FEMALE GAMETES FROM STEM CELLS?

Prof. Carlos Simón

POOR OVARIAN RESPONSE - ESHRE CAMPUS 2010

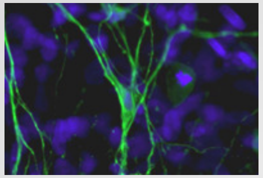
Ana I Marques-Mari, PhD

E-learning objectives

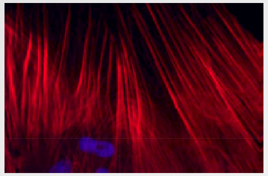
- To know about *in vivo* germline differentiation and different strategies used for *in vitro* differentiation.
- To learn about the State of the Art in germline differentiation:
  - From mouse and human ESC
  - From mouse and human SC
- To understand the main limitations when differentiation is carried out *in vitro*.
- To discuss about the future of synthetically generated gametes.

Differentiation Potential

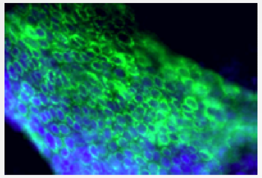
- Totipotent:
  - Zygote – Blastomeres
- Pluripotent:
  - **ESC**
  - GSC
  - iPS
- Multipotent:
  - **Somatic SC**



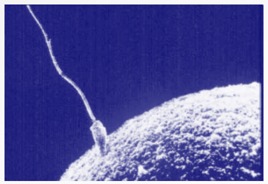
Mesoderm



Ectoderm

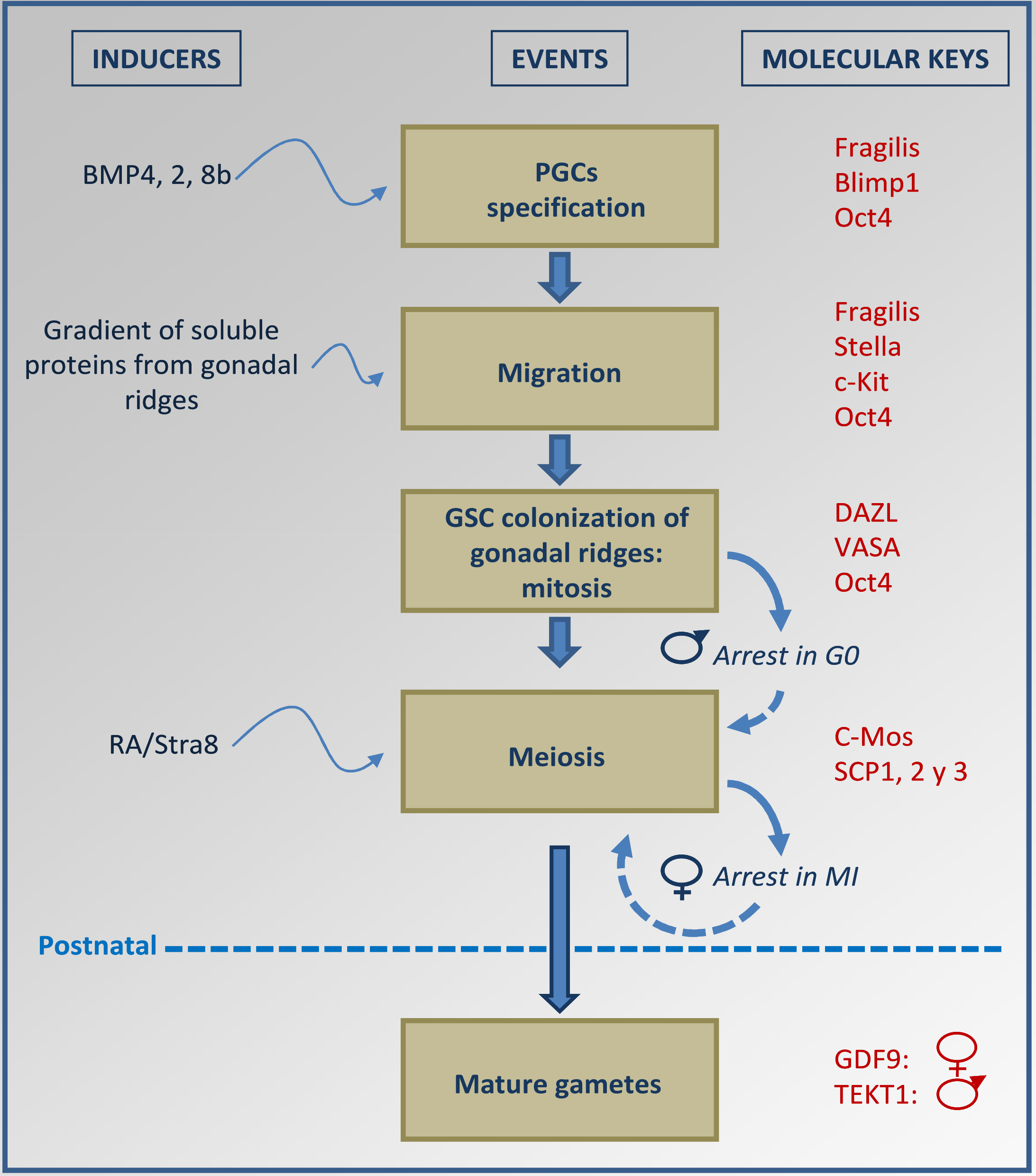


Endoderm



Germ cells

Genetics of the germline establishment *in vivo*

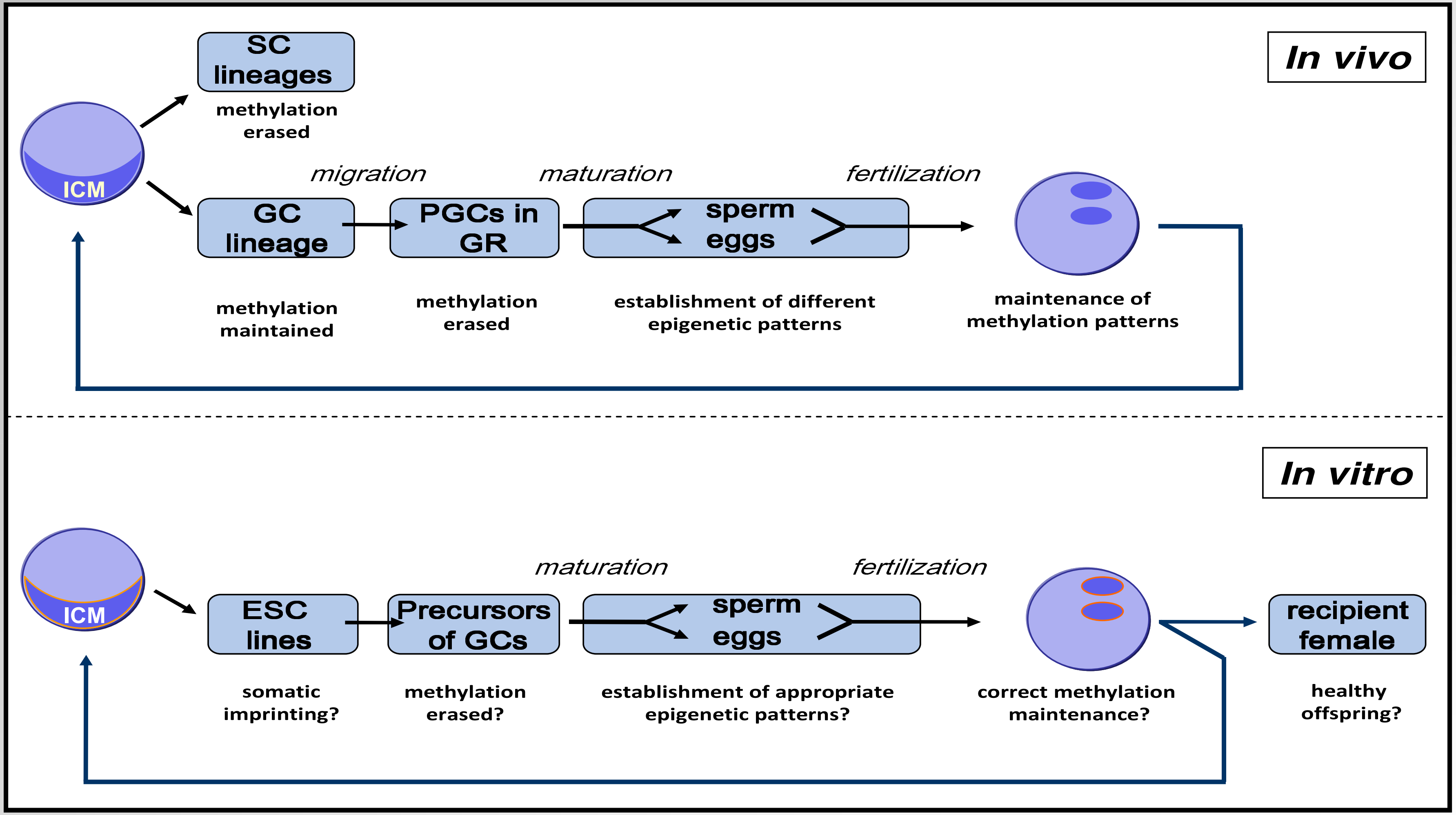


Critical step:

Meiosis

Modified from Marques-Mari et al., HRU 2009

Epigenetics of the germline establishment



Critical step: **Epigenetic modifications**

Modified from Marques-Mari et al., Hum Rep Update 2009

Strategies for germline differentiation *in vitro* from ESC

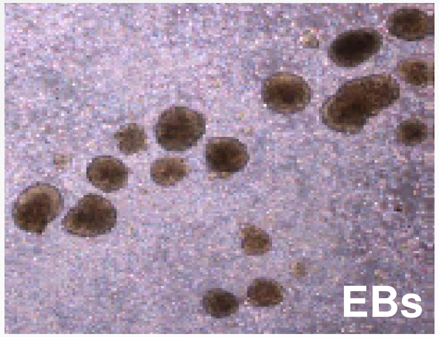
•**Spontaneous differentiation** in monolayer or through EBs

• **Addition of growth factors**.

• **Co-culture** with conditioned media or somatic cells.

• **Direct transfection**.

•**Transplant** into *in vivo* systems.



State of the Art in Germline Differentiation

GERM CELLS DIFFERENTIATION FROM EMBRYONIC STEM CELLS				
Authors	***Publication date***	***Source of SC***	***Cell type obtained***	***Viable offspring***
Hübner *et al.*	2003	mESCs – XX, XY	Oocytes	No, PB
Clark *et al.*	**2004**	**hESCs – XX, XY**	**Oocytes (although there was TEKT1 expression)**	**NT**
Lacham-Kaplan *et al.*	2006	mESCs - XY	Immature oocyte-like cells	NT
Novak *et al.*	2006	mESCs - XY	Ovarian follicles	NT
Kee *et al.*	**2006**	**hESCs - XX**	**Oocyte-like cells**	**NT**
Chen *et al.*	**2007**	**hESCs - XX**	**Oocyte-like cells**	**No, FD**
Qing *et al.*	2007	mESCs - XY	Oocytes	NT
Park *et al.*	**2009**	**hESCs - XX, XY hiPS - XY**	**PGCs**	**NT**
Kee *et al.*	**2009**	**hESC – XX, XY**	**Haploid gamete-like cells**	**NT**
Nicholas *et al.*	2009	mESC - XX	Immature oocyte-like cells	NT
Tilgner *et al.*	**2010**	**hESC – XX**	**VASA+ Germ-like cells**	**NT**

NT: not tested; PB: parthenogenic blastocyst; OF: oocyte fertilization; FD: follicles degeneration; hESCs: human embryonic stem cells; mESCs: mouse embryonic stem cells; PGCs: primordial germ cells

Differentiation in adherent culture

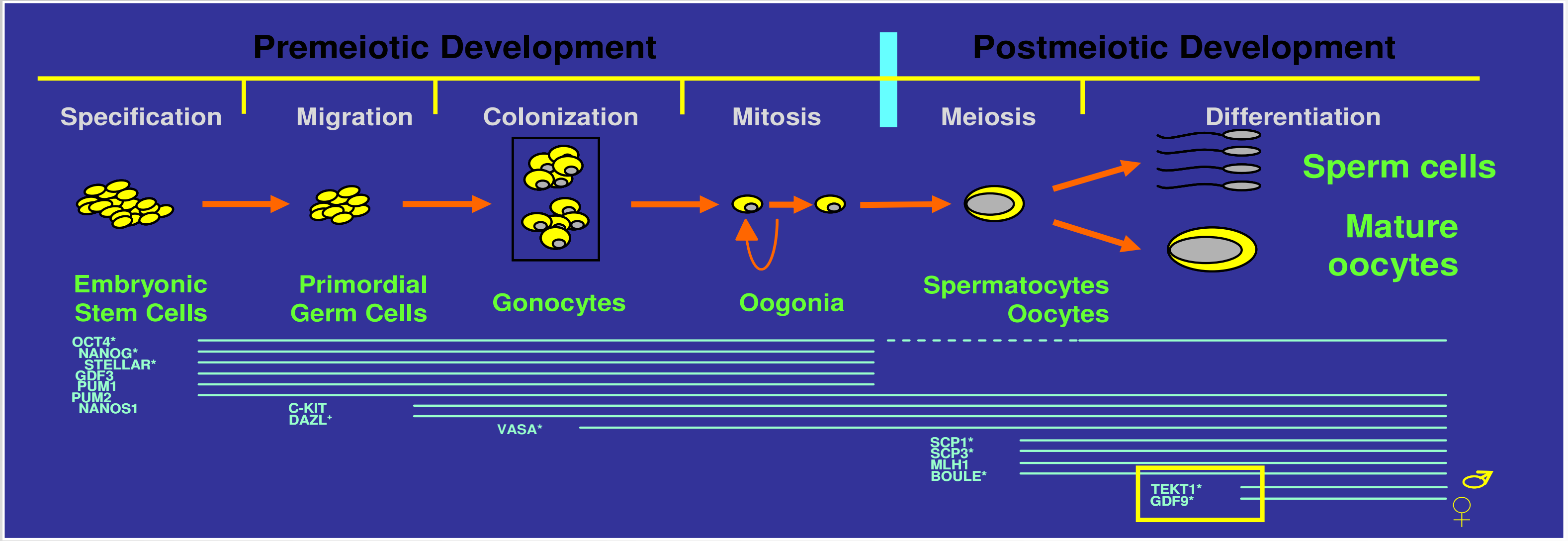
- Hübner *et al.*, Science 2003
  - Mouse ESC carrying a *gc*Oct4-GFP reporter were differentiated in monolayer.
  - Formation and characterization of follicle-like structures containing oocyte-like cells.
  - Presence of meiotic proteins (SCP3) and specific oocyte markers (ZP1-3).
  - Formation of pseudoblastocysts by parthenogenesis.

- Novak *et al.*, Stem Cells 2006
  - Lack of some meiotic proteins as SCP1 and SCP2. No evidences of chromosomal synapsis formation: unsuitable meiosis.

Differentiation of hESC through EBs and addition of Growth factors

- <u>Clark *et al*., Hum Mol Genet 2004</u>

  Spontaneous differentiation through EBs formation to obtain germ cells. Establishment of a gene expression sequence during germline specification in human.



Modified from Clark et al. 2004

- <u>Kee *et al*., Stem Cells Dev 2006</u>

  Addition of BMP4, 7 and 8 to the EBs medium increases VASA expression.

Differentiation through EBs in co-culture with CM or somatic cells

- Lacham-Kaplan *et al*., Stem Cells 2006
  - Co-culture of EBs with testicular cells conditioned medium to obtain oocyte-like cells in an early stage of development.
  - Lack of zona pellucida, but expression of specific markers as ZP3.

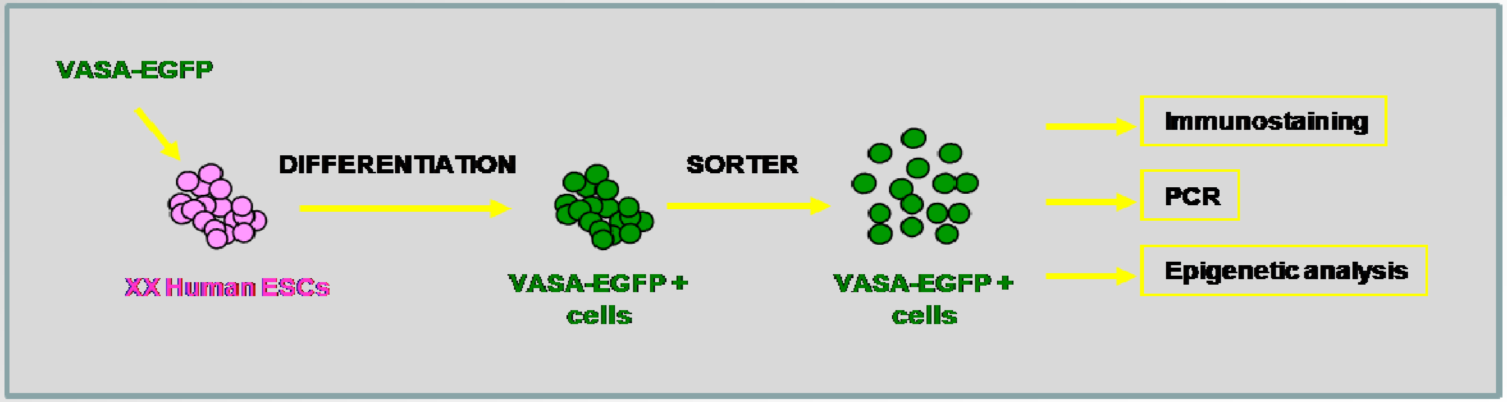
- Qing *et al*., Differentiation 2007
  - Co-culture of EBs onto ovarian granulosa cells.
  - Presence of premeiotic (Mvh), meiotic (SCP3) and postmeiotic (GDF9) markers, as well as oocyte specific markers (Figα, ZP1-3).

Differentiation of hESC by co-culture with somatic cells

- Park *et al.*, Stem Cells 2009
  - Co-culture of hESC with human fetal gonadal cells to obtain PGCs.
  - They also obtained PGCs from hiPS cells, although less efficiently.
  - Co-localization of the markers c-Kit/SSEA1 by FACS used as indicator or germ cell fate.
  - The obtained cells have initiated the imprinting erasure characteristic of germ cells.

Transfection of hESC lines + Adherent culture

- Tilgner *et al.*, Stem Cells 2010
  - Generation of hESC lines carrying a VASA-EGFP promoter construct.
  - Differentiation induction in adherent culture. Green fluorescent cells obtained after 14 days are putative germ cells.
  - Sorted VASA-GFP positive cells are enriched in some PGCs markers such as Oct4, Stella, DAZL, SSEA1, SSEA4 and c-Kit. A small number of cells showed SCP3 staining within the nucleus: indicative of meiosis.
  - VASA-GFP cells showed reduced methylation similar to PGCs *in vivo.*



Transfection of hESC lines + Growth factors

- <u>Kee *et al*., Nature 2009</u>
  - Differentiation of **<u>haploid gamete-like cells</u>** in vitro from hESCs expressing **meiotic and postmeiotic markers**.
  - The authors transfected hESCs XX and XY lines with a VASA-GFP reporter and differentiated them in adherent culture with addition of BMP-4, BMP-7 and BMP-8b to the culture medium.
  - The **<u>main limitation in *in vitro* differentiation of gametes</u>** is the **<u>meiosis</u>**.

    To induce entry and progression through meiosis of these cells, they genetically modified the hESC lines over-expressing the members of the DAZ gene family: DAZ, DAZL and BOULE.
  - The results suggest that these genes modulate formation and maturation of the germ cells:

    DAZL acts primarily in PGC formation.

    DAZ y BOULE in promoting progression to meiosis.

Transplant into *in vivo* systems

- Nicholas *et al*., Human Mol genetics 2009
  - Mouse ESC carrying an Oct4-GFP reporter were differentiated in EBs.
  - Cells displaying low intensity of GFP without SSEA1 expression were characterized as germ cells.
  - Markers of meiosis and oocyte maturation such as Stra8 and GDF9 were found in GFP+/SSEA1- cells. However, meiotic progression was incomplete as shown by abnormal alignment of SCP1 and SCP3.
  - DAZL is required for germ cell maturation *in vivo* as well as *in vitro* since DAZL null ESCs displayed a reduced percentage of differentiated GFP+/SSEA1- germ cells.
  - Co-aggregates of GFP+ germ cells and newborn ovarian tissue were transplanted into recipient mice. GFP+ oocytes arose from those grafts although with a very low efficiency (0.023%)

State of the Art in Germline Differentiation

GERM CELLS DIFFERENTIATION FROM SOMATIC STEM CELLS				
Authors	***Publication date***	***Source of SC***	***Cell type obtained***	***Viable offspring***
Johnson *et al.*	2004	mGSCs in OSE	Ovarian follicles after transplantation	NT
Johnson *et al.*	2005	mGSCs in female BM and PB	Ovarian follicles after transplantation	NT
Dyce *et al.*	2006	Fetal porcine skin	Oocytes	PB
Zou *et al.*	2009	Ovarian mGSCs	Follicles with oocytes after transplantation	Yes
Linher *et al.*	2009	Fetal porcine skin	PGCs that give rise to oocyte-like cells	NT

NT: not tested; PB: parthenogenic blastocyst; mGSCs: mouse germ stem cells; hGSCs: human germ stem cells; OSE: ovarian surface epithelium; BM: bone marrow; PB: peripheral blood

GSC in Bone Marrow and Peripheral Blood

- Johnson *et al*., Nature 2004 - Cell 2005

  - Suggested that GSCs in the adult mouse ovaries proliferate and restore the follicle pool: VASA+ cells in the ovary surface.
  - Proposed:
    - -GSC reside in the bone marrow: expression of germline markers. BM transplants rescue follicles population.
    - -Putative germ cells in peripheral blood: blood transfusions might collaborate in oocytes production.

- *Arguments against this hypothesis:*

Byskov et al., 2005. Crucial events in producing a functional oocyte has not been proven: meiosis and enclosure in a follicle.

Egan et al., 2006. Parabiotic mouse model. Failure of BM transplant and parabiosis to rescue ovulation.

Liu et al., 2007. No evidences of neo-oogenesis in the adult human ovary.

Differentiation of Oocyte-like cells from fetal porcine skin

- Dyce *et al*., Nat Cell Biol 2006
  - Obtaining of oocyte-like cells (OLC) from fetal porcine skin in co-culture with follicular fluid and with addition of gonadotropin.
  - Formation of follicular structures containing oocytes which underwent spontaneous cleavage forming pseudoblastocyst by parthenogenesis.
  - Presence of premeiotic (VASA) and meiotic markers (SCP3) and oocyte specific markers (ZP).

- Linher *et al*., Plos one 2009
  - Characterization of the PGCs that give rise to OLC from fetal porcine skin: Oct4, Stella, Dazl and VASA expression.

Differentiation of oocytes from ovarian cells with viable offspring

- Zou *et al*., Nature Cell Biology 2009
  - Generation of GSC lines from VASA+ neonatal and adult ovarian cells.
  - These cells were transfected with GFP and after transplantation into the ovaries of sterile mice generated follicles containing green oocytes (GFP +),
  - The GFP + oocytes were fertilized and produced normal and fertile offspring carrying the GFP gene.

Gametes from Stem Cells:

Present and Future

- Generation of gamete-like cells from embryonic and somatic SCs.
- Completion of meiosis *in vitro*, production of haploid cells and obtaining of successful progeny.
- Creation of *in vitro* models for gametogenesis study.

. . .

- Generation of gametes by **direct reprogramming**.
- Development of new strategies for proper meiosis completion and epigenetic pattern establishment in culture.
- Synthetic gametes as a tool for basic research.
- Potential use of oocytes in regenerative medicine with therapeutic cloning and nuclear transference techniques.
- Potential use of obtained gametes for assisted reproduction.

References:

- Byskov et al., Differentiation 2005; 73:438–446.
- Clark et al., Hum Mol Genet 2004; 13-727-739
- Dyce et al., Nat Cell Biol 2006; 8:384–390
- Eggan et al., Nature 2006; 441:1109–1114.
- Hübner et al., Science 2003; 300:1251-1256
- Johnson et al., Nature 2004; 428:145–150
- Johnson et al., Cell 2005; 122:303–315
- Kee et al., Stem Cells Dev 2006; 15-831-837
- Kee et al., Nature 2009; 462:222-225
- Lacham-Kaplan et al., Stem Cells 2006; 24:266-273
- Linher et al., Plos One 2009; 4(12):e8263
- Liu et al., Dev Biol 2007; 306:112–120.
- Marques-Mari et al., Hum Rep Update 2009;15(3):379-390
- Nicholas et al., Human mol genetics 2009; 18:4376-4389
- Novak et al., Stem Cells 2006; 24:1931–1936
- Park et al., Stem Cells 2009; 27:783-795
- Qing et al., Differentiation 2007; 75:902-911
- Tilgner et al., Stem Cells 2010; 28:84-92
- Zou et al., Nature Cell Biology 2009; 11:631–636