Stem cells to gametes

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Important for research

- Regulatory mechanisms in human gametogenesis

- To get human gametes for research
  - Somatic cell nuclear transfer
  - Parthenogenesis

- Toxicity tests in gametogenesis

- In the future maybe also treatment
PGC – oogonia - oocytes

- The epithelium of the gonadal ridge grows as primary sex cords to the mesenchyma of the gonadal ridge.
- The primary cords disappear, and the primordial germ cells develop to oogonia, which at first are dispersed in the mesenchyma.
- The oogonia start forming nest-like clusters at week 10.
- They divide intensively.
- Each oogonia will be surrounded by a single thin cell layer formed by the mesenchymal cells.
- By week 16, development of primordial follicles begins.
Which stem cells?

- Primordial germ cells (PGC) are pluripotent stem cells which normally form oocytes when residing in the ovary
  - Ovarian tissue is essential
  - Wnt 4 signalling is needed for the development of oocytes and ovary in the gonadal ridge.
- PGCs can form pluripotent stem cell lines (Shamblott et al. PNAS 1998)
- Embryonic stem cells (ESC)
- Induced pluripotent stem cells (iPSC)
- Ovarian stem cells?
- Other tissue-derived stem cells???
Primordial germ cells are the first step in gametogenesis (PGC)

- Real stem cells for gametogenesis
- Known in both mouse and human PGC (McLaren 2003, 2005, Shamblott et al. 1998)
- PGCs are the first step of gametogenesis from mESC and hESC
- Identified in cultures in the basis of gene and protein expression (VASA)
- Further development in vitro to postmeiotic cells
Expression of OCT4, SSEA4 and OCT4 + SSEA4 in sections from a human ovary, 18 wpc.

Bykova A et al. Hum. Reprod. 2011;humrep.der145
Expression of C-KIT, MAGE-A4 and OCT4, and morphology of perinatal human ovaries.

Expression of C-KIT, MAGE-A4 and OCT4, and morphology of perinatal human ovaries. (A) High magnification of peripheral part of a 13 day old ovary stained with HP. Three large diplotene oocytes and two smaller oogonia-like cells (arrows) appear to be enclosed in common cell cords. (B) Section close to (A) stained for C-KIT. The arrows point at the nucleus (no staining) of three small oogonia with stained plasma membranes. Stained plasma membranes of oocytes are seen in the lower part of the figure in connection with the oogonia. (C) Section of a 6 day old ovary stained with HP showing part of a medullary placed follicle containing many oogonia (arrows) in the granulosa layer. (D) Section adjacent to the one seen in (C) stained for C-KIT. The plasma membrane of the oogonia in the granulosa layer are all stained, whereas the granulosa cells are unstained. (E) Part of a WB from the medulla of an ovary of a fetus close to term with four oogonia stained for OCT4. (F) HP-stained section from medulla-cortex transition of a 32 wpc ovary with a large WB (marked by a stippled line) in the medulla. (G) A higher magnification of part of the WB in (F) with somatic cells, a diplotene oocyte partly enclosed in a follicle and abnormal oocytes at the pachytene stage (arrows). (H) Section adjacent to (G) stained for C-KIT showing a small follicle, in which the plasma membrane of the oocyte is stained, and smaller stained cells, probably oogonia, as well as an oocyte possibly in pachytene stage of the meiotic prophase (arrow) with unstained plasma membrane. (I) A tangential section of a follicle from a 5 week old ovary showing a few small oogonia and a larger cell stained for MAGE-A4

Byskov A et al. Hum. Reprod. 2011;humrep.der145
Byskov et al 2011:

No oogonia in ovaries of girls over 2 years

MAGE4 not after 3 months of age, only in tumours

Perinatally often follicles containing more than one oocyte
Fertilisable oocytes from neonatal mouse ovaries

- Female germline stem cells (FGSCs) in postnatal mouse ovaries after 15 months culture
- Transduction with GFP
- Transplantation into infertile mice ovaries, GFP-positive oocyte production and offspring
- This has not repeated by anybody else so far
Oocytes from mouse ESC, Hubner et al. 2003

- Follicle-like structures developed spontaneously
- Oocytes up 10 diameter of 100µ
- Oestradiol secretion
- Steroidogenic enzymes
- GDF9 secretion increase up to 7-fold
- ZP proteins, weak ZP could be identified
- Later, meiotic markers (SNP3 etc)
- Addition of gonadotrophin (pregnant mare serum), resulted in polar body extrusion like event
Hubner et al. Science 2003, Derivation of oocytes from mouse embryonic stem cells, d26 culture
Characterisation of the mES-derived oocytes, Hubner et al.
Meiotic markers, polar body extrusion
Spontaneous parthenogenetic activation, Hubner et al. Science 2003
Viable offspring from mESC derived sperm

- A two-step culture
- Reporter gene (Stra8-GFP) expressing mESC
- FACS sorting after initial mESC culture
- RA-induction, selection by Prm1-dsred expression
- Microinjection of cell after a few days
- Offspring obtained, which had several abnormalities, died from 5 days to 5 months
- These experiments have not been repeated

Nayernia et al. Developmental Cell 2006
hESC to gametes

- Embryoid bodies, suspension, 3D spheres
- Retinoic acid
- BMP 2, BMP4, BMP8b
- Both female and male hESC, both male and female gametogenesis from both
Germ cell differentiation from human embryonic stem cells


- Aflatoonian et al 2005

- Early postmeiotic cells as shown by marker expression
Human *DAZL, DAZ* and *BOULE* genes modulate primordial germ-cell and haploid gamete formation from human ES cells

Germ-cell properties of VASA–GFP+ cells.

Instructing an embryonic stem cell-derived oocyte fate: lessons from endogenous oogenesis.
Nicholas CR, Chavez SL, Baker VL, Reijo Pera RA.
Table 1
Human germ cell differentiation protocols

<table>
<thead>
<tr>
<th>Reports (Ref.)</th>
<th>Clark et al. (2)</th>
<th>Kee et al.(3)</th>
<th>West et al.(214)</th>
</tr>
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<tbody>
<tr>
<td>ESC-derived</td>
<td>Germ cells</td>
<td>Germ cells</td>
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<tr>
<td>Species</td>
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<td>ESC sex</td>
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<td>Time course (up to)</td>
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<td>Enrichment</td>
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<td>MEFs and bFGF</td>
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<td>Identification</td>
<td>VASA, SCP1, GDF9, TEKT1</td>
<td>VASA, SCP3</td>
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<td>Isolation</td>
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<td>Maturation</td>
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<td>Germ cell profile</td>
<td>Early and late germ cell markers detected</td>
<td>BMP cocktail increases germ cell marker expression</td>
<td>90% of germ-like cells express meiotic markers</td>
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<tr>
<th>Tilgner et al.</th>
<th>Bucay et al.</th>
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<td>PGCs</td>
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<tr>
<td>Human</td>
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<td>SSEA1 + FACS</td>
<td>CXCR4 + FACS</td>
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<tr>
<td>PGC-like cells with partial imprint erasure and histone modification</td>
<td>PGC-like cells along with sertoli-like cells</td>
</tr>
</tbody>
</table>
Specification of PGCs

Blimp1
BMP4  Stella
BMP8b  Fragilis
BMP2  Oct4
SCF  Nanog
TNAP
C-Kit

Migration to genital ridge

Gamete determination

RA  VASA
    DAZL
    Stra8
    SCP3

Gametogenesis

H1t  GDF9
TNP1  ZP1
TNP2  ZP3
    PRM1
    PRM2

SPERM  EGG

Gonadal development

Moore and Aflatoonian 2011
Ovarian niche transplantation surmounts the in vitro ESC-derived oocyte maturation bottleneck.
Development of a human ovarian niche via human fetal ovary re-aggregation and transplantation.

Human ESC to gametes
HS360

Kjartansdottir et al. unpublished
Gametes need their somatic niche for proper maturation from stem cells

- The niches develop from developing pluripotent stem cells at the same time as the gametes do?
- The developing gametes are transplanted to ovarian/testicular tissue?
- An artificial niche?
Oocyte-like cells (OCT)

- There are several articles describing establishment of "oocyte-like" cells from various tissues and cells
  - Ovarian surface epithelium etc
- No convincing morphology
- No functional evidence, final functional evidence would be fertilisation
Human adult germ line stem cells are not pluripotent

- They express markers which are common also in testicular fibroblast lines
- They can form tumours, which are not teratomas
- No proven teratoma formation as a sign of pluripotency

Induced germ cells are not pluripotent

- We have two induced cell lines (Nanog, Sox2, Lin 28. Oct4) which form germ cells tumours when injected to SCID mice, but are not pluripotent and do not form germ cells in vitro.

ChiPSC 22

Gametes from iPS cells

- Regulatory mechanisms and toxicity testing
- If functional and safe would solve the ethical problems related to gamete donation, and paucity of donors
- The epigenetic normality has to be shown
- Fertilisation and normal blastocyst development needs to be shown
- Excellent model to study such events in human
- Primordial germ cells, and postmeiotic spermatocytes form human iPSC (Panula, Bergström, Kee, Hovatta, Reijo Pera, muuta 2010)
Germ cell differentiation of iPS cells

Panula et al. Mol Hum Genet 2010
Human fetal gonad (7.5 weeks dpc) differentiation for 14 days *in vitro*

PAS staining (paraffin embedded tissue)

Stukenborg et al. unpublished
**In vitro cultures**

**In vitro cultures**

Organ and tissue culture


3D-culture

Many thanks

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Golden eagle, Owen Valley, California 2011