



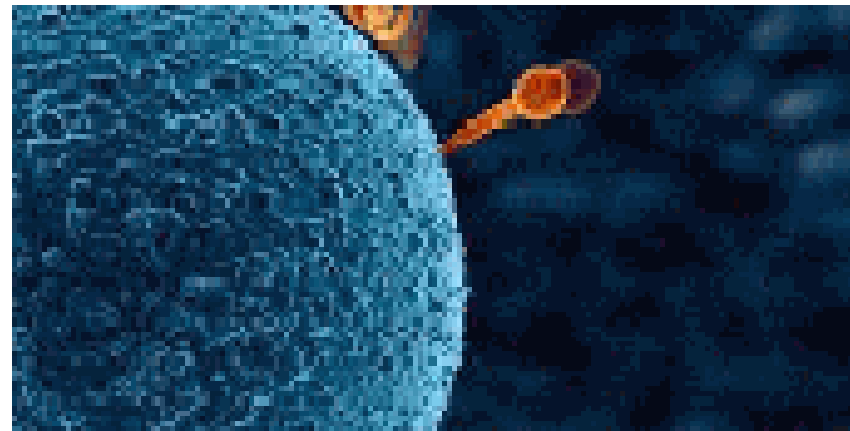
# De toekomst van fertiliteitspreservatie

## *In vitro oogenese*

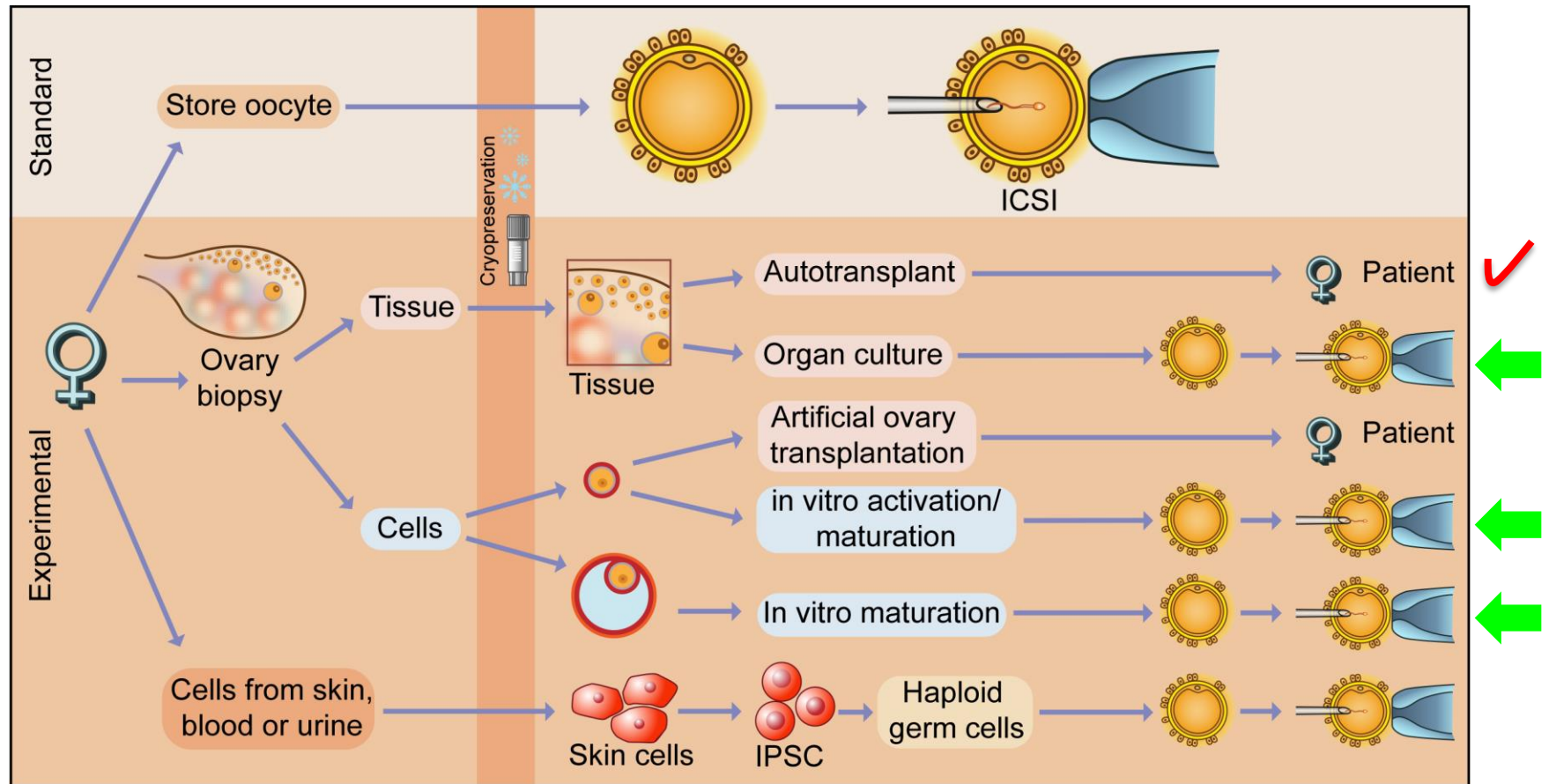
**S.M. Chuva de Sousa Lopes**

Leiden University Medical Center  
Department of Anatomy and Embryology  
Leiden, The Netherlands

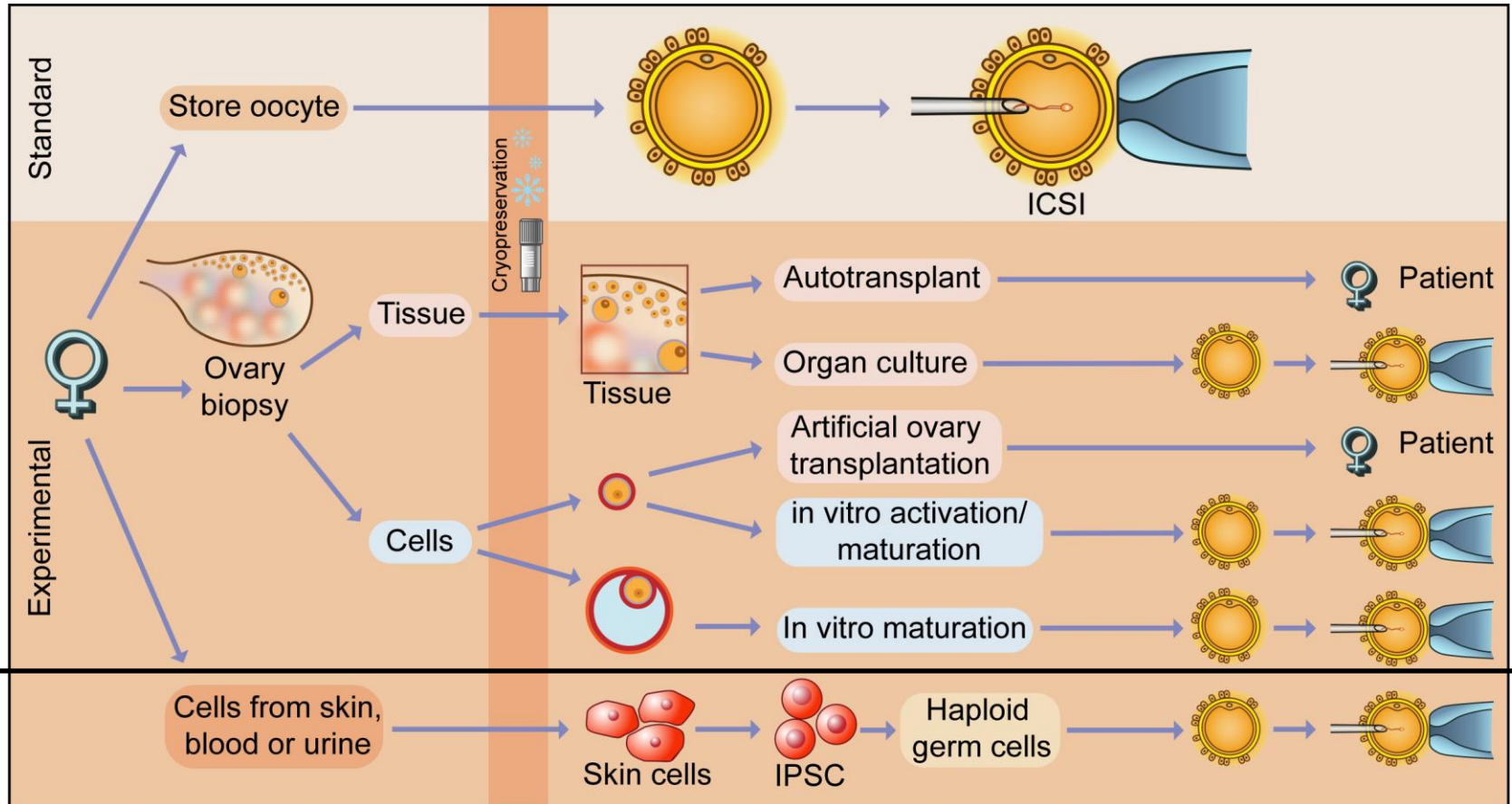
**Lopes@Lumc.nl**



# Fertility preservation - women

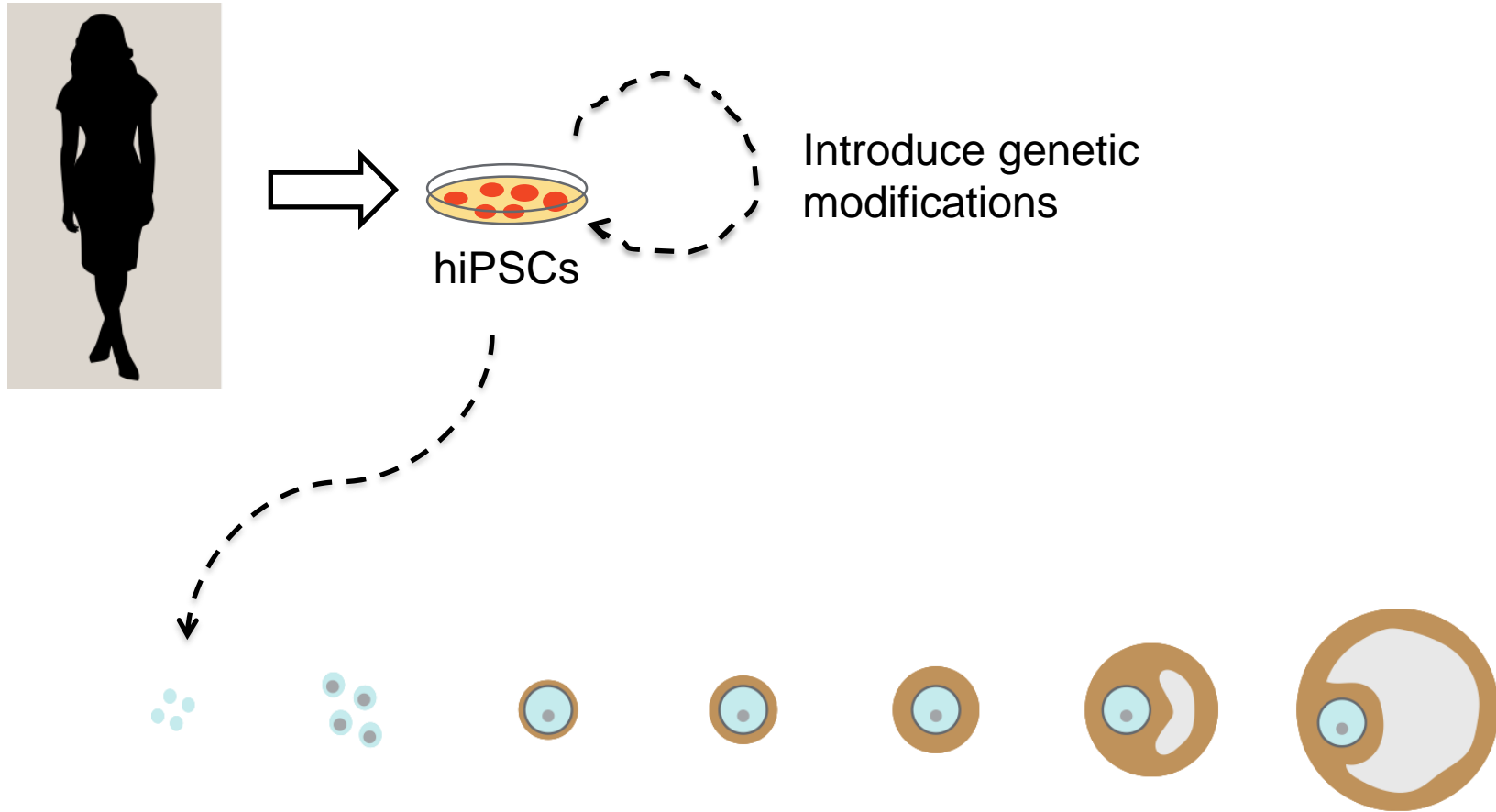


# Fertility preservation - women

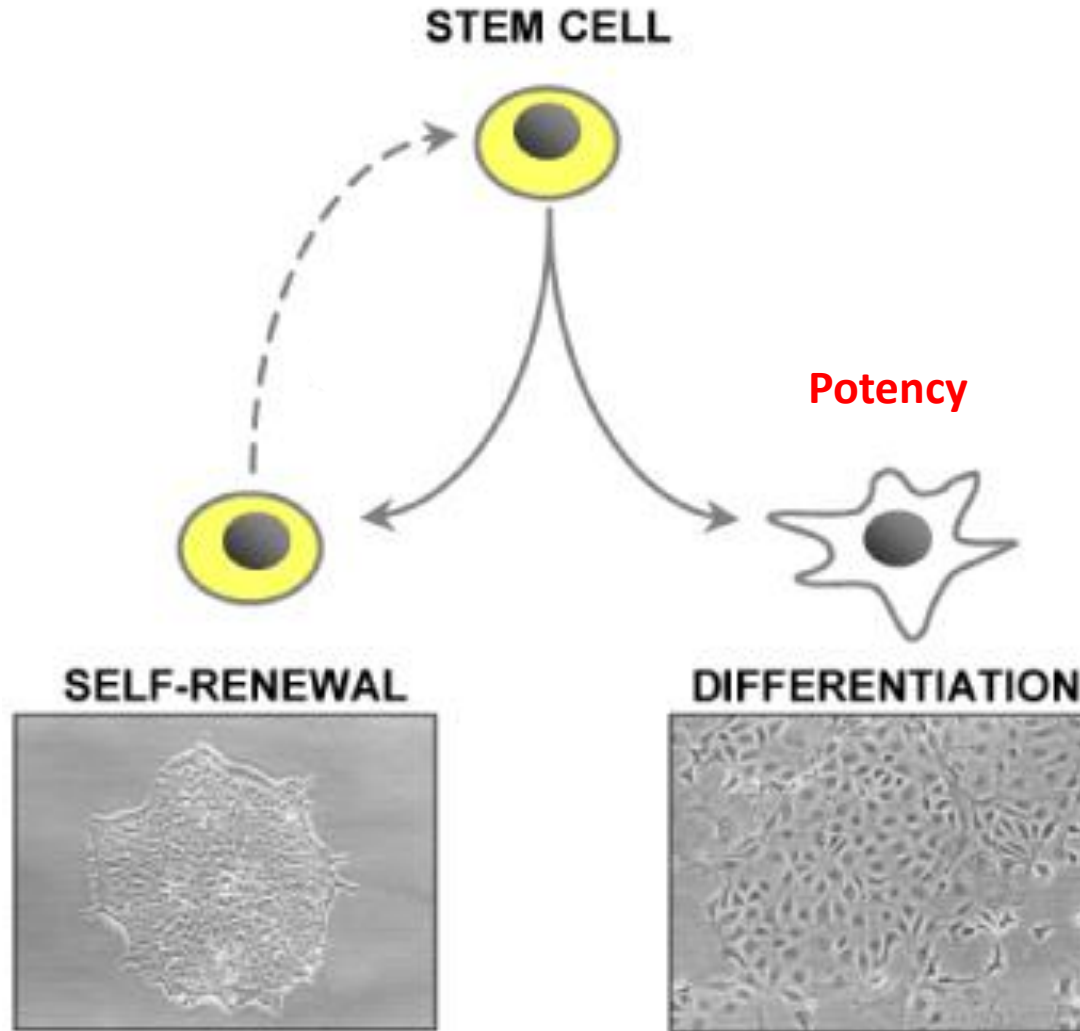


- Source of oocytes
- Independent of age – biological clock
- Not invasive

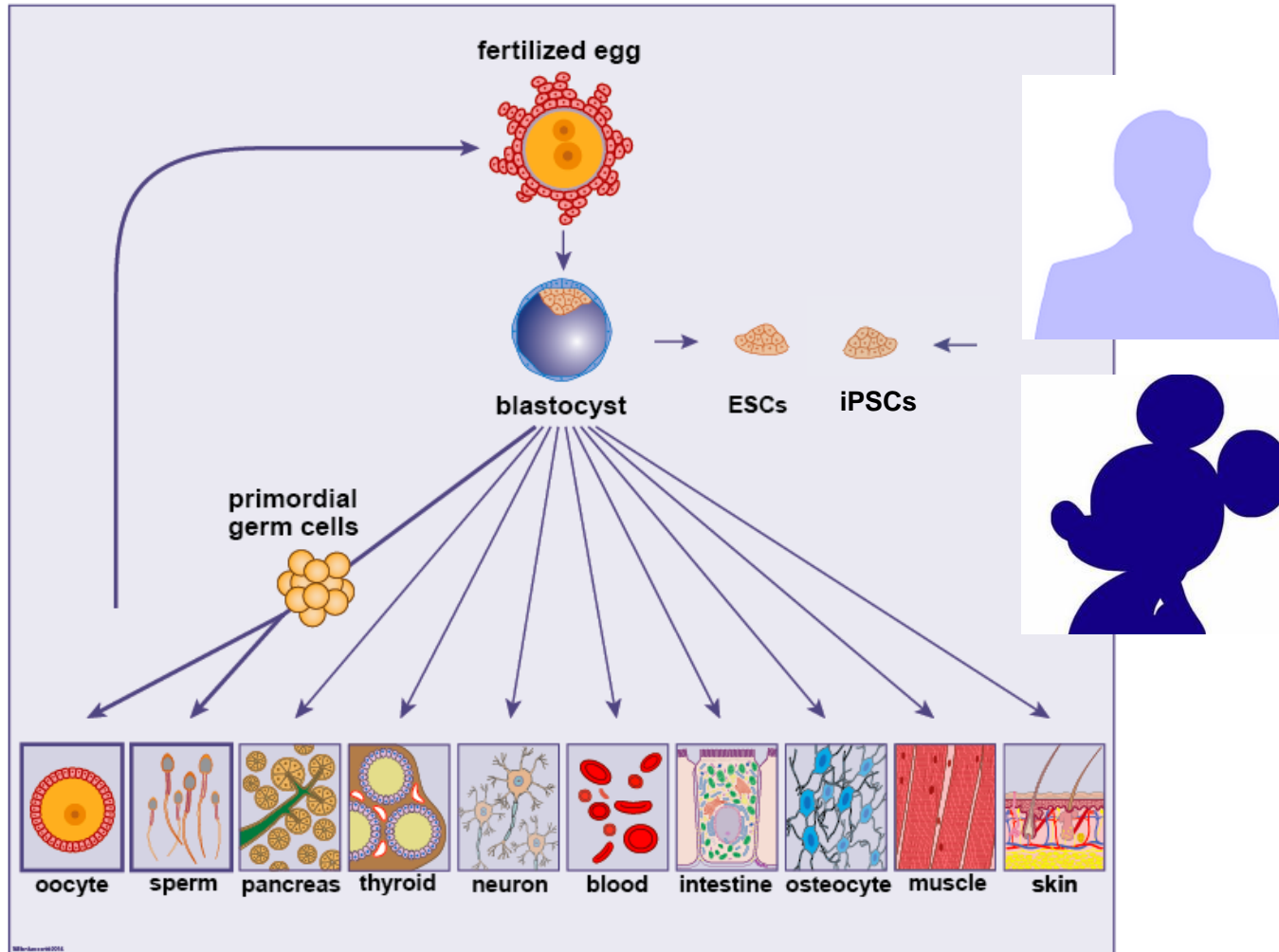
# In the future - oocytes



# Human pluripotent stem cells



# Human pluripotent stem cells

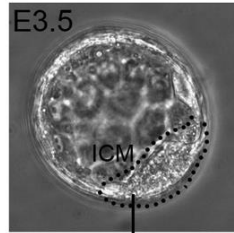


# Human pluripotent stem cells

## Pluripotency

### Naive

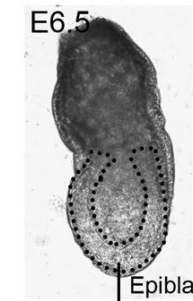
Mouse blastocyst



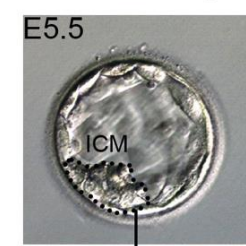
mESCs

### Primed

Mouse epiblast



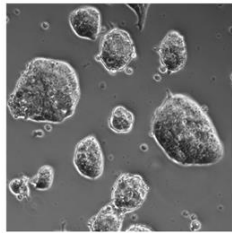
Human blastocyst



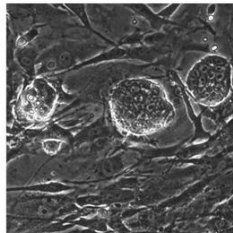
mEpiSCs

hESCs

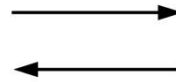
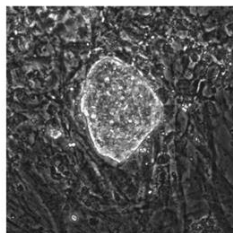
2i + LIF



serum + LIF



naive hESCs



media  
substrate  
oxygen

...



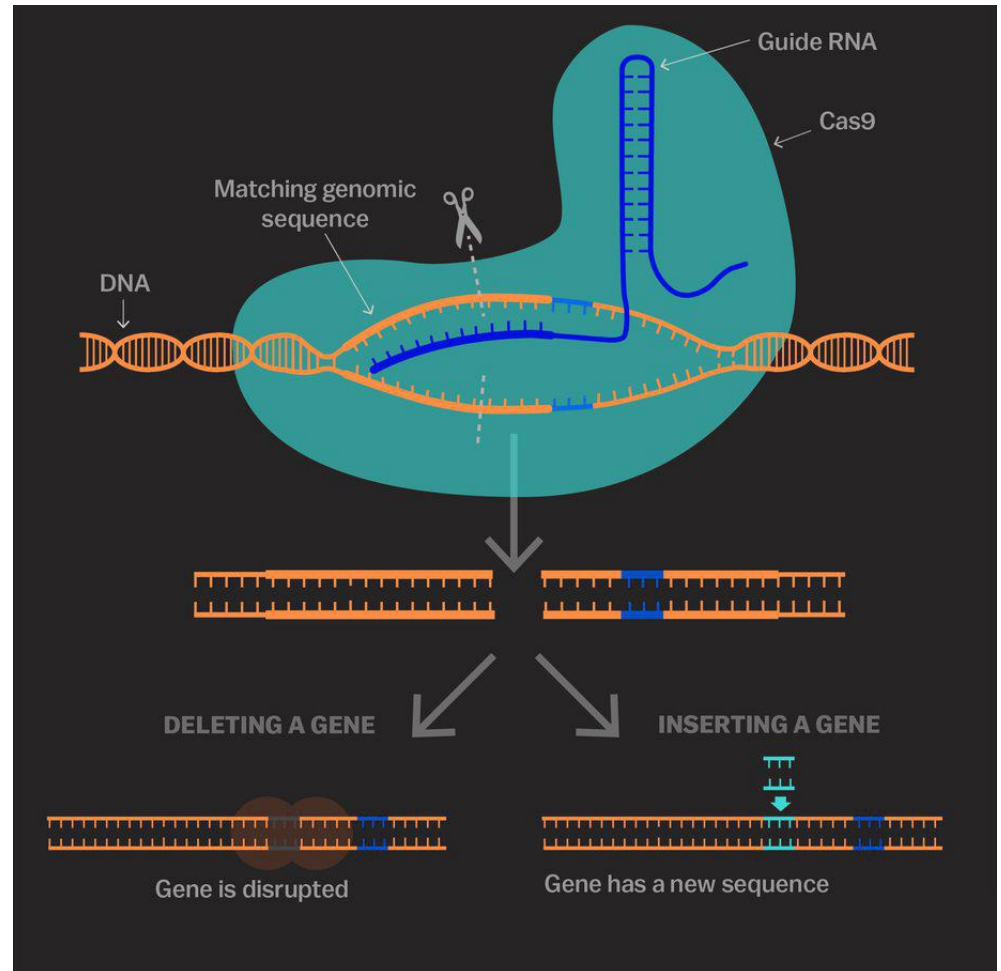
# GENE EDITING: what is it?

- Controlled change in the DNA of an organism
- GMO – genetically modified organism
- Repair/introduce a gene/characteristic
- Plants or animals (or bacteria) + progeny
- Industry: food or medication
- Academia: disease models



# GENE EDITING: CRISPR-CAS9

- New gene-editing
- Simple
- Cheap
- Fast
- Cut = precise
- Repair
  - NHEJ – indels
  - HDR - template



# GENE EDITING: CRISPR-CAS9 in food

NATURE | VOL 560 | 2 AUGUST 2018

In the EU, gene-edited crops and food will be treated in the same way as genetically modified organisms.

GENE EDITING

## EU law deals blow to CRISPR crops

Jansson also has practical concerns about the ruling. He developed a 'CRISPR cabbage' that he has consumed, and which was growing in his home garden as he spoke to *Nature*. "I took a photo yesterday, and I took another after the ruling. It's still the same plant. Yesterday it wasn't a GMO, and now it's a GMO. I'm a bit curious what I have to do. Do I have to remove it?"

### FOOD FACT:

50 countries, including France, Germany and Italy have banned or restricted GMOs, while the US doesn't even require labeling.



source: LabelGMOs.org

UrbanOrganicGardener.com

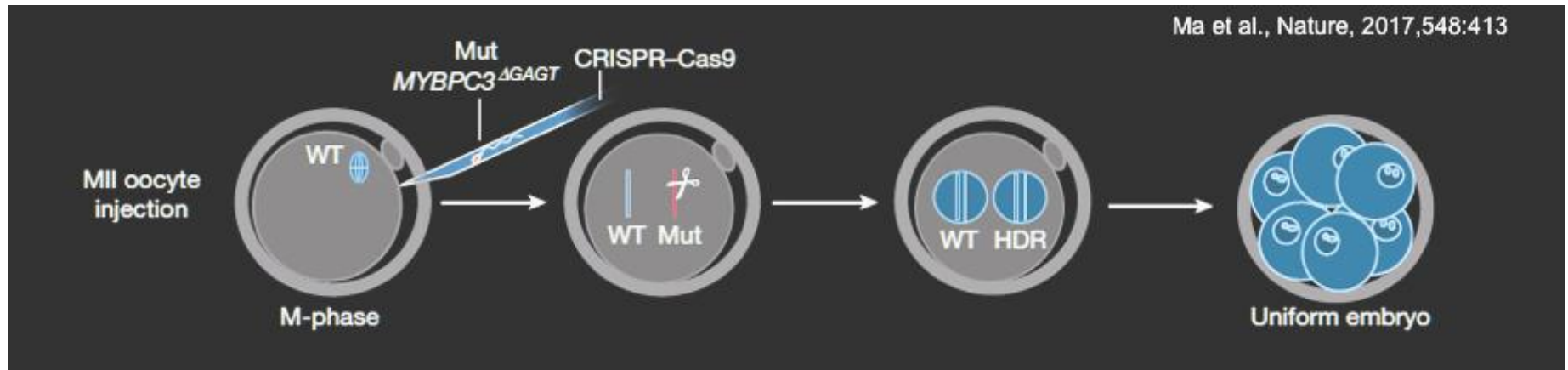
GMOs are found in 80% of packaged food in the US

Percentage of each Genetically Modified Crop that is grown in the United States



# GENE EDITING: CRISPR-CAS9 in reproduction

- Repair monogenetic diseases
- Include in IVF treatments
- Off-target? Safe?
- Where to stop?



# From stem cells to oocytes? – Part I



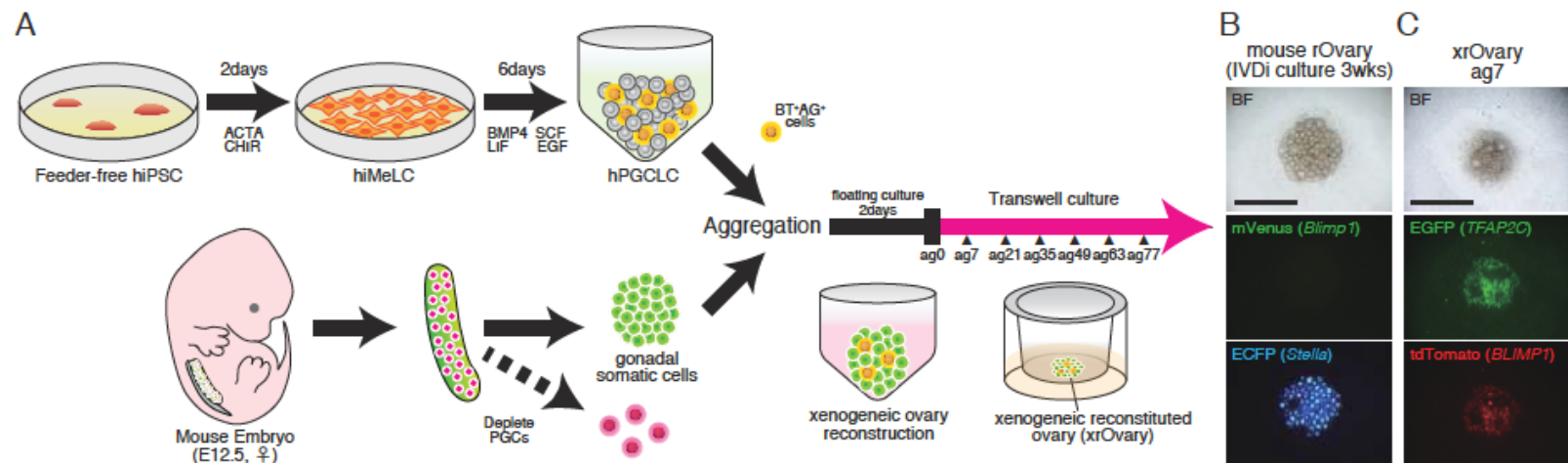
Rienzi et al., Human Reproduction, 2012

# From stem cells to oocytes?

Cite as: C. Yamashiro *et al.*, *Science*  
10.1126/science.aat1674 (2018).

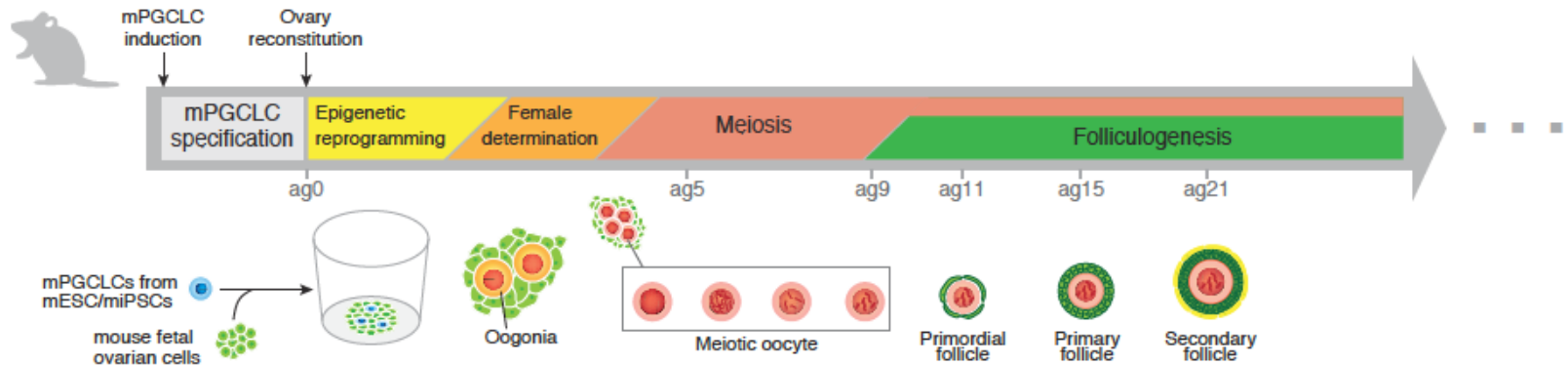
## Generation of human oogonia from induced pluripotent stem cells in vitro

Chika Yamashiro<sup>1,2</sup>, Kotaro Sasaki<sup>1,2</sup>, Yukihiro Yabuta<sup>1,2</sup>, Yoji Kojima<sup>1,2,3,4</sup>, Tomonori Nakamura<sup>1,2</sup>, Ikuhiro Okamoto<sup>1,2</sup>, Shihori Yokobayashi<sup>1,2,4</sup>, Yusuke Murase<sup>1,2</sup>, Yukiko Ishikura<sup>1,2</sup>, Kenjiro Shirane<sup>5,6</sup>, Hiroyuki Sasaki<sup>5,6</sup>, Takuya Yamamoto<sup>3,4,7</sup>, Mitinori Saitou<sup>1,2,3,4\*</sup>

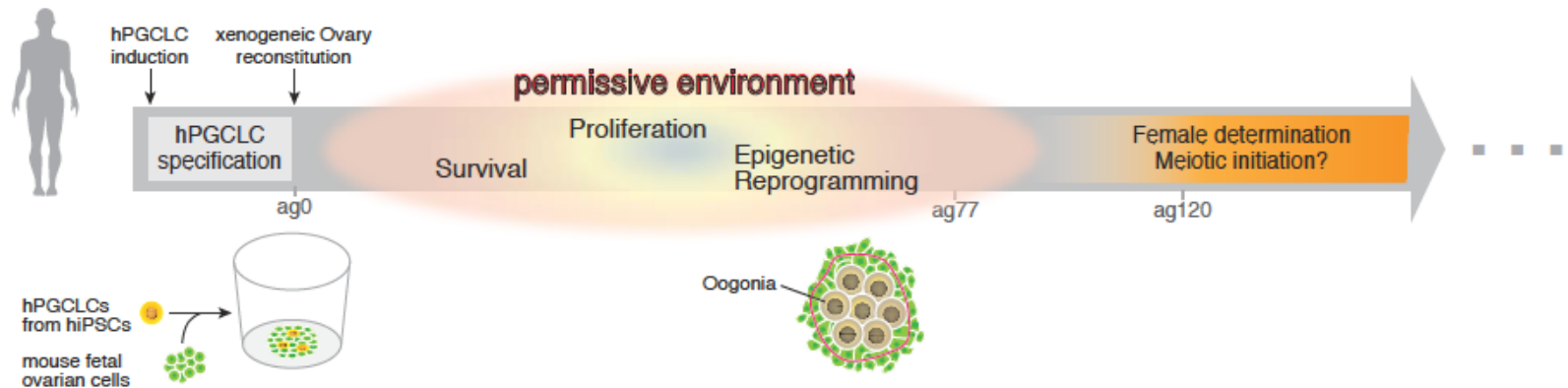


# From stem cells to oocytes?

## Mouse-Mouse rOvary

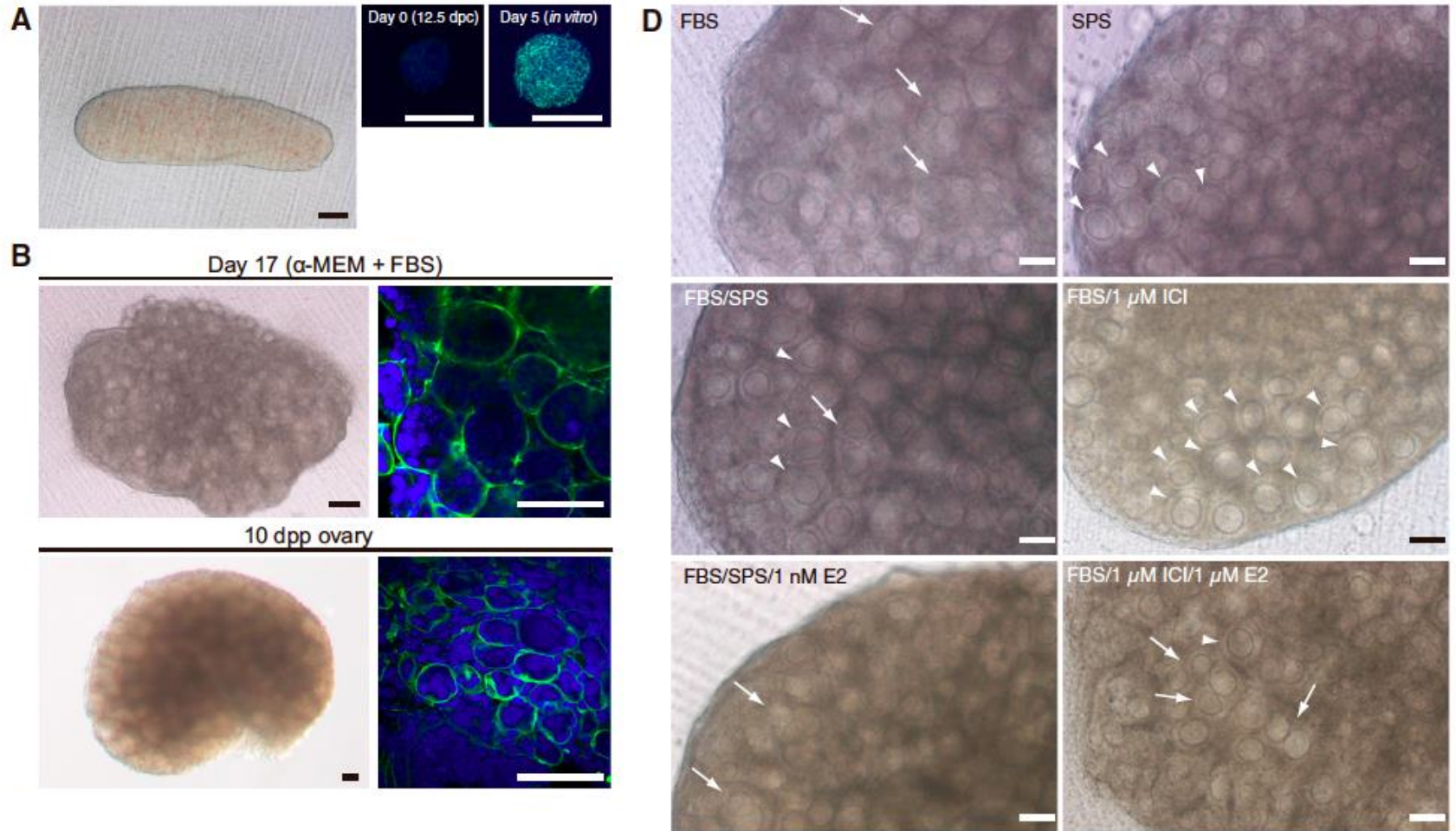


## Human-Mouse xenogeneic rOvary





# From stem cells to oocytes?

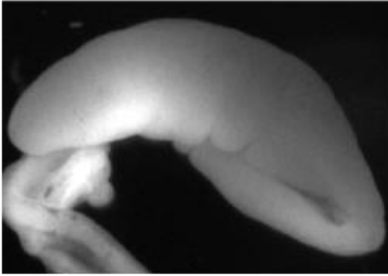


Morohaku et al., PNAS, 2016

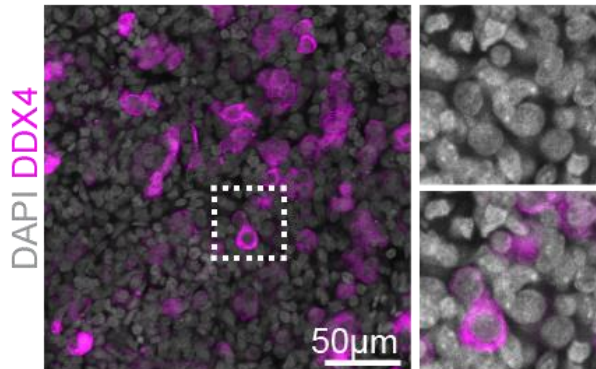


# From stem cells to oocytes?

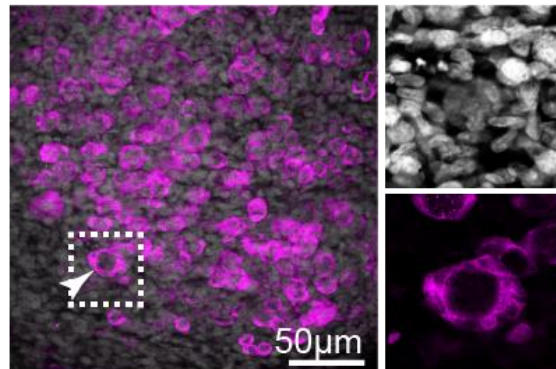
W17 ovary



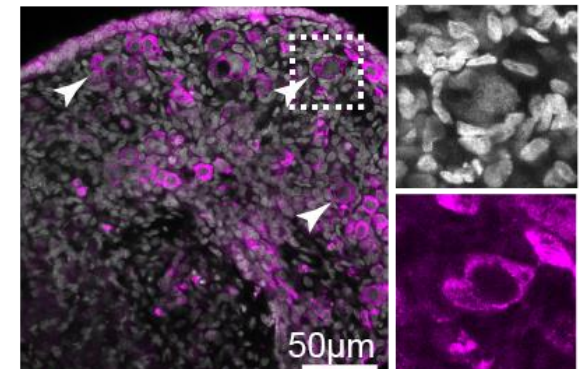
W17 ovary



W17 + 7days



W17 + 14days



# From stem cells to oocytes? – Part II



Rienzi et al., Human Reproduction, 2012

# Future – fusion of gametes and stem cells?

Cell Stem Cell

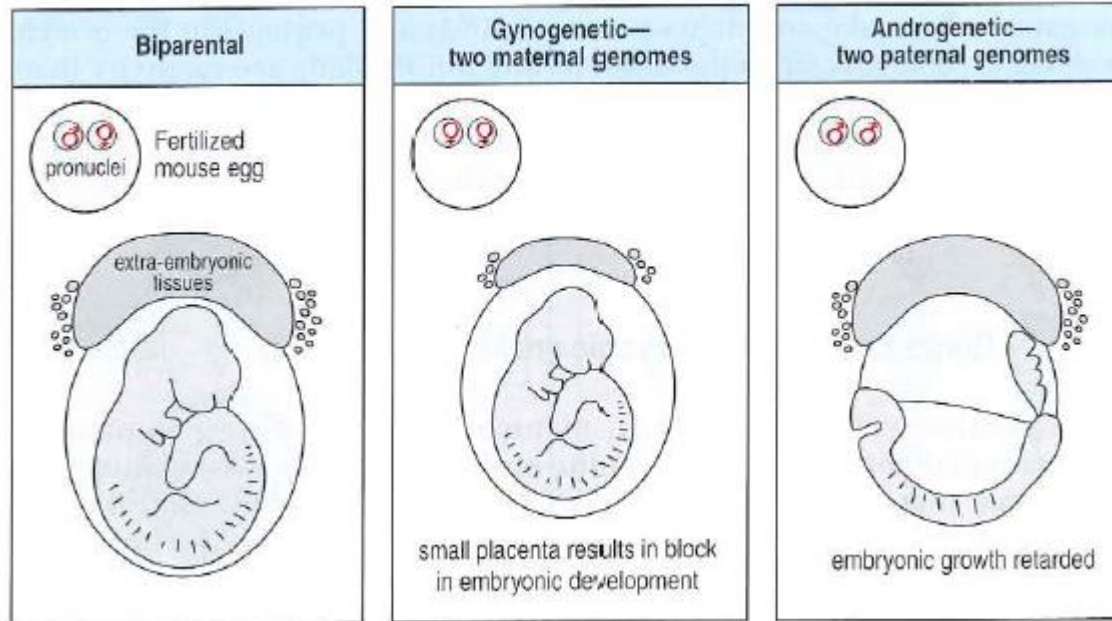
Article

CellPress

## Generation of Bimaternal and Bipaternal Mice from Hypomethylated Haploid ESCs with Imprinting Region Deletions

Zhi-Kun Li,<sup>1,2,5</sup> Le-Yun Wang,<sup>1,2,5</sup> Li-Bin Wang,<sup>1,2,3,5</sup> Gui-Hai Feng,<sup>1,2,5</sup> Xue-Wei Yuan,<sup>1,2,4,5</sup> Chao Liu,<sup>1,2,3</sup> Kai Xu,<sup>1,2,3</sup> Yu-Huan Li,<sup>1,2,3</sup> Hai-Feng Wan,<sup>1,2</sup> Ying Zhang,<sup>1,2</sup> Yu-Fei Li,<sup>1,2,3</sup> Xin Li,<sup>1,2</sup> Wei Li,<sup>1,2,3,\*</sup> Qi Zhou,<sup>1,2,3,6,\*</sup> and Bao-Yang Hu<sup>1,2,3,\*</sup>

# Imprinting?

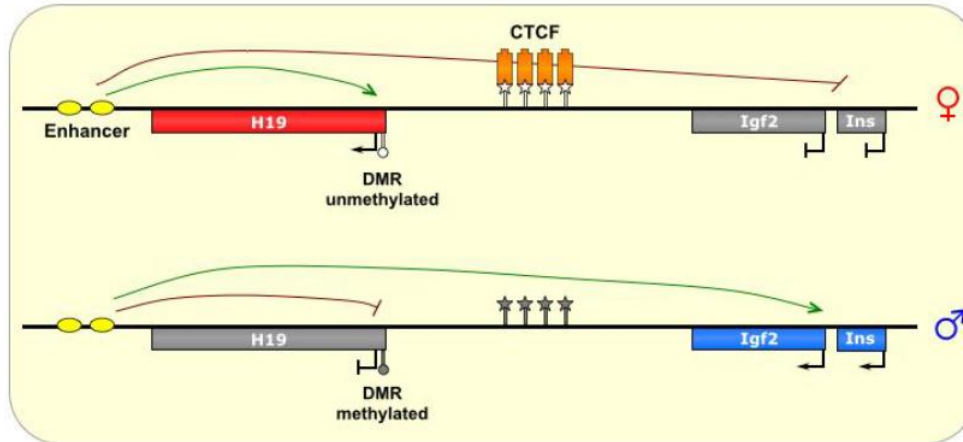


“Battle of the sexes”:

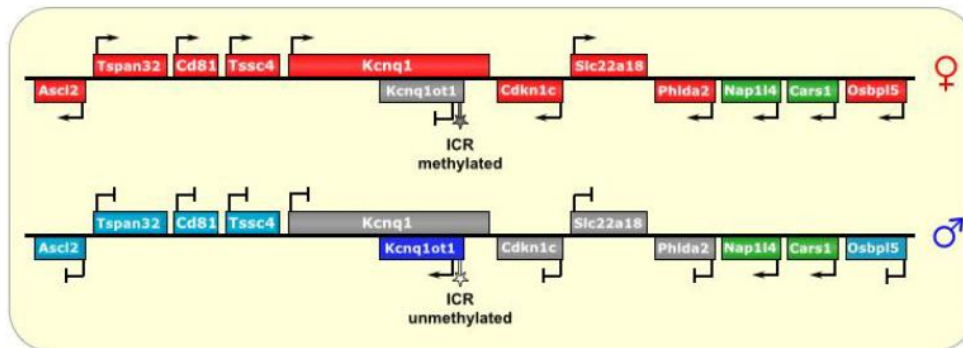
Maternal imprints: smaller placenta

Paternal imprints: smaller embryo

# Imprinting?



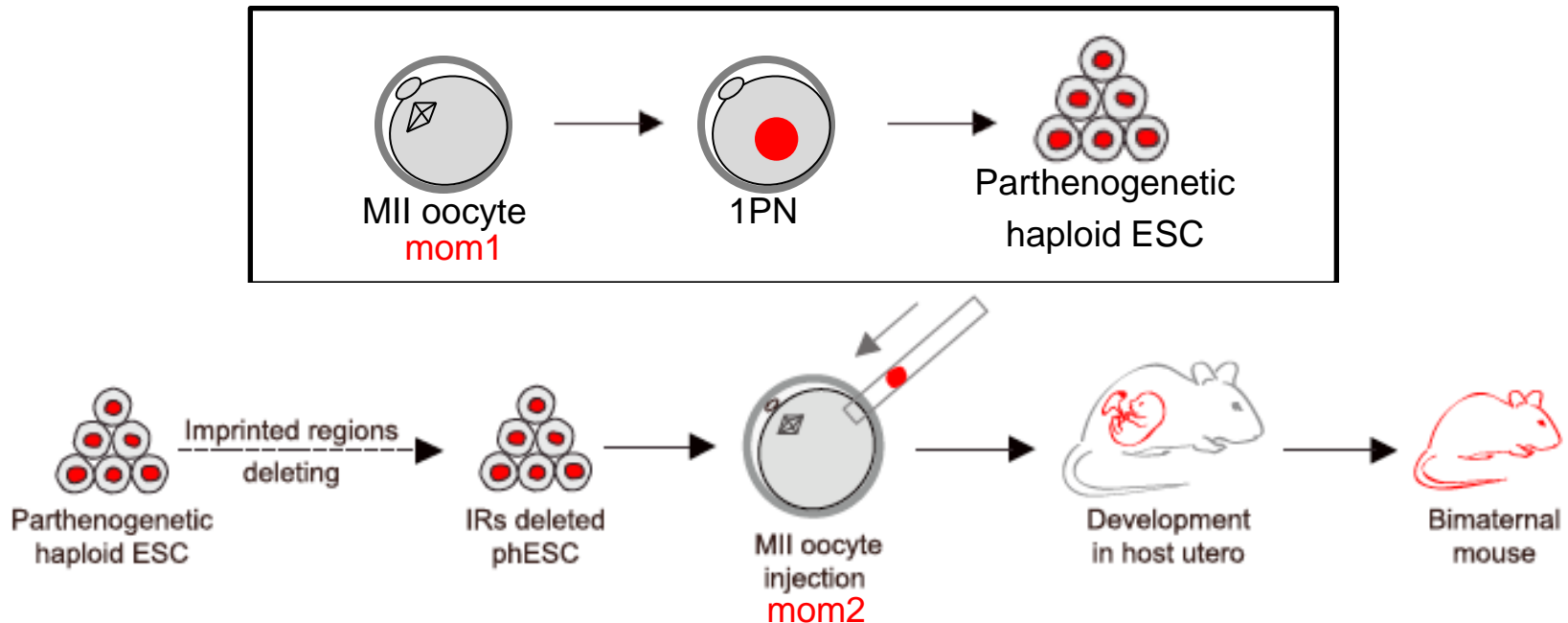
Imprint erasure?



Artuoro et al., Biomolecules, 2014

## Generation of **Bimaternal** and Bipaternal Mice from Hypomethylated Haploid ESCs with Imprinting Region Deletions

Zhi-Kun Li,<sup>1,2,5</sup> Le-Yun Wang,<sup>1,2,5</sup> Li-Bin Wang,<sup>1,2,3,5</sup> Gui-Hai Feng,<sup>1,2,5</sup> Xue-Wei Yuan,<sup>1,2,4,5</sup> Chao Liu,<sup>1,2,3</sup> Kai Xu,<sup>1,2,3</sup> Yu-Huan Li,<sup>1,2,3</sup> Hai-Feng Wan,<sup>1,2</sup> Ying Zhang,<sup>1,2</sup> Yu-Fei Li,<sup>1,2,3</sup> Xin Li,<sup>1,2</sup> Wei Li,<sup>1,2,3,\*</sup> Qi Zhou,<sup>1,2,3,6,\*</sup> and Bao-Yang Hu<sup>1,2,3,\*</sup>



# Bipaternal mice

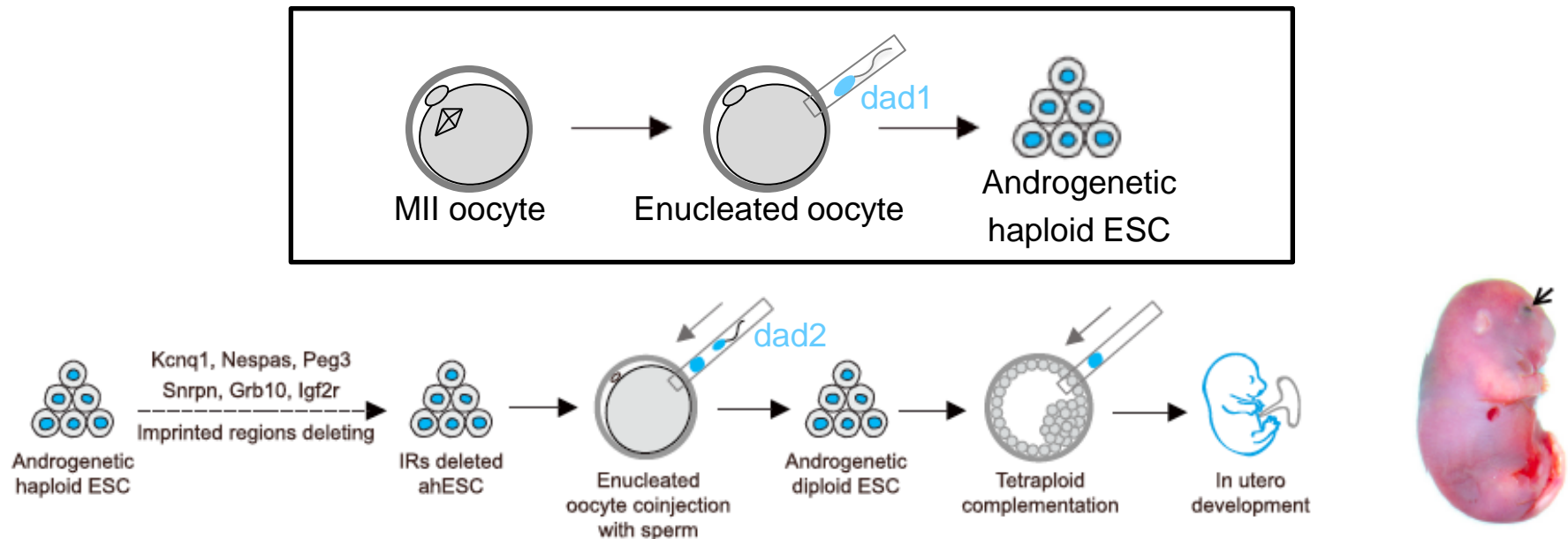
Cell Stem Cell

Article

CellPress

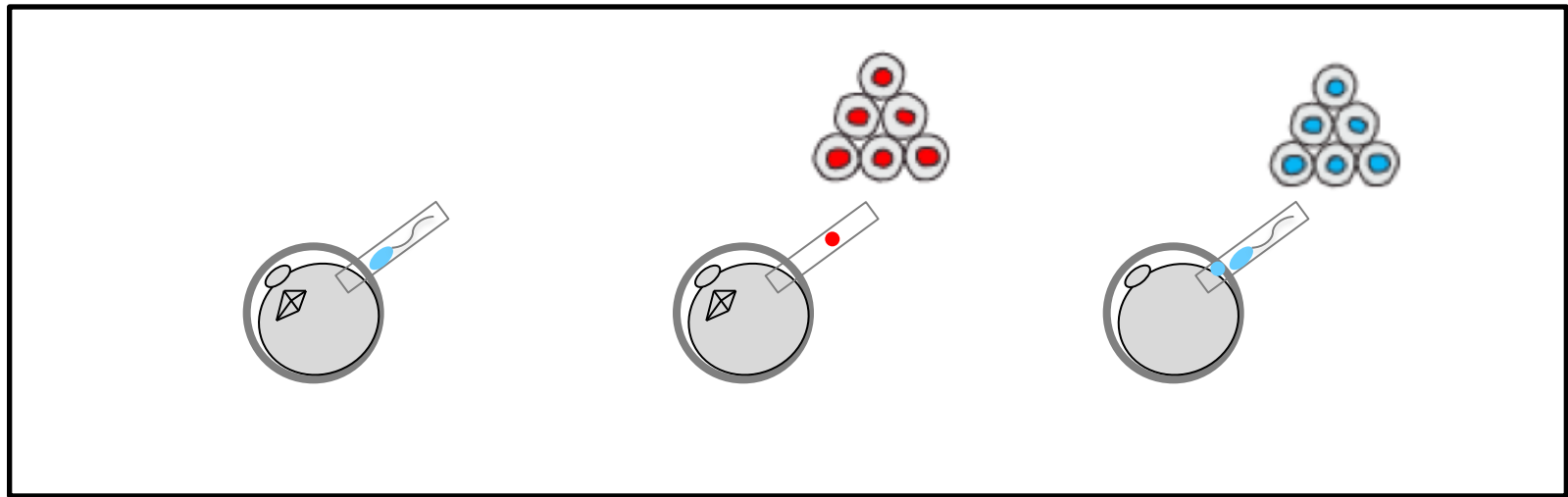
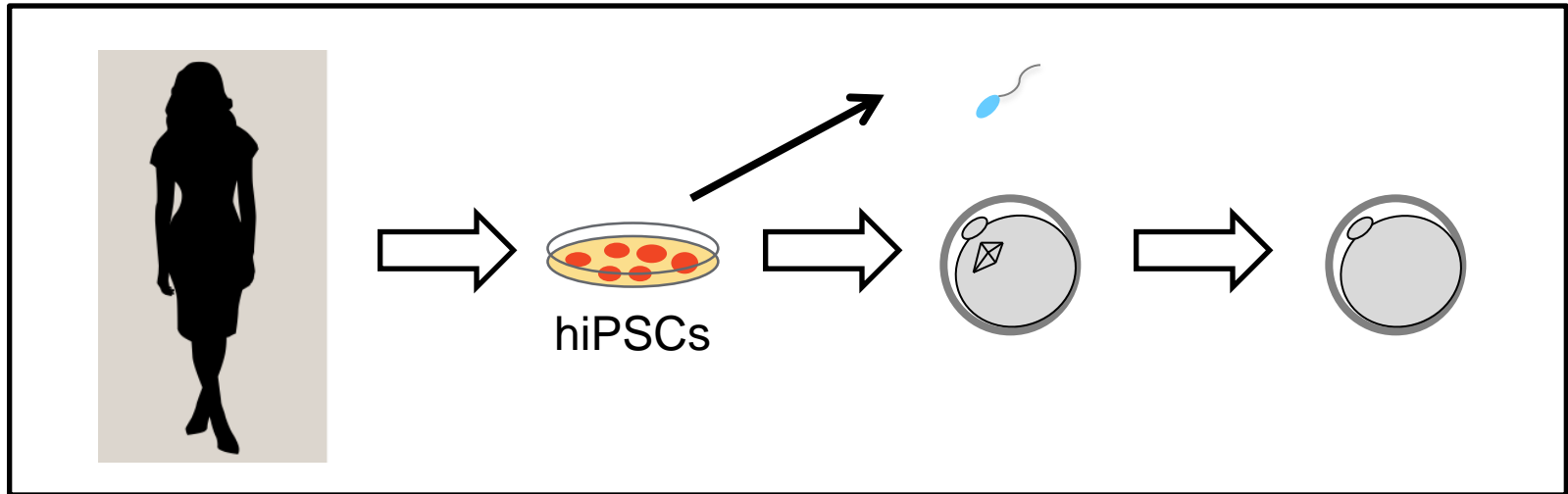
## Generation of Bimaternal and **Bipaternal** Mice from Hypomethylated Haploid ESCs with Imprinting Region Deletions

Zhi-Kun Li,<sup>1,2,5</sup> Le-Yun Wang,<sup>1,2,5</sup> Li-Bin Wang,<sup>1,2,3,5</sup> Gui-Hai Feng,<sup>1,2,5</sup> Xue-Wei Yuan,<sup>1,2,4,5</sup> Chao Liu,<sup>1,2,3</sup> Kai Xu,<sup>1,2,3</sup> Yu-Huan Li,<sup>1,2,3</sup> Hai-Feng Wan,<sup>1,2</sup> Ying Zhang,<sup>1,2</sup> Yu-Fei Li,<sup>1,2,3</sup> Xin Li,<sup>1,2</sup> Wei Li,<sup>1,2,3,\*</sup> Qi Zhou,<sup>1,2,3,6,\*</sup> and Bao-Yang Hu<sup>1,2,3,\*</sup>





# Future – fusion of gametes and stem cells?



Gene editing?

# Thank you!



LEIDS UNIVERSITAIR MEDISCH CENTRUM

## **Dept. Anatomy and Embryology, LUMC**

**Susana Chuva de Sousa Lopes**

Maaïke Nieveen

Monika Bialecka

Jasin Taelman

Xueying Fan

Ioannis Moustakas

## **Dept. Gynaecology, LUMC**

Lucette van der Westelaken

Gonneke Pilgram

Leoni Louwe

## **Dept. Reproductive Medicine, UZ Gent**

**Petra De Sutter**

Susana Chuva de Sousa Lopes

Bjorn Heindryckx

Mina Popovic



European  
Research  
Council