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# Reprogramming somatic cells into rabbit and avian iPSC

Marielle Afanassieff, Bertrand Pain

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Submitted on 5 Jun 2020

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# Infrastructures nationales en biologie et santé

## Réunion de lancement du projet CRB-Anim

Vendredi 9 novembre 202, de 9h30 à 17h00

Amphithéâtre du siège de l'INRA - 147, rue de l'Université – 75 007 Paris

**9h00 – 9h30** Accueil des participants

**9h30 – 9h45** Séance d'ouverture

*INRA*

**9h45 – 10h15** L'Appel « Infrastructures nationales en biologie et santé » des projets  
« Investissements d'Avenir » :

*ANR*

*CGI*

*Ministère de la Recherche*

**10h15 – 10h45** Les enjeux du CRB-Anim pour l'agriculture française et les ressources génétiques

*Ministère de l'Agriculture*

**10h45 – 11h15** L'exemple européen du Centre for Genetic Resources, the Netherlands (CGN)

*Sipke-Joost Hiemstra (Wageningen University)*

**11h45 – 12h15** Présentation du projet CRB-Anim

*Michèle Tixier-Boichard (INRA)*

12h15 – 13h30 Buffet déjeuner

**Session 1 : Les Centres de Ressources Biologiques : Rôles et missions pour la recherche**

**13h30 – 14h00** Le projet Investissements d'Avenir « Biobanques »

*(Bruno Clément, INSERM)*

**14h00 – 14h30** L'exemple du European Marine Biological Resource Centre (EMBRC)

*(Bernard Kloareg, UPMC)*

**14h30 – 15h00** Management of genetic diversity in genebanks with genomics and other tools

*(Jack Windig, CGN, WUR)*

**15h00 – 15h30** Pause café

**Session 2 : Illustration des objectifs spécifiques du CRB-Anim**

**15h30 – 16h00** Du centre de ressources biologiques aux résultats scientifiques et économiques :  
l'exemple du modèle canin

*(Catherine André, CNRS)*

**16h00 – 16h30** L'impact des CRB sur l'innovation en biotechnologies de la reproduction

*(Marielle Afanassieff, Bertrand Pain, INSERM)*

**16h30 – 17h00** Discussion finale

*Table ronde avec les représentants des différents partenaires*

Entrée libre sur inscription sur le site : <https://colloque.inra.fr/kickoff-meeting-crb-anim>

Contact : Olivier Ruetsch ([olivier.ruetsch@paris.inra.fr](mailto:olivier.ruetsch@paris.inra.fr), 01 42 75 93 25)

# *Reprogramming somatic cells into rabbit and avian iPSC*

**Marielle AFANASSIEFF & Bertrand PAIN**

**AgroBioStem - INSERM U846 - INRA USC 1361 - Bron  
CRB-Anim 9/11/2012**

# *Developments for somatic samples to be used for reproductive biology (WP2.2)*

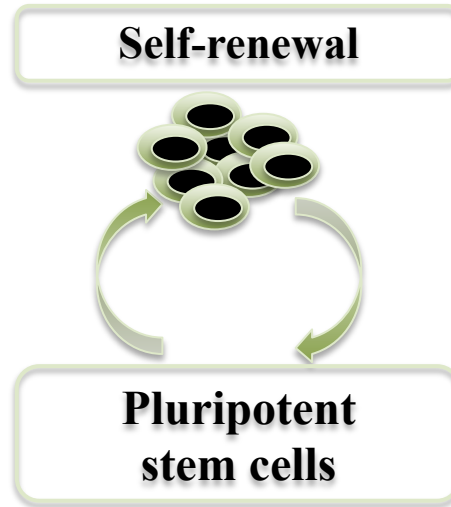
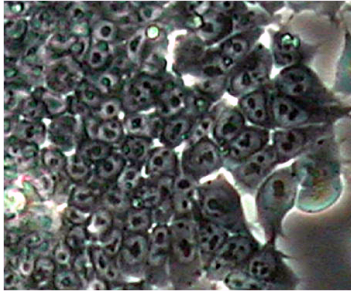
**Goals: To increase the type and the number  
of samples for cryobanking**



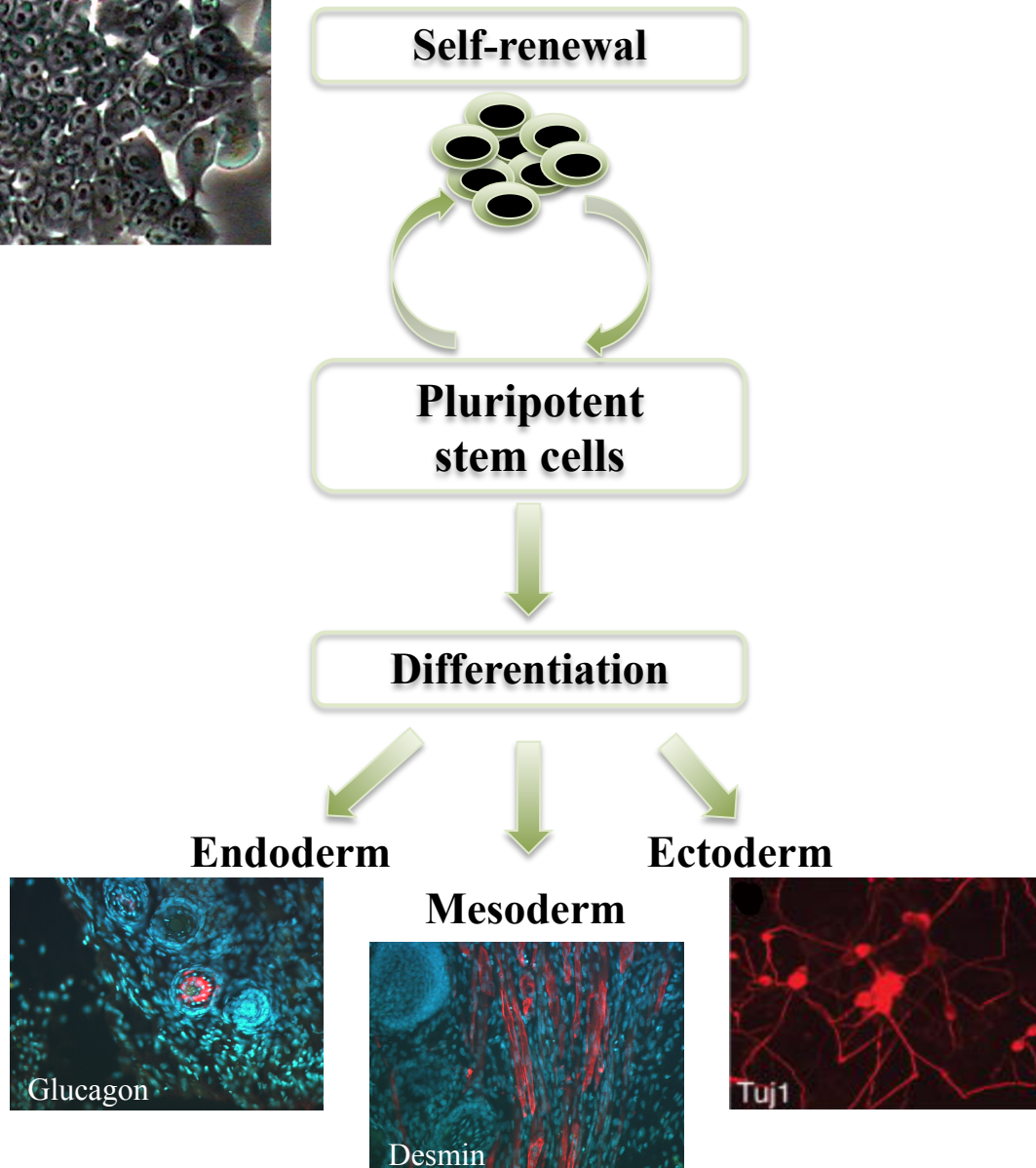
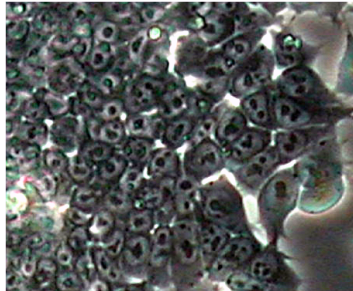
# *Properties of pluripotent stem cells*

**Pluripotent  
stem cells**

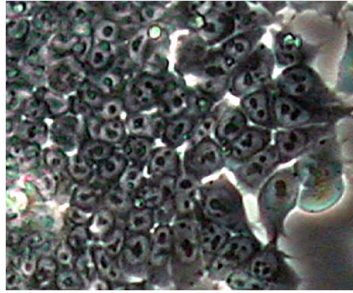
# *Properties of pluripotent stem cells*



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**Self-renewal**



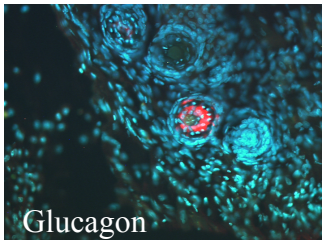
**Pluripotent stem cells**

**Oncogenesis**

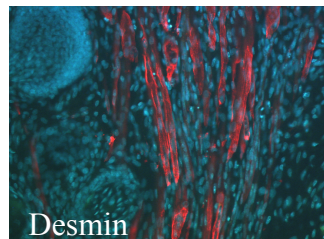


**Differentiation**

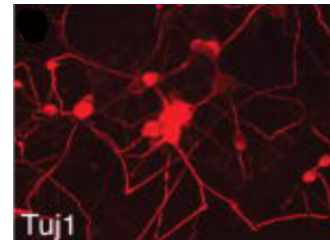
**Endoderm**



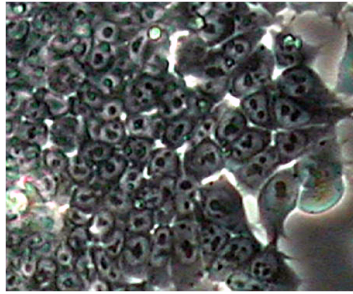
**Mesoderm**



**Ectoderm**



# Properties of pluripotent stem cells



Self-renewal



Oncogenesis

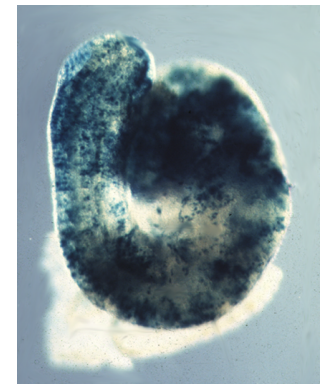
Pluripotent stem cells

Embryo colonization



Differentiation

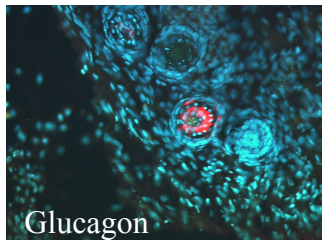
Chimeras



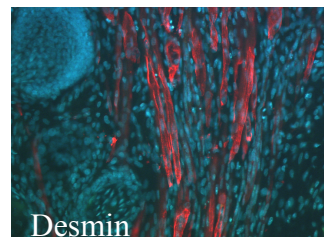
Endoderm

Ectoderm

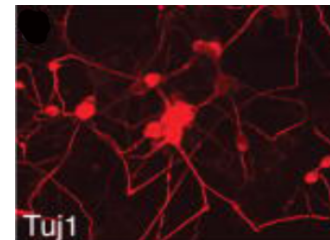
Mesoderm



Glucagon

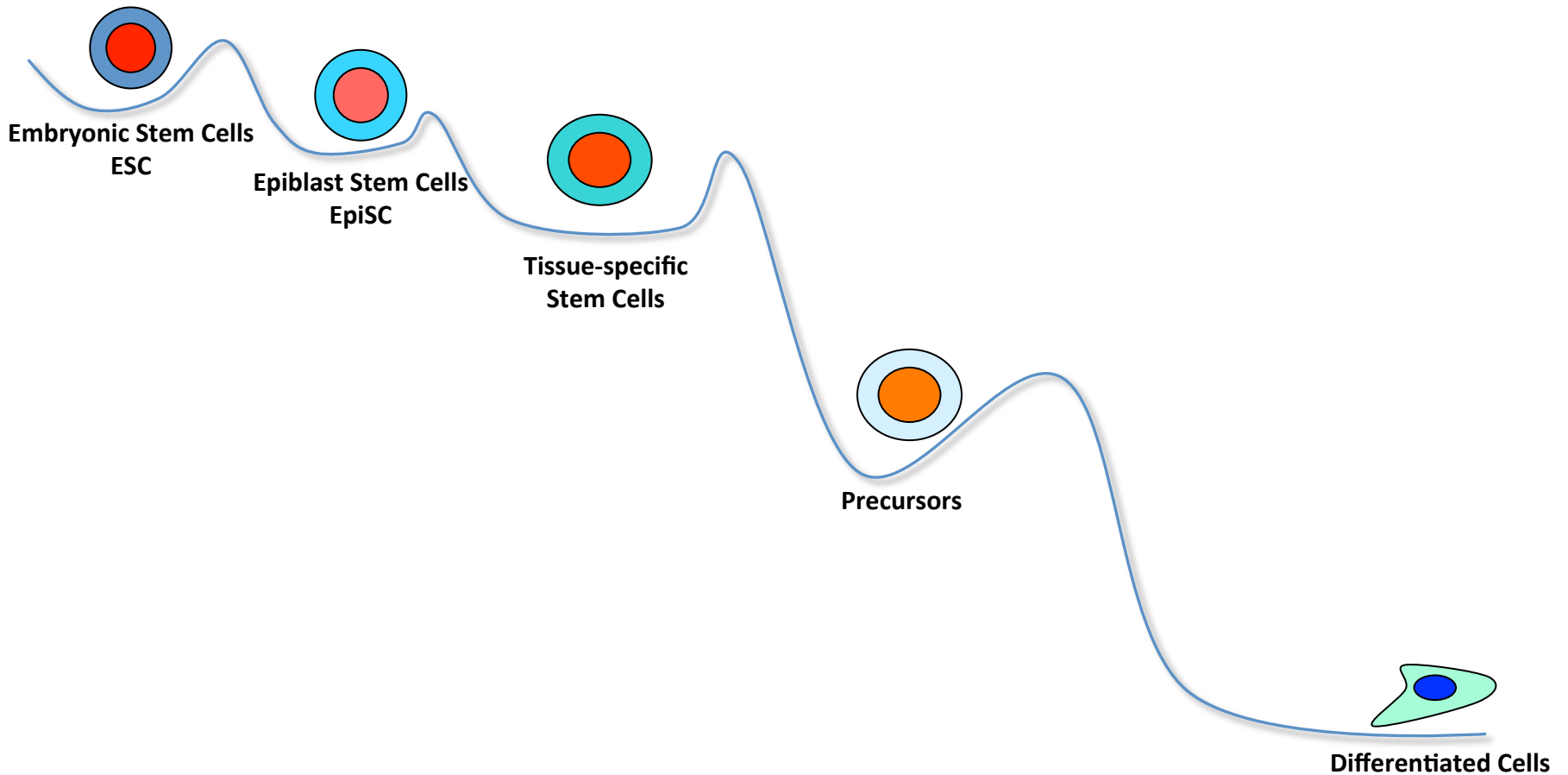


Desmin

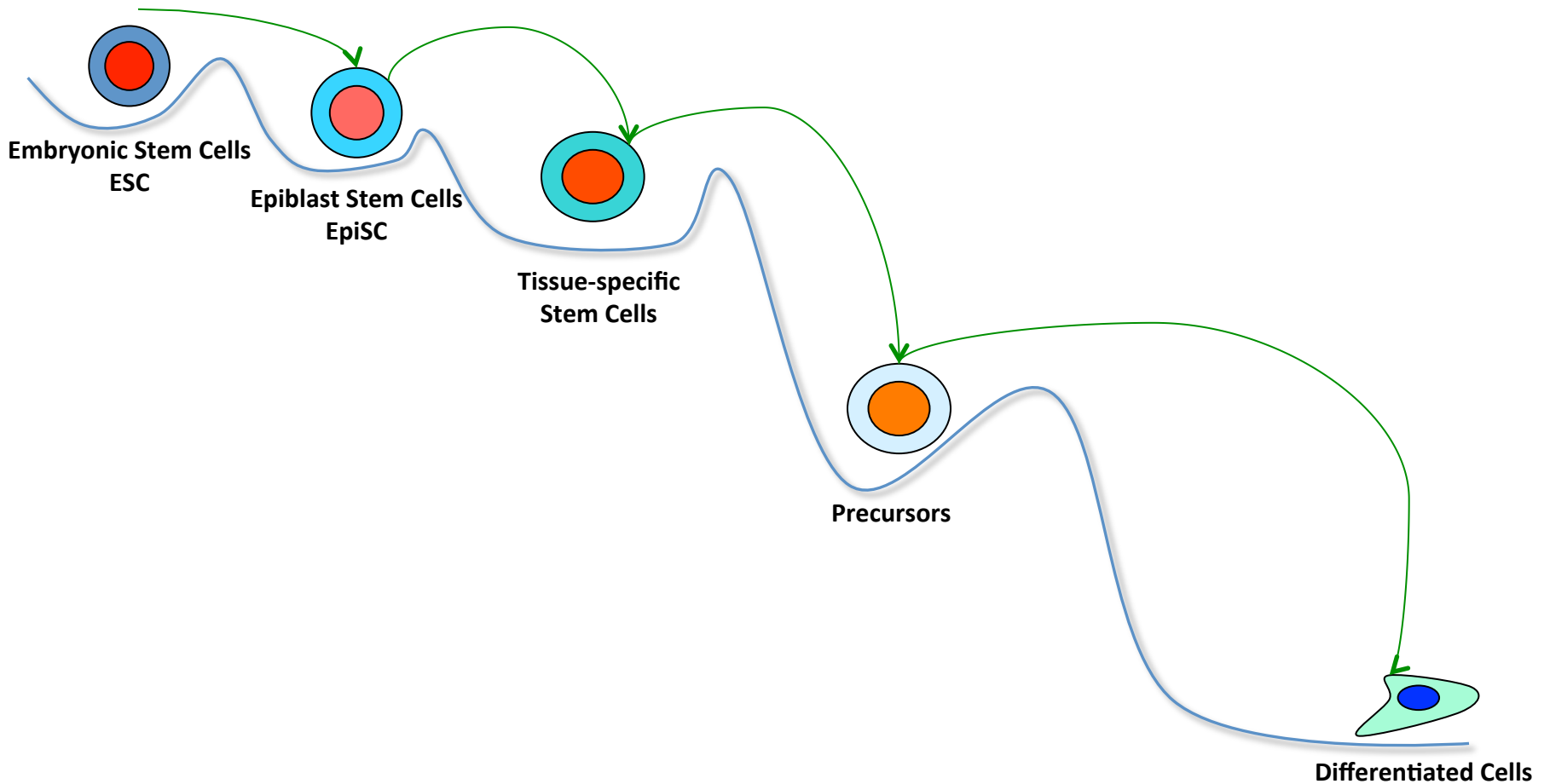


Tuj1

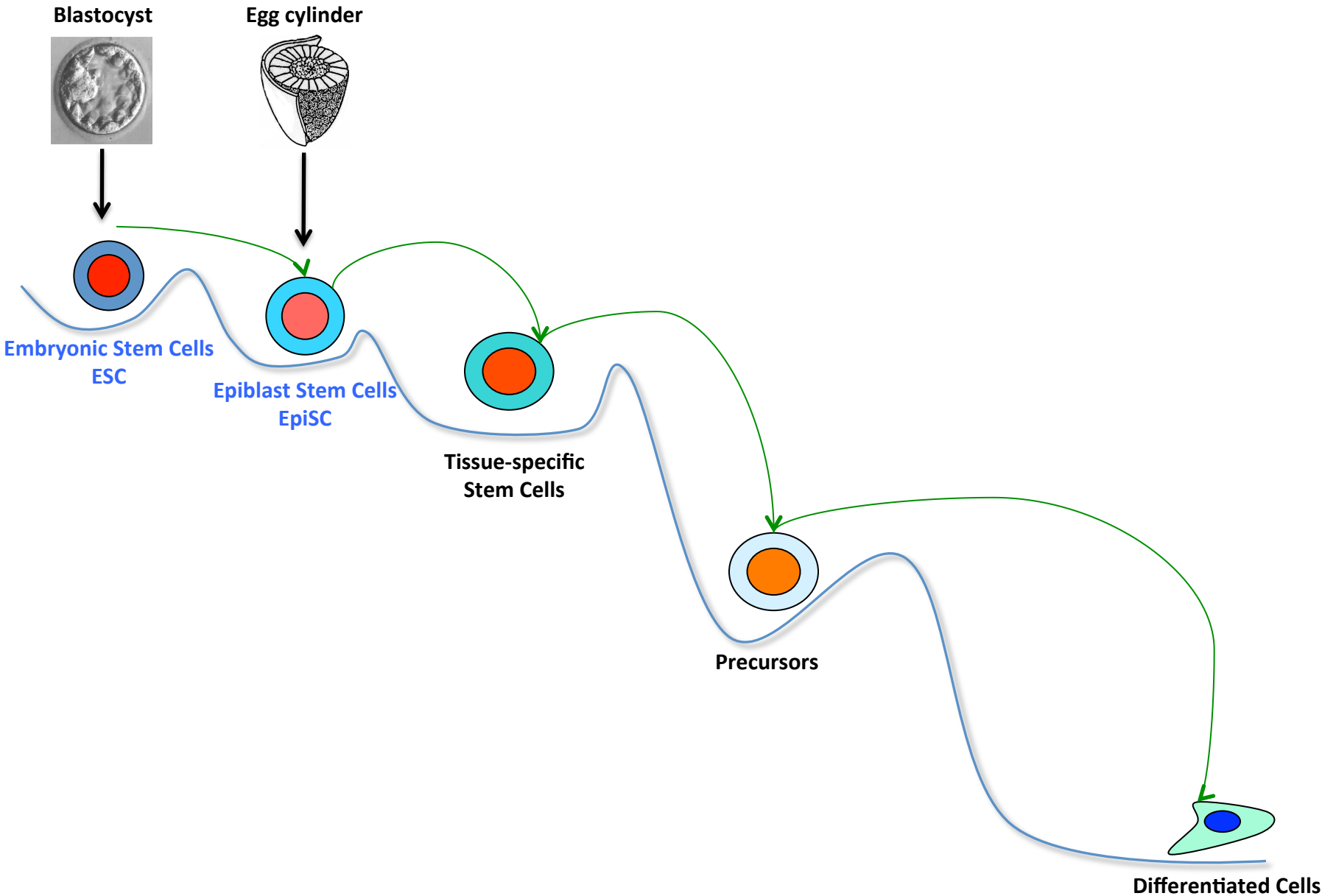
# *Types of pluripotent stem cell*



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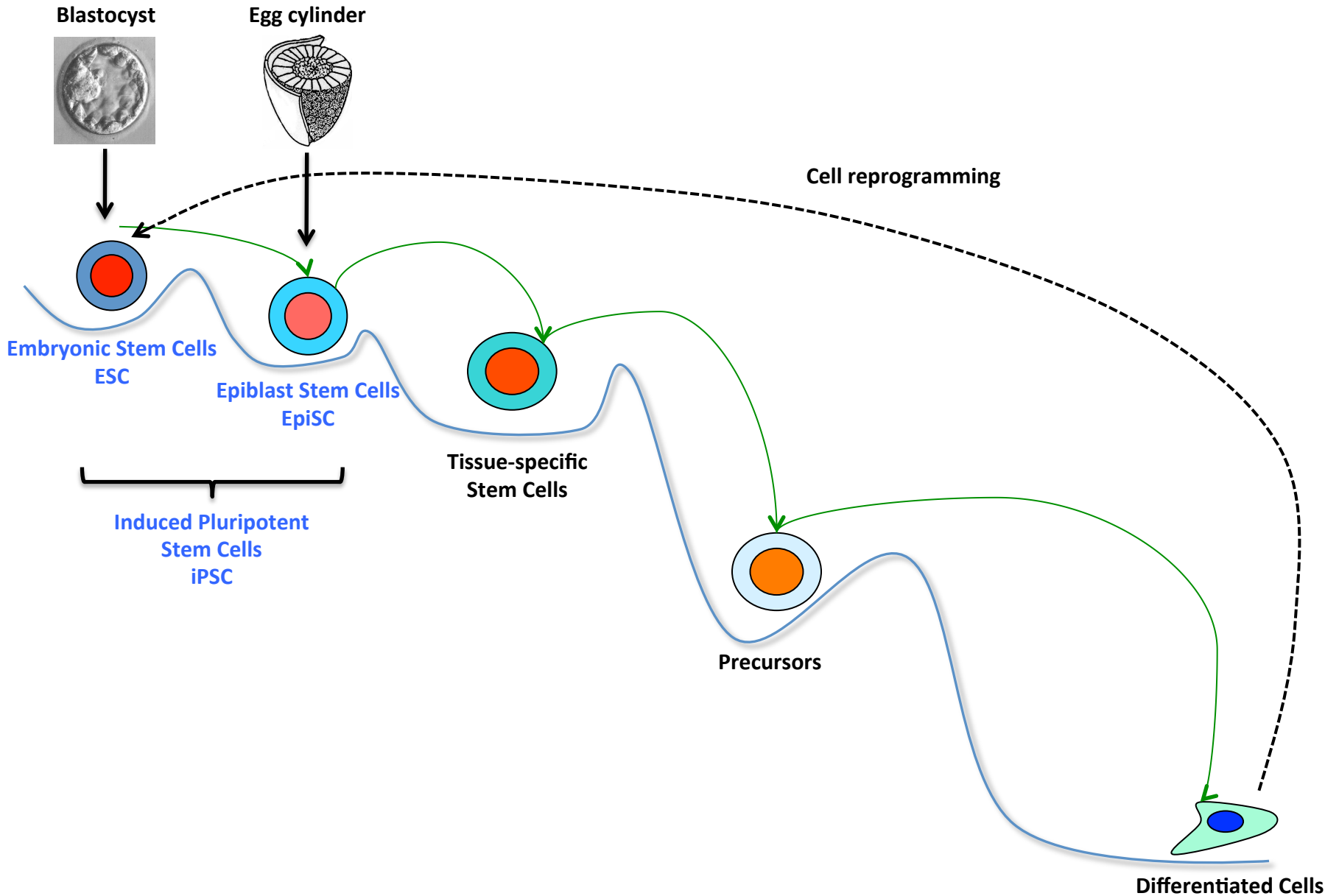


# *Types of pluripotent stem cell*





# Types of pluripotent stem cell



# *Reprogramming: the stem cell revolution – the iPSC*

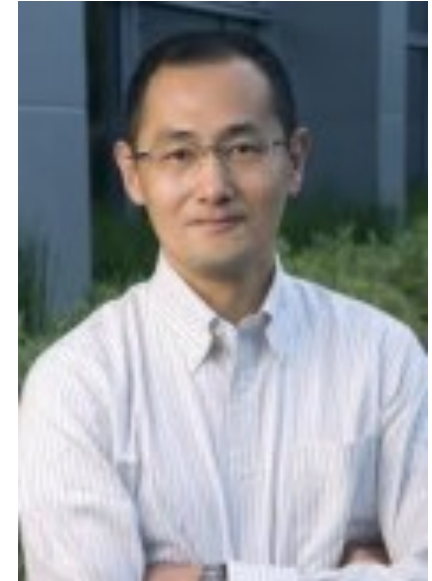


The Nobel Prize in Physiology or Medicine 2012

jointly to

John B. Gurdon and Shinya Yamanaka

for the discovery that mature cells can be reprogrammed to become pluripotent



The Nobel Prize recognizes two scientists who discovered that mature, specialised cells can be reprogrammed to become immature cells capable of developing into all tissues of the body. Their findings have revolutionised our understanding of how cells and organisms develop.

John B. Gurdon discovered in 1962 that the specialisation of cells is reversible. In a classic experiment, he replaced the immature cell nucleus in an egg cell of a frog with the nucleus from a mature intestinal cell. This modified egg cell developed into a normal tadpole. The DNA of the mature cell still had all the information needed to develop all cells in the frog.

Shinya Yamanaka discovered more than 40 years later, in 2006, how intact mature cells in mice could be reprogrammed to become immature stem cells. Surprisingly, by introducing only a few genes, he could reprogram mature cells to become pluripotent stem cells, i.e. immature cells that are able to develop into all types of cells in the body.

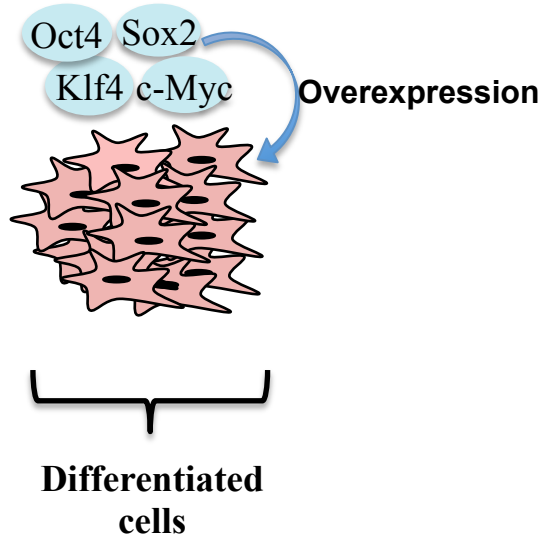
These groundbreaking discoveries have completely changed our view of the development and cellular specialisation. We now understand that the mature cell does not have to be confined forever to its specialised state. Textbooks have been rewritten and new research fields have been established. By reprogramming human cells, scientists have created new opportunities to study diseases and develop methods for diagnosis and therapy.

# *Cell reprogramming in Mouse*

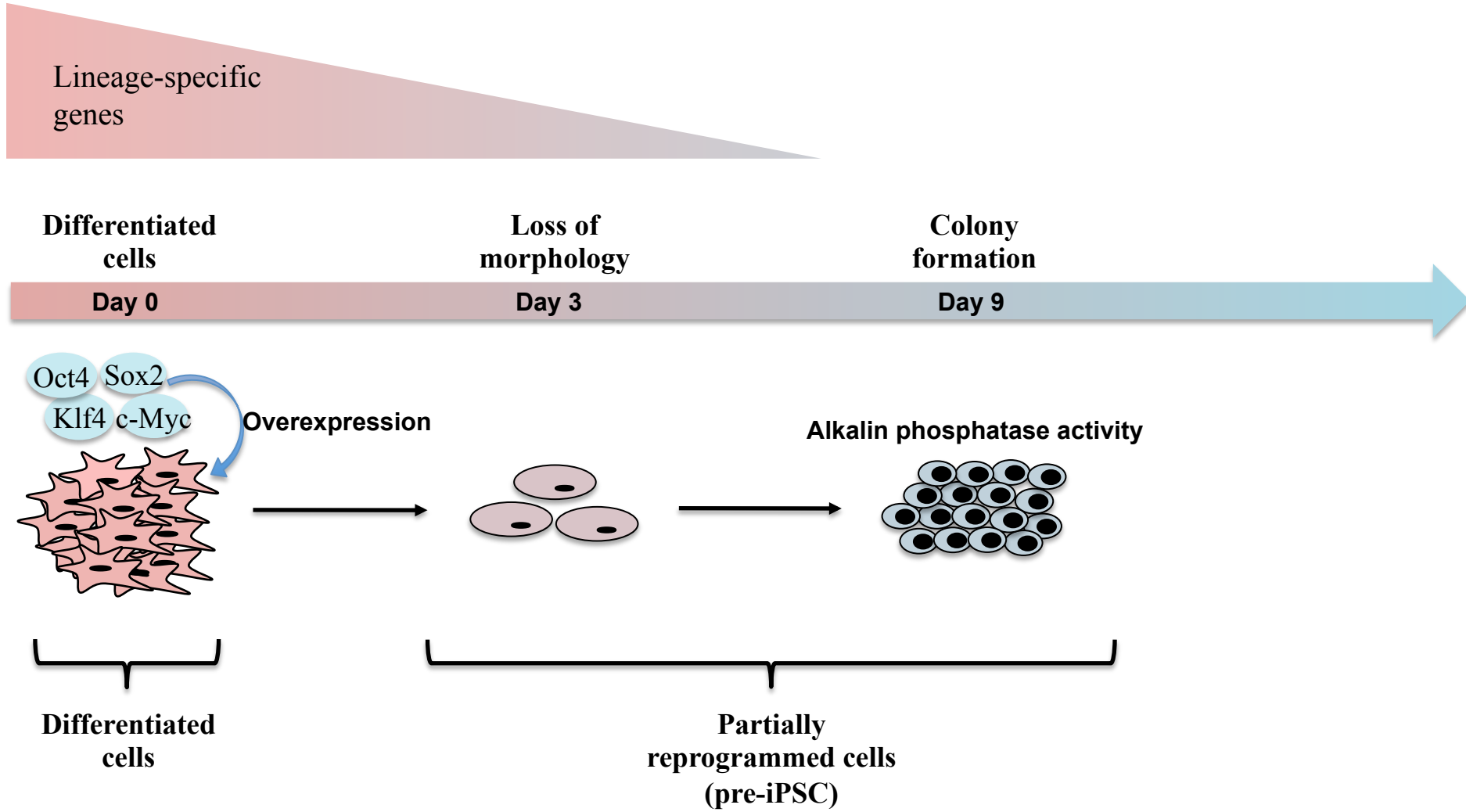
Lineage-specific genes

**Differentiated cells**

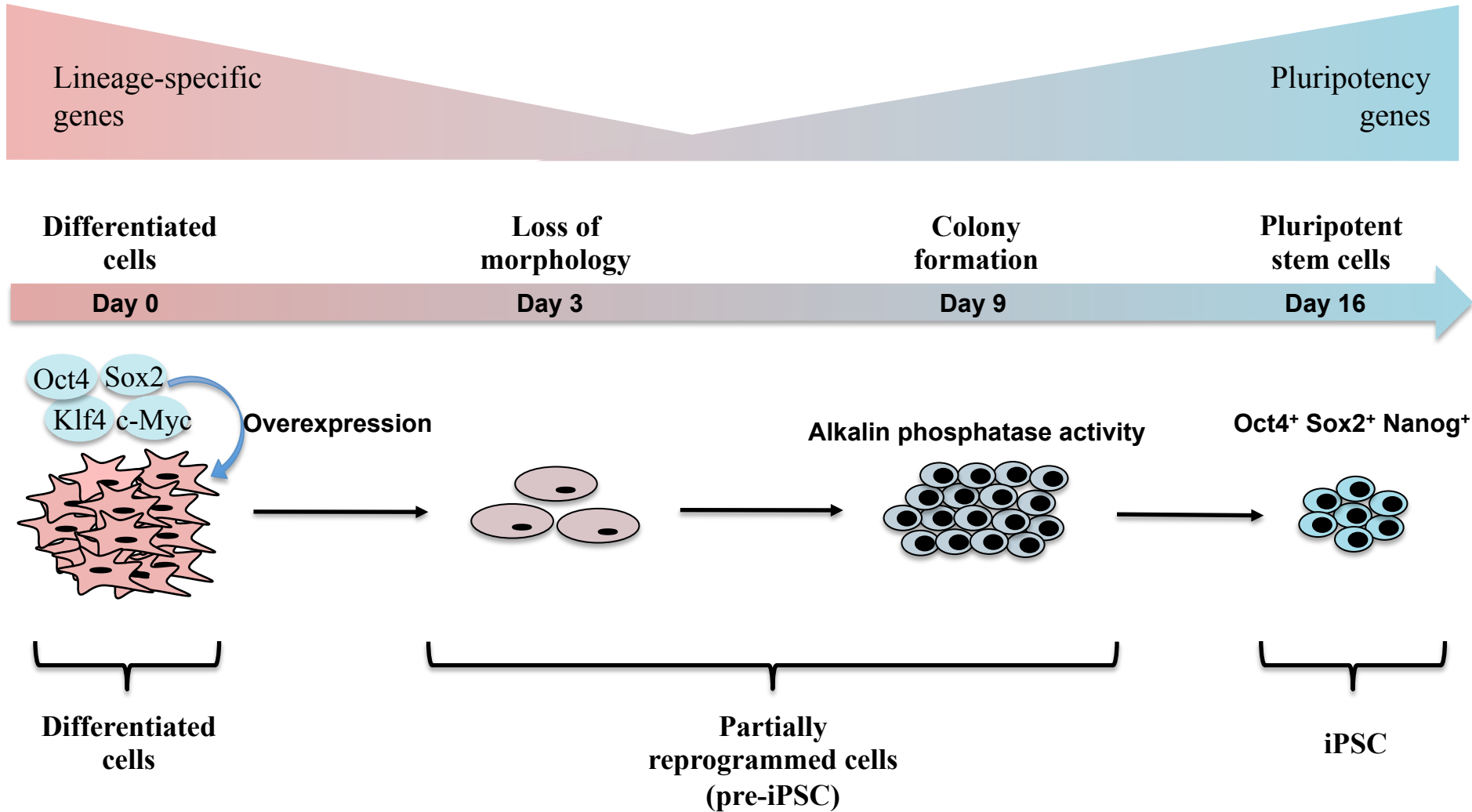
**Day 0**



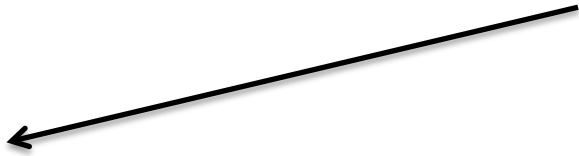
# Cell reprogramming in Mouse



# Cell reprogramming in Mouse



# *Interest of iPSC for conservation of species*

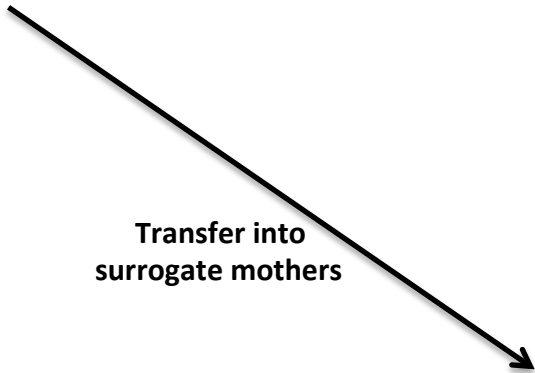


**Embryo**

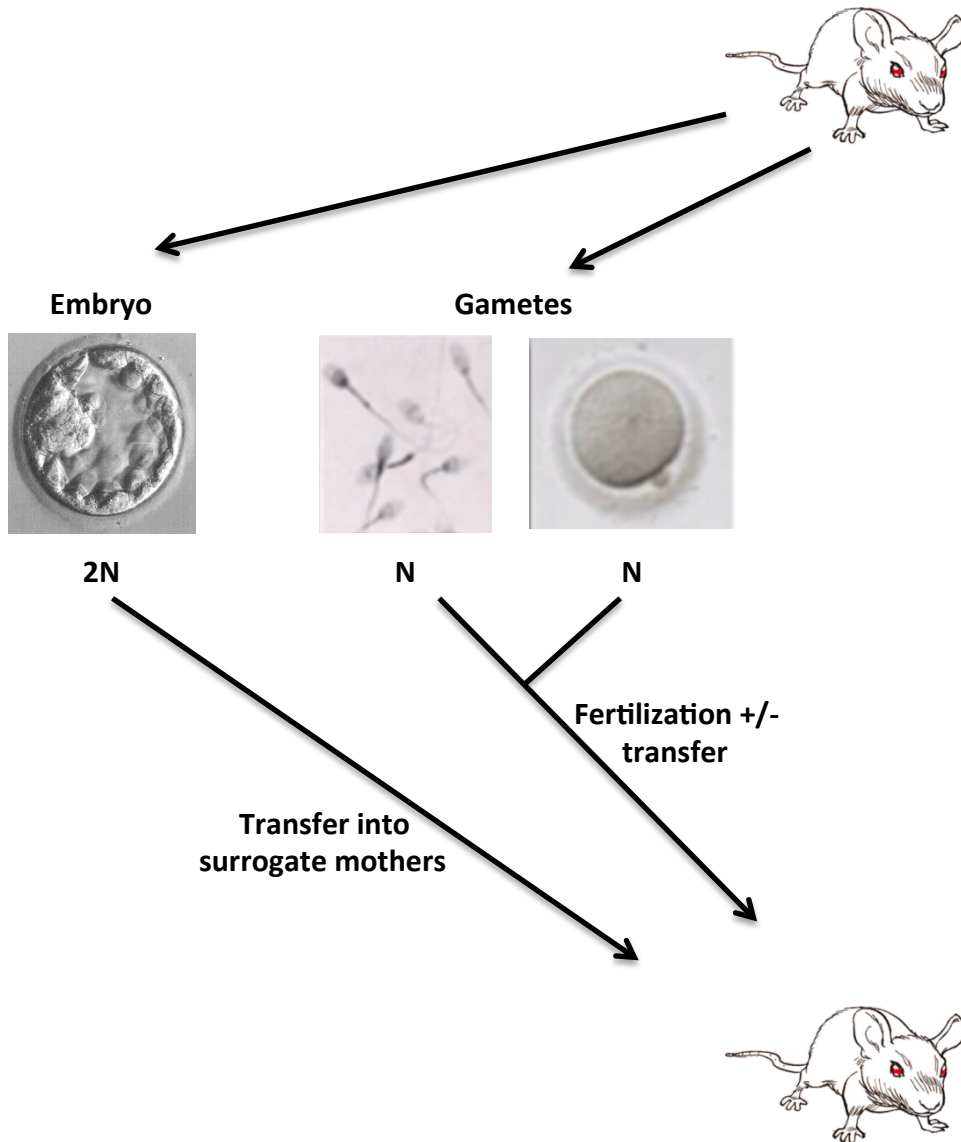


**2N**

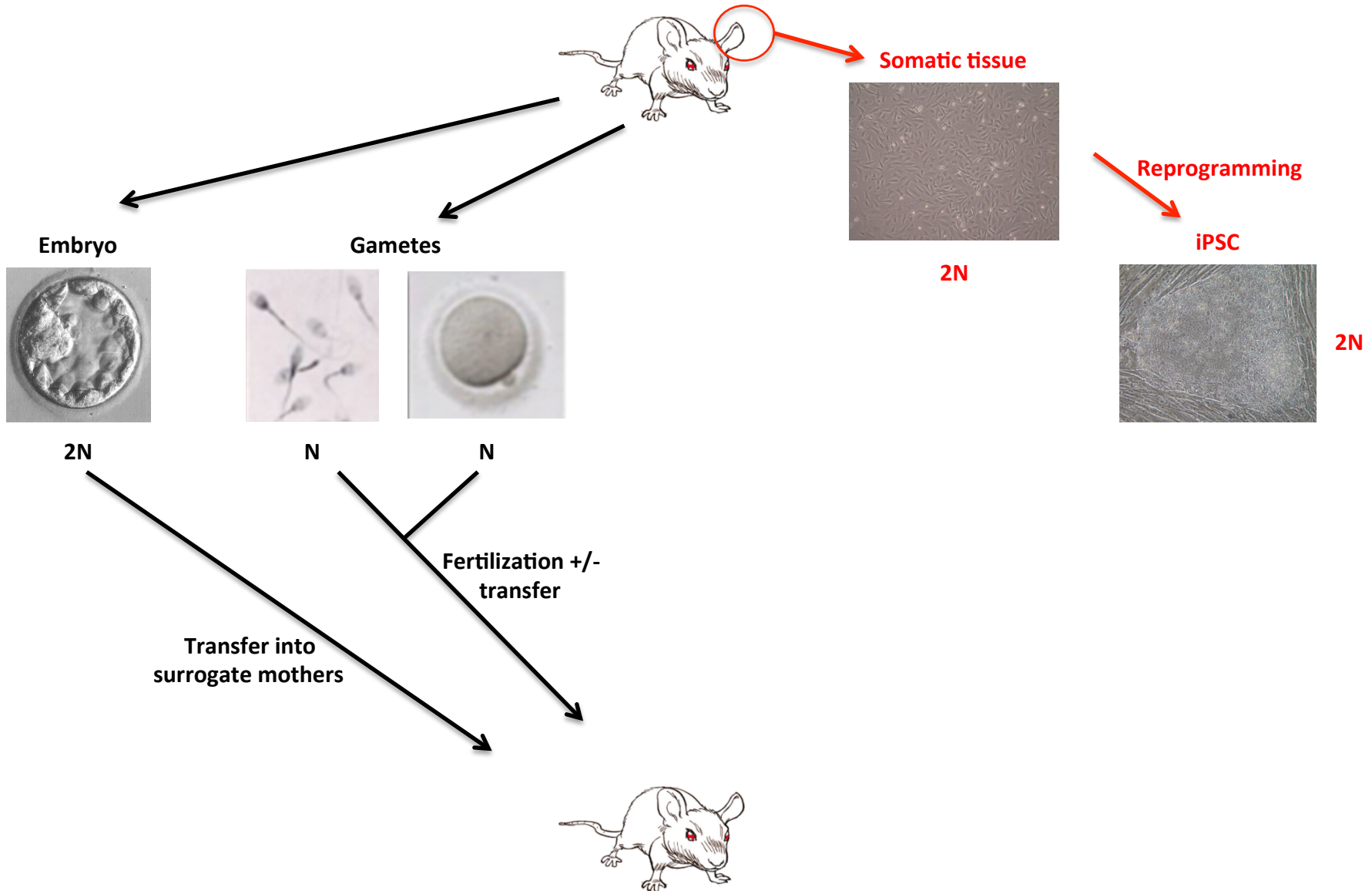
**Transfer into  
surrogate mothers**



# *Interest of iPSC for conservation of species*

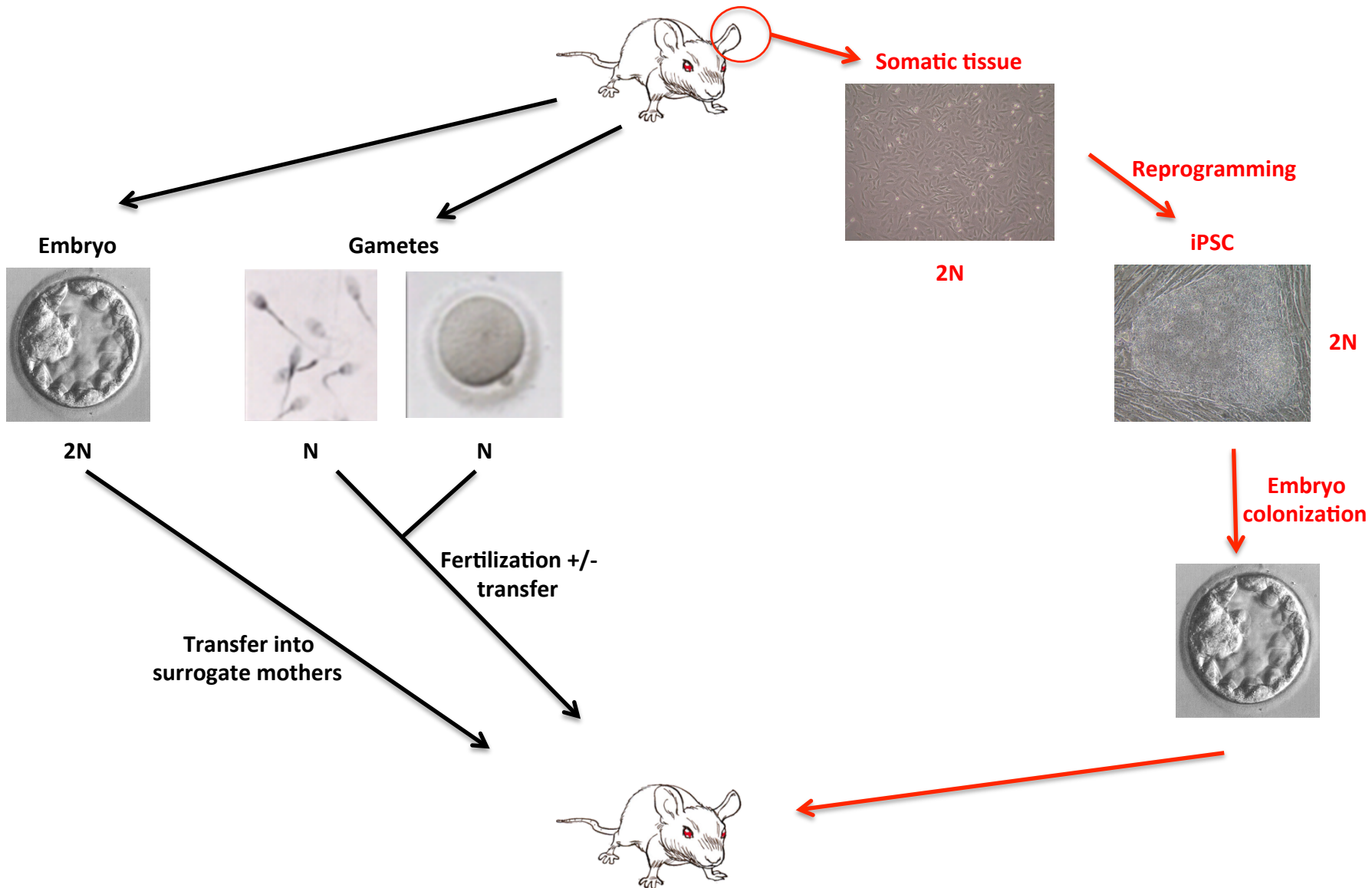


# *Interest of iPSC for conservation of species*

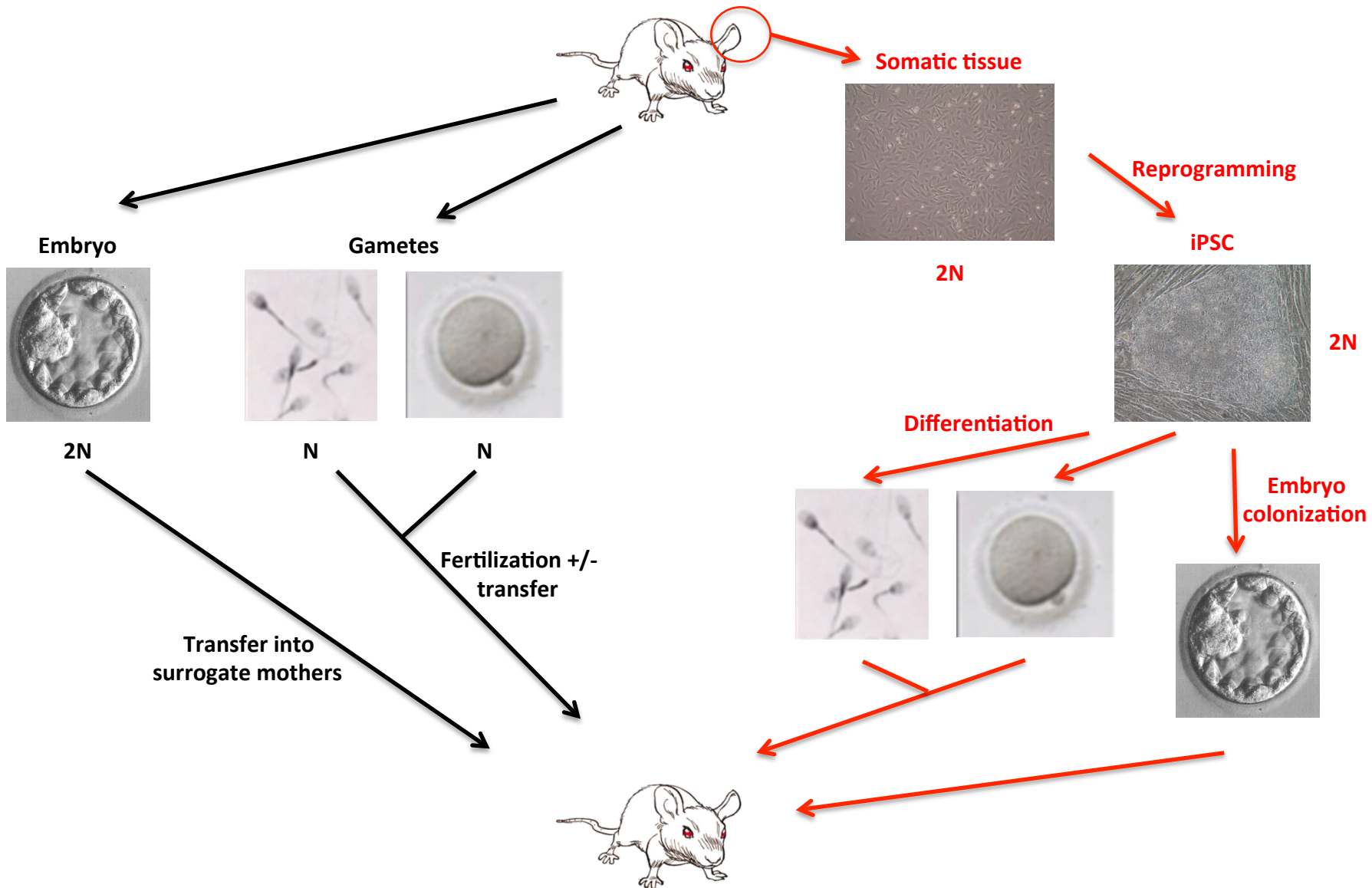




# *Interest of iPSC for conservation of species*



# *Interest of iPSC for conservation of species*



# *Reprogramming somatic cells into rabbit iPSC*

## ✓ *Advantages of the rabbit ?*

small animal, easy and cheap to breed

prolific animal with a short reproductive interval

physiologically and genetically closer to other mammals

# *Reprogramming somatic cells into rabbit iPSC*

## ✓ *Advantages of the rabbit ?*

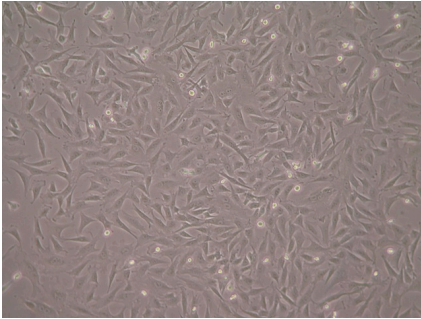
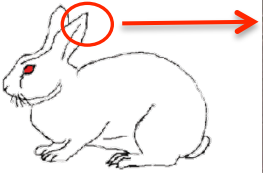
small animal, easy and cheap to breed  
prolific animal with a short reproductive interval  
physiologically and genetically closer to other mammals

## ✓ *Development of rabbit cryobanking*

iPSC technology is available for rabbit  
determination of somatic tissues/cells to be used  
establishment of cryoconservation conditions  
adaptation of reprogramming techniques

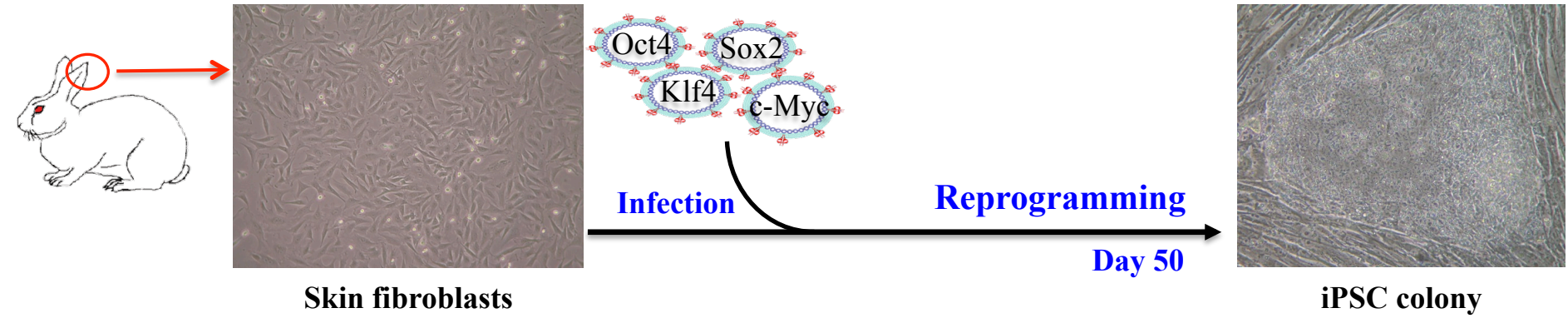
## ✓ *Good model for farm animals*

# *Rabbit iPSC*

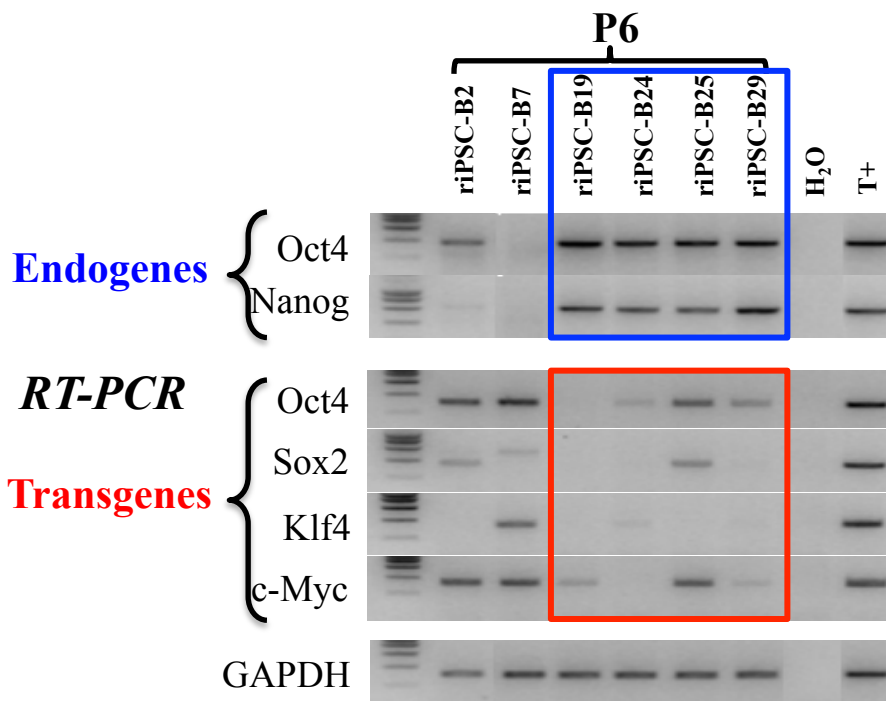
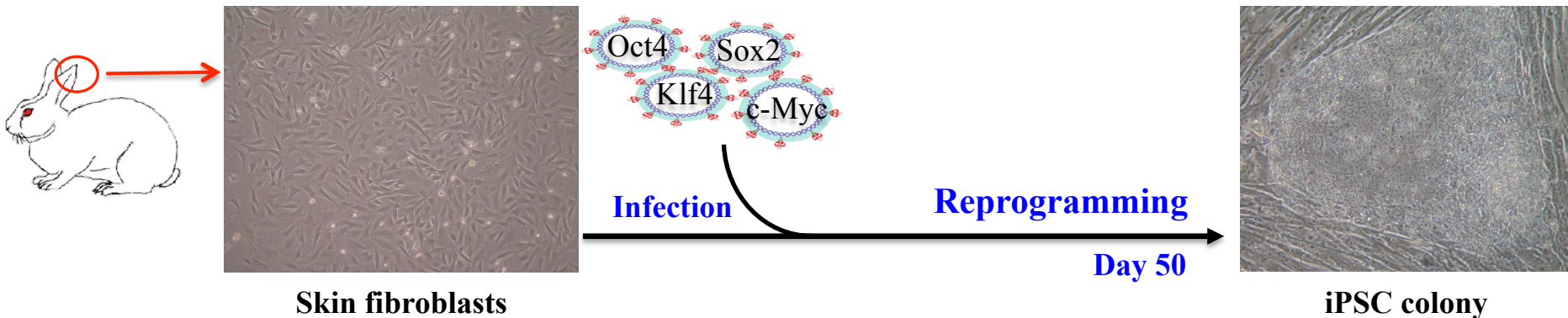


**Skin fibroblasts**

# *Rabbit iPSC*



# Rabbit iPSC

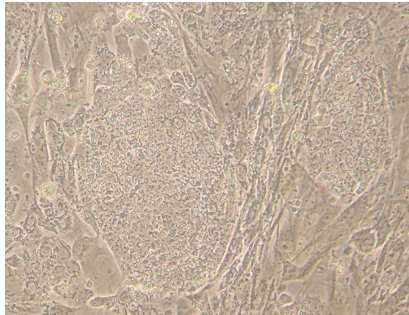






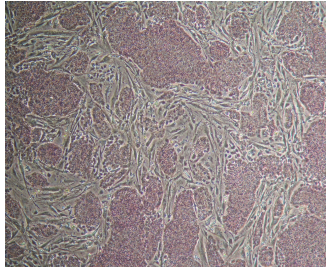


# *Characterization of rabbit iPSC*



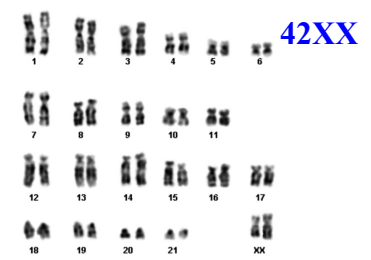
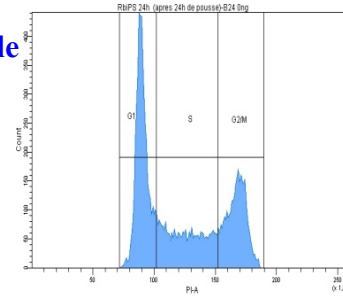
**Rabbit iPSC**

# Characterization of rabbit iPSC

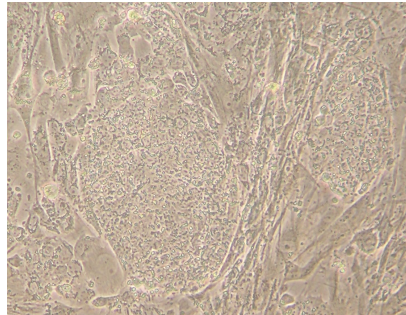


AP activity

Cell cycle

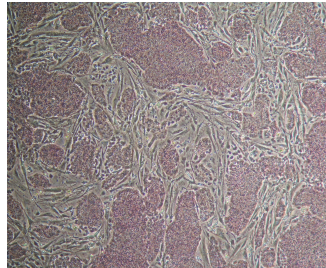


**Self-renewal**



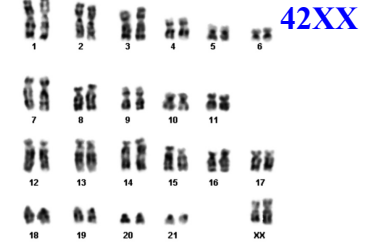
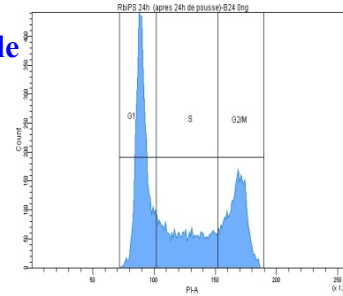
Rabbit iPSC

# Characterization of rabbit iPSC

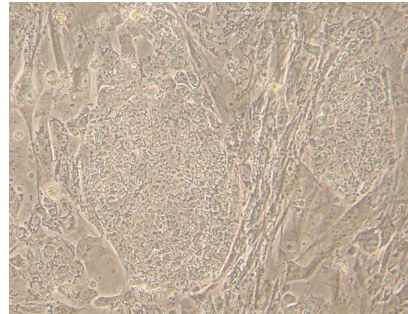


AP activity

Cell cycle



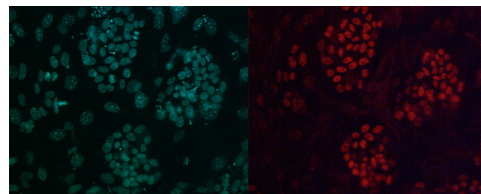
Self-renewal



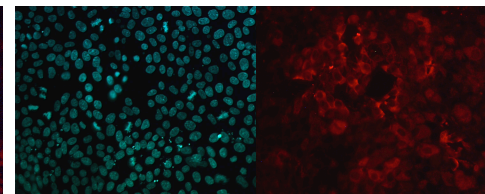
Rabbit iPSC



Pluripotency markers

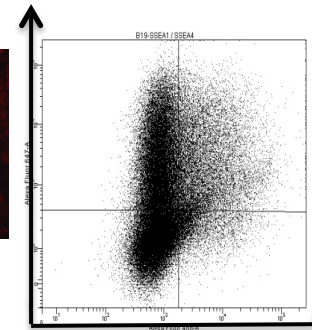


Oct4



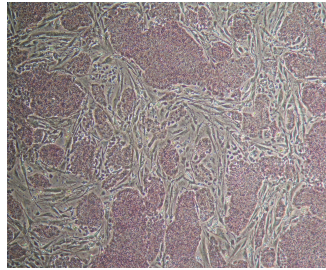
E-Cadherin

SSEA1



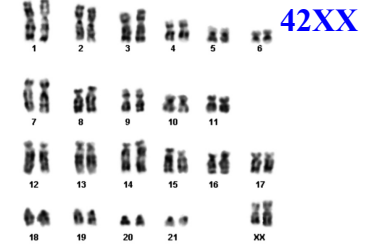
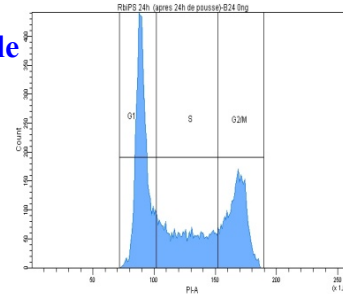
SSEA4

# Characterization of rabbit iPSC

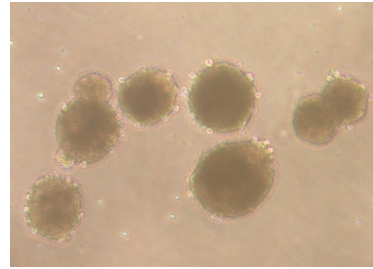


AP activity

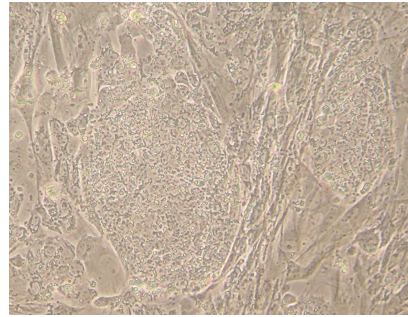
Cell cycle



Self-renewal



Embryoid bodies



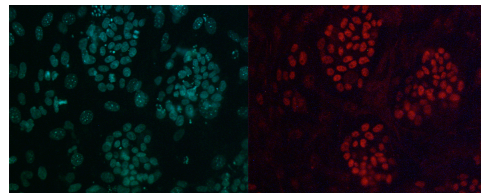
Rabbit iPSC

Differentiation and oncogenesis

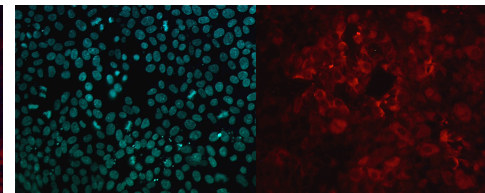


Teratoma

Pluripotency markers

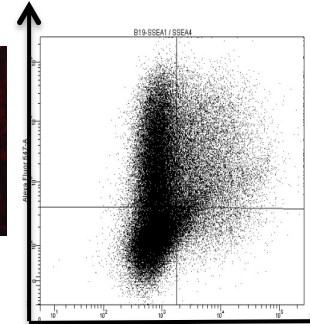


Oct4



E-Cadherin

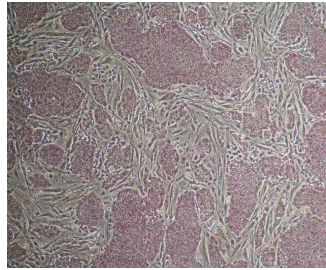
SSEA1



SSEA4

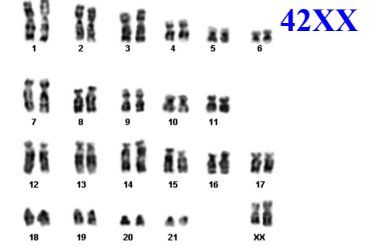
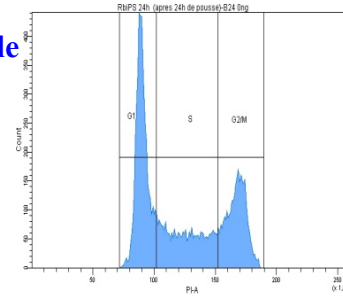


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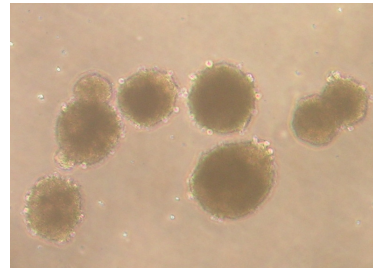


AP activity

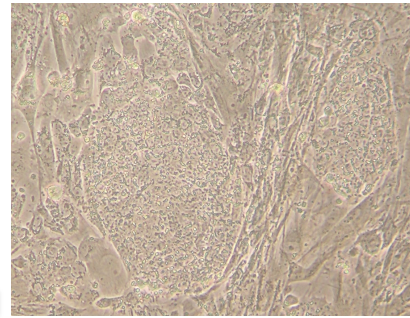
Cell cycle



Self-renewal

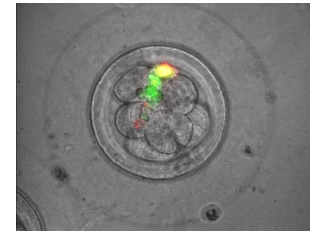


Embryoid bodies

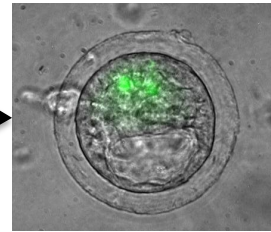


Rabbit iPSC

Embryo colonization ?



8-cell stage embryo



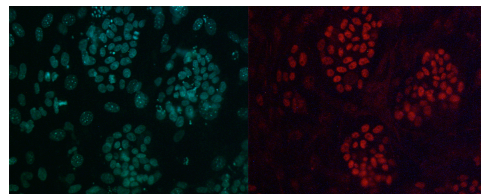
Blastocyst

Differentiation and oncogenesis

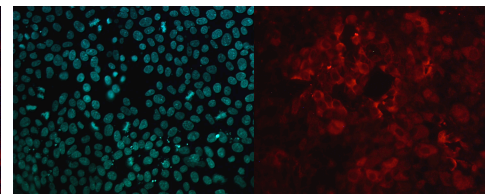


Teratoma

Pluripotency markers

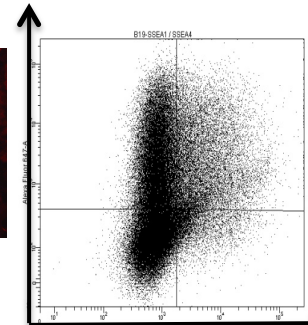


Oct4



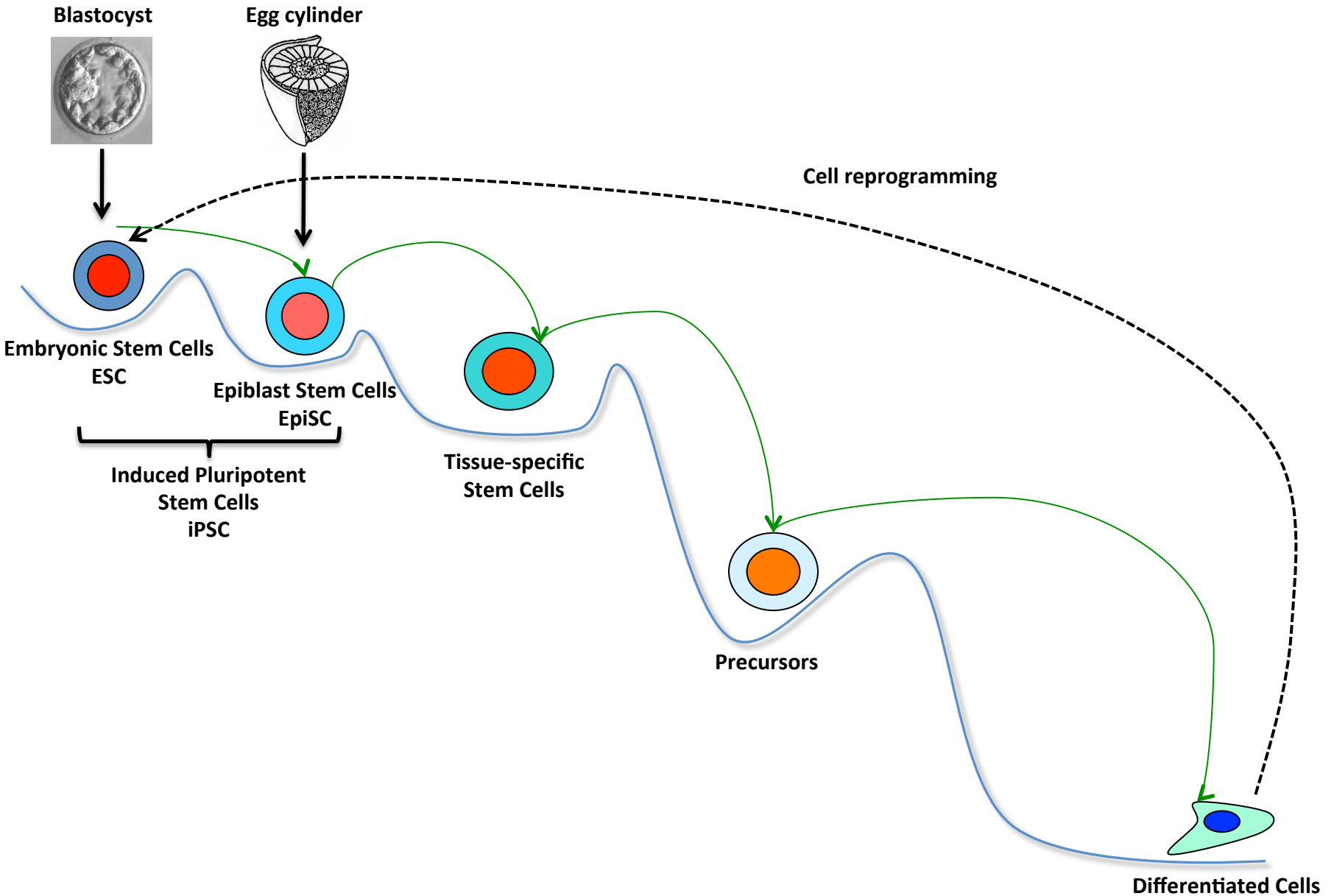
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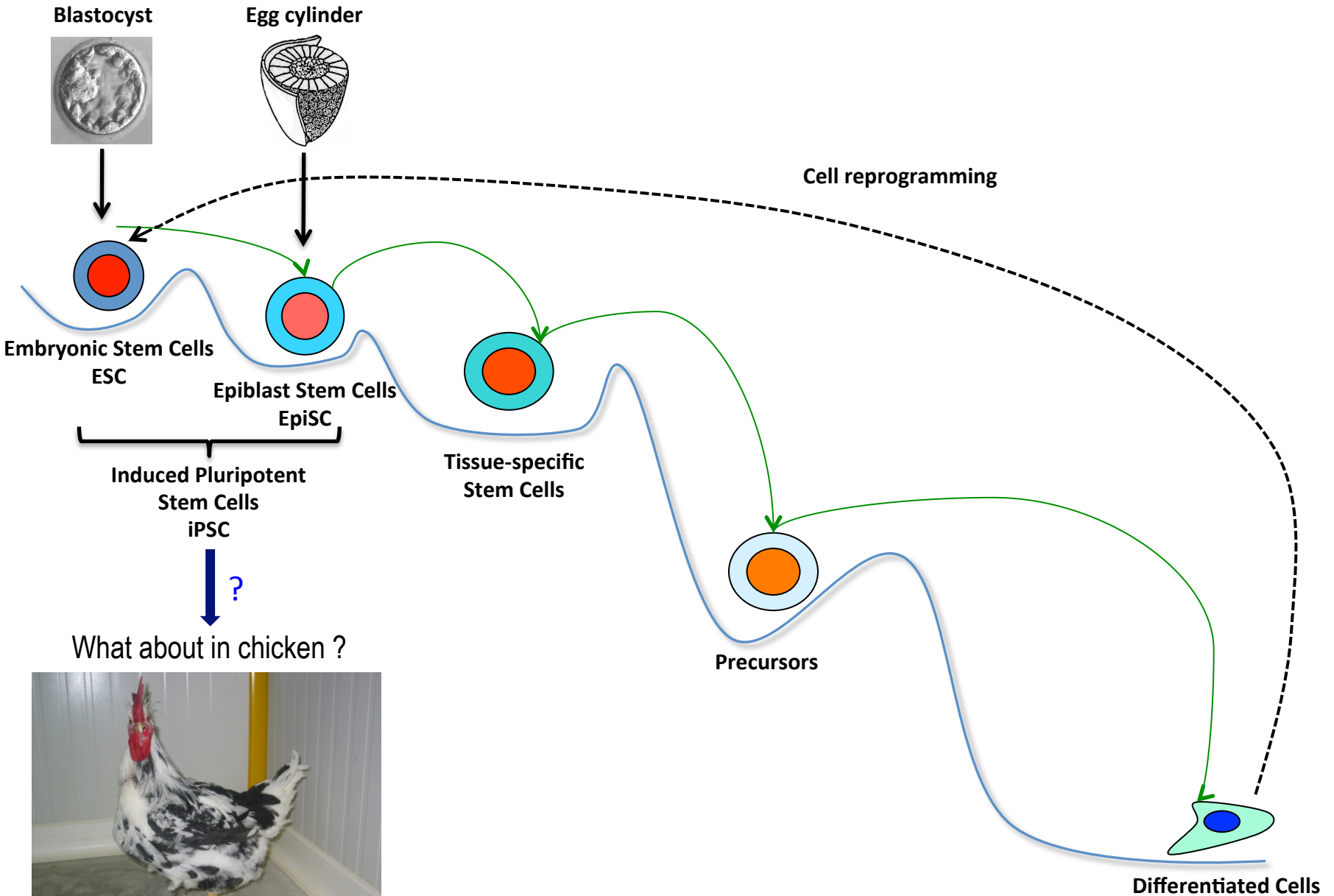


SSEA4

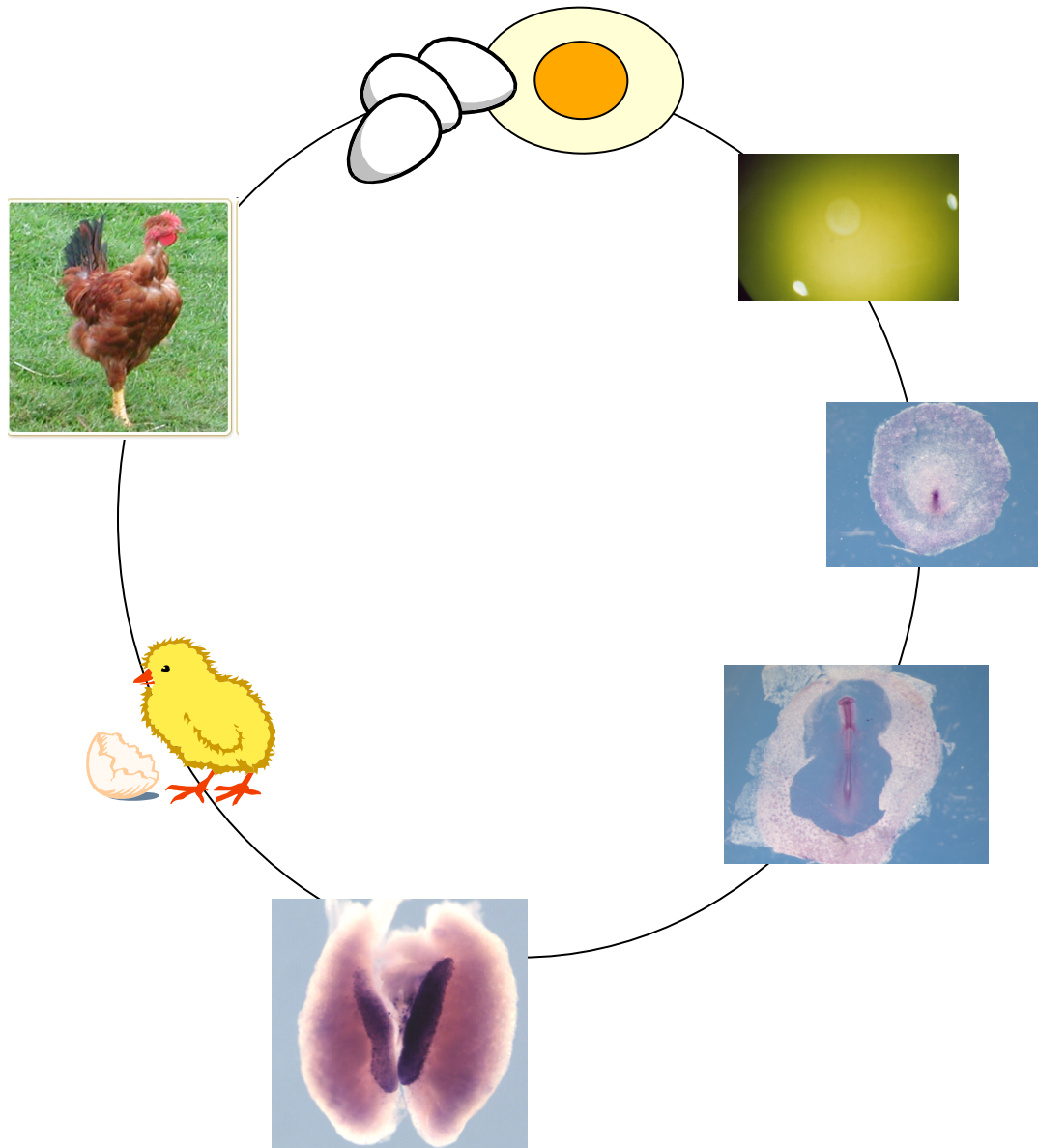
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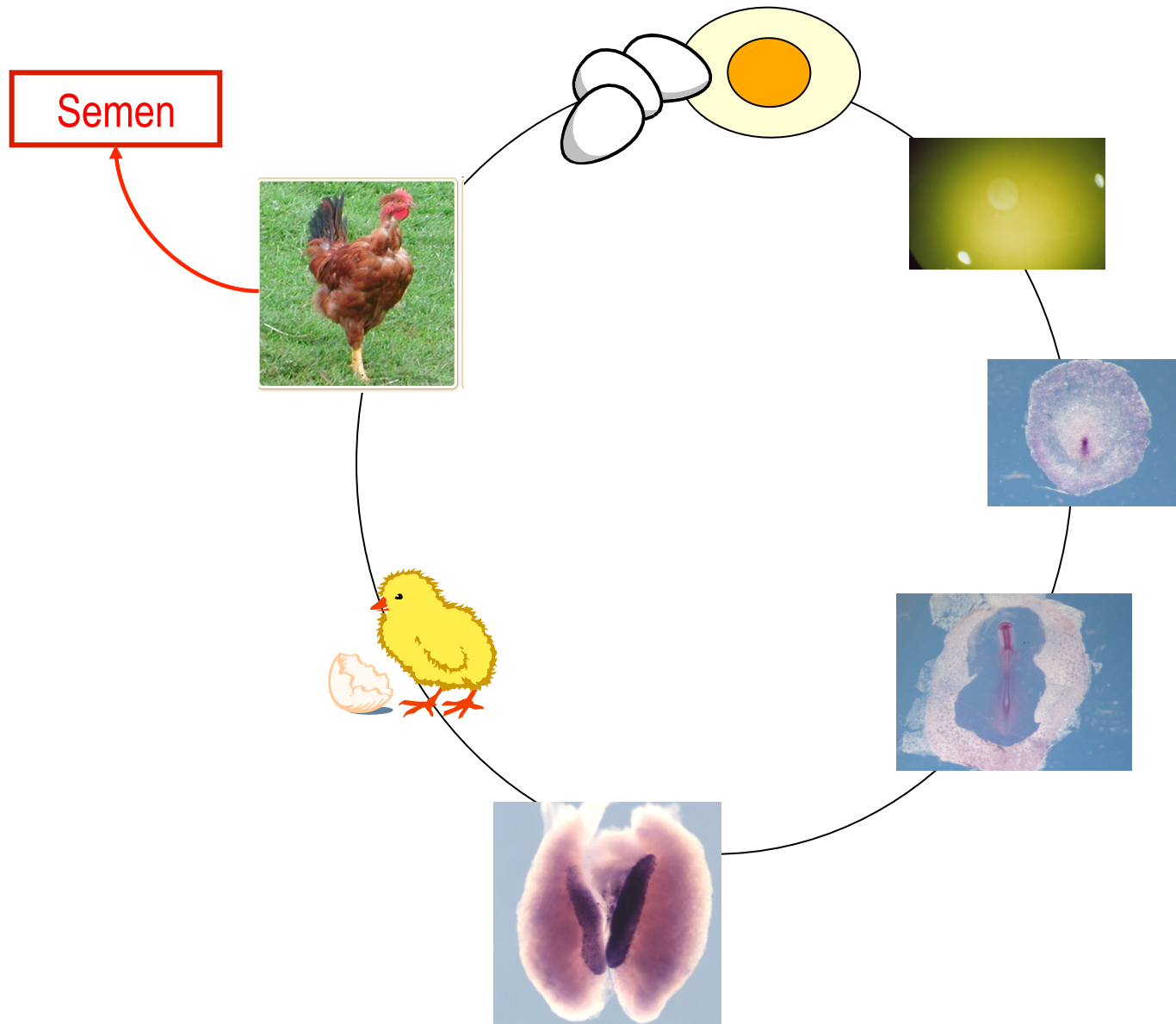


# *Stem cells in chicken*

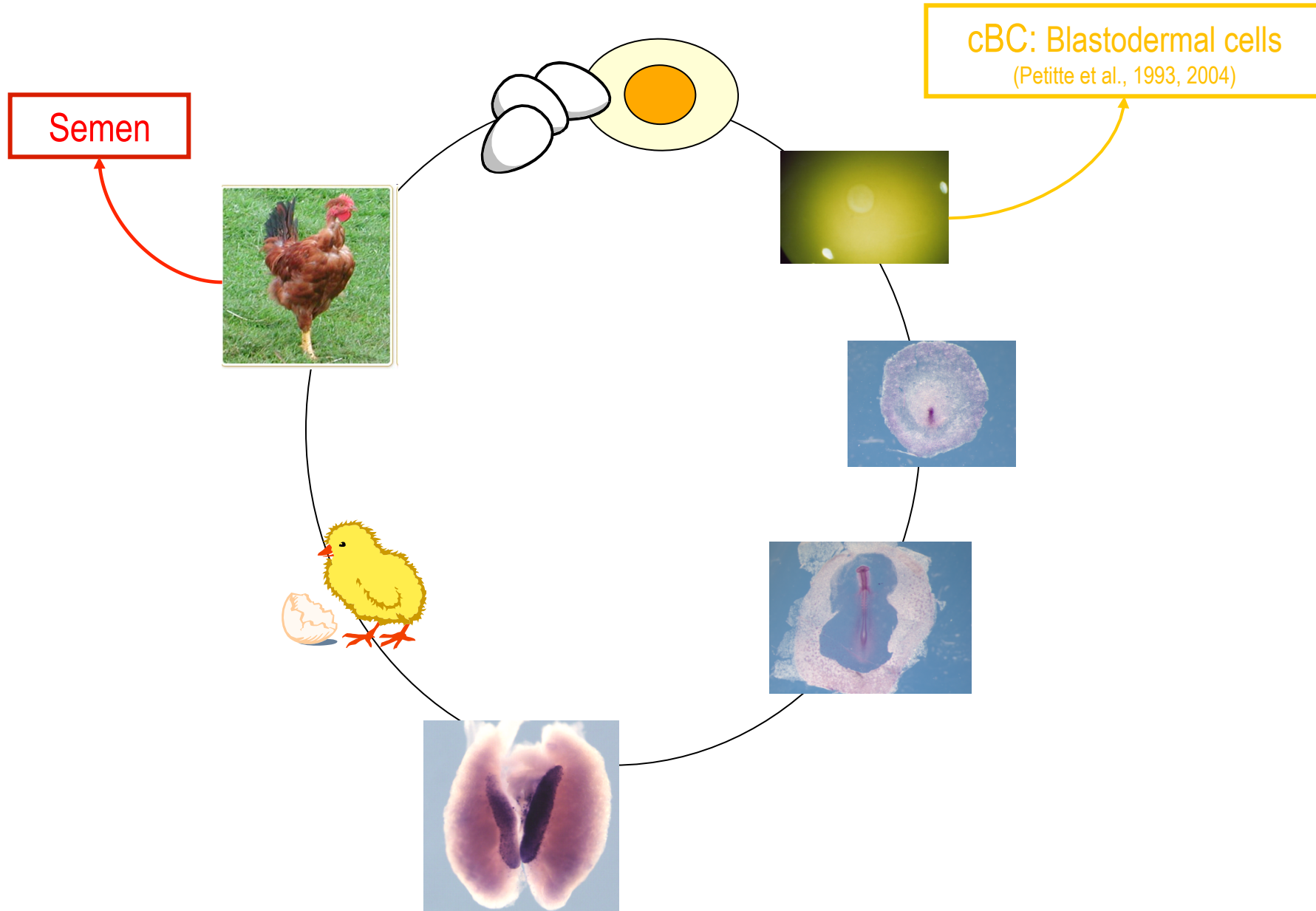




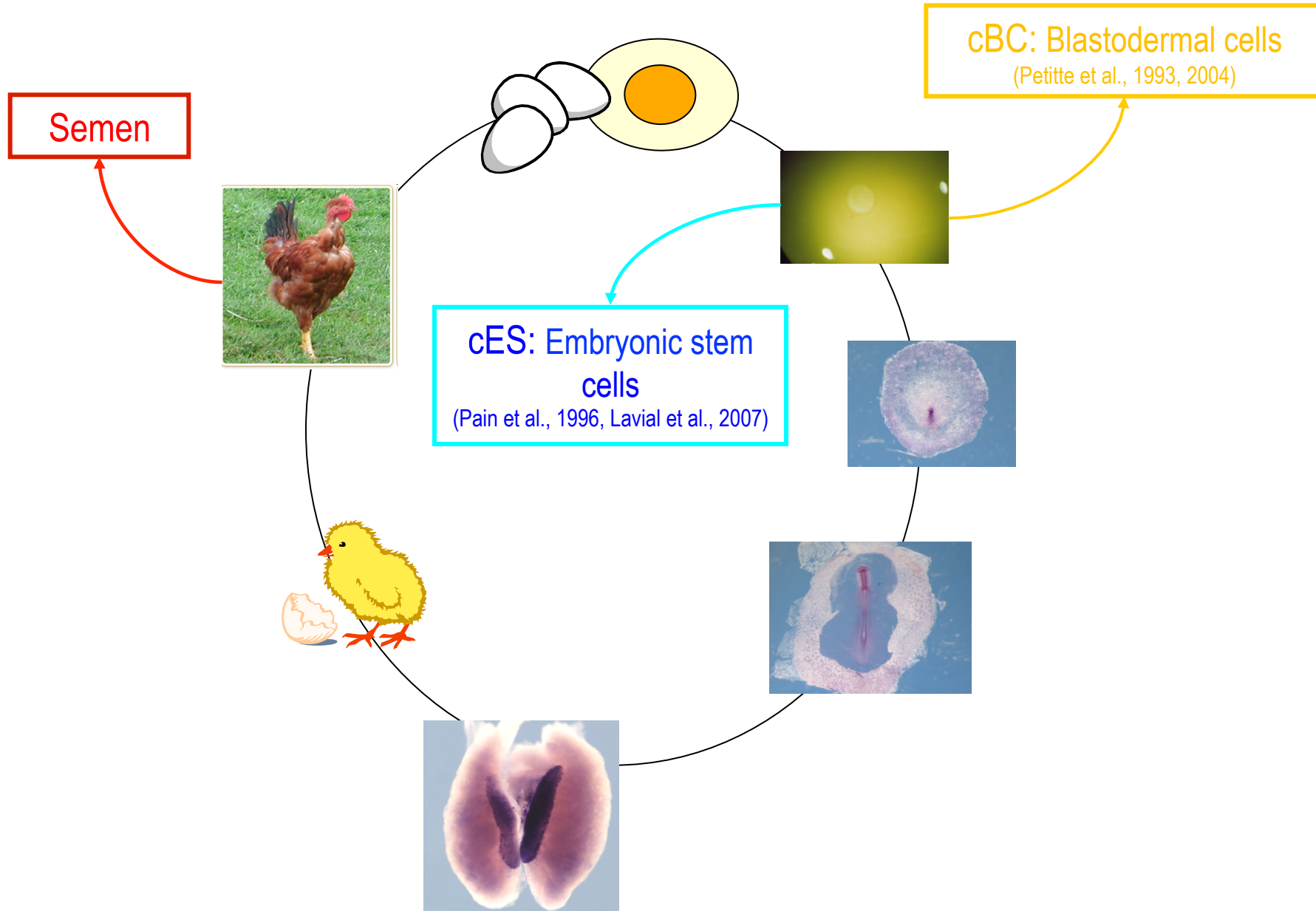
# *Stem cells in chicken*



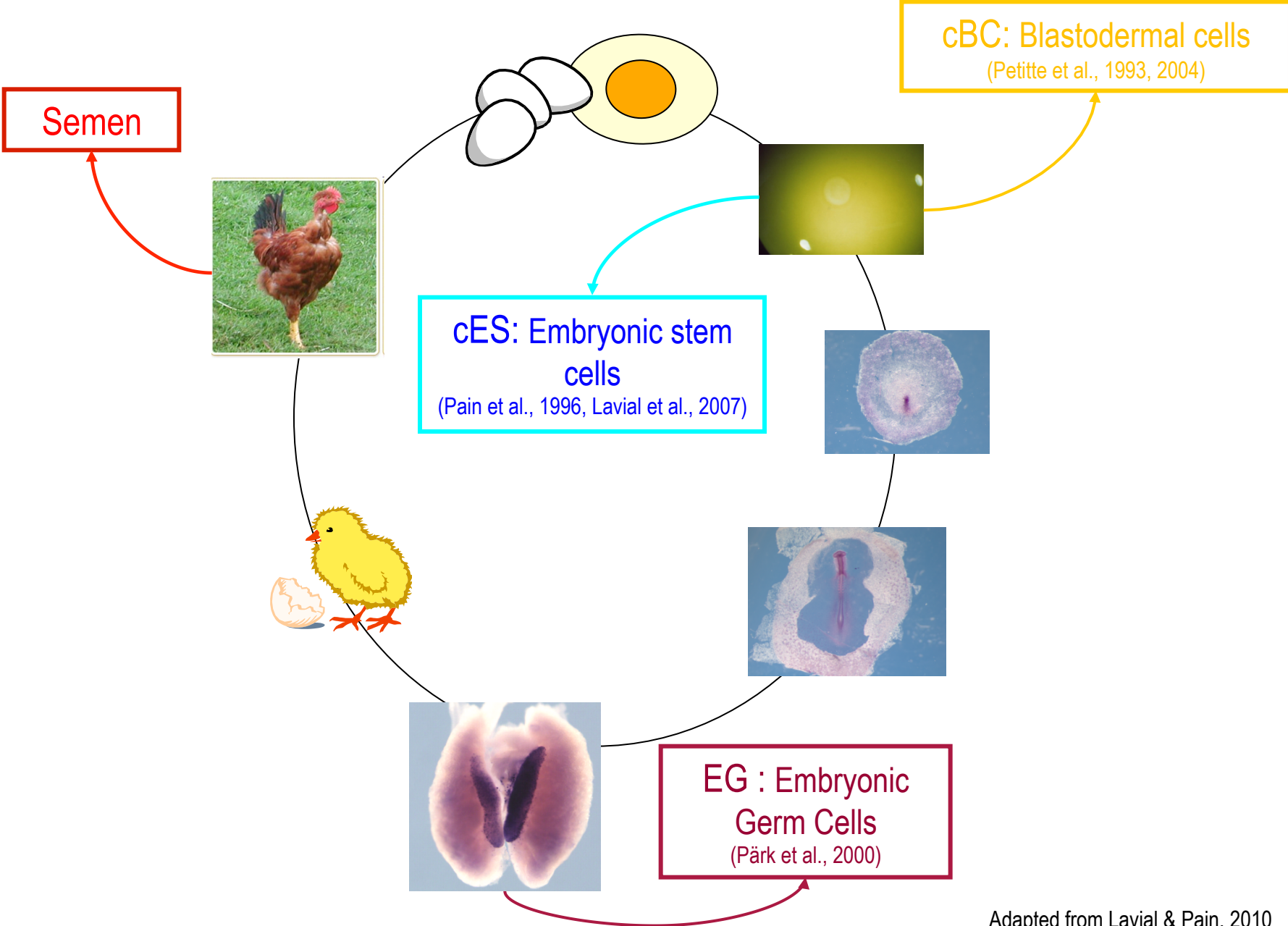
# Stem cells in chicken



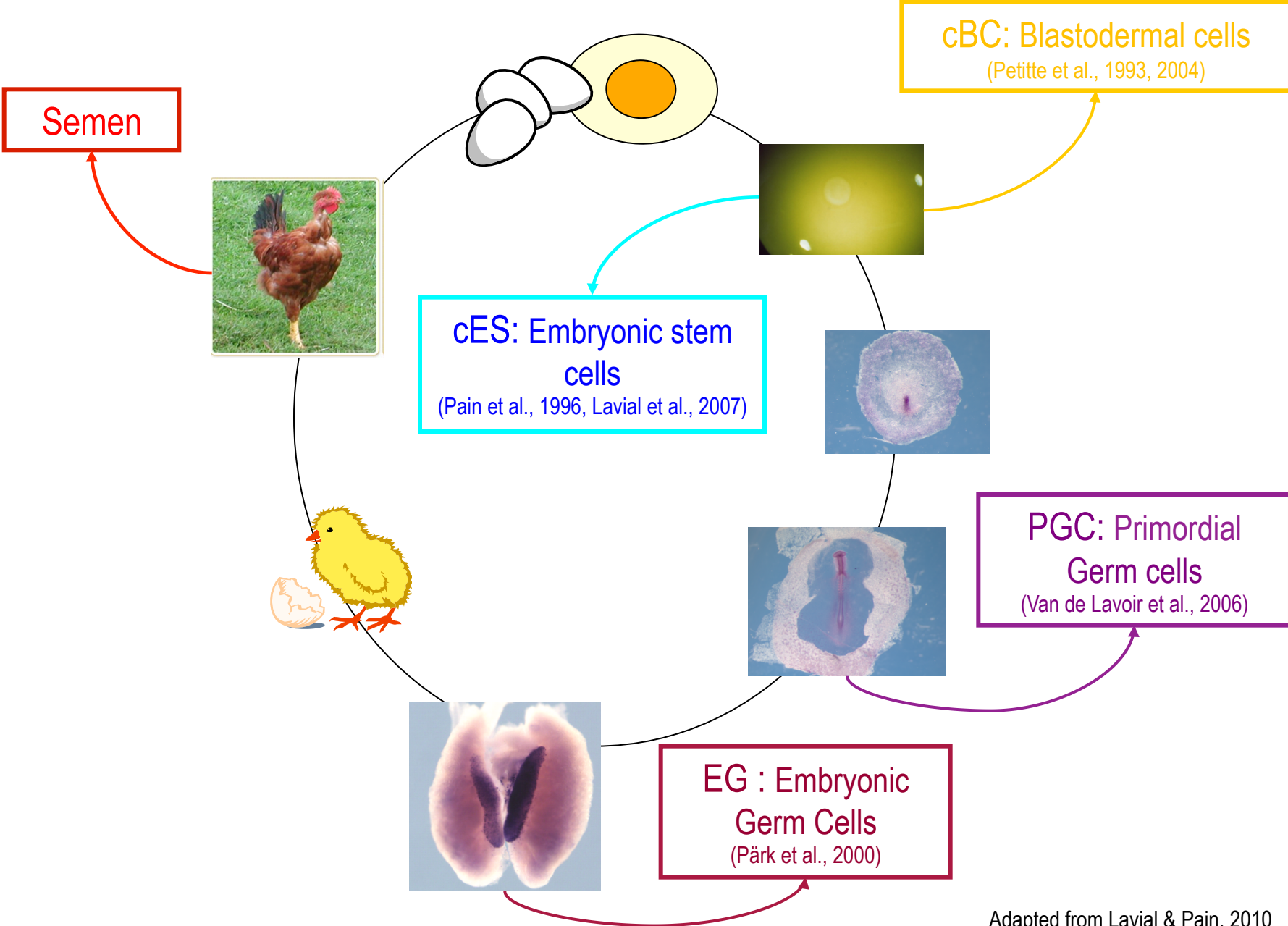
# Stem cells in chicken



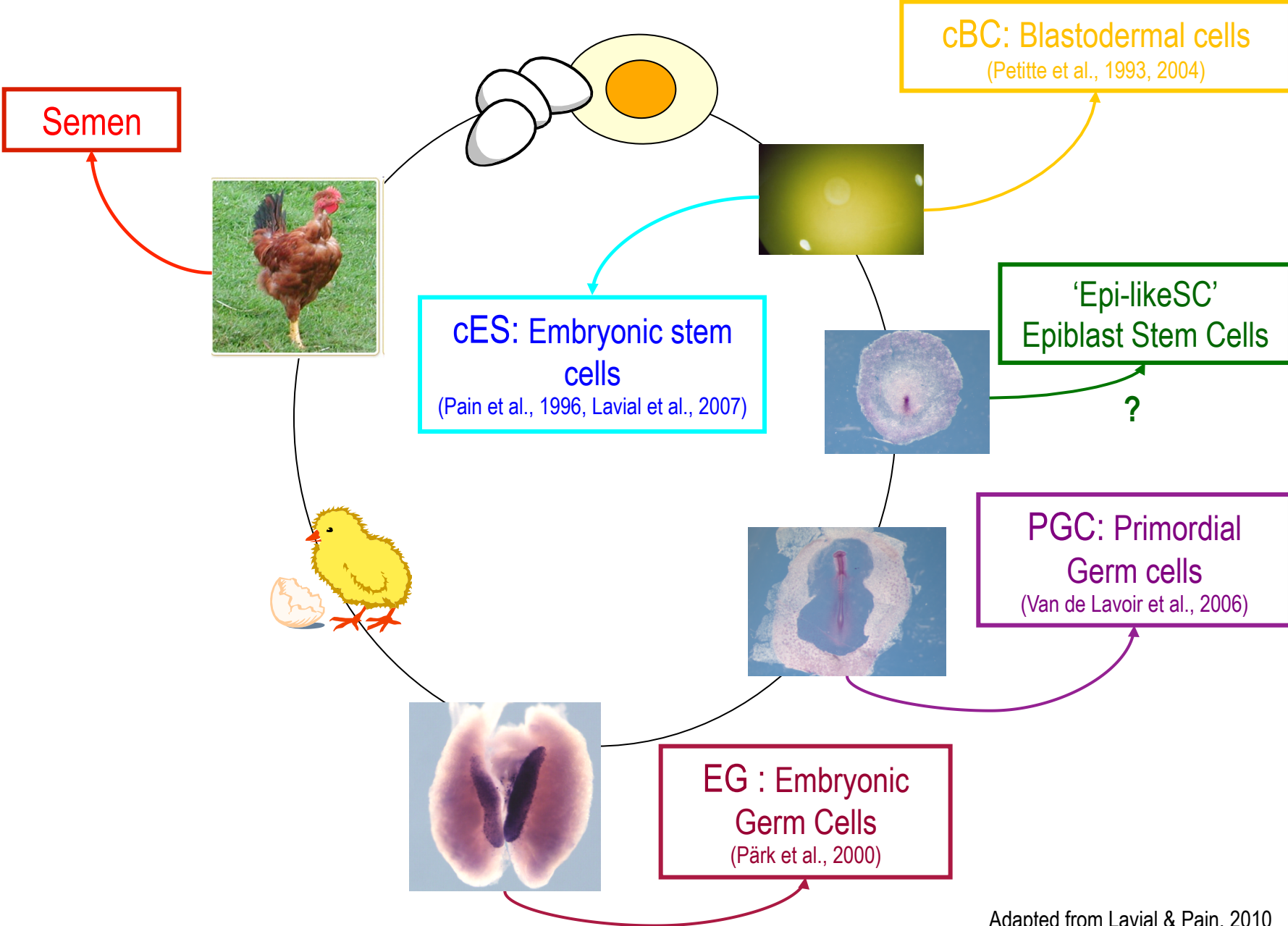
# Stem cells in chicken



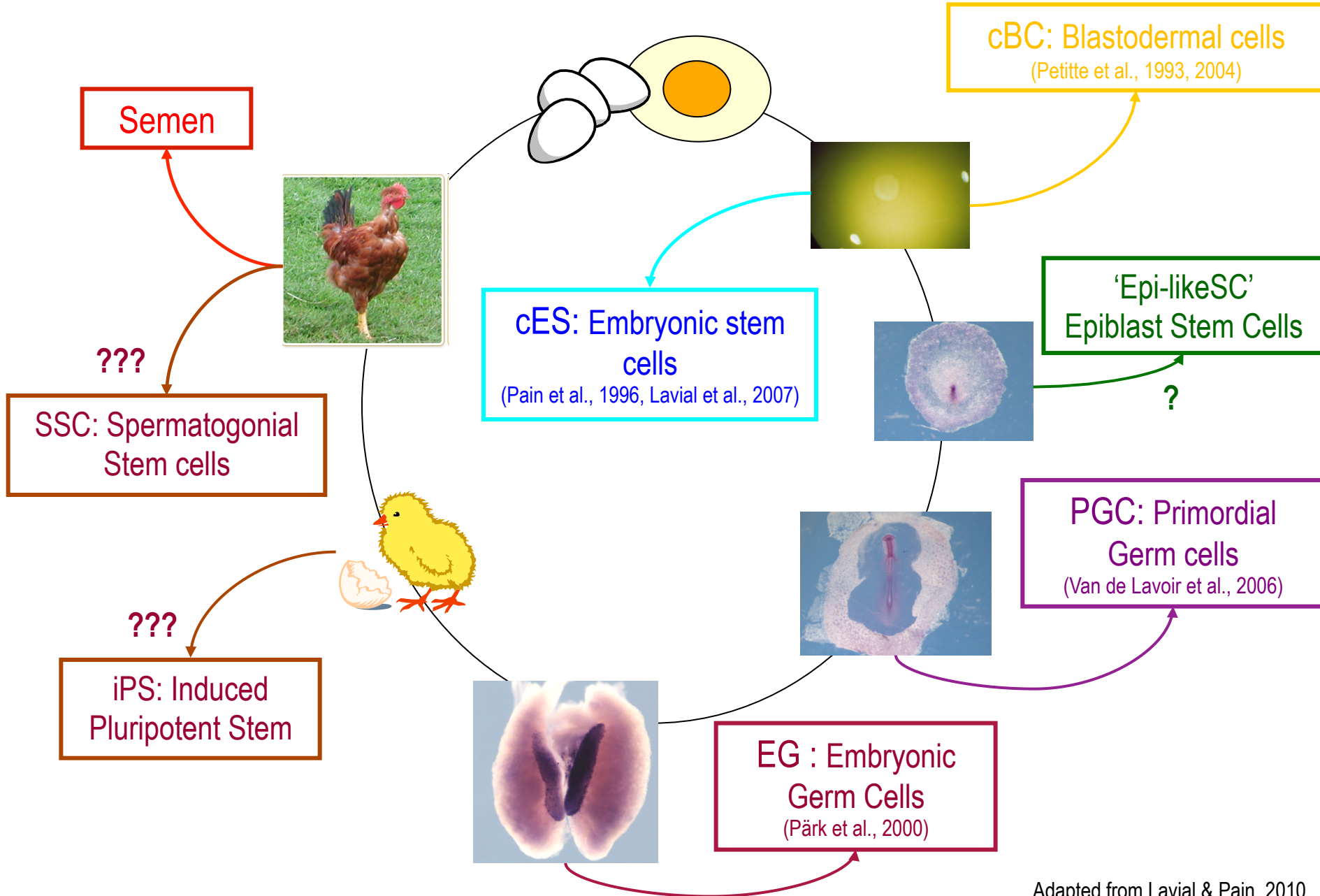
# Stem cells in chicken



# Stem cells in chicken

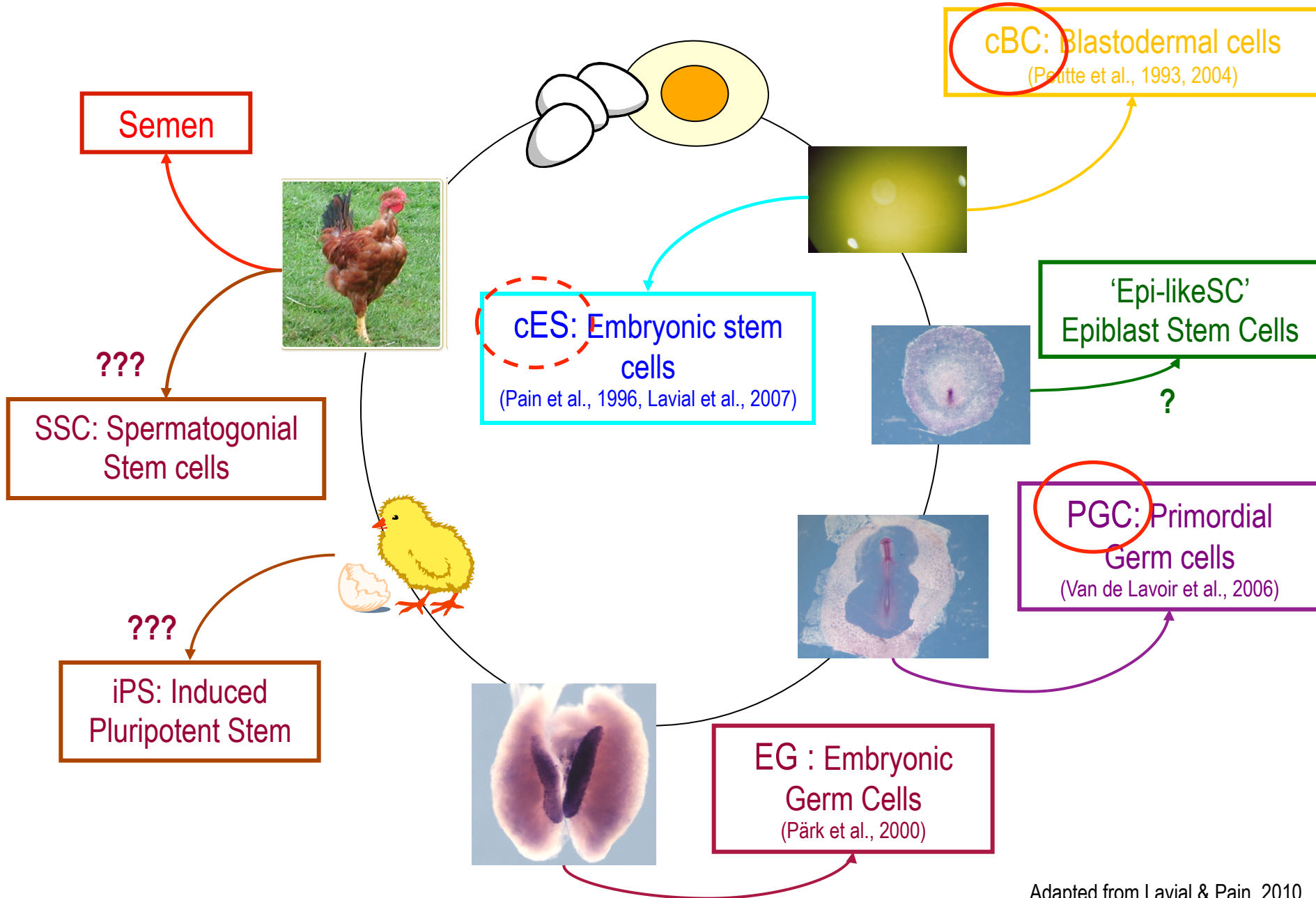


# Stem cells in chicken



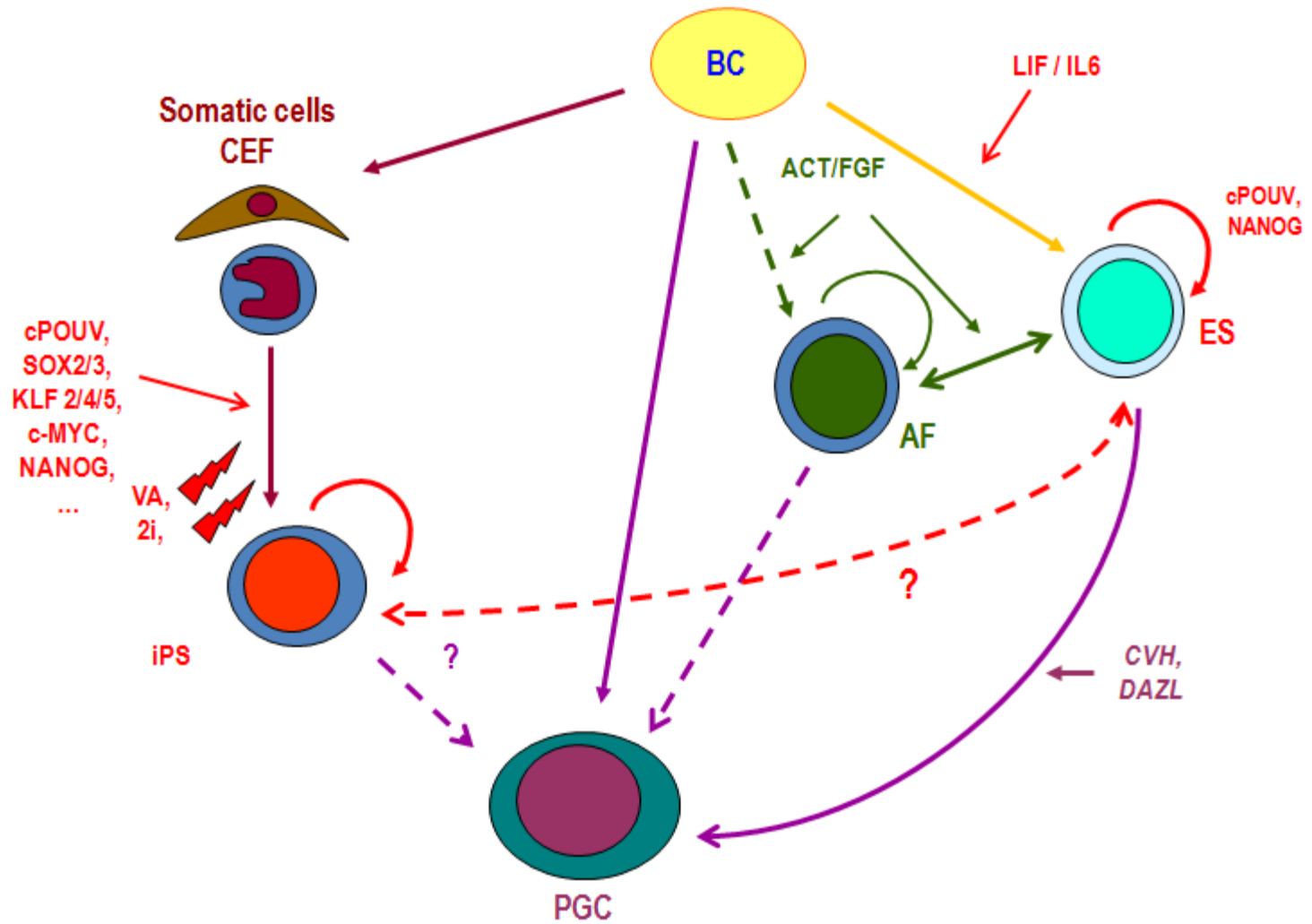


# Stem cells in chicken

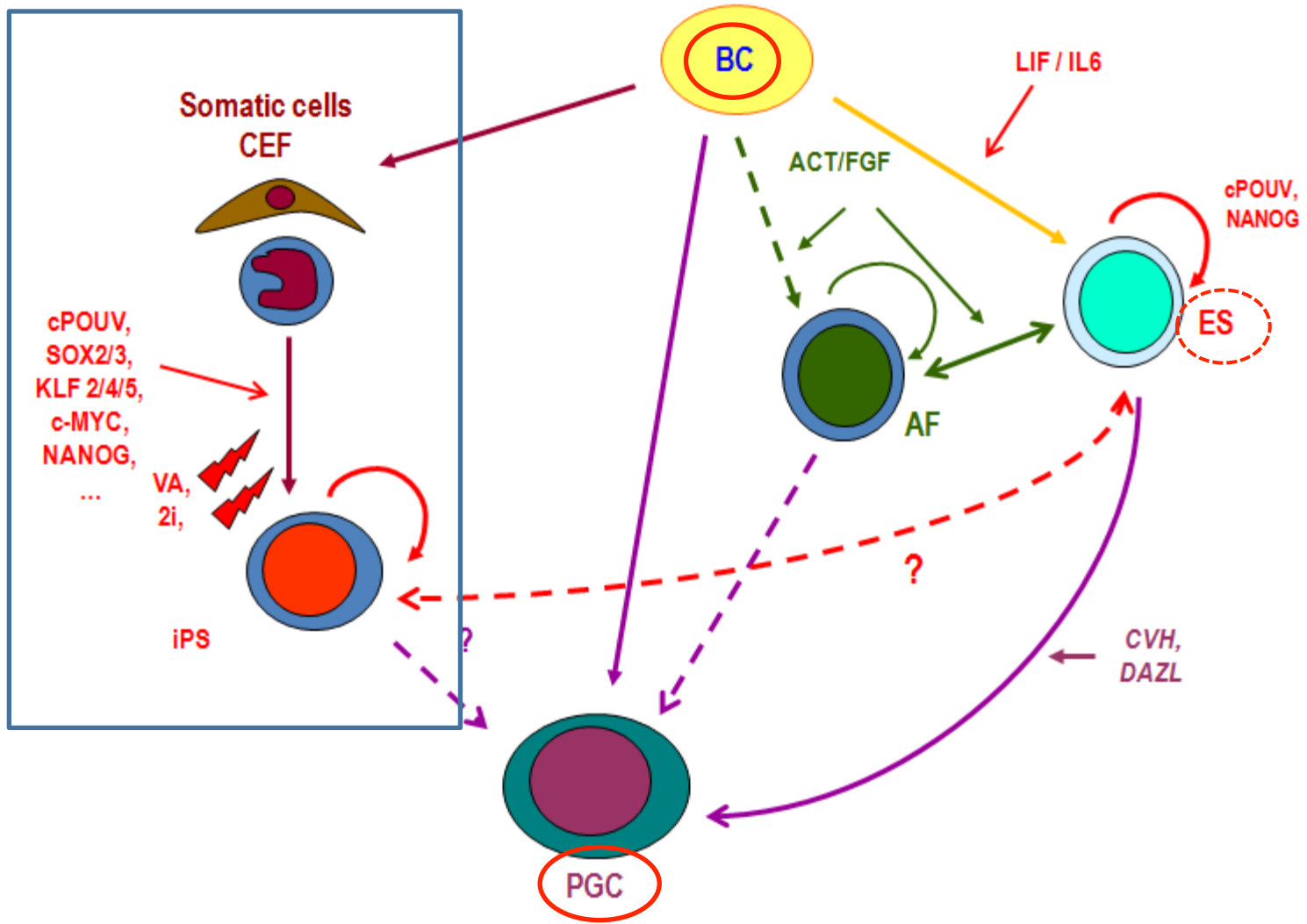




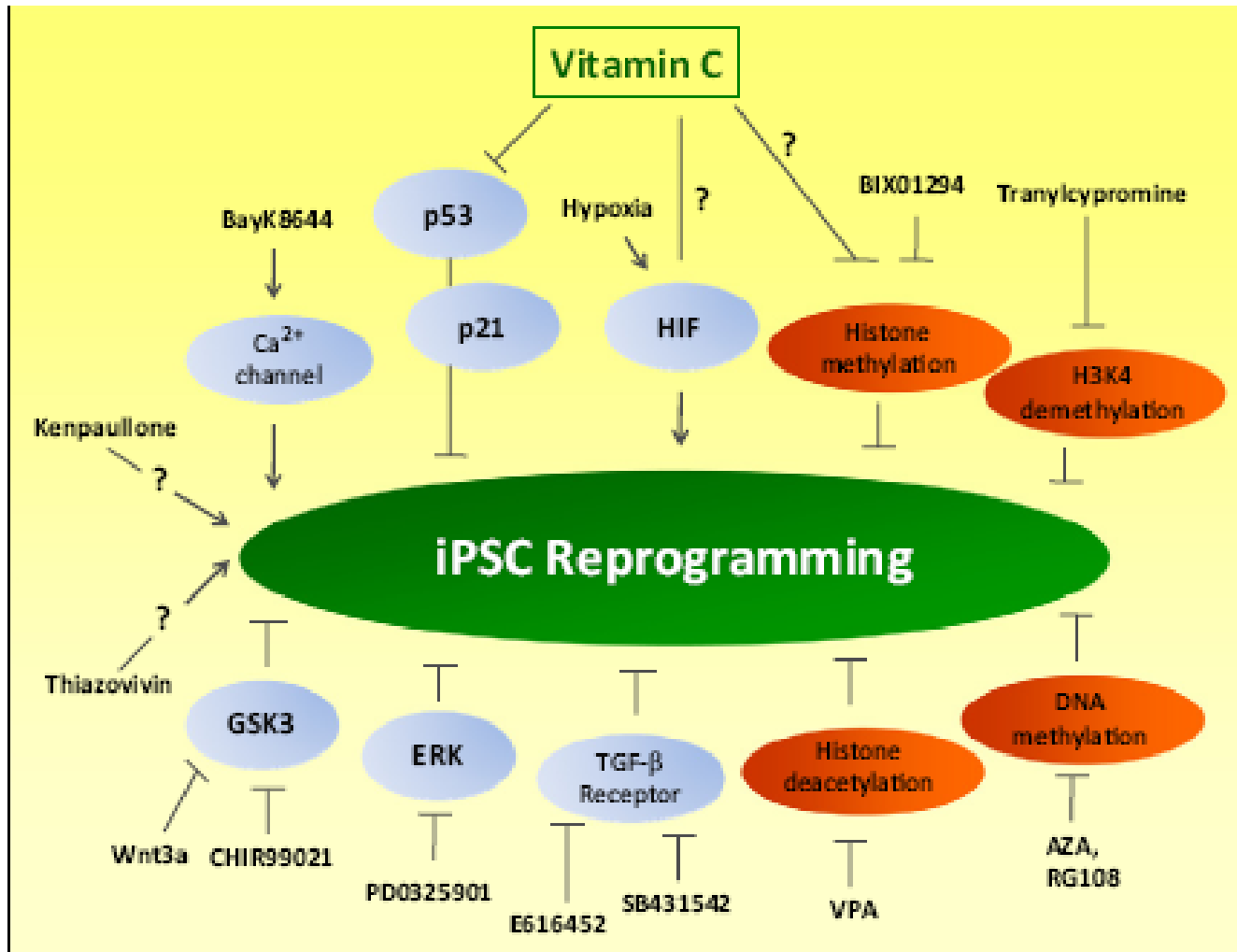
# Stem cells in chicken



# Stem cells in chicken

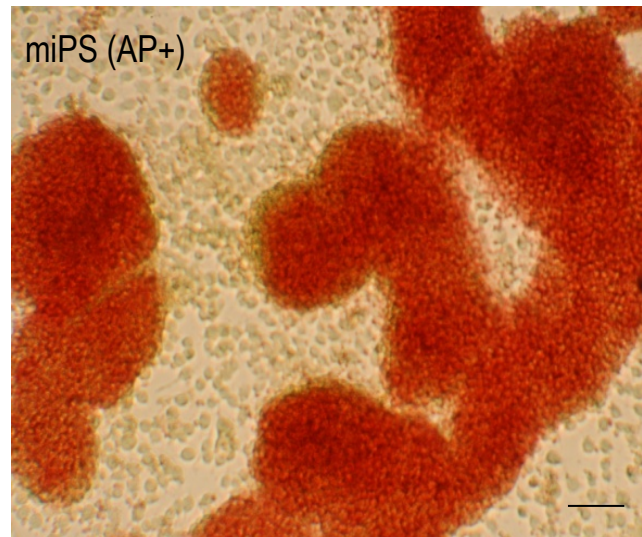
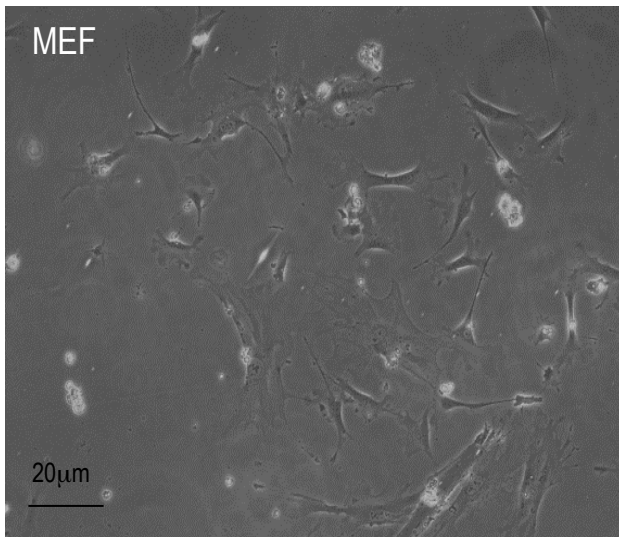
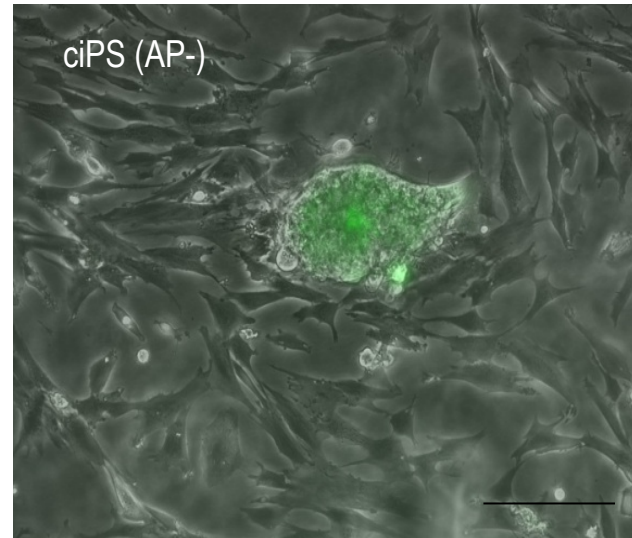
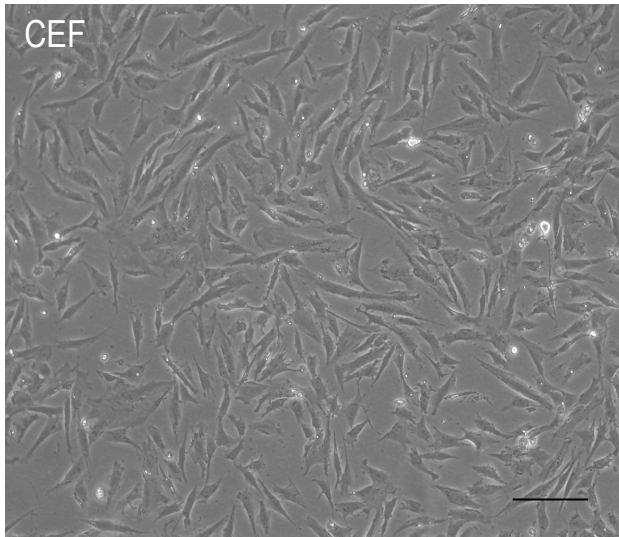


# Reprogramming: a stochastic and complex process



# *First tests*

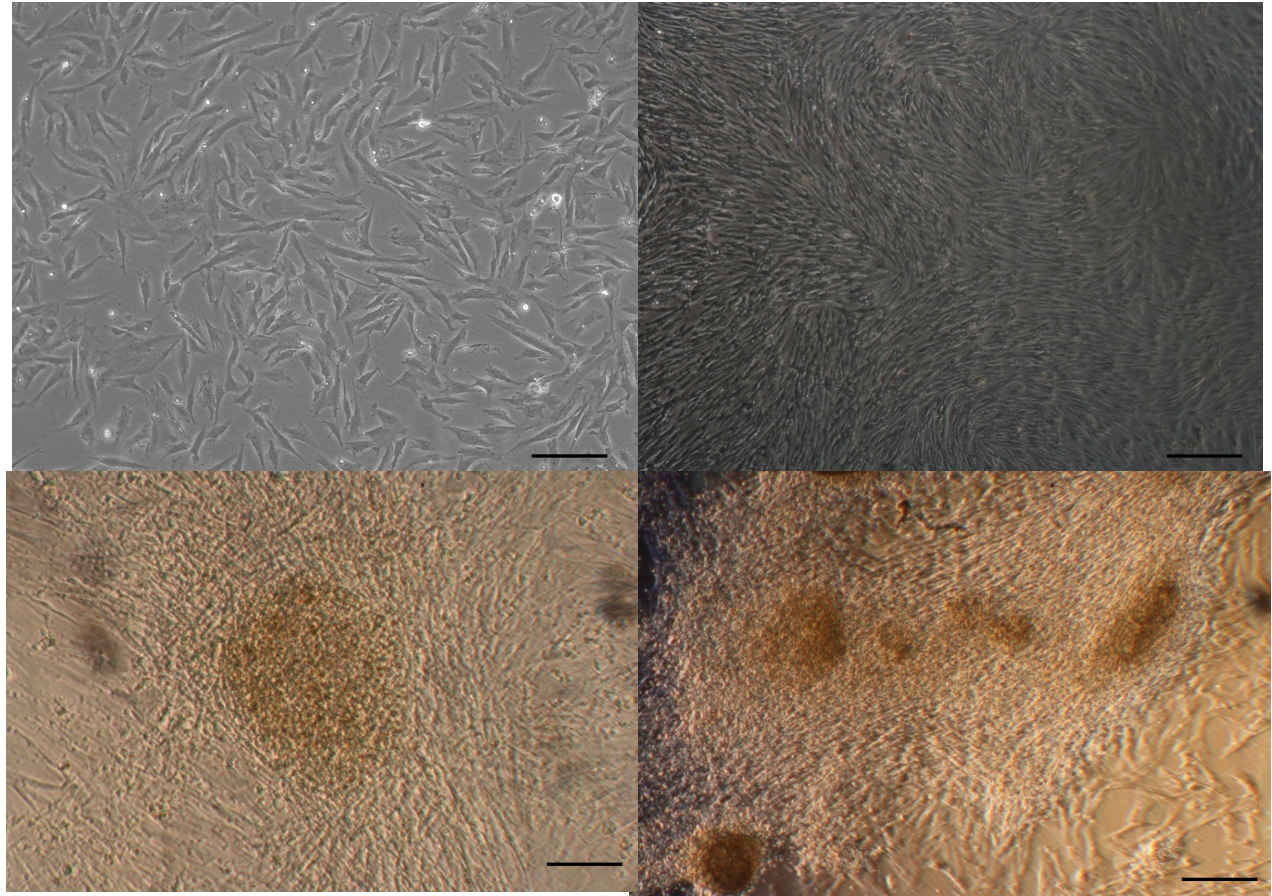
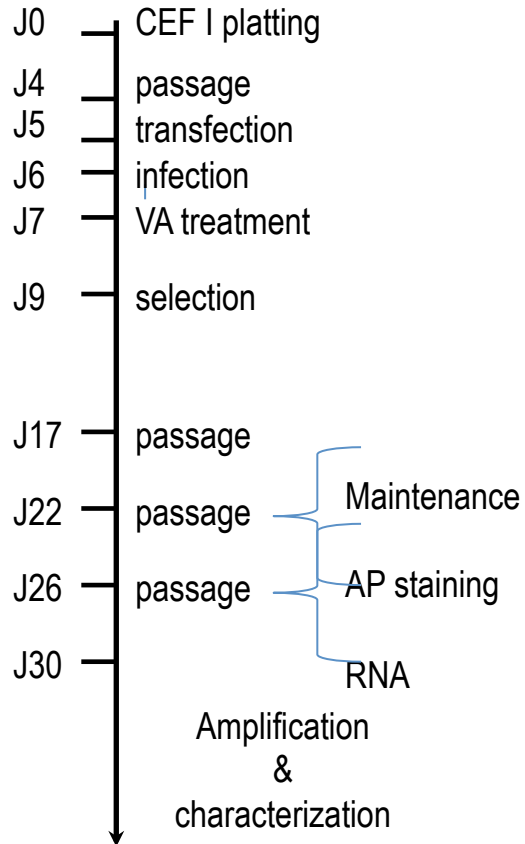
The direct infection with the polycistronic vector (pLent-KOSM) leads to small colonies unable to proliferate



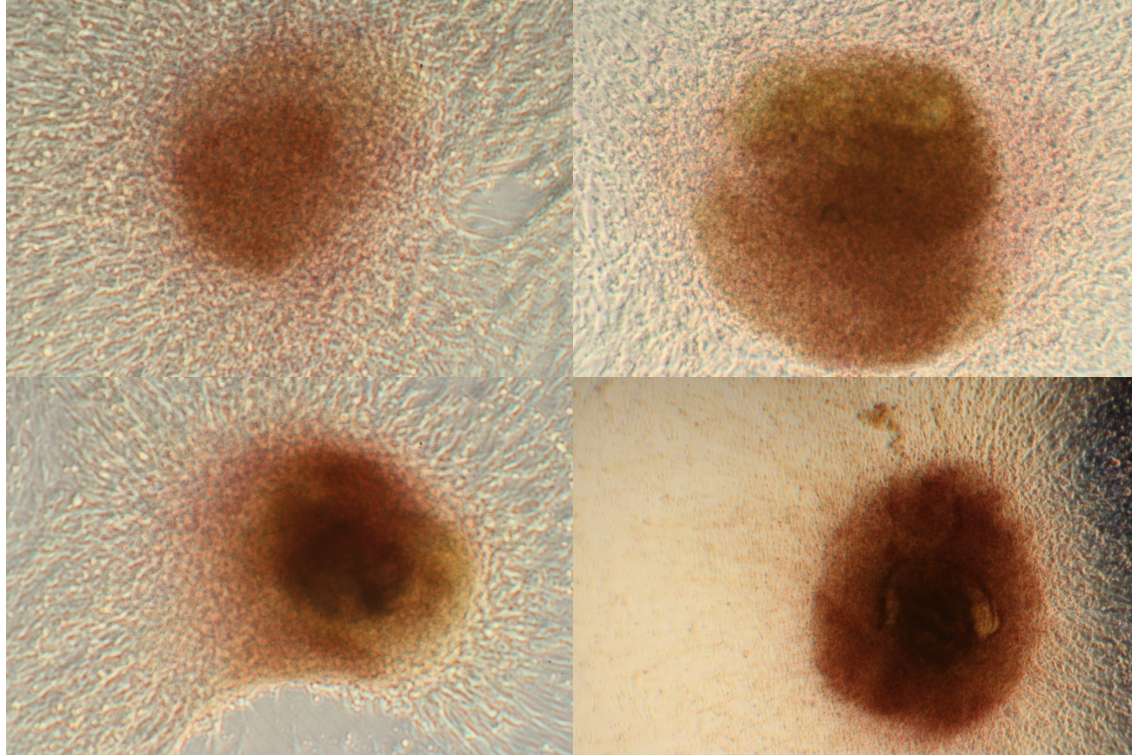


# *New strategy*

➔ Development of a two step procedure using pPB and lentiviral vectors

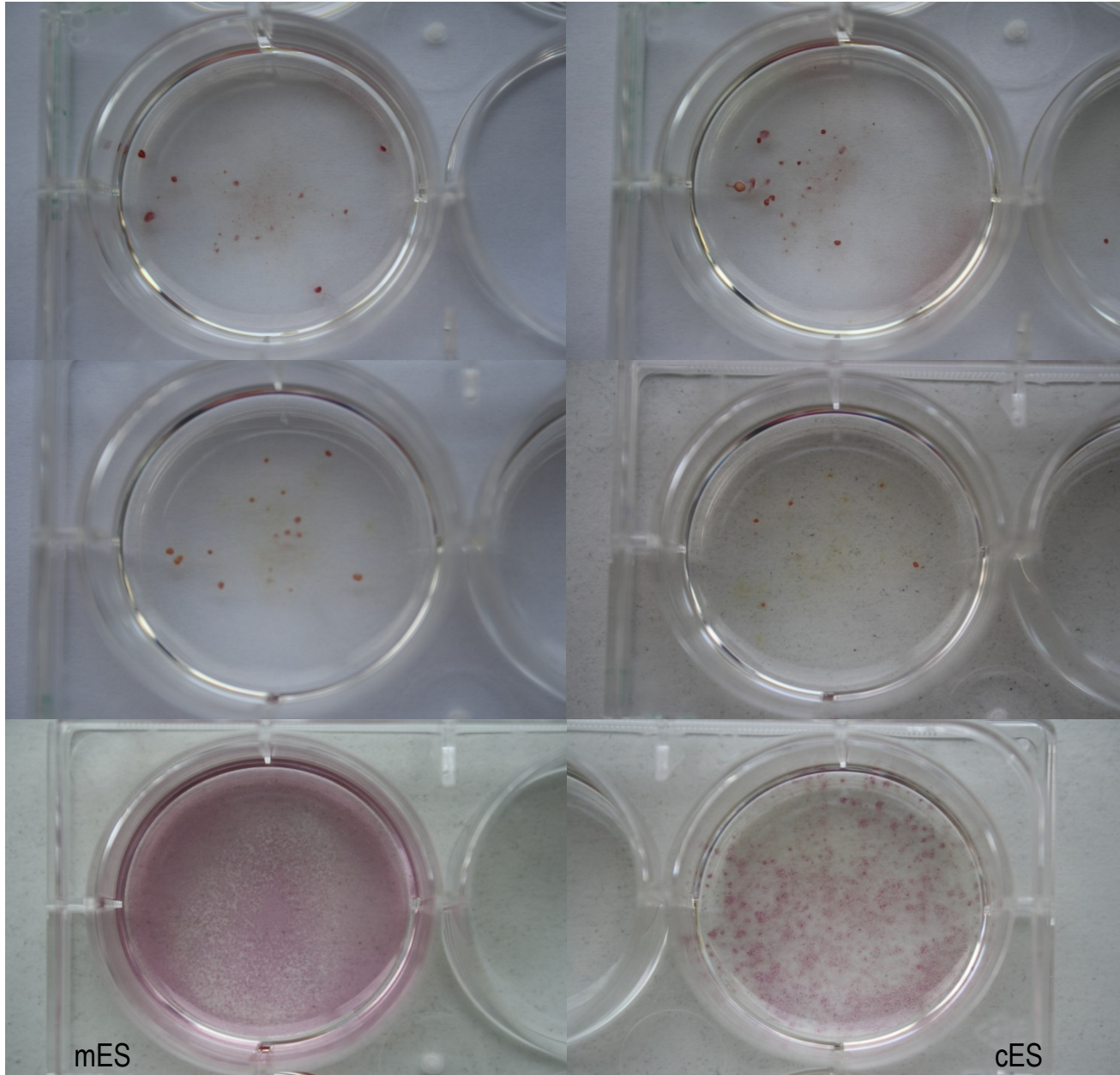


# *New strategy*

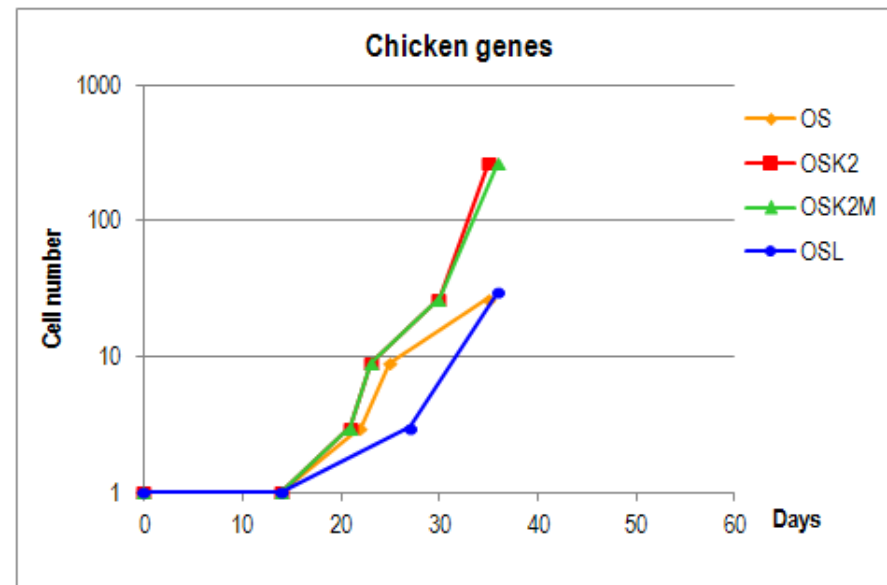
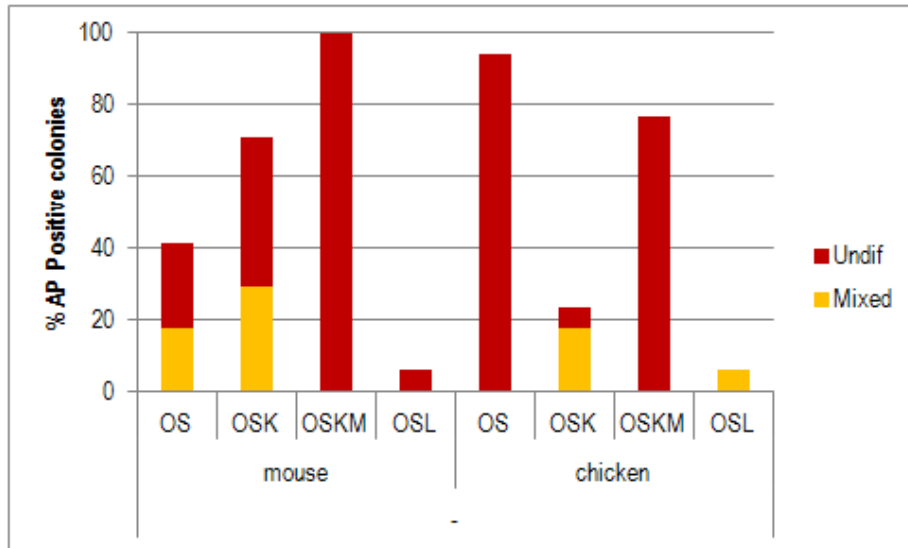




# *New strategy*

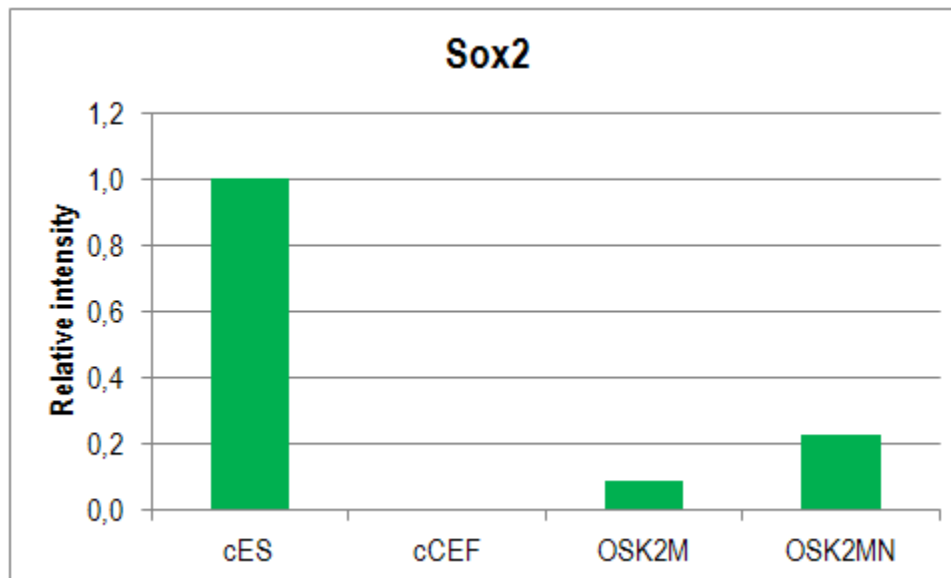
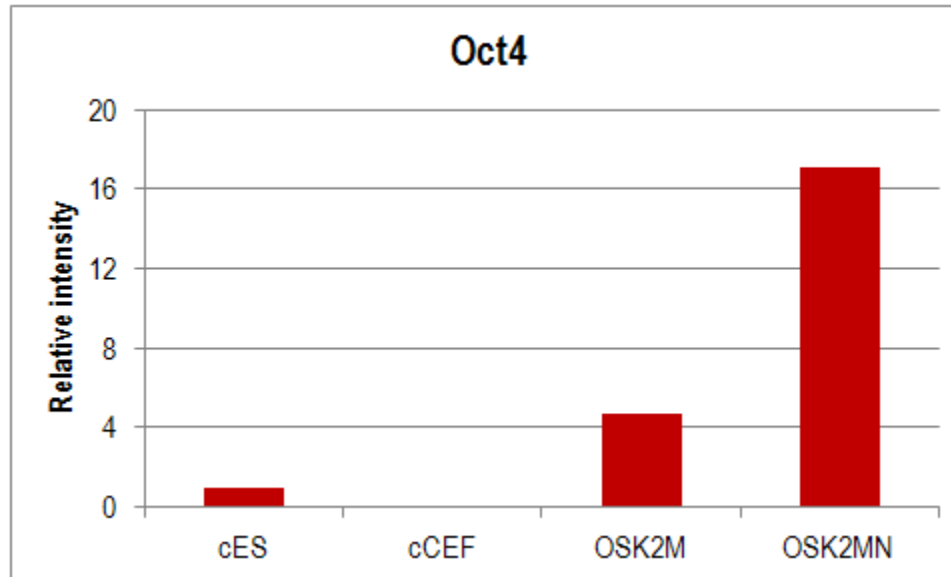


# New strategy





# *New strategy*



## *Still to be done...*

- To standardize the reprogramming protocol → the optimal combination
- To fully characterize the reprogrammed cells
- To inject the reprogrammed cells into recipient embryos
- To evaluate their developmental potential
- To determine the somatic tissues/cells to be used
- To establish the optimum cryobanking conditions

# Thanks to

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<http://http/www.sbri.fr>

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