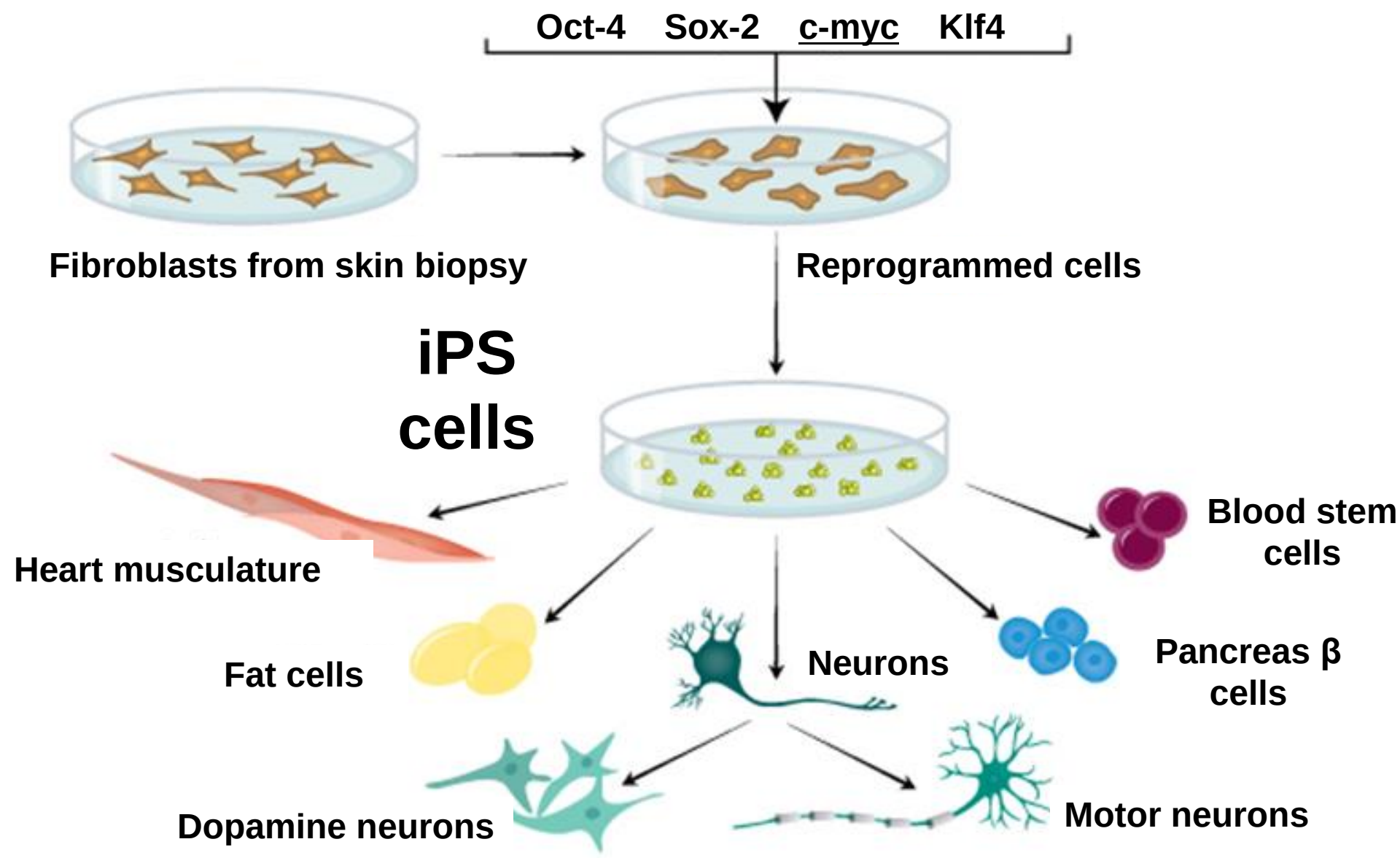
2. SCNT somatic cell nuclear transfer

3. iPS cells induced pluripotency

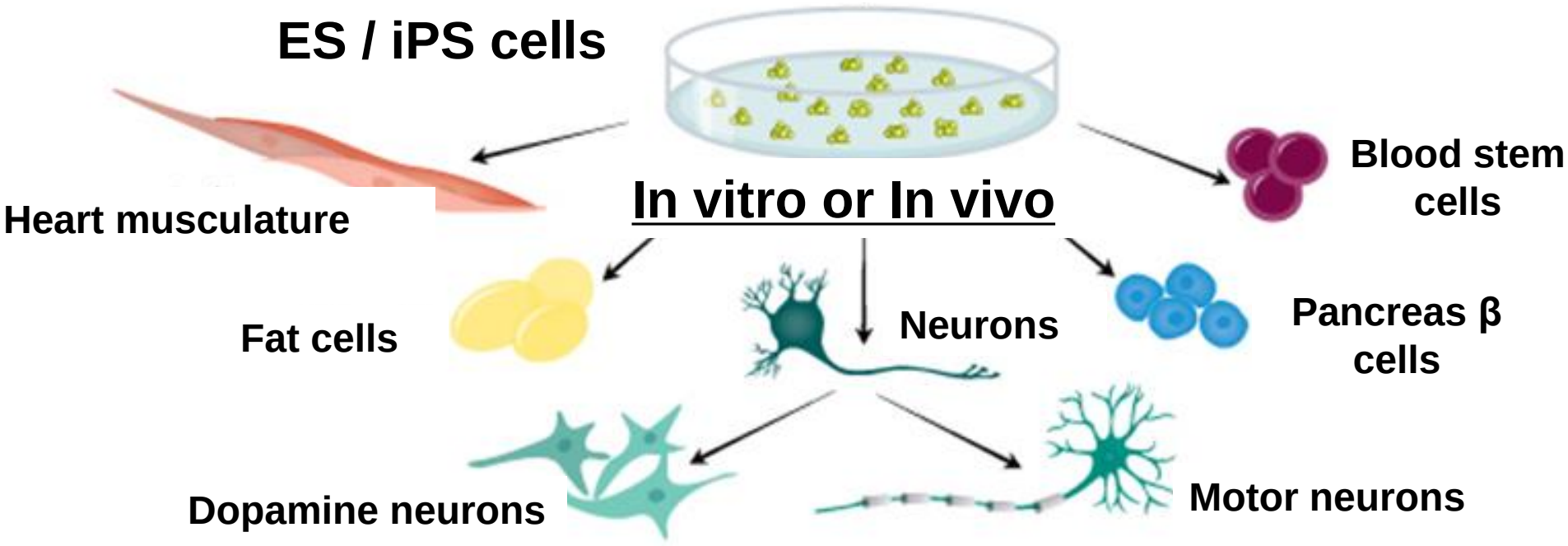
Embryonic stem cells: pluripotent



iPS cells: induced pluripotent stem cells



Pluripotent stem cells



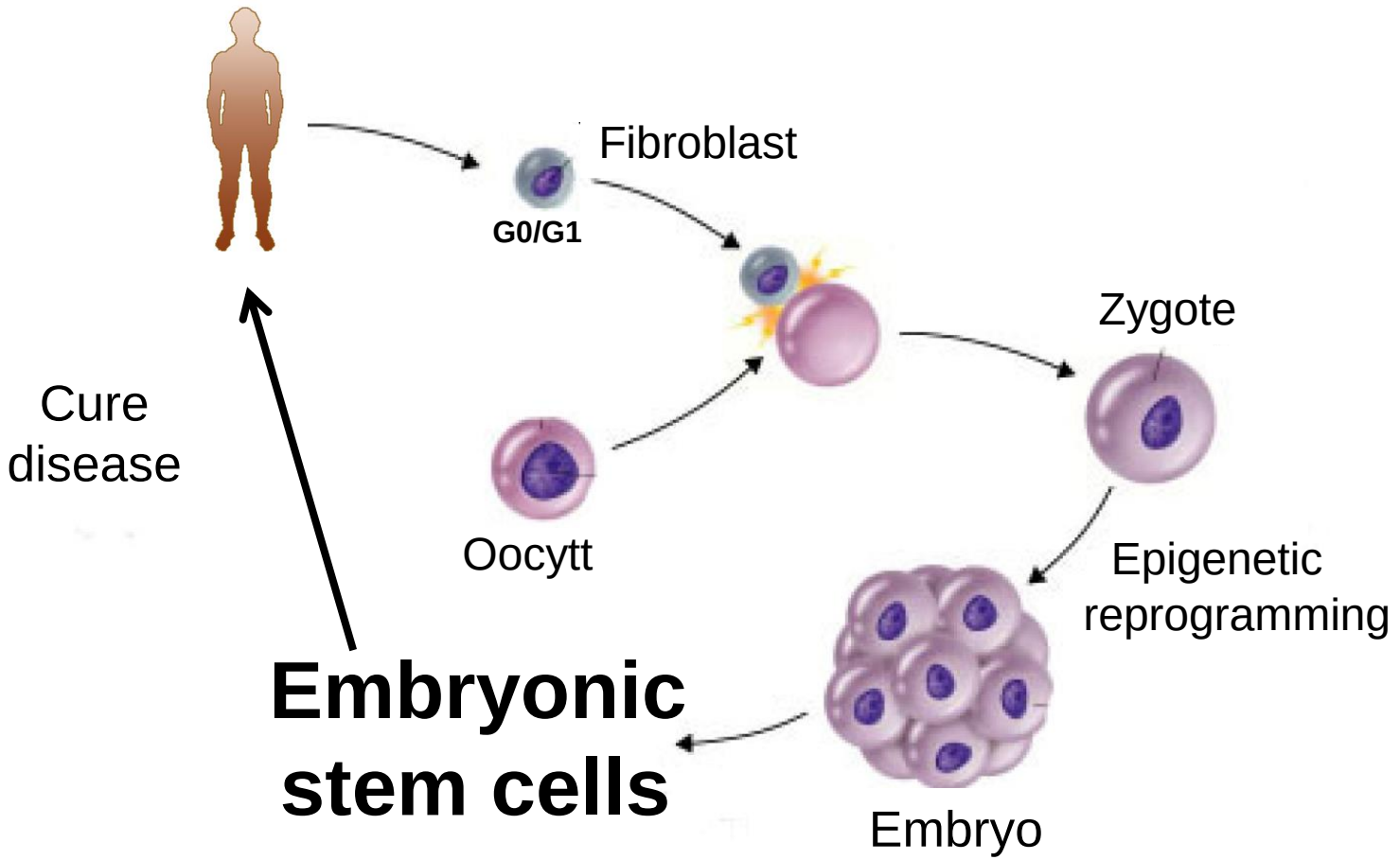
Pluripotent stem cells and tissue compatibility:

1. ES cells from embryo
   – limited immunological match
2. iPS cells from skin cells
   – complete immunological match

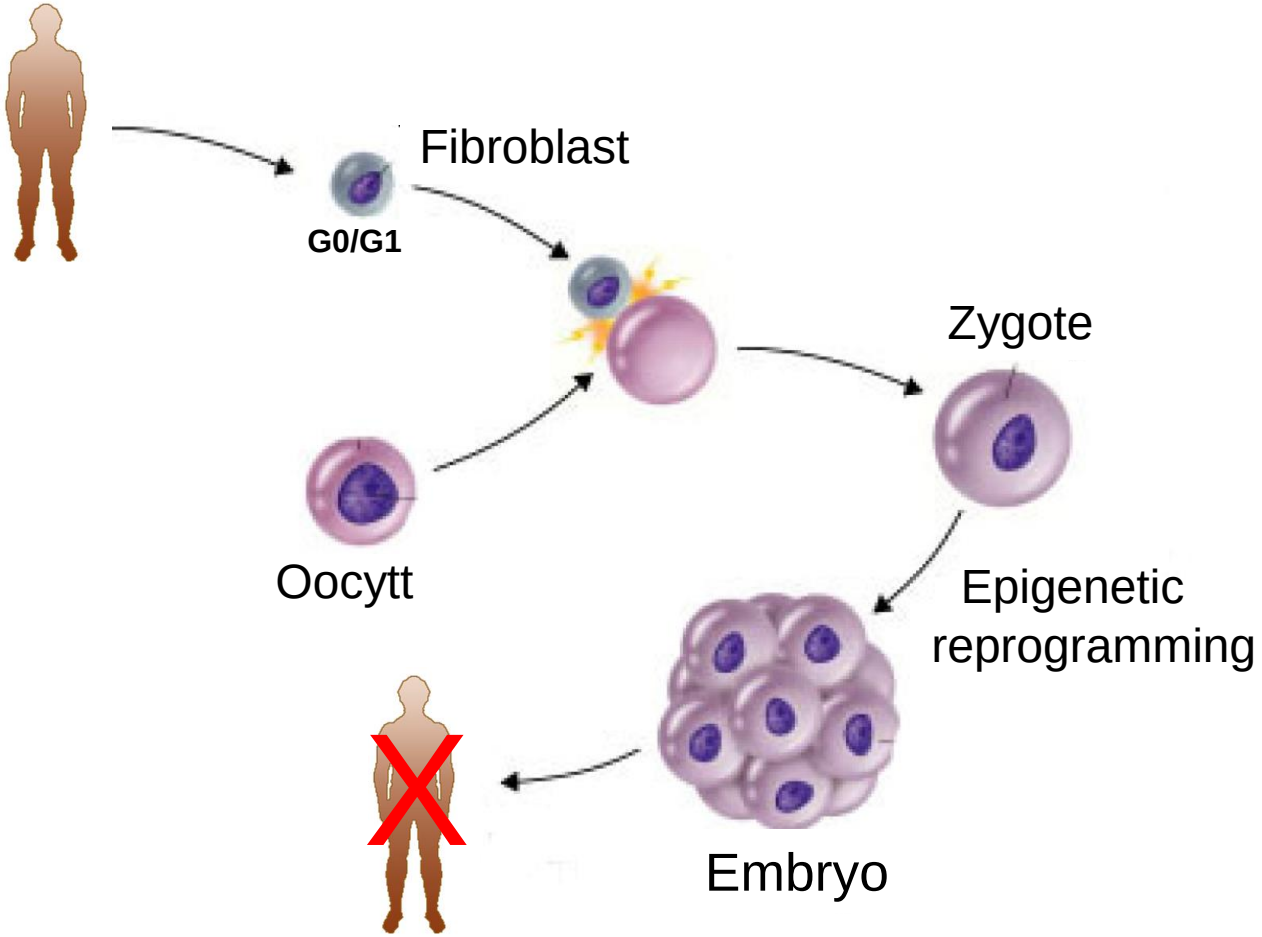
Therapeutic cloning:

Production of patient specific embryonic stem cells with complete immunological match

Therapeutic cloning



Reproductive cloning



Cell

Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer

Masahito Tachibana,[1] Paula Amato,[2] Michelle Sparman,[1] Nuria Marti Gutierrez,[1] Rebecca Tippner-Hedges,[1] Hong Ma,[1] Eunju Kang,[1] Alimujiang Fulati,[1] Hyo-Sang Lee,[1,6] Hathaitip Sritanaudomchai,[3] Keith Masterson,[2] Janine Larson,[2] Deborah Eaton,[2] Karen Sadler-Fredd,[2] David Battaglia,[2] David Lee,[2] Diana Wu,[2] Jeffrey Jensen,[1,4] Phillip Patton,[2] Sumita Gokhale,[5] Richard L. Stouffer,[1,2] Don Wolf,[1] and Shoukhrat Mitalipov[1,2,*]

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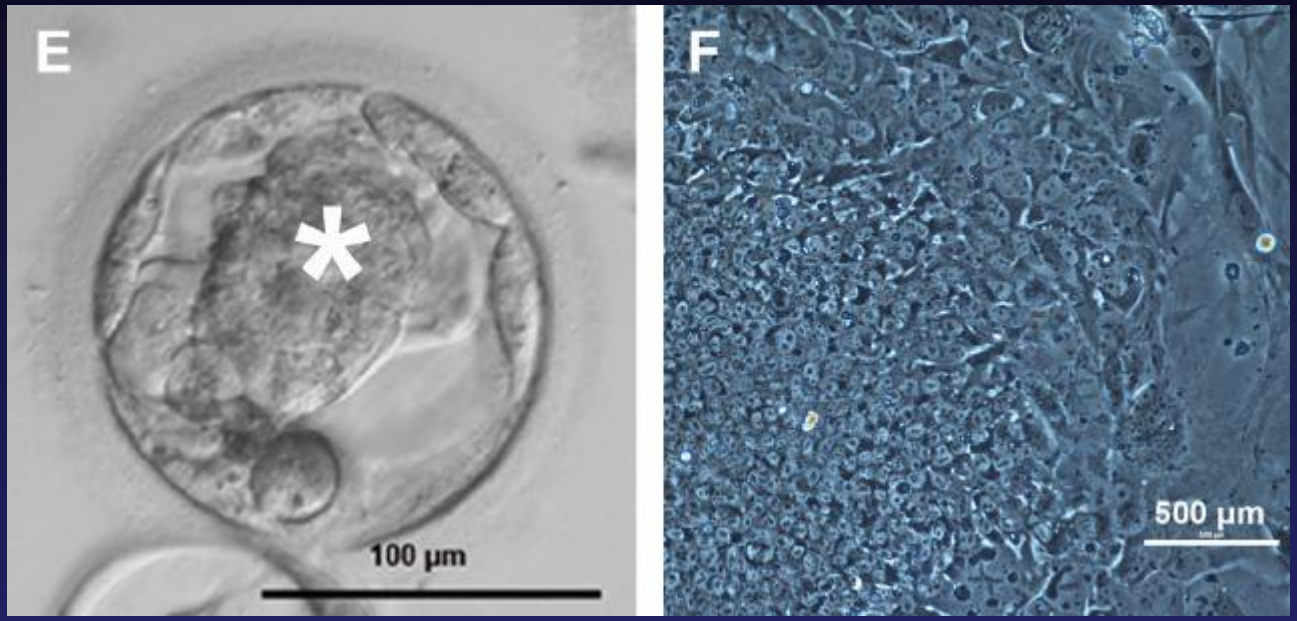
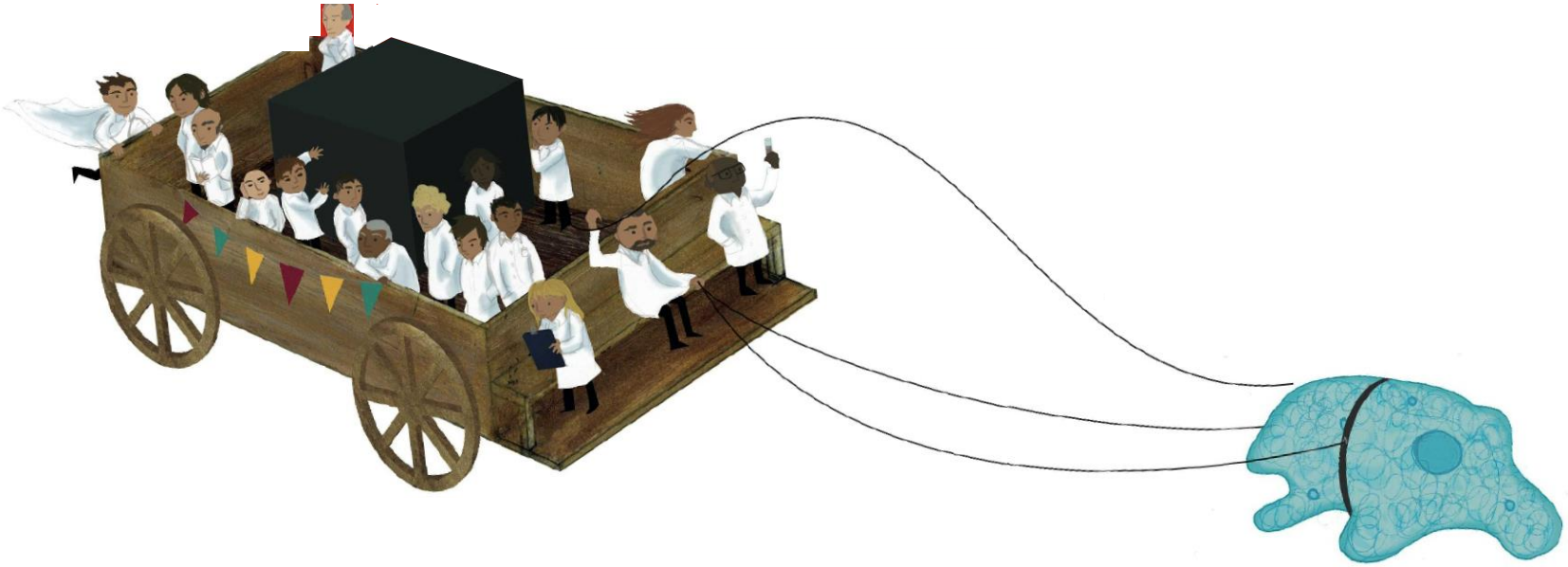
Cell, 2013

-23% of cloned embryos developed into blastocysts
-ES cells were derived from 50% of cloned blastocyst

Mitalipov et al 2013: human therapeutic cloning



Patient specific stem cells in regenerative medicine



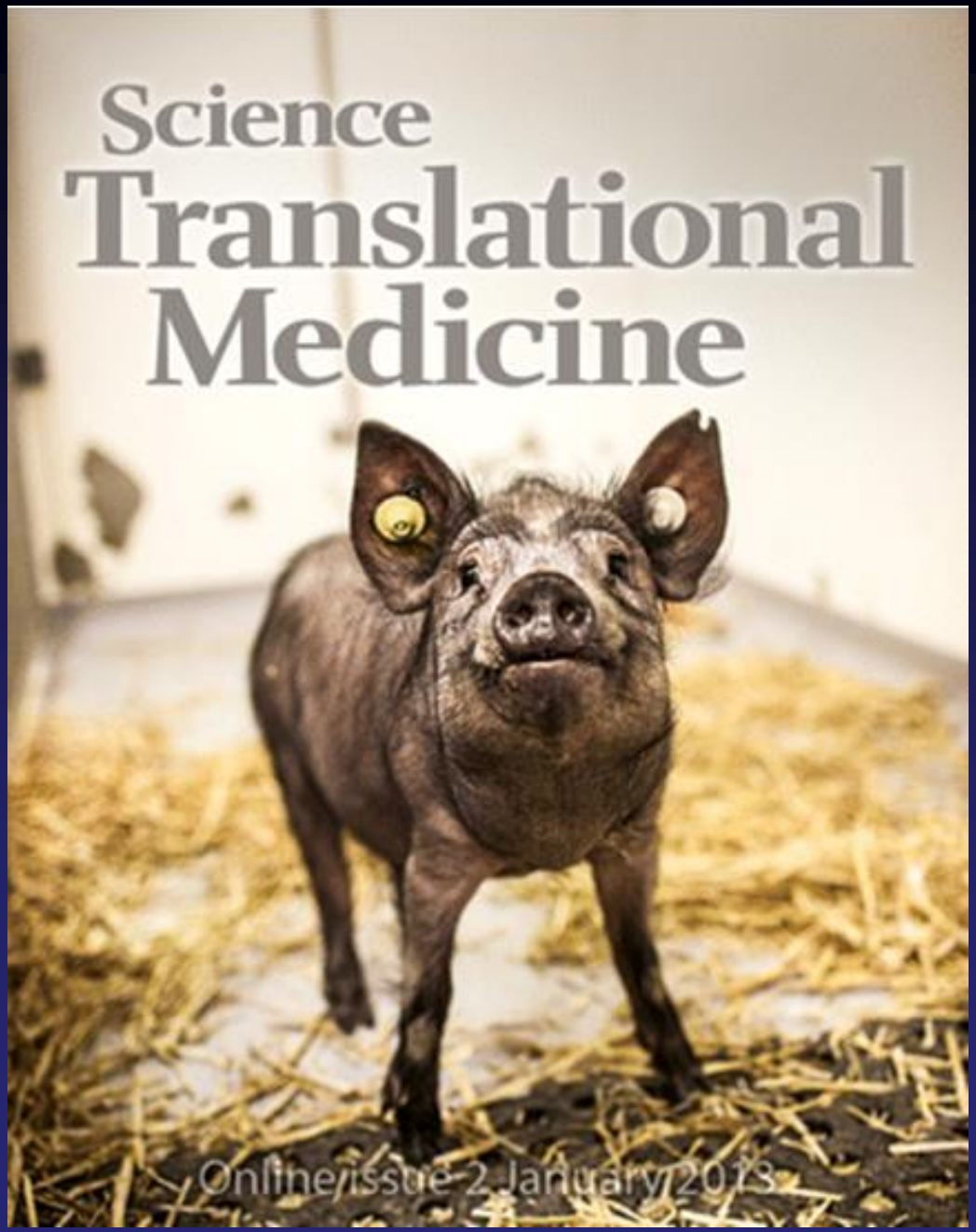
Still, limited clinical use of…

Cloning by somatic cell nucear transfer 1997: Dolly...

sheep
pig
cattle
mouse
horse
mule
goat
rabbit
cat
dog.....

Department of Animal Science, Reproductive Biology
University of Aarhus

Varberg
Hallands Lä
Falker
Anholt
Skive
Viborg
Randers
Holstebro
Denmark
Silkeborg
(Arhus)
Arhus
Syddjurs
Herning
Skanderborg
Sejerø
Hovedstaden
Horsens
Samsø
Hedensted
Endelave
Holbæk
(Copenhagen)
Vejle
Kalundborg
Roskilde
Varde
Esbjerg
Vejen
Kolding
Sjælland
Slagelse


Science
Translational
Medicine
Online Issue 2 January 2013

SCNT: Reproductive cloning in animals

Fibroblasts

Genetic modification

PCSK9: gene for hypercholesterolemia

Transgenic fibroblasts

G0/G1

Reconstructed embryo

Pig oocyte

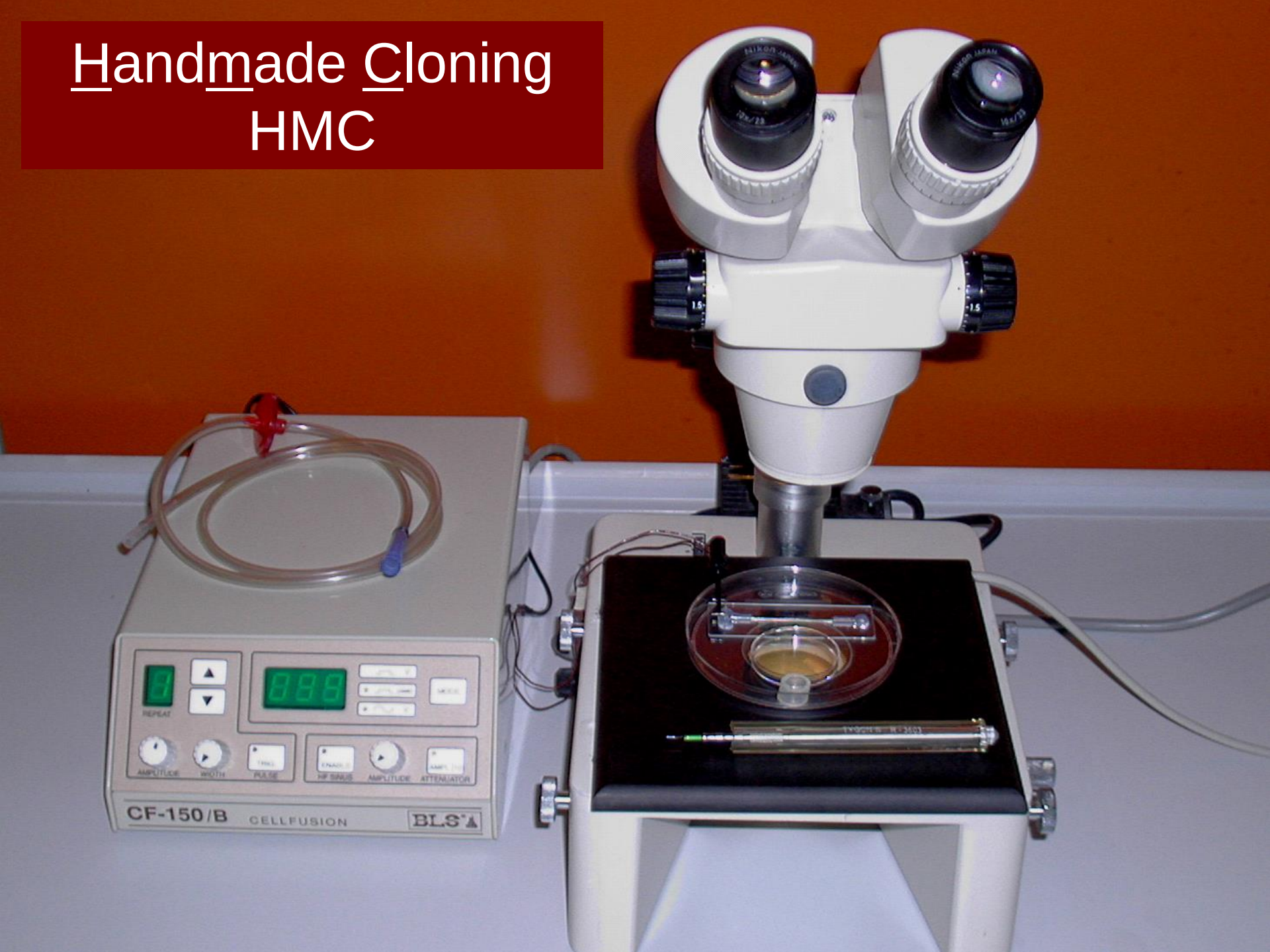
Epigenetic reprogramming

Embryo

Foster sow

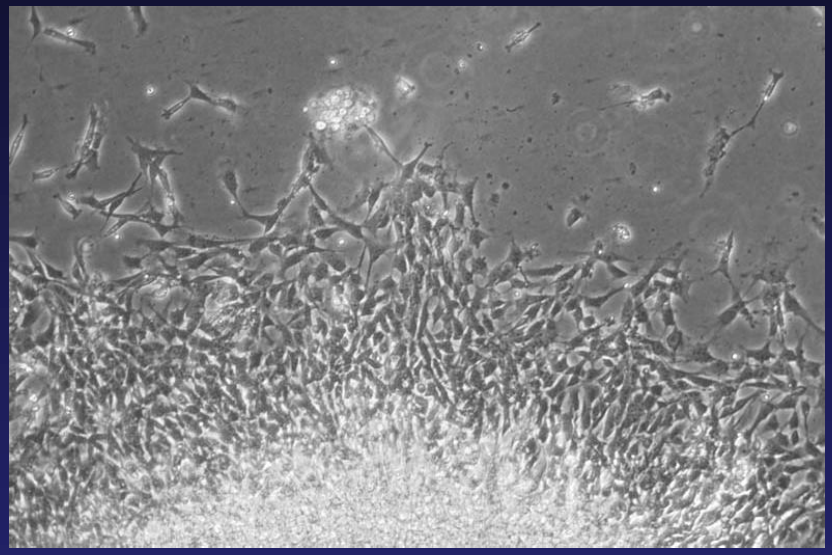
Transgenic Pigs

Handmade Cloning HMC



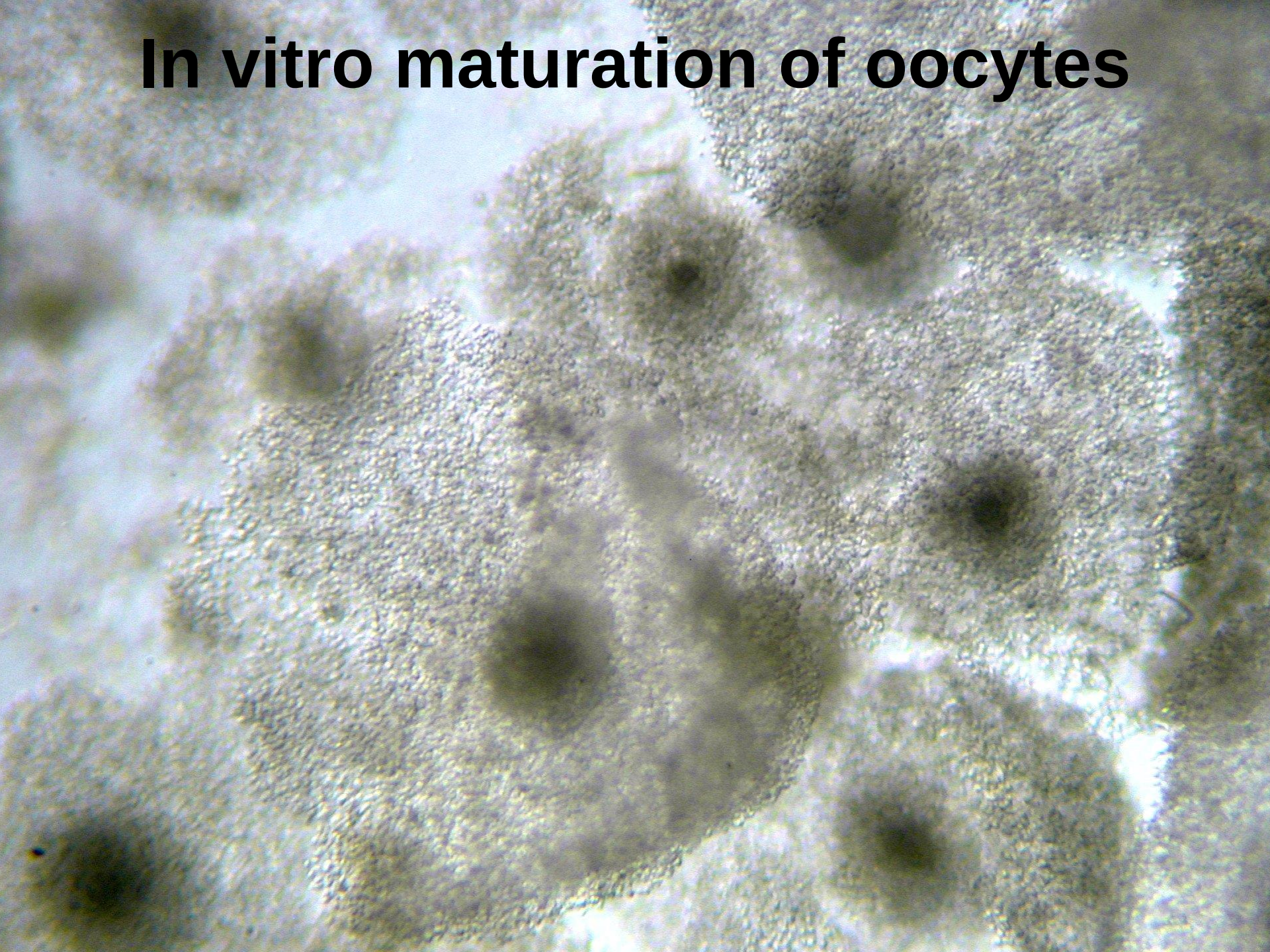
The first Handmade cloned pigs….

Fibroblasts from the donor pig

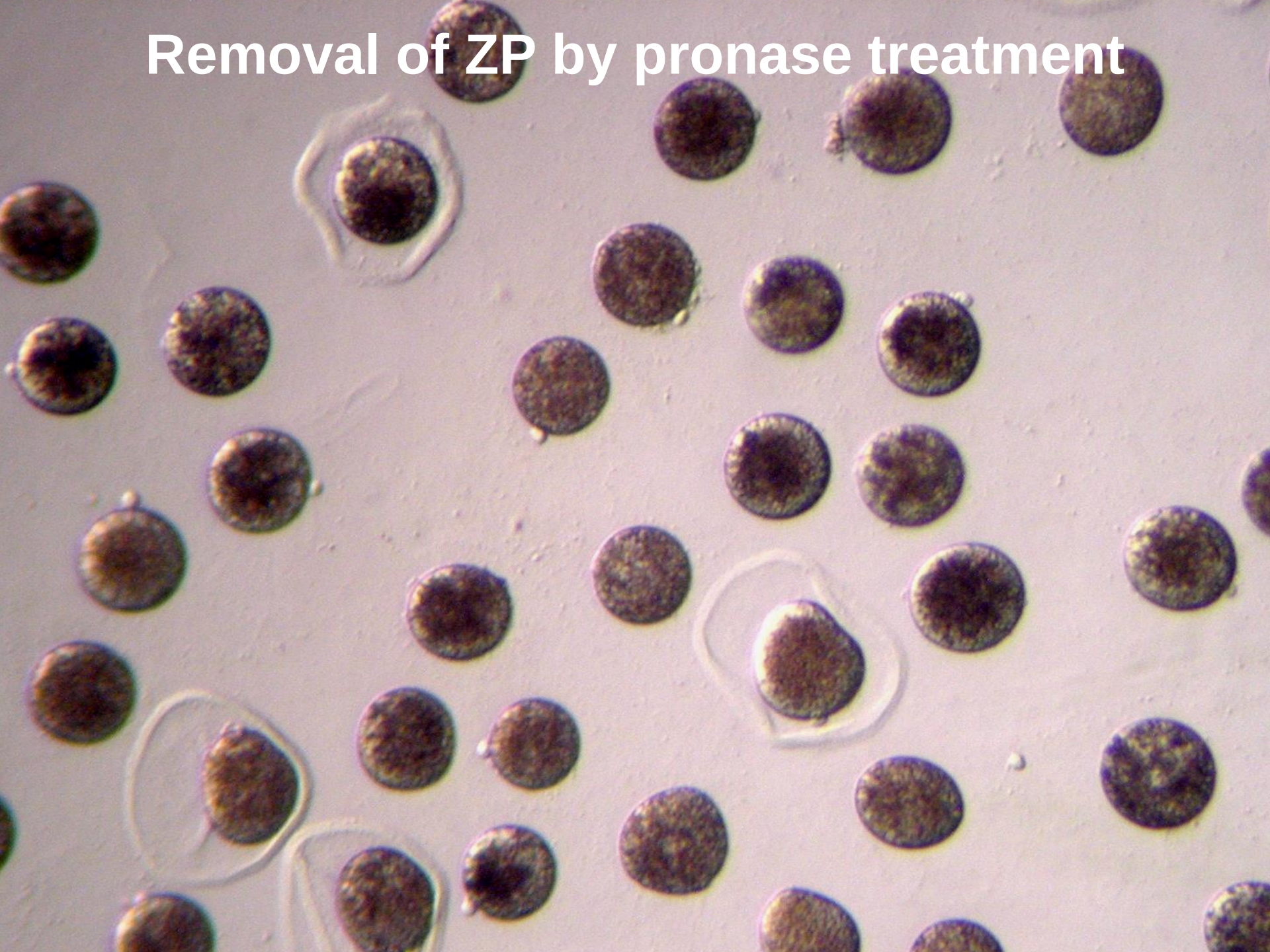


Pig oocytes from slaughterhouse ovaries

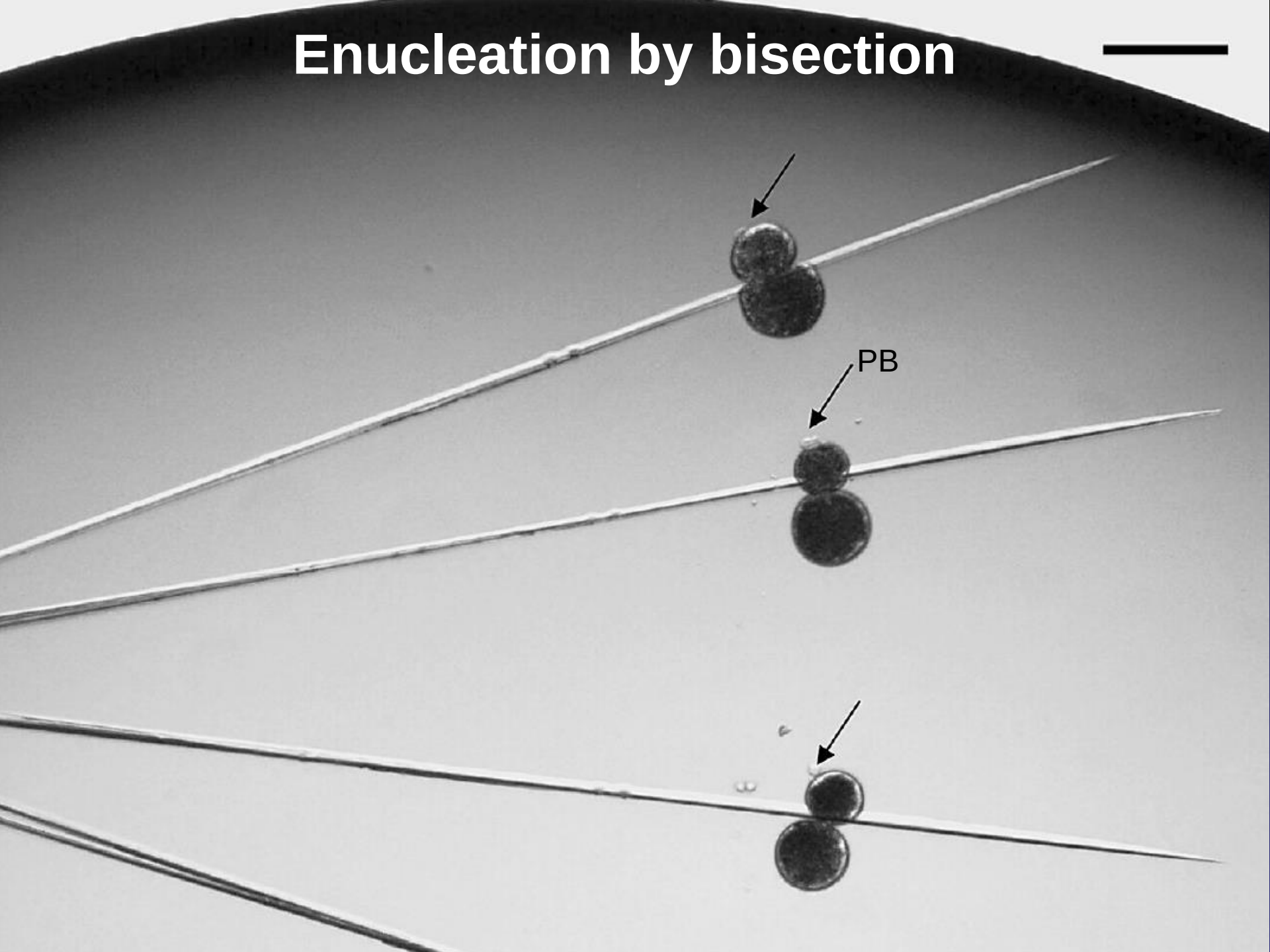
In vitro maturation of oocytes



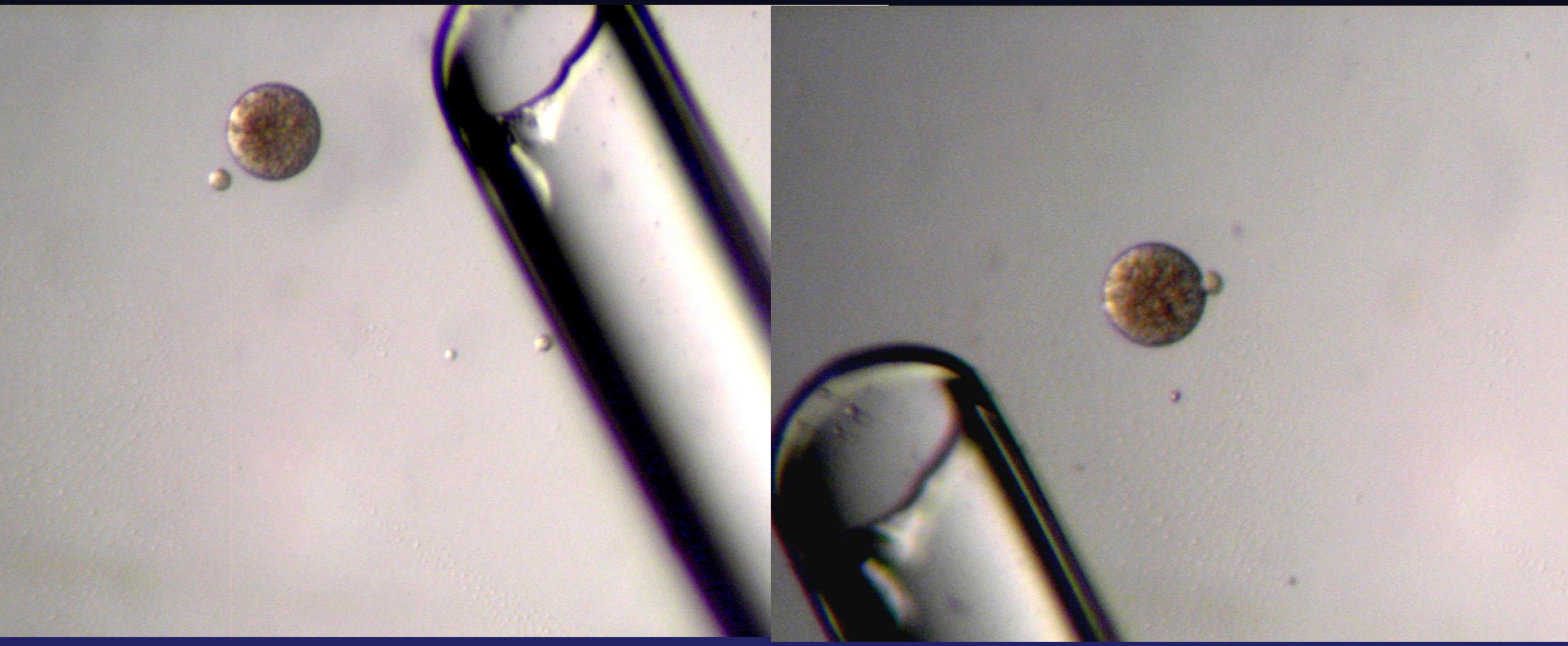
Removal of ZP by pronase treatment



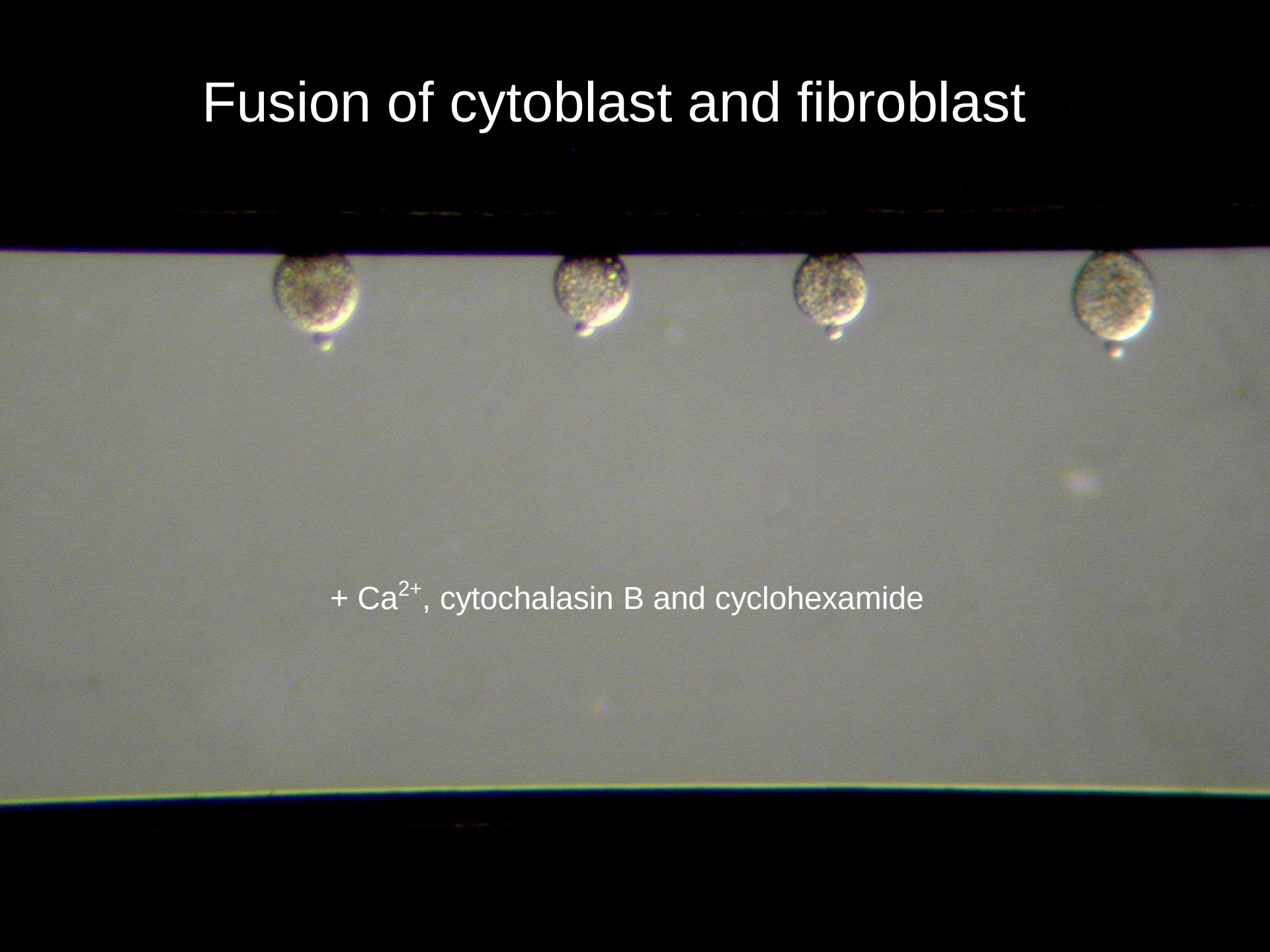
Enucleation by bisection



Fibroblast attached to cytoblast

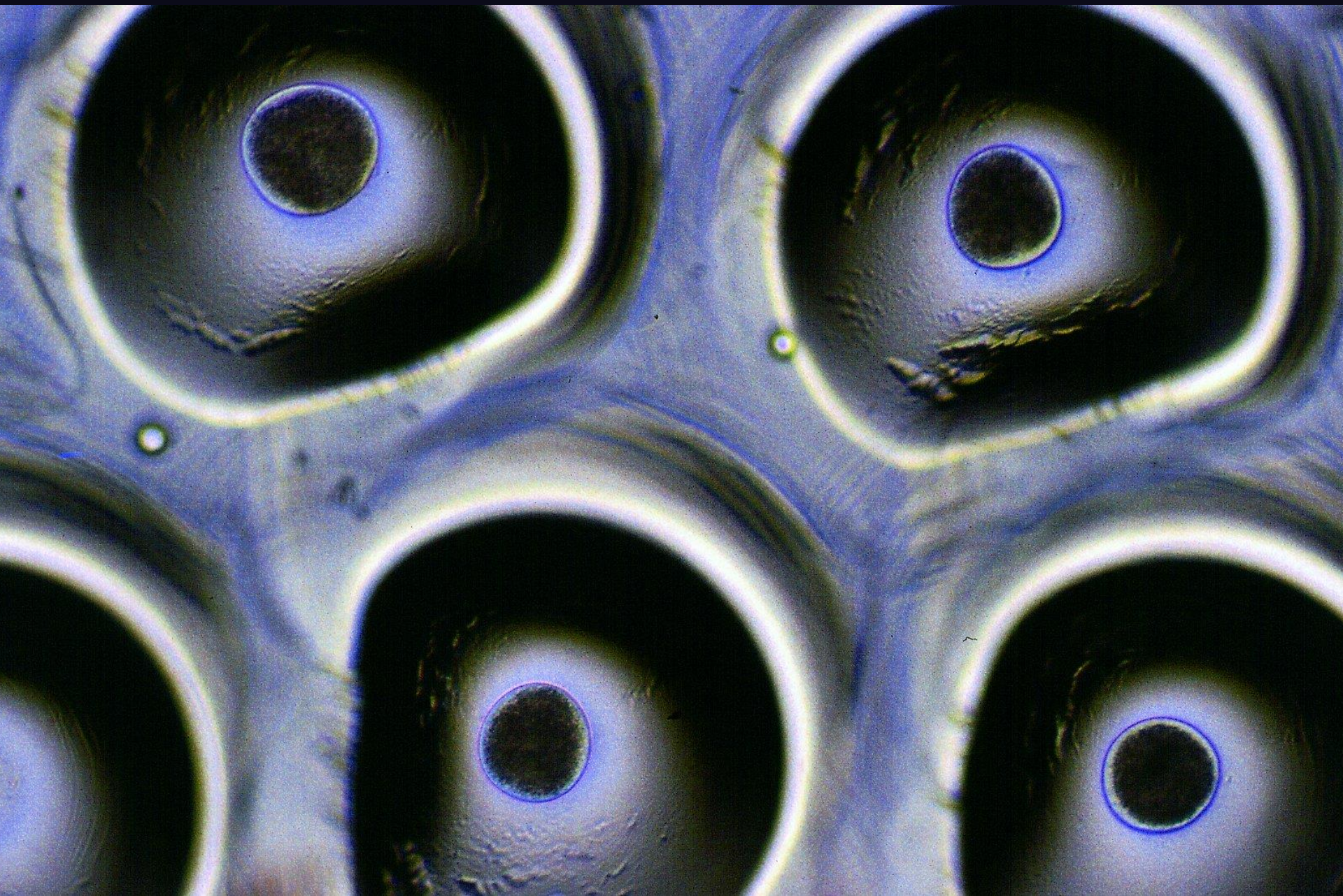


Fusion of cytoblast and fibroblast

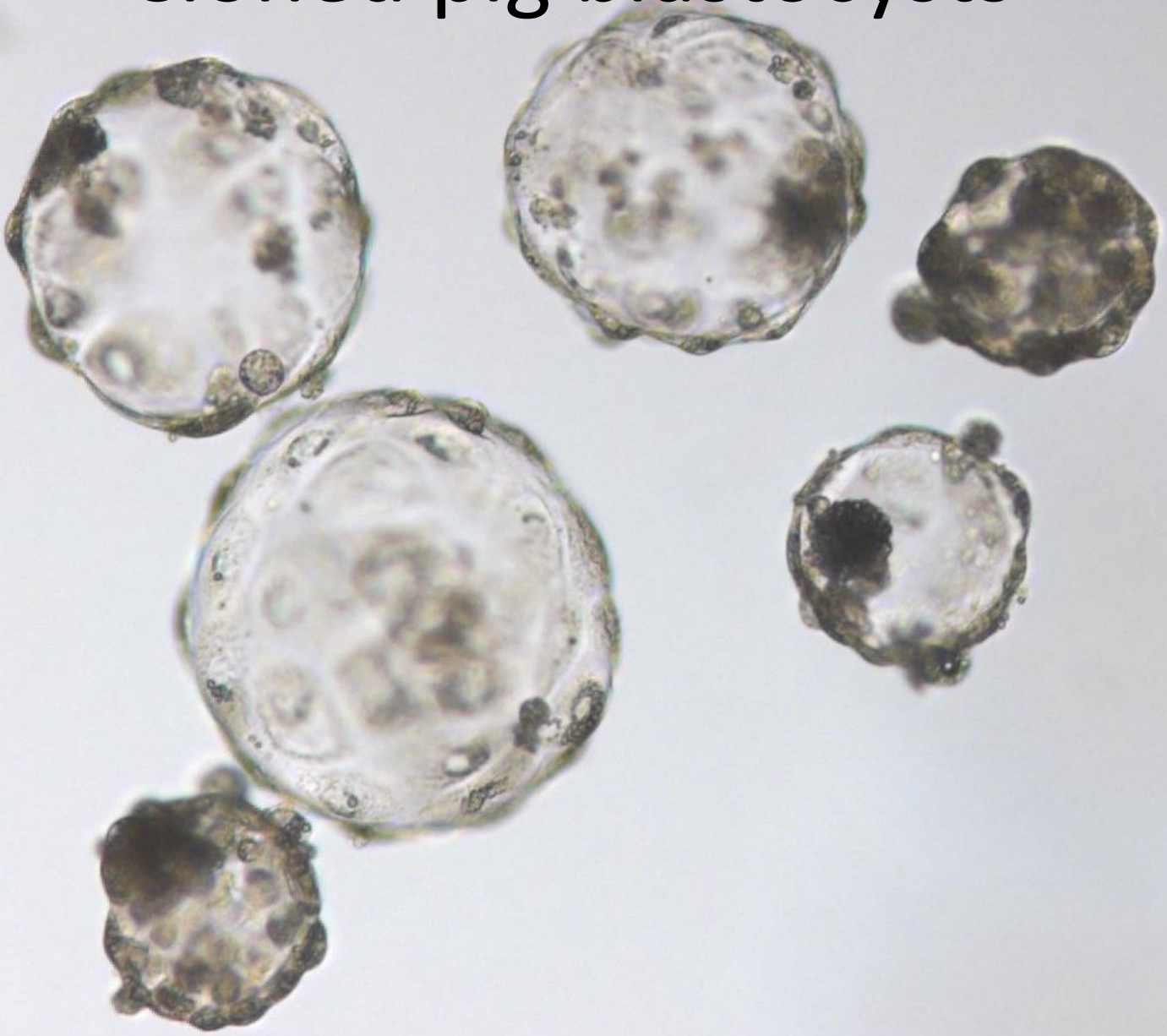


+ Ca^{2+}, cytochalasin B and cyclohexamide

In vitro culture in micro-wells (WOW)



Cloned pig blastocysts



Embryo transfer to recipient uterus

3 months and 3 weeks later…

1000 MII oocytes

2x70 blastocysts

1 litter.

“epigenetic reprogramming errors”

Embryology group at University of Århus:

Henrik Callesen, Ying Liu, Rong Li, Janne Adamsen, Klaus Villemoes, Anette Pedersen, Ruth Kristensen, Gábor Vajta, Peter Kragh

Embryo Transfer, Copenhagen University:

Mette Schmidt

Atherosclerosis group at University of Århus:

Jacob Bentzon, Charlotte Sørensen, Erling Falk, Lars Bolund

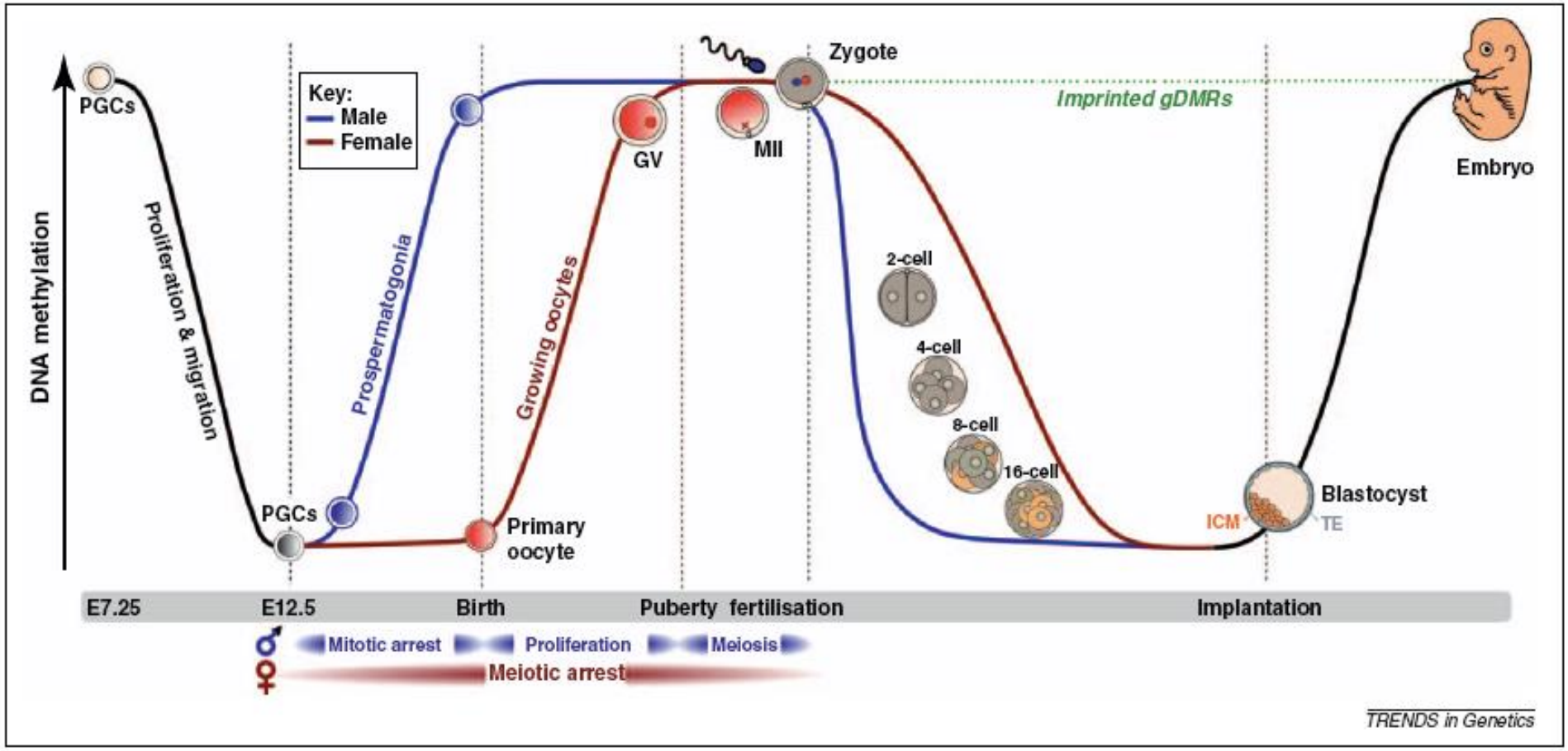
Generation of egg and sperm from pluripotent stem cells:

1. MEIOSIS

2. EPIGENETIC REPROGRAMMING

3. In vitro differentiation of stem cells to gametes?

DNA methylation and development

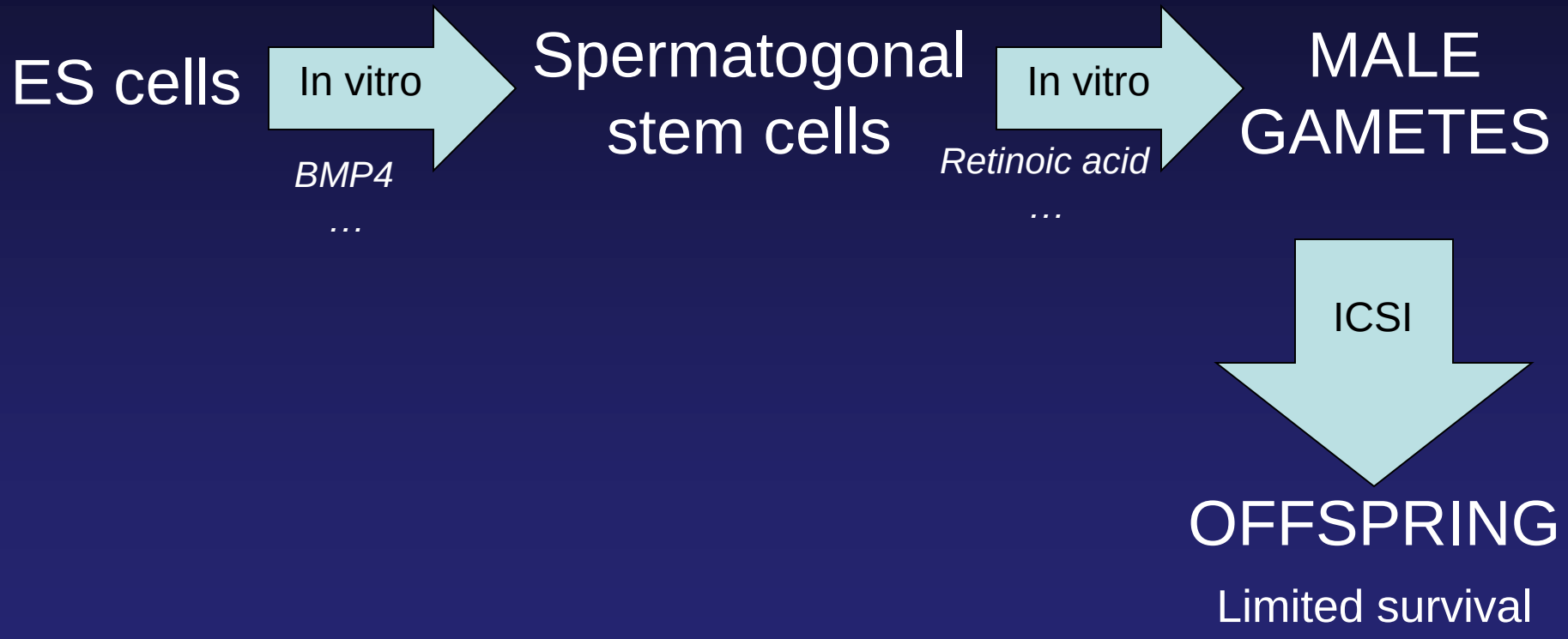


Developmental Cell *11*, 125–132, July, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.devcel.2006.05.010

In Vitro-Differentiated Embryonic Stem Cells Give Rise to Male Gametes that Can Generate Offspring Mice

Short Article

Nayernia et al. 2006



Cell

Reconstitution of the Mouse Germ Cell Specification Pathway in Culture by Pluripotent Stem Cells

Katsuhiko Hayashi,[1,3] Hiroshi Ohta,[1,3] Kazuki Kurimoto,[1,3] Shinya Aramaki,[1] and Mitinori Saitou[1,2,3,*]
[1]Department of Anatomy and Cell Biology, Graduate School of Medicine
[2]Institute for Integrated Cell-Material Sciences
Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan

Cell *146*, 519–532, August 19, 2011

iPS / ES cells → In vitro → PGC → In vivo → SPERMATOZOA

Activin
bFGF
SCF
LIF
EGF
BMP4/8b

ICSI

OFFSPRING

Offspring from Oocytes Derived from in Vitro Primordial Germ Cell–like Cells in Mice

Katsuhiko Hayashi,[1,2,3]* Sugako Ogushi,[1,4] Kazuki Kurimoto,[1,5] So Shimamoto,[1] Hiroshi Ohta,[1,5] Mitinori Saitou[1,2,5,6]*

SCIENCE VOL 338 16 NOVEMBER 2012

iPS / ES cells → In vitro → PGC → In vivo → OOCYTES → IVM & IVF → OFFSPRING

Activin
bFGF
SCF
LIF
EGF
BMP4/8b

Cell PRESS
Open ACCESS

Cell Reports
Report

Direct Differentiation of Human Pluripotent Stem Cells into Haploid Spermatogenic Cells

Charles A. Easley IV,[1,2,5] Bart T. Phillips,[1,2] Megan M. McGuire,[2] Jennifer M. Barringer,[2] Hanna Valli,[1,2] Brian P. Hermann,[3] Calvin R. Simerly,[1,2] Aleksander Rajkovic,[1,2] Toshio Miki,[4] Kyle E. Orwig,[1,2] and Gerald P. Schatten[1,2,*]

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http://dx.doi.org/10.1016/j.celrep.2012.07.015

Easley et al. 2012

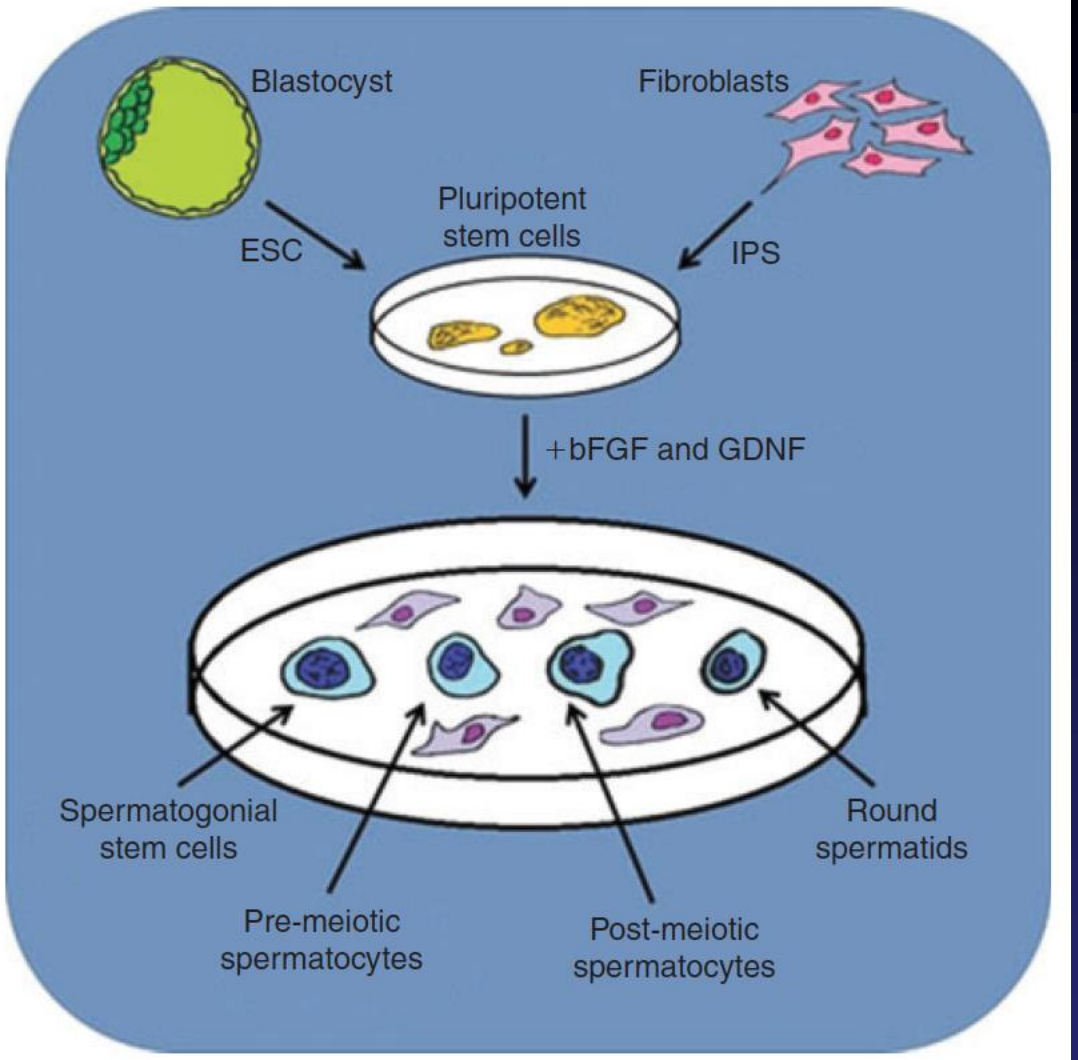

Fig. 1. *In vitro* culture induces germ cell differentiation of human pluripotent stem cells (hPSCs). The hPSCs differentiate into spermatogonia, spermatocytes, and haploid spermatids. Haploid spermatids have uniparental imprints similar to fertile human spermatozoa (Easley *et al.* 2012*b*).

Easley et al. 2012

Pluripotent stem cells in ART – how far are we?

-Regenerative medicine:

- ES / iPS cells are produced and differentiated in vitro.
- Still, limited clinical use.

-Production of germ cells from pluripotent stem cells

- Promising results in mice.
- Still, therapeutic application is not possible.

- Ethical issues….