Production of sperm and oocytes in vitro

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The germ cell cycle

Chuva de Sousa Lopes & Roelen 2010 Differentiation 79, 131-140
Extra-embryonic BMP4 induces the formation of primordial germ cells (PGCs) in the proximal epiblast.

All cells of the epiblast have the capacity to adopt a PGC fate.

Generation of germ cells from pluripotent cells would need to follow a similar program.

Lawson et al., 1999 Genes Dev 13, 424-436
Chuva de Sousa Lopes et al., 2004 Genes Dev 18, 1838-1849
PGCs are first recognized as a small cluster of ALP-positive cells.

Fig. 7. (A) Stage 7-IV embryo stained as whole-mount for ALP activity.

Ginsburg et al., 1990, Development 110: 521-528

Lawson et al., 1999 Genes Dev 13, 424-436
PGCs migrate through the hindgut to colonize the developing gonads.
The cells that form the inner cell mass are *pluripotent*. 
Embryonic stem (ES) cells are derived from blastocysts

An egg is fertilized and forms an embryo

A blastocyst is being formed with an inner mass of pluripotent cells

The embryo cleaves

The cells of the inner cell mass can be cultured in vitro

...for many years!

Nerve cells can be used to treat Parkinson’s disease

Pancreatic cells can be used to cure diabetes

Cardiomyocytes can repair a damaged heart

Mummery, vd Stolpe, Roelen & Clevers 2014, Stem Cells
Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

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iPS cells are pluripotent
ES and iPS cells can form functional germ cells in vivo: chimaera
Differentiation to pre-gastrulating epiblast-like cells (EpiLC)

Hayashi et al., 2011, Cell 146: 519-532
Germ cell formation and differentiation

Kojima et al., 2017, Cell Stem Cell 21: 517-532

Hayashi et al., 2011, Cell 146: 519-532
Somatic gonadal cells direct sex determination

PGC formation

- migrating PGCs
- prospermatogonia
- spermatogonial stem cells
- spermatocytes
- round spermatids
- spermatozoa

entering meiosis
primordial follicle
primary follicle
secondary follicle
antral follicle
MII oocyte
zygote
Somatic tissue is needed for the final differentiation

Cells sorted from aggregates for Blimp1-Venus
Transplantation into the seminiferous tubules of W/Wv mice lacking endogenous spermatogenesis

Hayashi et al., 2011, Cell 146: 519-532

Hayashi et al., 2012, Science 338: 971-975
Reconstitution *in vitro* of the entire cycle of the mouse female germ line

Timing of oocyte development differs between species

- Human ~175 days
- Mouse ~45 days
- Cattle ~100 days
Differences in germ cell specification

Kojima et al., 2017, Cell Stem Cell 21: 517-532
Generation of human oogonia from hiPS cells in vitro

Yamashiro et al., 2018 Science 362, 356-360
Genetic improvement can go much faster
Reproduction of critically endangered species

Saragastu et al., 2016 Zoo Biology 35, 280-292
Numbers of healthy eggs decrease with age
Thank you