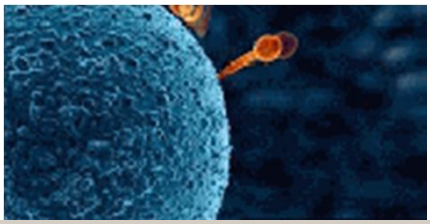
VERENIGING VOOR
KLINISCHE EMBRYOLOGIE

Create and modify your own GERM CELLS

S.M. Chuva de Sousa Lopes

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

Lopes@Lumc.nl



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Why is this important? - Infertility

Problem:

- 1 in 8 women face involuntary infertility (increase)
- Heavy emotional burden

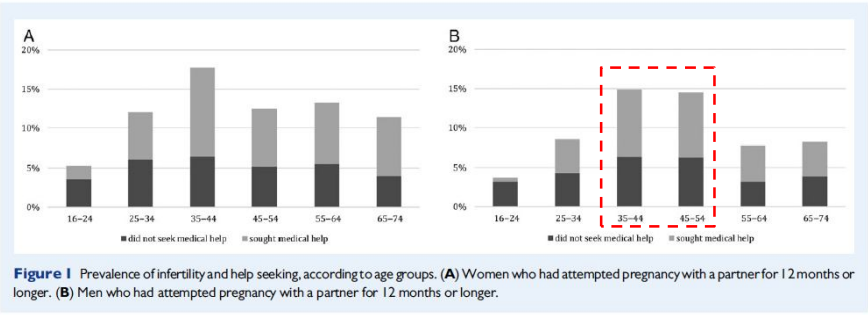

Figure 1 Prevalence of infertility and help seeking, according to age groups. (**A**) Women who had attempted pregnancy with a partner for 12 months or longer. (**B**) Men who had attempted pregnancy with a partner for 12 months or longer.

Datta et al., Human Reprod 2016

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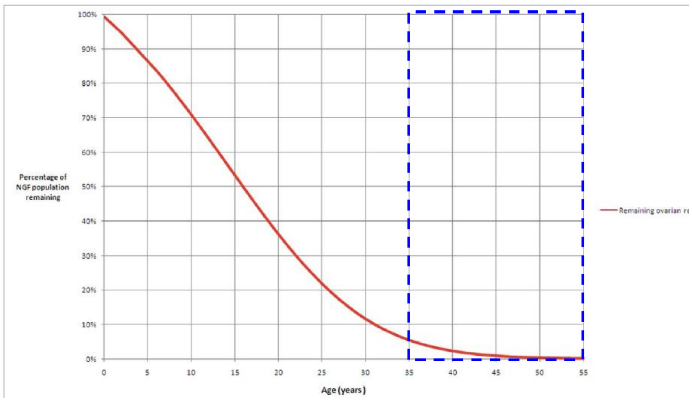


Wallace and Kelsey, Plos One 2010

Why is this important? - Cancer

Problem:

- **Growing number of survivor cancer patients**

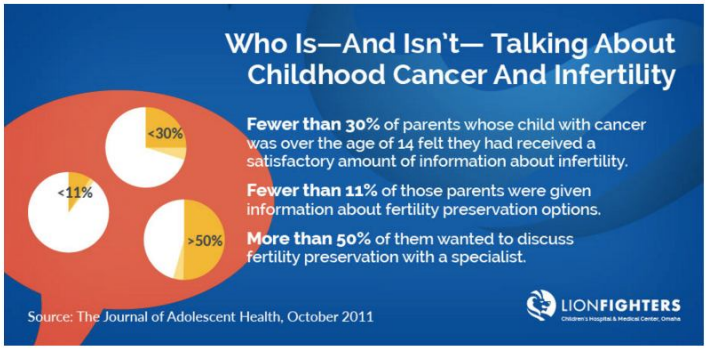
Fertility Preservation Options For Pediatric Cancer Patients

Why is this important? - Cancer

Problem:

- **Growing number of survivor cancer patients**

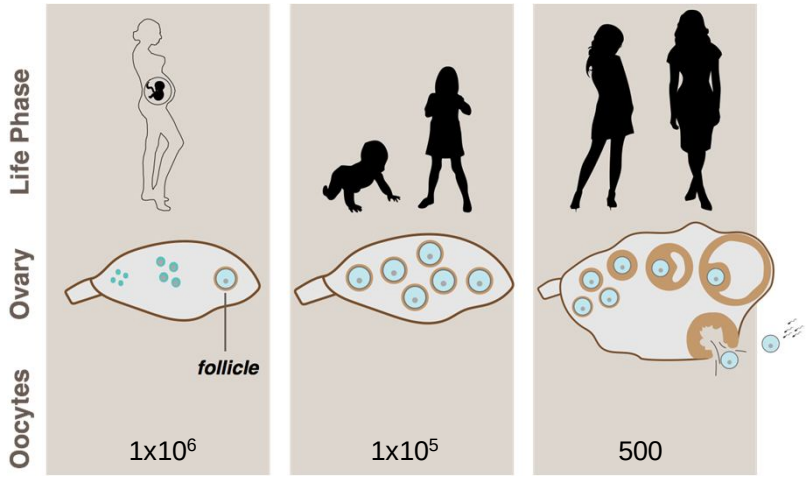
Why Infertility Is Sometimes Overlooked In Pediatric Cancer Patients



Why is this important? - Disease modelling

Problem:

- **No (human) *in vitro* models to study oogenesis**



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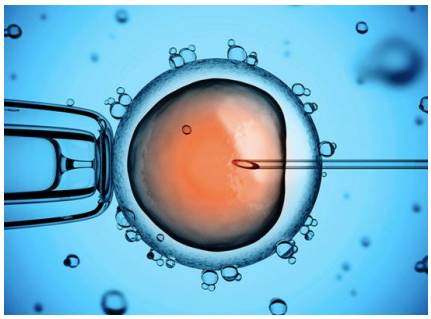
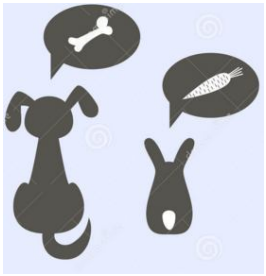


- **Causes?**
- **Treatments?**

Why is this important? - Industry

Problem: • **No (human) *in vitro* models to study oogenesis**

Reproductive toxicity

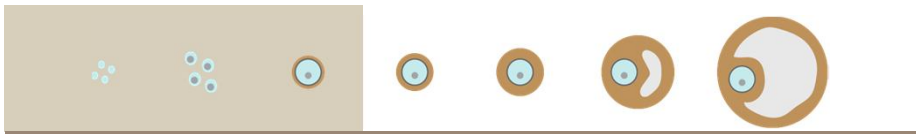


Can we **create** own germ cells?

Problem: • **How far are we?**

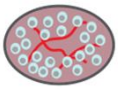
Oogenesis spotlighted: making mature human oocytes

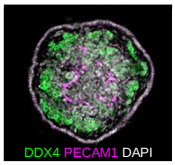
Tissue engineering - human follicles



Functional assays (in progress)

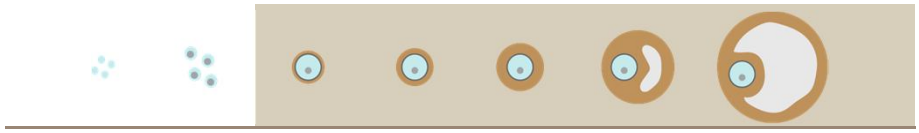
Formation of follicles
mini-ovary





Chuva de Sousa Lopes 11

Tissue engineering - human follicles



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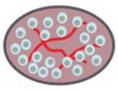
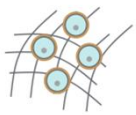
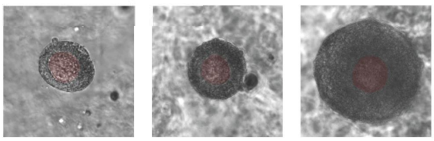


Growth of follicles
3D-scaffold





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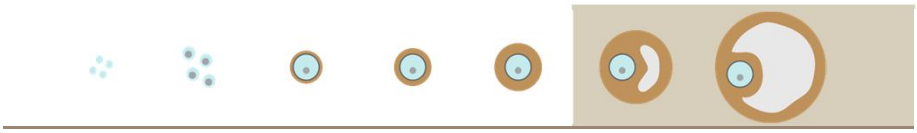
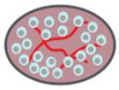
Tissue engineering - human follicles



Functional assays (in progress)

Formation of follicles
mini-ovary



Growth of follicles
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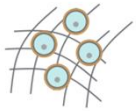
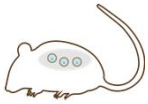
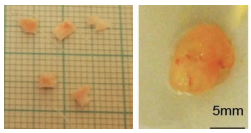
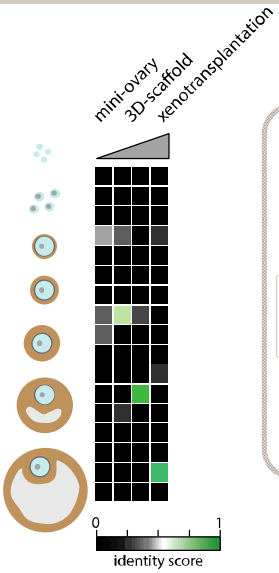


Maturation of follicles
xenotransplantation

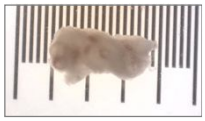


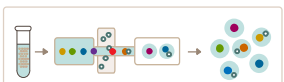


In vivo template - single-cell & bioinformatics



ovary





Single-cell transcriptomics:
- cellular identities
- signalling pathways

Evaluate differentiation:
- quantitative
- systematic
- fast

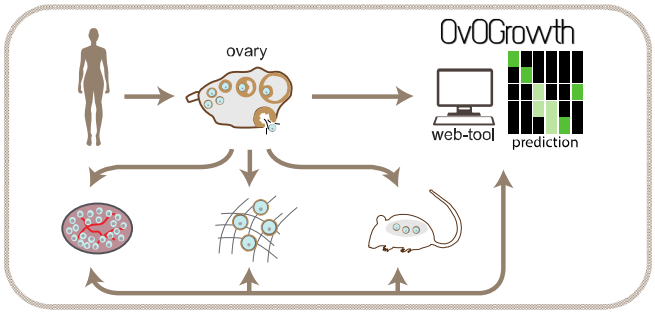
Impact

OvOGrowth

Scientific
- Human model oogenesis
- Single-cell – ovary
- OvOGrowth – platform

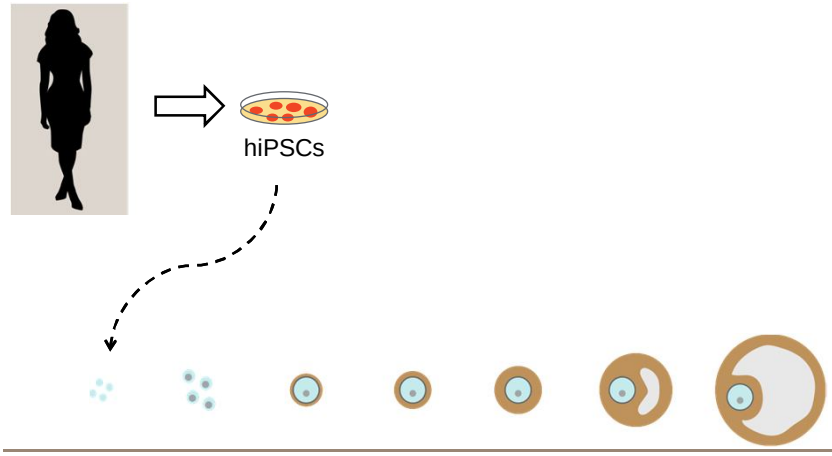
Clinical
- In vitro maturation
- Fertility preservation
- Diagnostic tools

Societal
- Quality of life
- Industry – reproductive toxicity
- Fewer animals



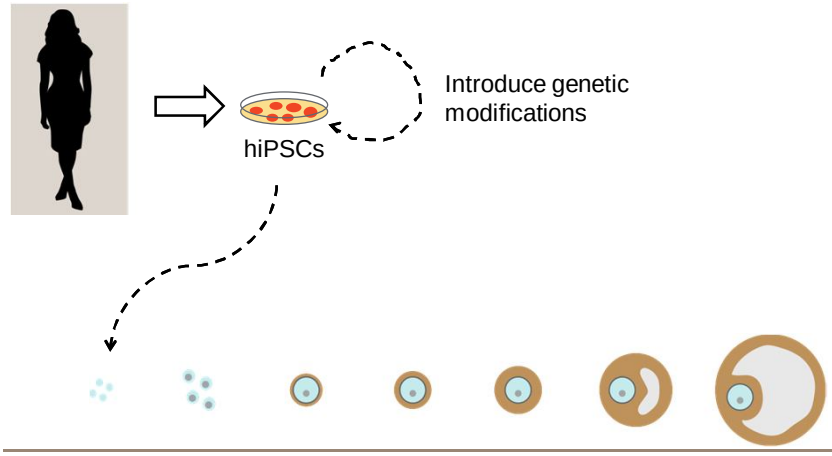
erc European Research Council

Can we **create** own germ cells?



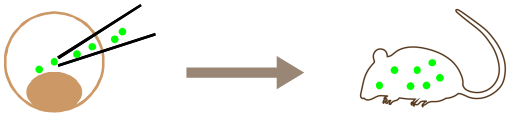
Can we **create** and **modify** own germ cells?

Problem: • **How far are we?**



Can stem cells make gametes?

- Embryonic stem cells (ESC/EpiSC/etc) ✓
- Induced pluripotent stem cells ✓
- Adult stem cells (less potent, in vivo, clinical)



stem cells <> (4N) embryo

Can stem cells make gametes?

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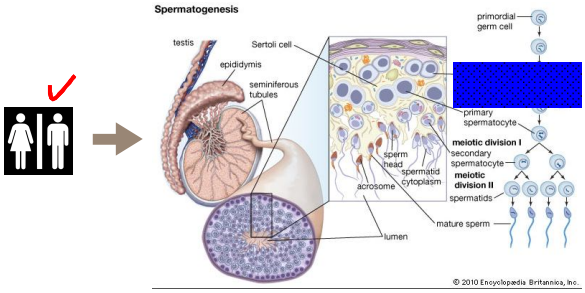
- Adult stem cells (less potent, in vivo, clinical) ✓

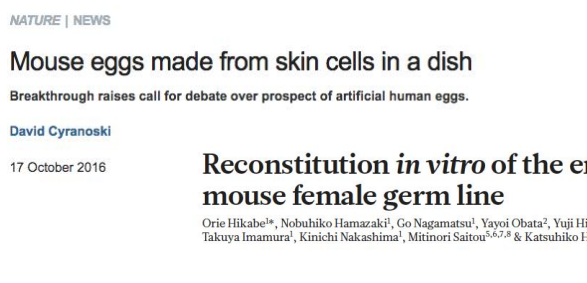


PLZF

Yan et al., J Cell Sci, 2016, 129:492

Can stem cells make gametes *in vitro*?

- Depends on the definition of ***in vitro***
- Bottleneck: **meiosis** (recombination – half chr)
- Mice: co-culture with gonadal tissue (embryos/babies)



NATURE | NEWS

Mouse eggs made from skin cells in a dish

Breakthrough raises call for debate over prospect of artificial human eggs.

David Cyranoski

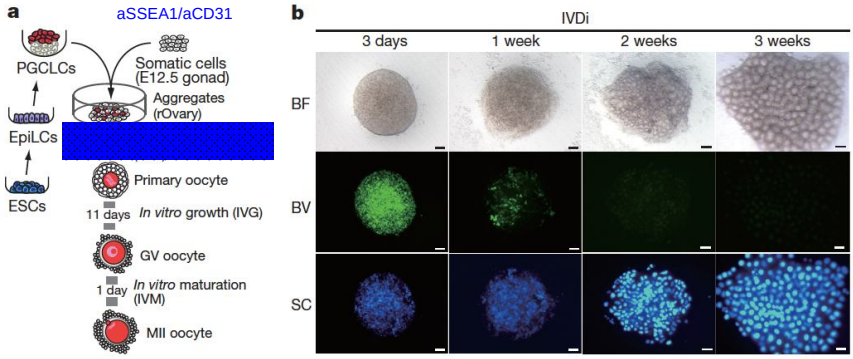
17 October 2016

Reconstitution *in vitro* of the entire cycle of the mouse female germ line

Orie Hikabe[1*], Nobuhiko Hamazaki[1], Go Nagamatsu[1], Yayoi Obata[2], Yuji Hirao[3], Norio Hamada[1,4], So Shimamoto[1], Takuya Imamura[1], Kinichi Nakashima[1], Mitinori Saitou[5,6,7,8] & Katsuhiko Hayashi[1,9*]

Hibake et al., Nature 2016

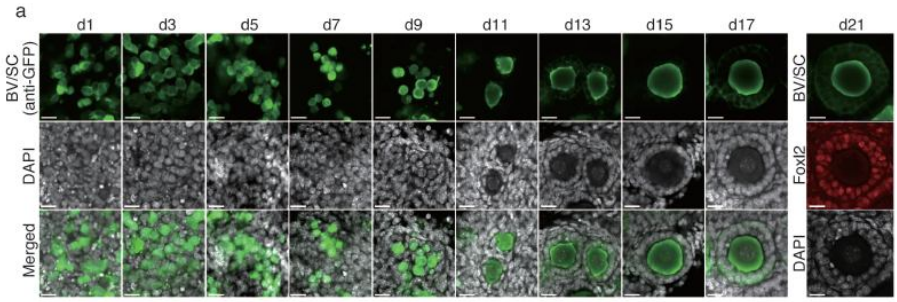
From PSCs to mature oocytes *in vitro*?



IVDi = day0-11: aMEM, 2%FCS, 150uM AA, bME
day4-6: StemPro34, 10% FCS, 150uM AA bME
day7-10: add 500nM ICI182780 (oestrogen inhibitor)
day10-21: StemPro

Hibake et al., Nature 2016

From PSCs to mature oocytes *in vitro*?



Hibake et al., Nature 2016

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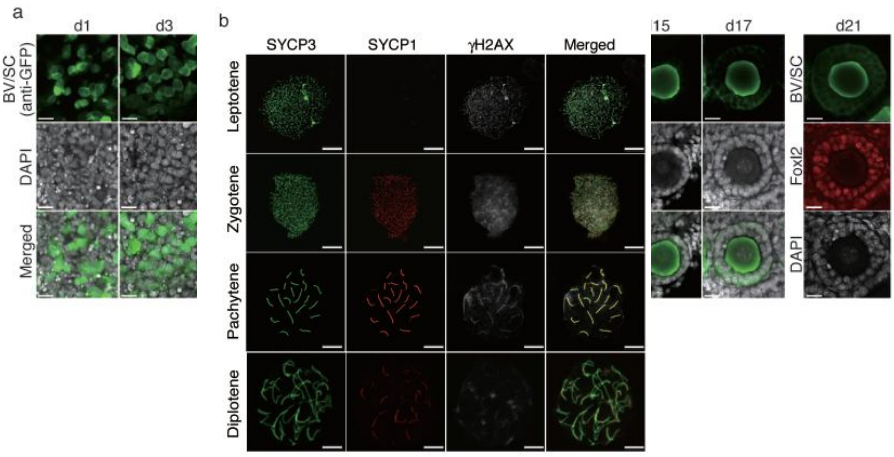

Hibake et al., Nature 2016

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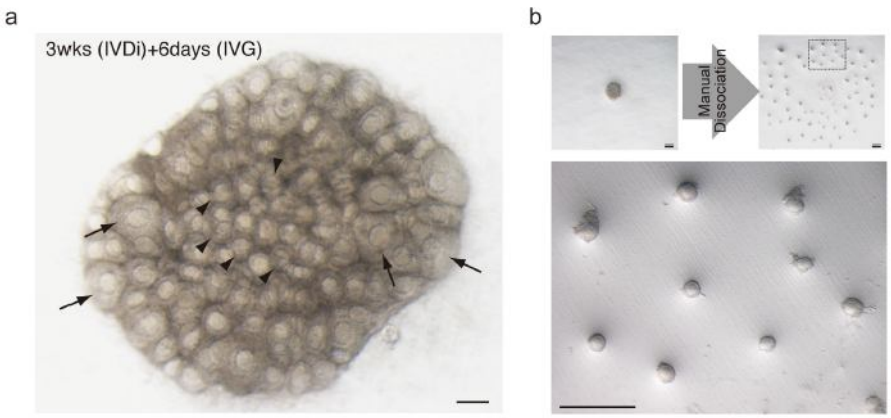

Hibake et al., Nature 2016

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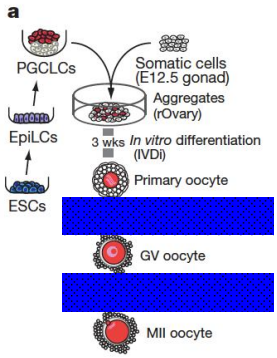
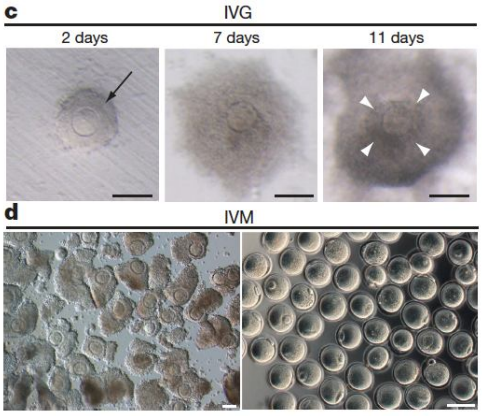


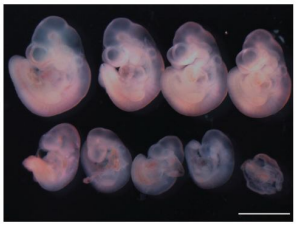


IVG = day0-2: aMEM, 5%FCS, 2%PVP, 150uM AA, bME, NaPyr, 0.1 IU FSH, 15ng/ml BMP15, 15ng/ml GDF9
day3-11: remove BMP15 and GDF9

IVM = day1: aMEM, 5%FCS, NaPyr, 0.1 IU FSH, 4ng/ml EGF, 1.2 IU hCG GDF9

Hibake et al., Nature 2016

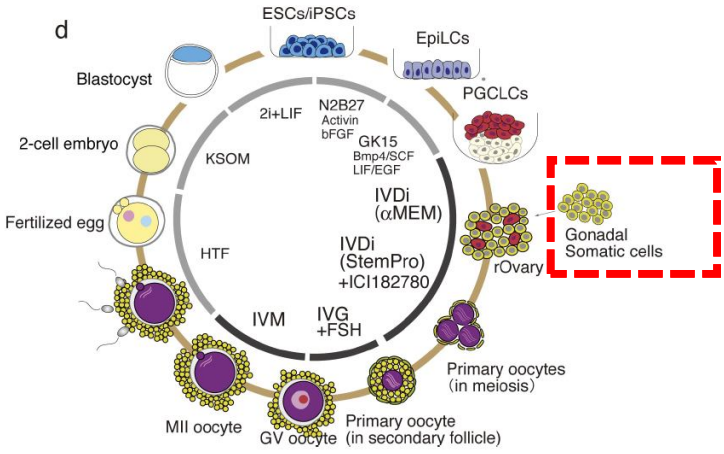
From PSCs to mature oocytes *in vitro*?



2cell -> blastocyst -> embryo -> pup
316 11 (3.5%)

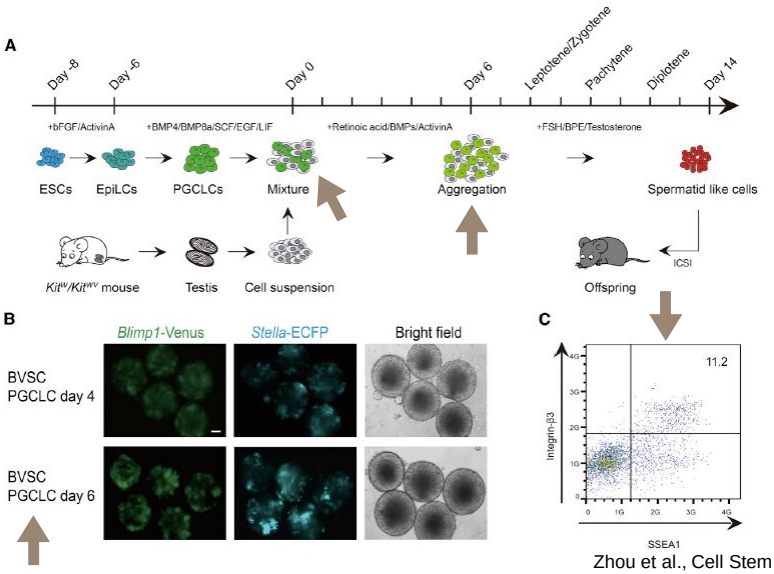
Hibake et al., Nature 2016

From PSCs to mature oocytes *in vitro*?



Hibake et al., Nature 2016

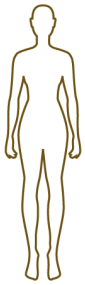
From PSCs to mature sperm *in vitro*?



Zhou et al., Cell Stem Cell 2016

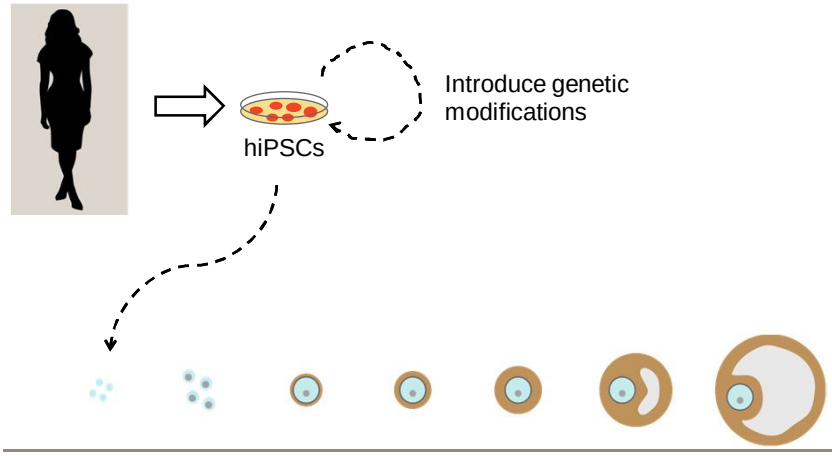
From PSCs to mature gametes *in vitro*?

- Co-culture step (embryo or baby gonads)
- Use of serum; very inefficient
- We are getting there!



- Issue of naïve/primed (what to use?)
- Co-culture step is challenging
- Gametogenesis is different from mouse
- Adult stem cells: male is ongoing, female unclear

Can we **create** and **modify** own germ cells?



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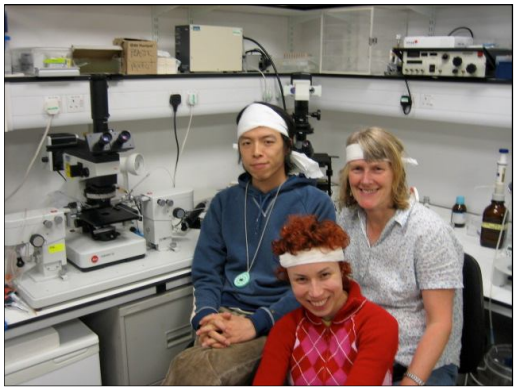

Can we **create** and **modify** own germ cells?

Thank you!

Dept. Anatomy and Embryology, LUMC
Susana Chuva de Sousa Lopes
Lisbeth van Iperen
Monika Bialecka
Fand Wang
Nannan He

Dept. Gynaecology, LUMC
Lucette van der Westelaken
Leoni Louwe

Dept. Reproductive Medicine, UZ Gent
Petra De Sutter
Susana Chuva de Sousa Lopes
Bjorn Heindryckx
Jasin Taelman
Sharat Warrier
Mina Popovic