





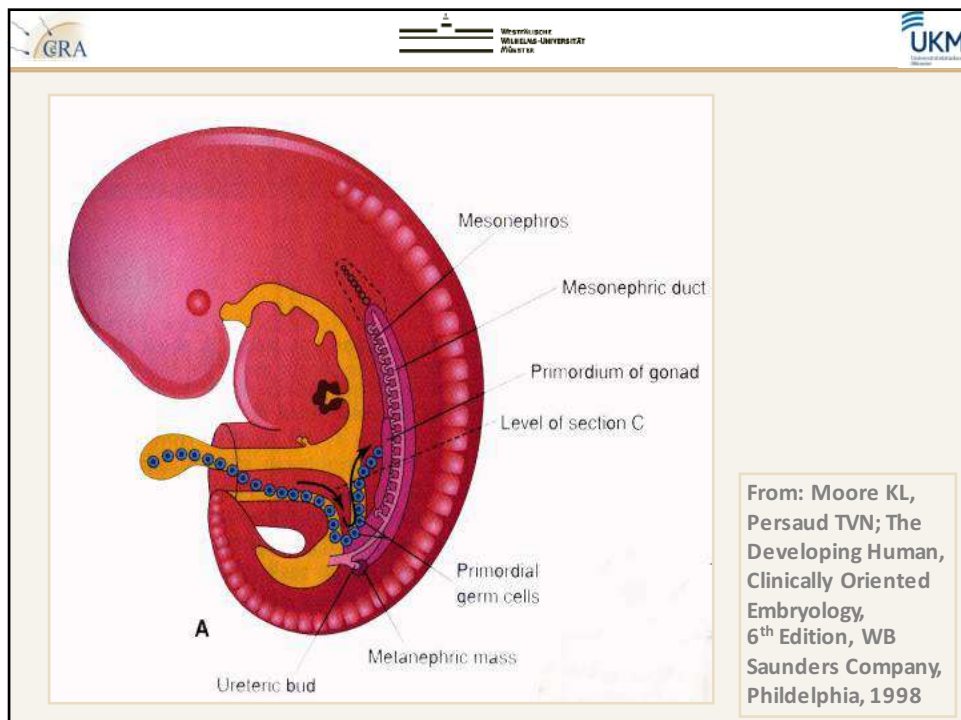
**5<sup>th</sup> World Congress of the**  
**International Society for Fertility Preservation**  
 Vienna, Austria. November 16-18; 2017  
 Session 2: Stem cells and *in vitro* growth of gametes

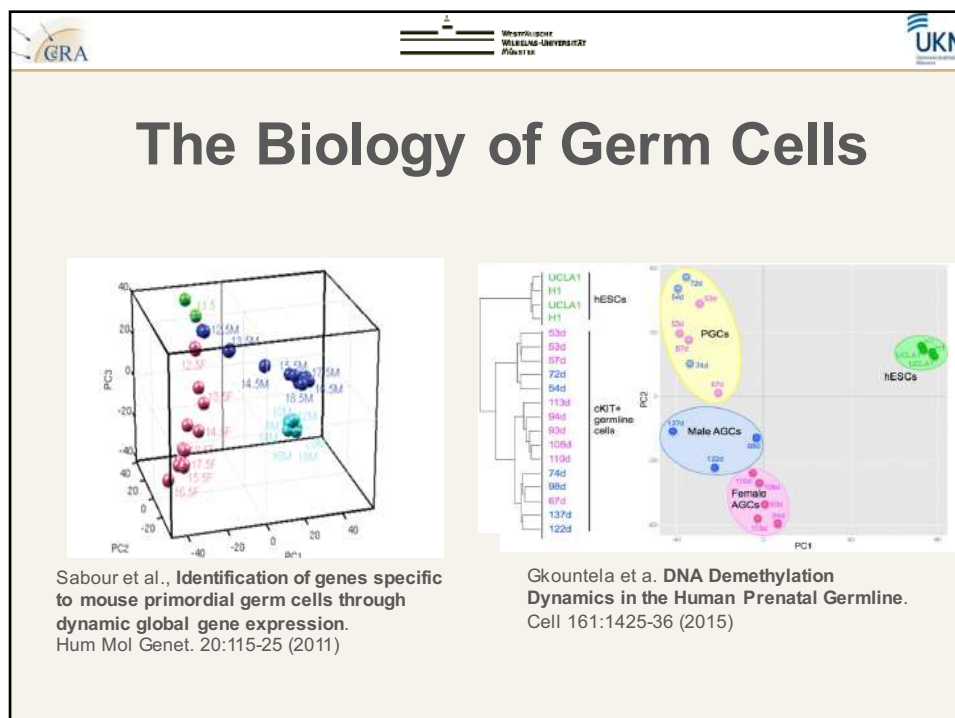
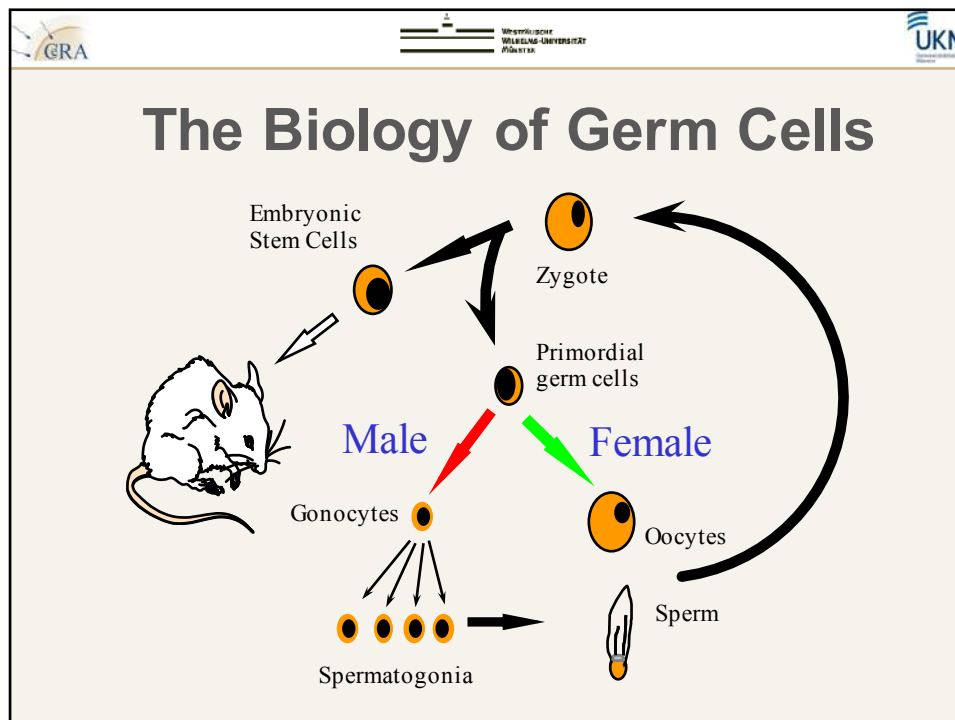
## Development, sex differentiation and clonal expansion of PGCs to create primordial follicles and spermatogonia

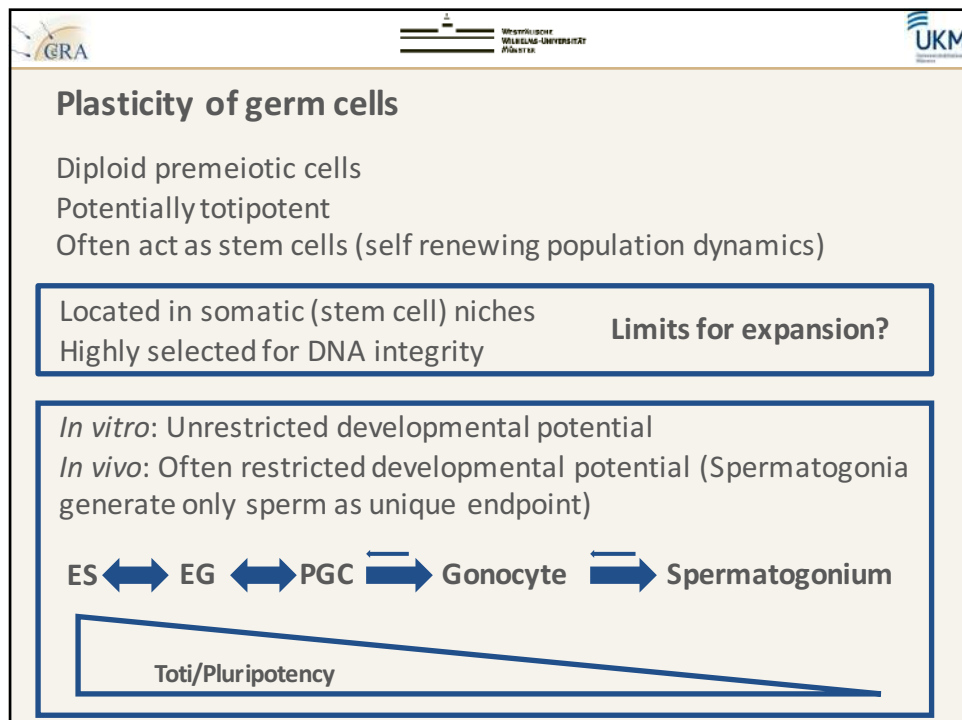
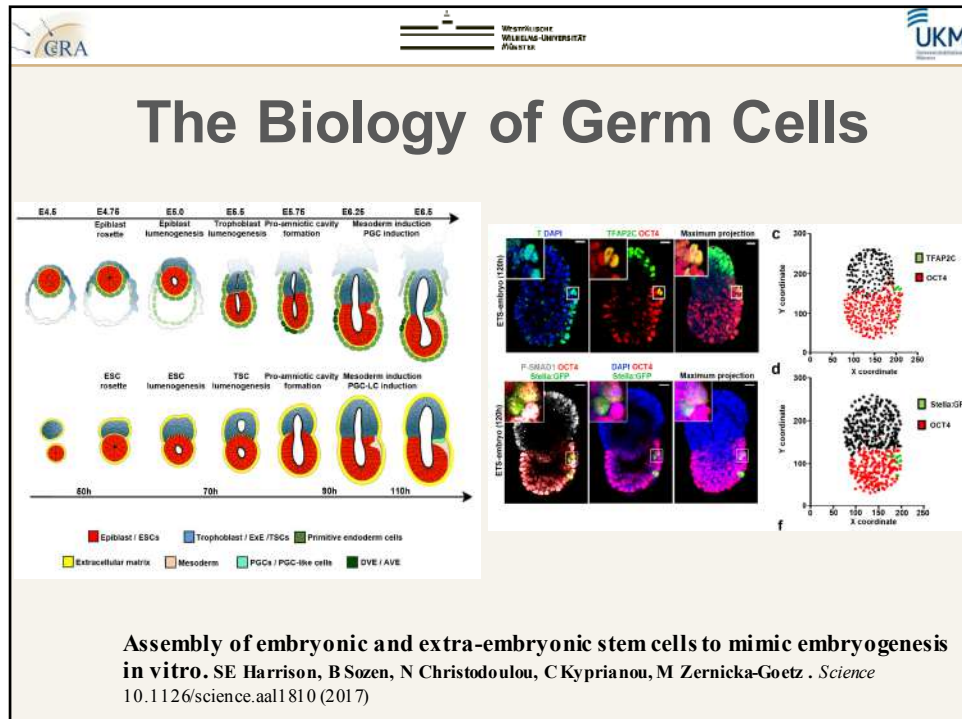
### *Scenarios for in vitro gametogenesis*

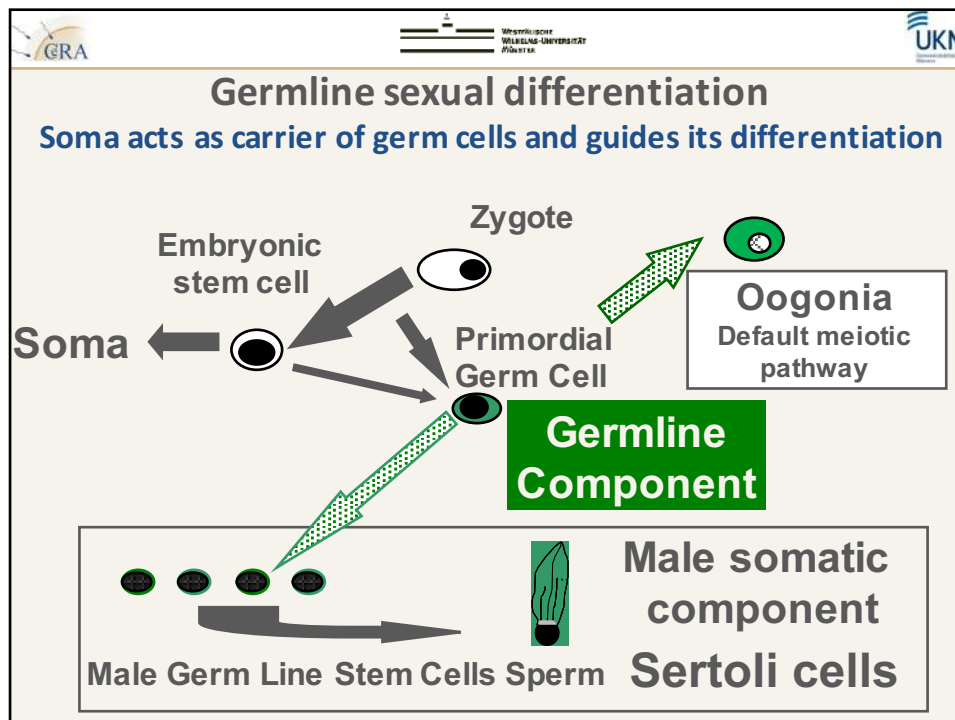
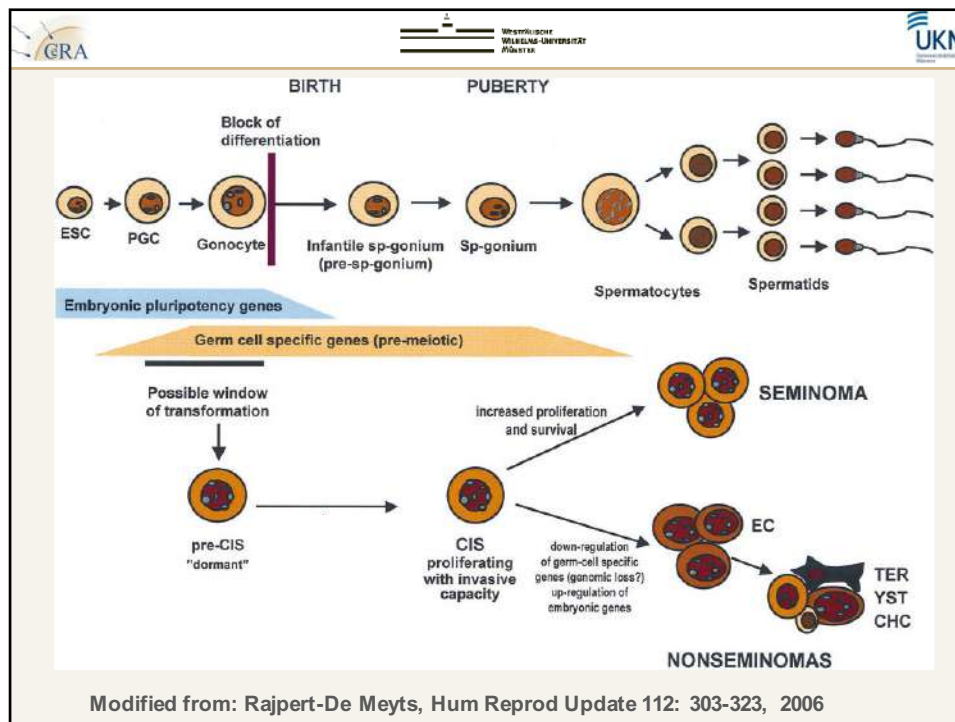


Stefan Schlatt  
 Centre of Reproductive Medicine and Andrology  
 University Münster, Germany









**Gonadal stem cell niches: Worm and Fly**

*Phenotype, number and function of germline stem cells depends on the gonadal niche*

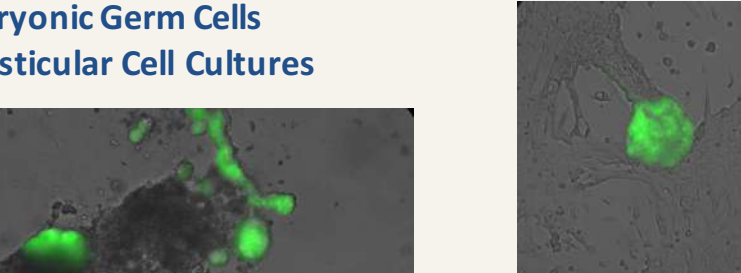
The diagram illustrates the self-renewal of germline stem cells in the *Drosophila* ovary. It shows a cross-section of the ovary with a distal tip cell (green) at the base, which is the source of germline stem cells (red). These stem cells divide to produce mitotic stem cells (yellow) and meiotic cells (white). The diagram also shows the self-renewal of somatic stem cells (blue) in the somatic region. The diagram is labeled with 'Distal tip cell', 'Germ line stem cell', 'Mitotic stem cell', 'Meiotic stem cell', 'Hub cell', 'Germ line stem cell', 'Somatic stem cell', 'Gonialblast', and 'Cyst cell'.

GRA

UNIVERSITÄT  
WÜRZBURG  
FÜR MEDIZIN

UKM  
UNIVERSITÄT  
KÖLN

## Embryonic Germ Cells in Testicular Cell Cultures



GFP-expression in mouse embryonic germ cells

Mouse testis (day 7) cultured in DMEM  
No matrix substance





Klein AM, Nakagawa T, Ichikawa R, Yoshida S, Simons BD.

## Mouse germ line stem cells undergo rapid and stochastic turnover.

Cell Stem Cell. 2010 7:214-24.  
doi: 10.1016/j.stem.2010.05.017

Klein AM, Simons BD.

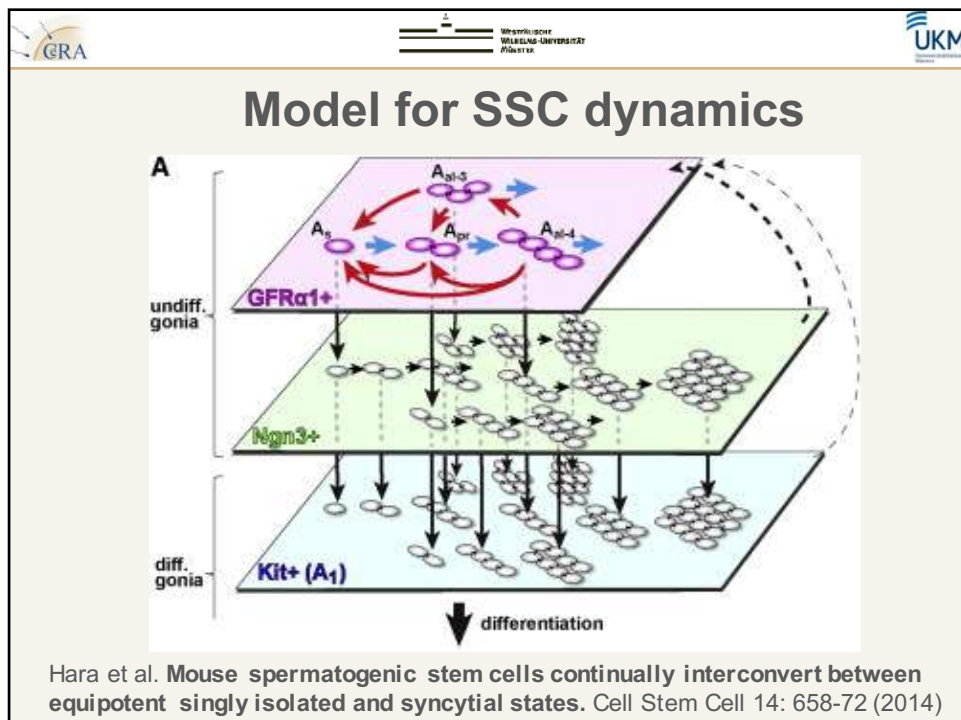
## Universal patterns of stem cell fate in cycling adult tissues.

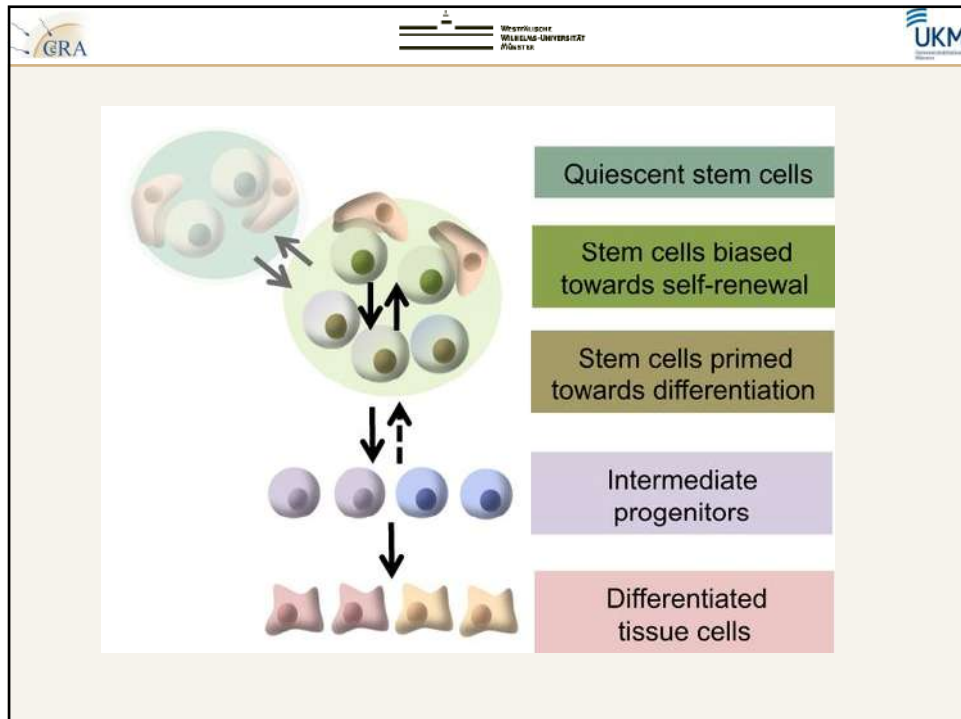
Development. 2011 138:3103-11.  
doi: 10.1242/dev.060103.

Krieger T, Simons BD.

## Dynamic stem cell heterogeneity.

Development. 2015 142: 1396-406.  
doi: 10.1242/dev.101063. Review





### Scenarios for *in vitro* gametogenesis

(PubMed: *in vitro* spermatogenesis: > 3000 hits)

**Some experiments on spermatogenesis in vitro.**  
Goldschmidt. PNAS 1: 220-222 (1915)

**Initiation of spermatogenesis in vitro.**  
Steinberger et al. Endocrinol 74: 788-792 (1964)




**In vitro differentiation of germ cells from nonobstructive azospermic patients using three-dimensional culture in a collagen matrix.**  
Lee et al., Fertil Steril 87: 824-833 (2007)

**In vitro post-meiotic germ cell development from human embryonic stem cells.**  
Aflatoonian et al., Hum Reprod 24: 3150-3159 (2009)

**PSCDGs of mouse multipotent adult germline stem cells can enter and progress through meiosis to form haploid germ cells in vitro.**  
Nolte et al., Differentiation 80:184-94 (2010)

**In vitro production of functional sperm in cultured neonatal mouse testes.**  
Sato et al. Nature 471: 504-507 (2011)

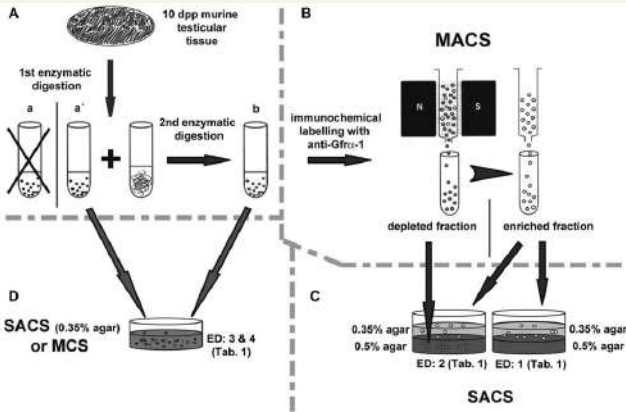


## New horizons for in vitro spermatogenesis?




### An update on novel three-dimensional culture systems as tools for meiotic and post-meiotic differentiation of testicular germ cells

Stukenborg et al. Mol Hum Reprod 15: 521–529, 2009

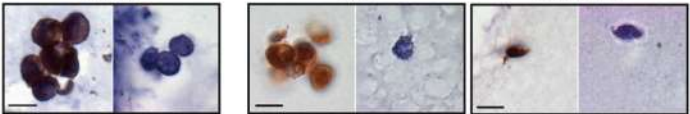


Abu Elhija M, Lunenfeld E, Schlatt S, Huleihel M.  
**Differentiation of murine male germ cells to spermatozoa in a soft agar culture system.**  
 Asian J Androl. 14:285-93 (2012)

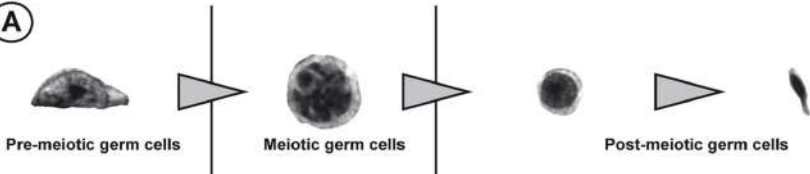
Stukenborg JB, Wistuba J, Luetjens CM, Elhija MA, Huleihel M, Lunenfeld E, Gromoll J, Nieschlag E, Schlatt S.  
**Coculture of spermatogonia with somatic cells in a novel three-dimensional soft-agar-culture-system.** J Androl 29: 312-29 (2008)

**Boule**      **Crem**      **Protamine**

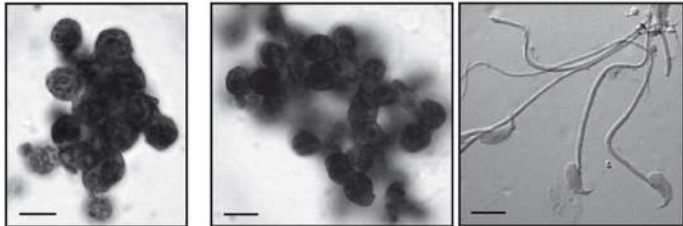


**(A)**



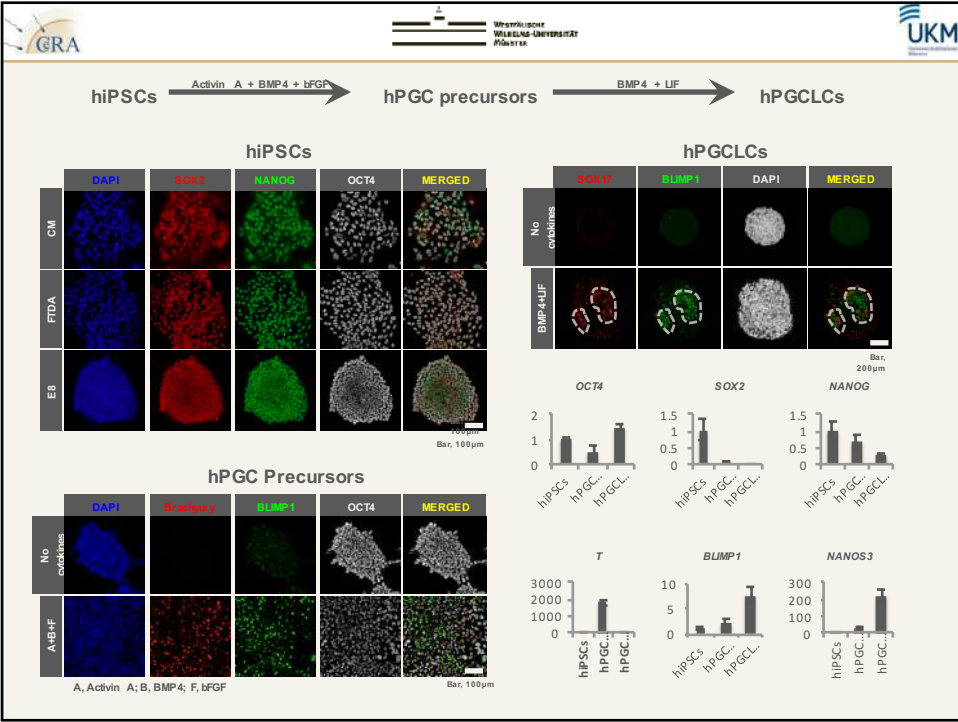
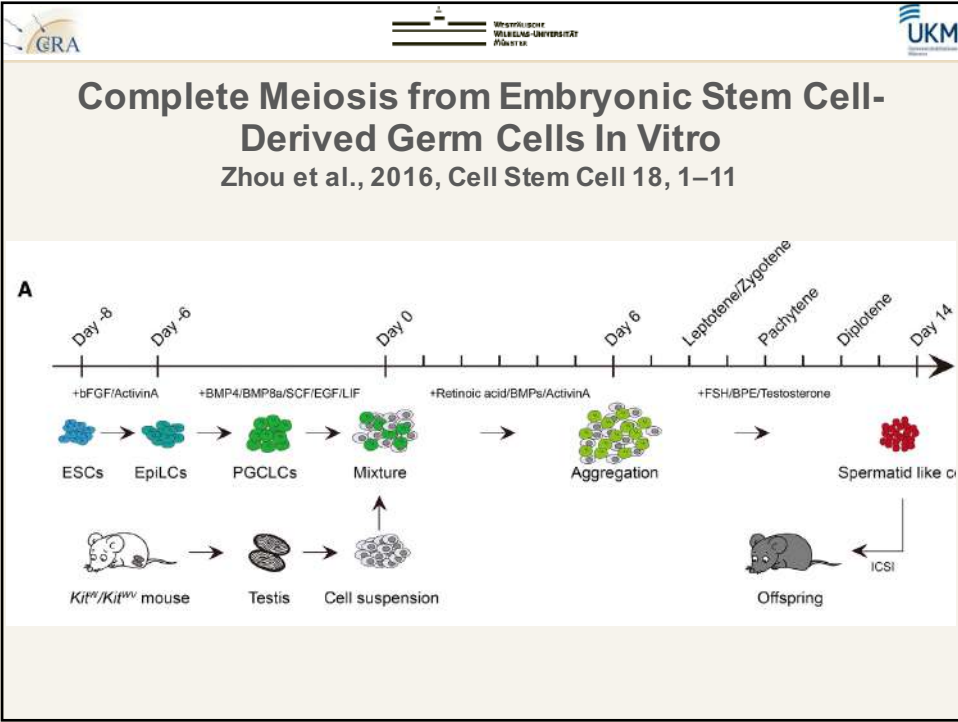
Pre-meiotic germ cells      Meiotic germ cells      Post-meiotic germ cells

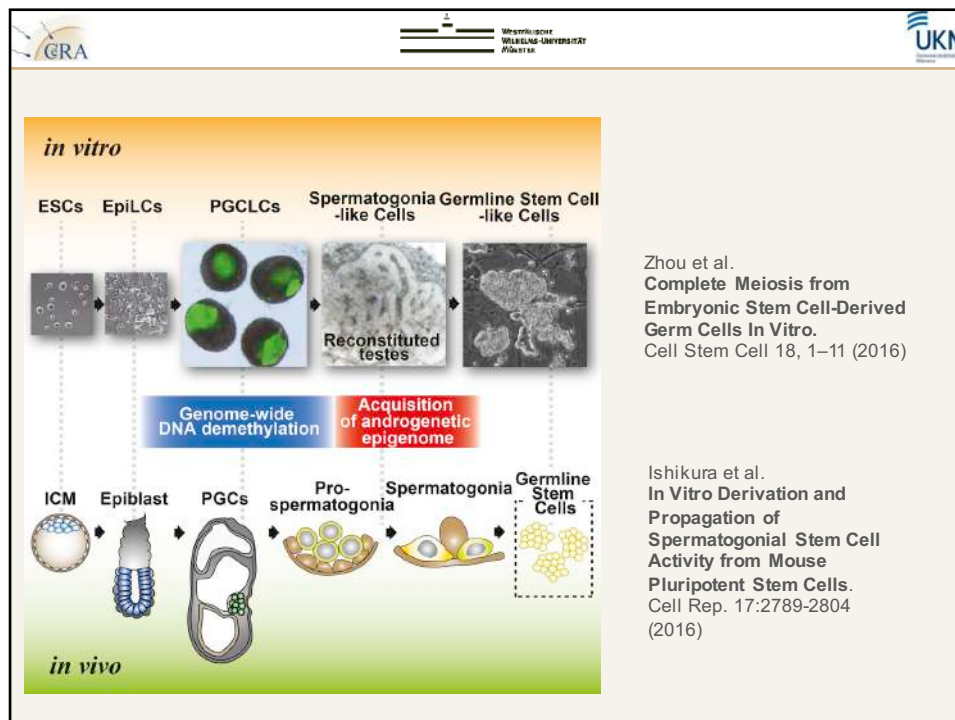
**(C)**



pachytene spermatocytes      round spermatids      elongated spermatids







## How to create a testis in vitro?

*In vitro* cord formation: Sertoli cell recapitulate organogenesis

**Day 1-2**




Pan F, Chi L, Schlatt S. Effects of nanostructures and mouse embryonic stem cells on *in vitro* morphogenesis of rat testicular cords. PLoS One 8:e60054. (2013)

Observe germ cell homing and expansion

Bright Field

GFRα1


Bar. 100μm

## How to create a testis in vitro?

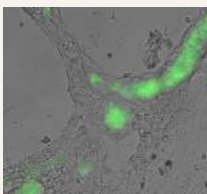

*In vitro* cord formation: Sertoli cell recapitulate organogenesis

**Day 3-4**

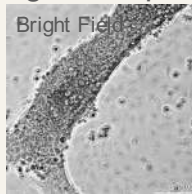


Pan F, Chi L, Schlatt S. Effects of nanostructures and mouse embryonic stem cells on *in vitro* morphogenesis of rat testicular cords. PLoS One 8:e60054. (2013)

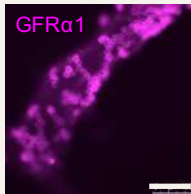
### Observe germ cell homing and expansion




Bright Field



GFR $\alpha$ 1



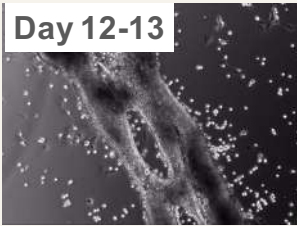
Bar. 100 $\mu$ m

## How to create a testis in vitro?

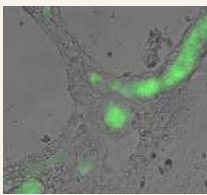
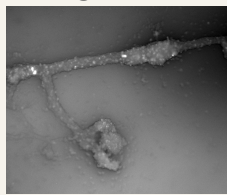
*In vitro* cord formation: Sertoli cell recapitulate organogenesis

**Day 12-13**

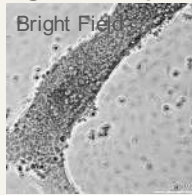


Pan F, Chi L, Schlatt S. Effects of nanostructures and mouse embryonic stem cells on *in vitro* morphogenesis of rat testicular cords. PLoS One 8:e60054. (2013)

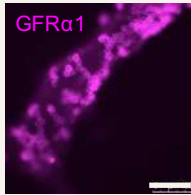
### Observe germ cell homing and expansion

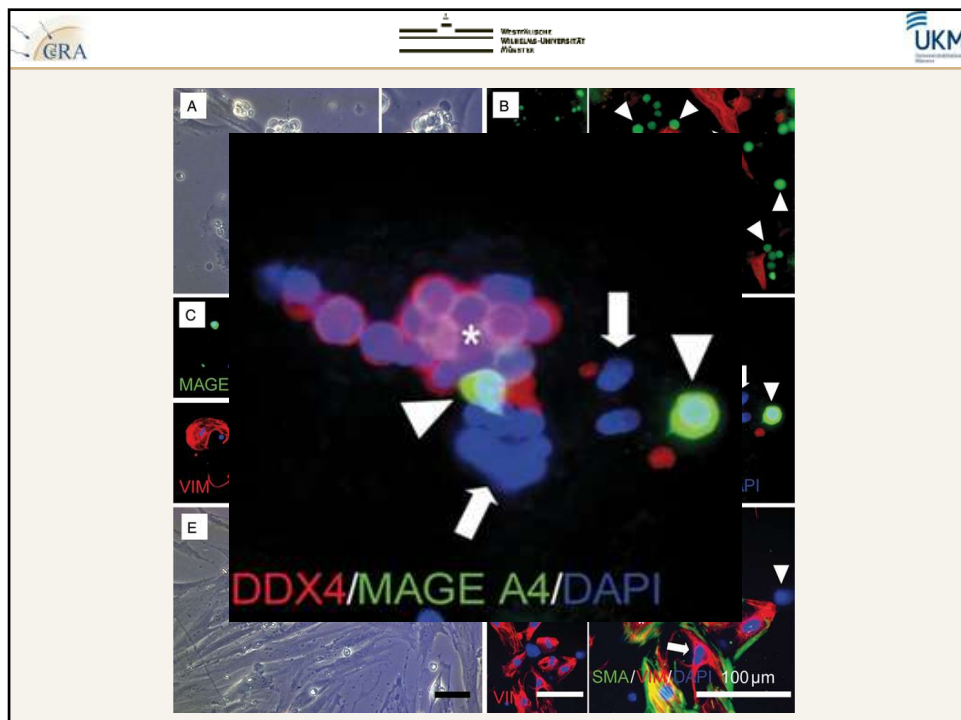
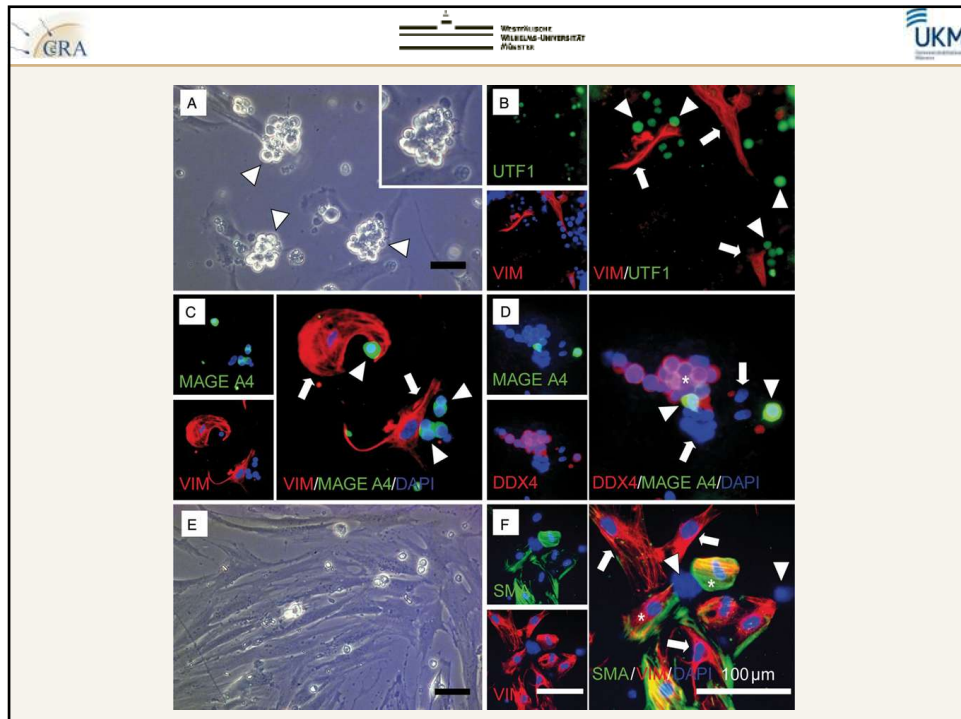
Bright Field



GFR $\alpha$ 1



Bar. 100 $\mu$ m



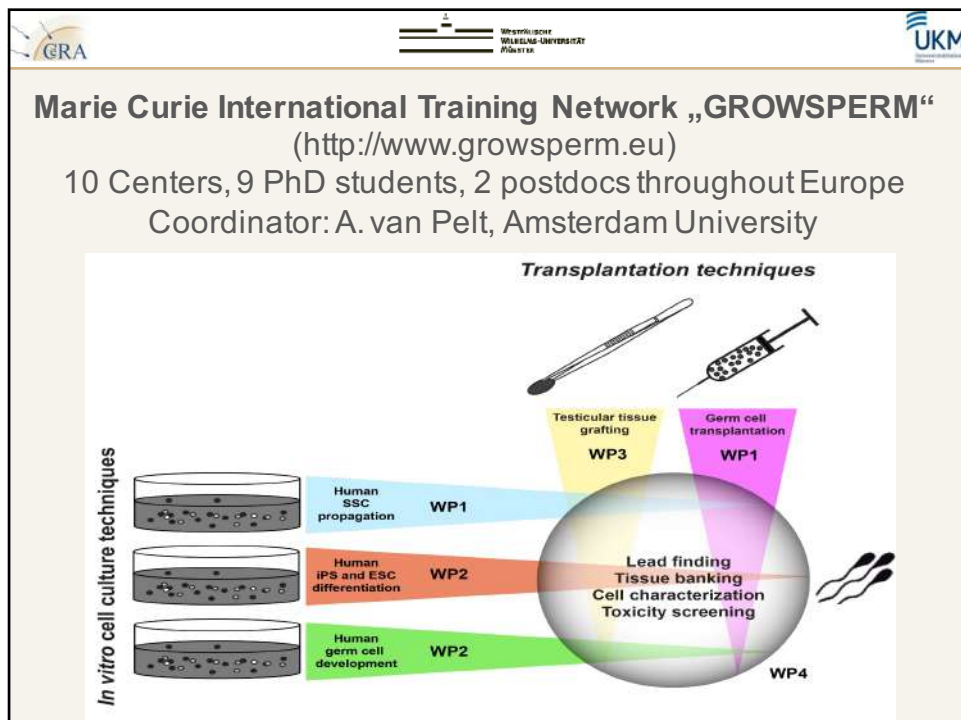
**Hypothesis**  
**on *in vitro* gametogenesis**

Will rely on better understanding of

- germ cell segregation/establishment
- germ cell plasticity
- soma/germline interactions and communication
- stem cell niche (clonal expansion)

Gamete production can be achieved by *in vitro* recapitulation of the gonadal microenvironment

- Female: embryonic/fetal stages of ovarian development
- Male: Adult seminiferous epithelium (Sertoli cells)







## Acknowledgements

**Centre for Research in Reproductive Physiology, University of Pittsburgh School of Medicine, PA, USA**

Kirsi Jahnukainen  
Bhavika Joshi  
Tony Plant  
Suresh Ramaswamy  
David Simorangkir  
Scott Hergenrother  
Kathrin Gassei

**Centre of Reproductive Medicine and Andrology, University Münster, Germany**

|                  |                      |
|------------------|----------------------|
| Jens Ehmcke      | Birgit Westernströer |
| Jörg Gromoll     | Jan-Bernd Stukenborg |
| Sabine Kliesch   | Joachim Wistuba      |
| Michael Zitzmann | Eberhard Nieschlag   |







**GIF**

German Israeli Foundation  
for Scientific Research and  
Development