Gametes from human pluripotent stem cells

"The maternal embryonic interface"

Valencia, December 2-3, 2010

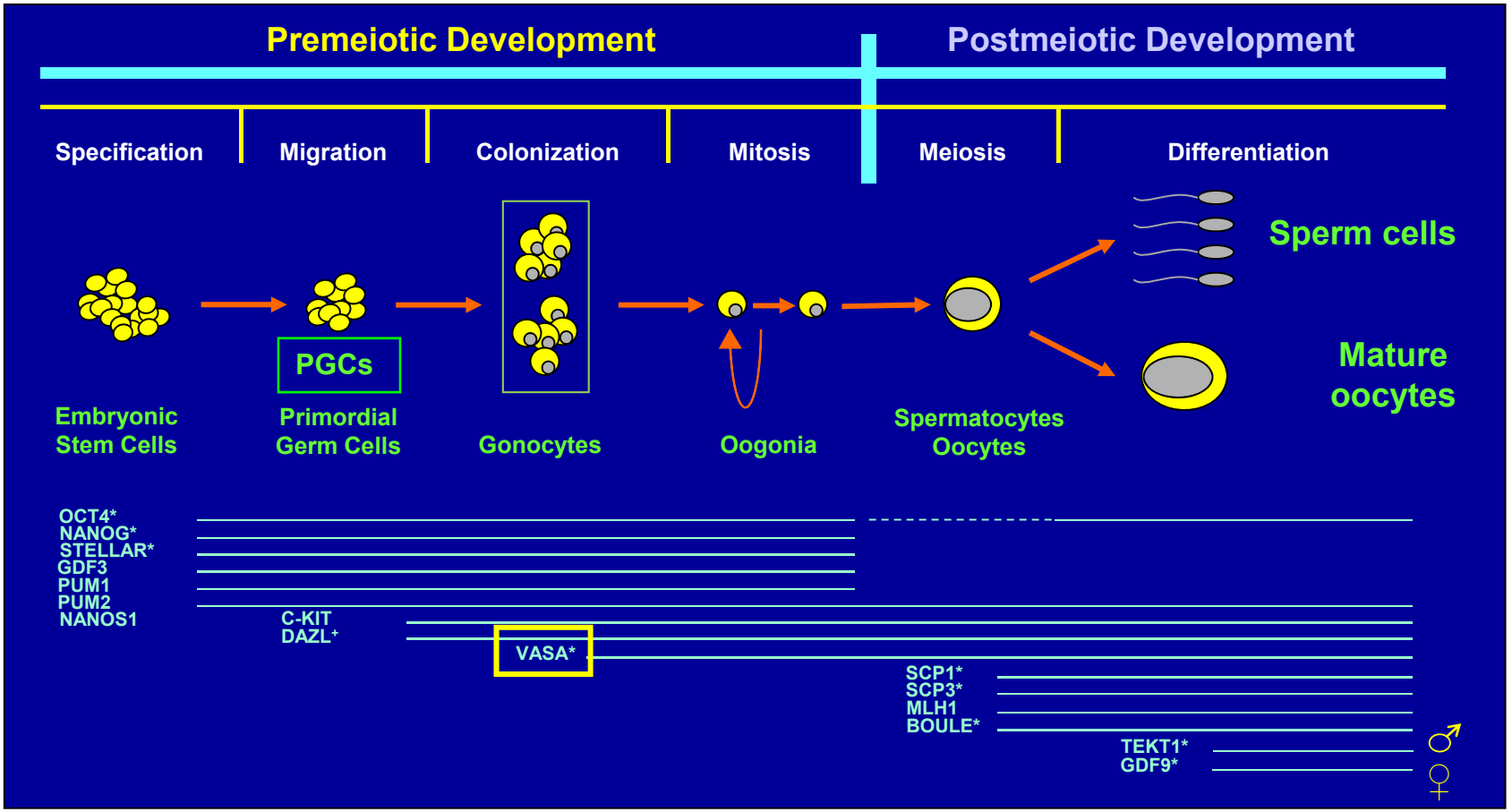
Ana I. Marqués-Marí, PhD

Differentiation of germ cells from hESCs

GERM CELLS DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS				
Authors	***Publication date***	***Source of SC***	***Cell type obtained***	***Viable offspring***
Clark *et al*.	2004	hESCs – XX, XY	Oocytes (although there was TEKT1 expression)	NT
Kee *et al*.	2006	hESCs - XX	Oocyte-like cells	NT
Chen *et al*.	2007	hESCs - XX	Oocyte-like cells	NT, FD
Bucay *et al.*	2008	hEsc - XX	PGCs	NT
West *et al.*	2008	hESCs - XY	Germ-like cells	NT
Park *et al.*	2009	hESCs - XX, XY hiPSC - XY	PGCs	NT
Kee *et al.*	**2009**	**hESC – XX, XY**	**Haploid gamete-like cells**	**NT**
Tilgner *et al.*	2010	hESC – XX	VASA+ Germ-like cells	NT
Panula *et al.*	**2011**	**hiPSC – XX, XY**	**Haploid gamete-like cells**	**NT**

NT: not tested; FD: follicles degeneration; hESCs: human embryonic stem cells; PGCs: primordial germ cells

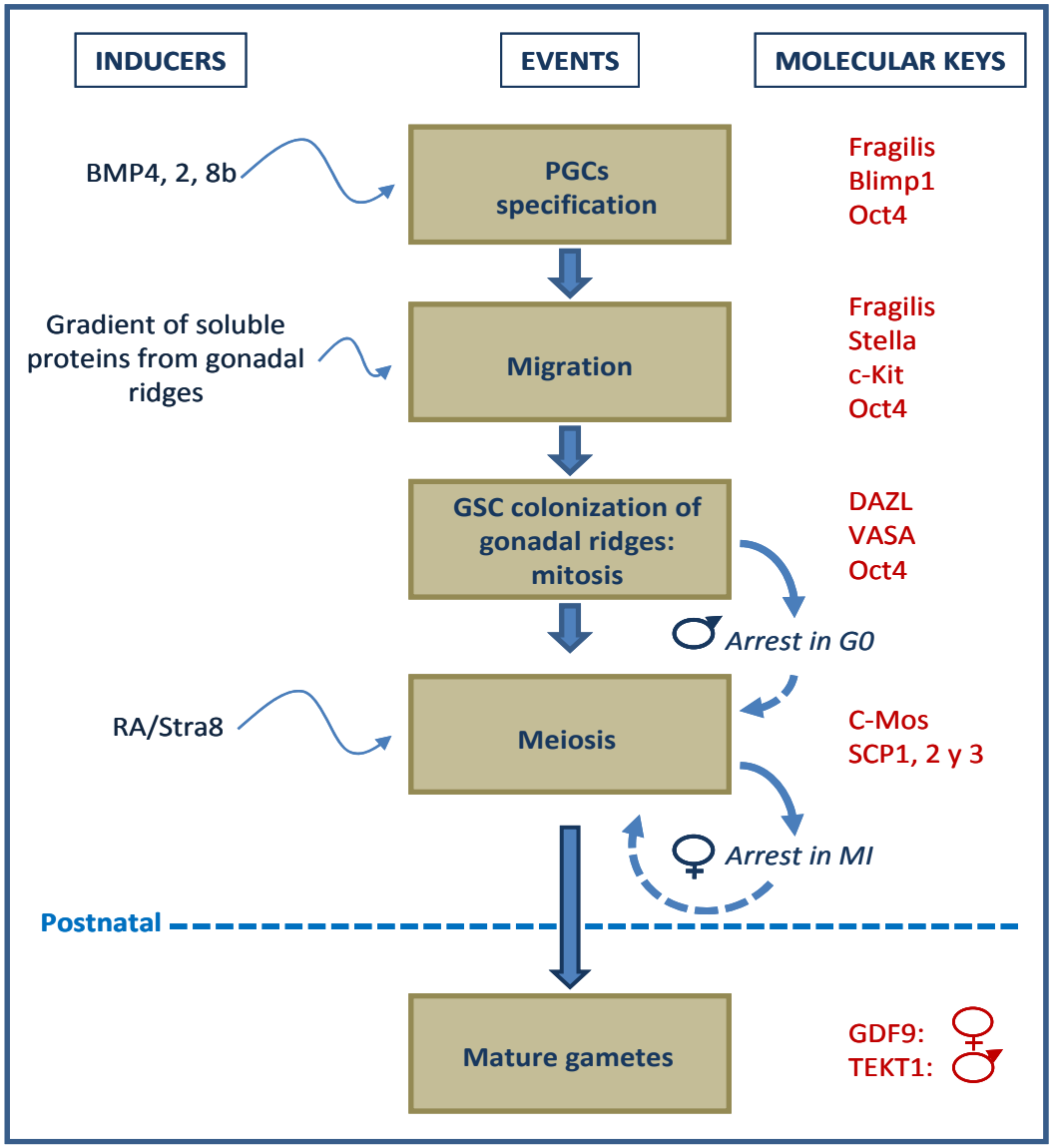
Germ line key molecular markers



Modified from Clark et al., 2004 – Human Molecular Genetics 13:7, 727-739

Spontaneous differentiation of germ cells from hESCs in vitro

Germline establishment and development *in vivo*



Critical step:

Meiosis

Modified from Marques-Mari et al., HRU 2009

Strategies to germline differentiation from hESCs

- **Spontaneous differentiation** in monolayer or through EBs
- **Addition of growth factors**.
- **Co-culture** with conditioned media or somatic cells.
- **Transplant** into *in vivo* systems.
- **Genetic modification:** DNA transfer by virus infection.

Spontaneous differentiation in monolayer or EBs

Remove factors that maintain pluripotency: feeders, bFGF and LIF*

- ***Hübner et al., Science 2003***
- ***Novak et al., Stem Cells 2006***

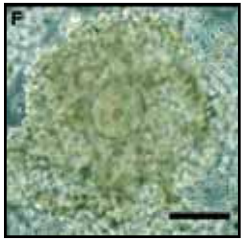
–Mouse ESC were differentiated in monolayer.

–Formation of follicle-like structures containing oocyte-like cells.

–Presence of meiotic proteins (SCP3) and specific oocyte markers (ZP1-3).

–Formation of pseudoblastocysts by parthenogenesis.

Lack of some meiotic proteins as SCP1 and SCP2. No evidences of chromosomal synapsis formation: unsuitable meiosis.



Follicle-like

Putative Oocyte


Pseudoblastocyst

Differentiation of hESC with EBs and addition of GF

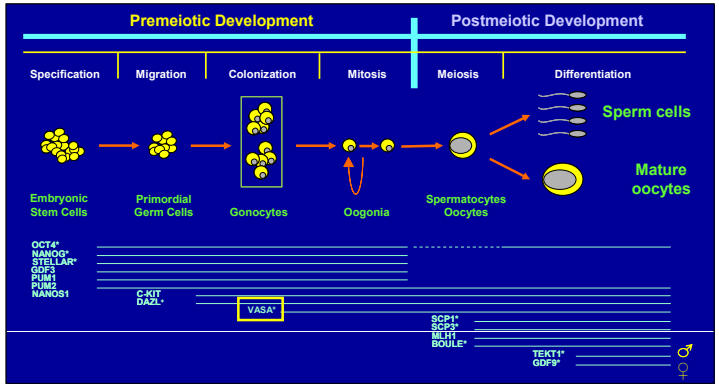
Remove factors that maintain pluripotency to form EBs in suspension
Addition of BMP4, 7 and 8 to the EBs medium

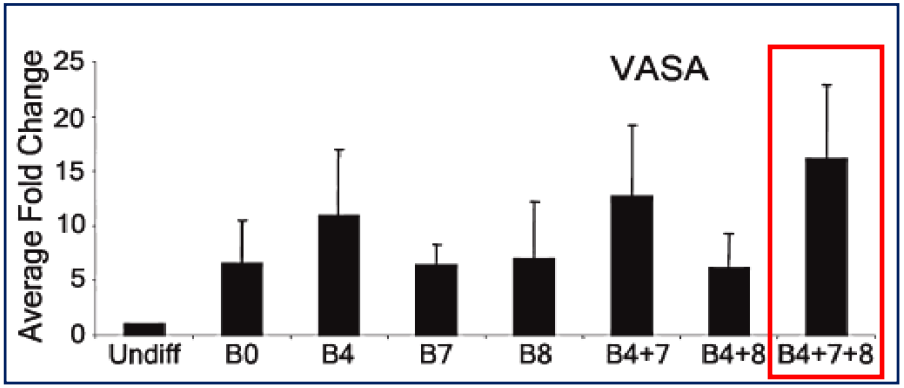
- ***Clark et al., Hum Mol Genet 2004***
- ***Kee et al., Stem Cells Dev 2006***

–Human ESC were differentiated through EBs.

–Establishment of a gene expression sequence during human germline specification.

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





Differentiation in co-culture with CM or somatic cells

Remove factors that maintain pluripotency to form EBs in suspension
Seed the EBs onto a monolayer formed by somatic cells

- ***Lacham-Kaplan et al., Stem Cells 2006***

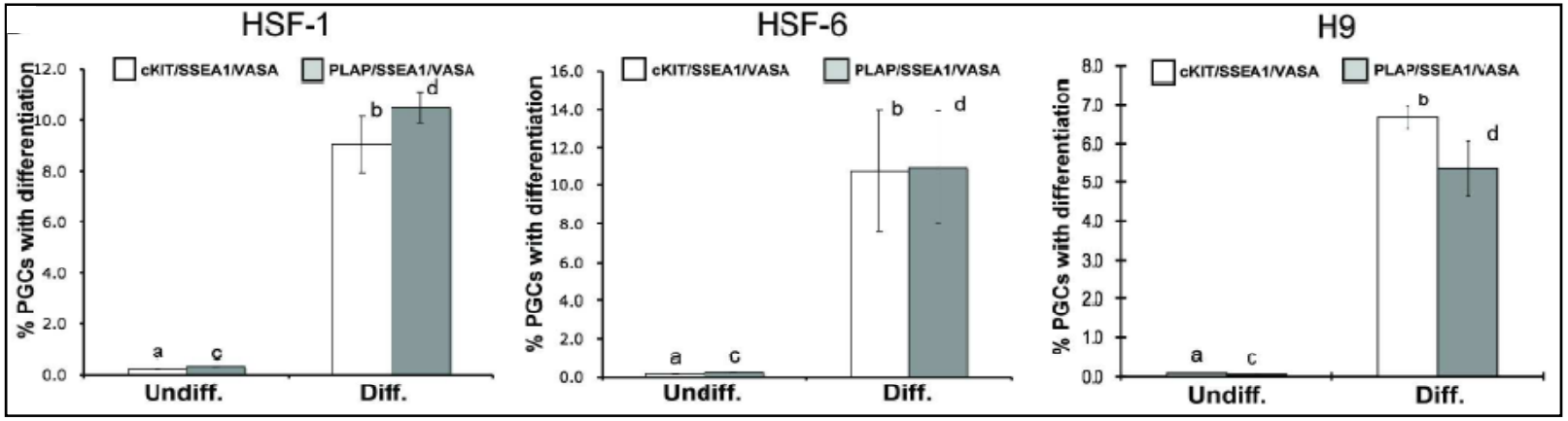
Co-culture of EBs from mESCs with testicular cells CM and formation of oocyte-like cells.

- ***Qing et al., Differentiation 2007***

Co-culture of EBs from mESCs onto ovarian granulosa cells. Premeiotic, meiotic and postmeiotic markers.

- ***Park et al., Stem Cells 2009***

Co-culture of hESC with human fetal gonadal cells to obtain PGCs.



7 days differentiation of hESC lines in co-culture with stromal cells from fetal gonad

Transplant into in vivo systems

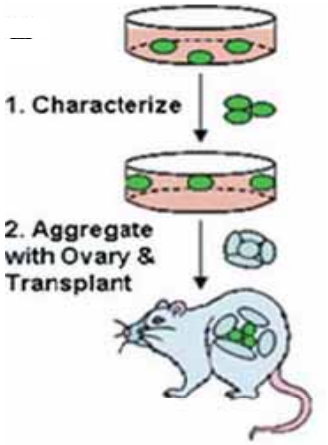
Remove factors that maintain pluripotency to form EBs in suspension
Transplant the aggregates into the ovary of recipient mice

- ***Nicholas et al., Human Mol genetics 2009***

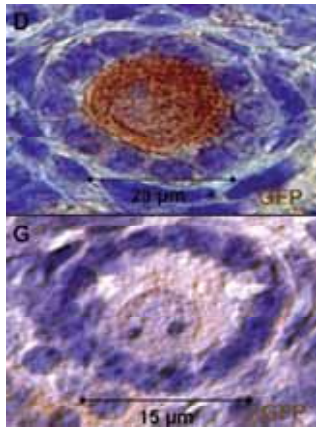
–mESC were differentiated through EBs to germ cells: incomplete meiotic progression and no folicle formation.

–Further differentiation was checked by transplantation into the ovary.

–GFP+ oocytes arose from those grafts although with a very low efficiency (0.023%)



In vitro differentiation and transplant



Very few Oocytes from graft

Genetic modification: virus-mediated DNA transfer

Transfect undifferentiated cells to overexpress some germline specific genes:
DAZL, DAZ and BOULE

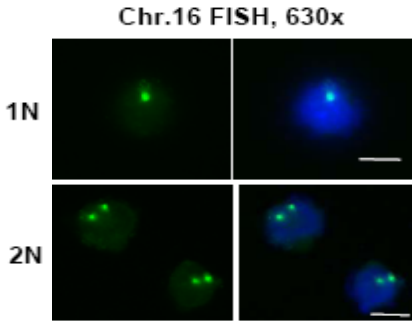
Remove factors that maintain pluripotency: feeders and bFGF

- ***Kee et al., Nature 2009***

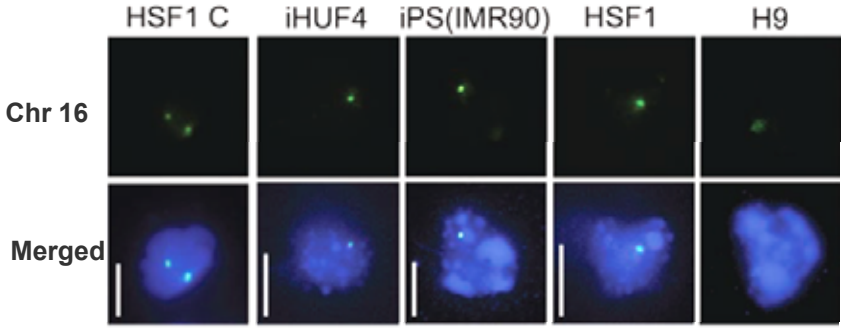
–Obtaining of haploid gametes after overexpression of the DAZ gene family in hESCs

- ***Panula et al., Human molecular genetics 2011***

–Obtaining of haploid gametes after overexpression of the DAZ gene family in iPSCs



Haploid cells from hESCs



Haploid cells from human iPSCs

Haploid gametes from hESCs

nature

doi:10.1038/nature08562

nature

LETTERS

Human *DAZL*, *DAZ* and *BOULE* genes modulate primordial germ-cell and haploid gamete formation

Kehkooi Kee[1], Vanessa T. Angeles[1], Martha Flores[1], Ha Nam Nguyen[1] & Renee A. Reijo Pera[1]

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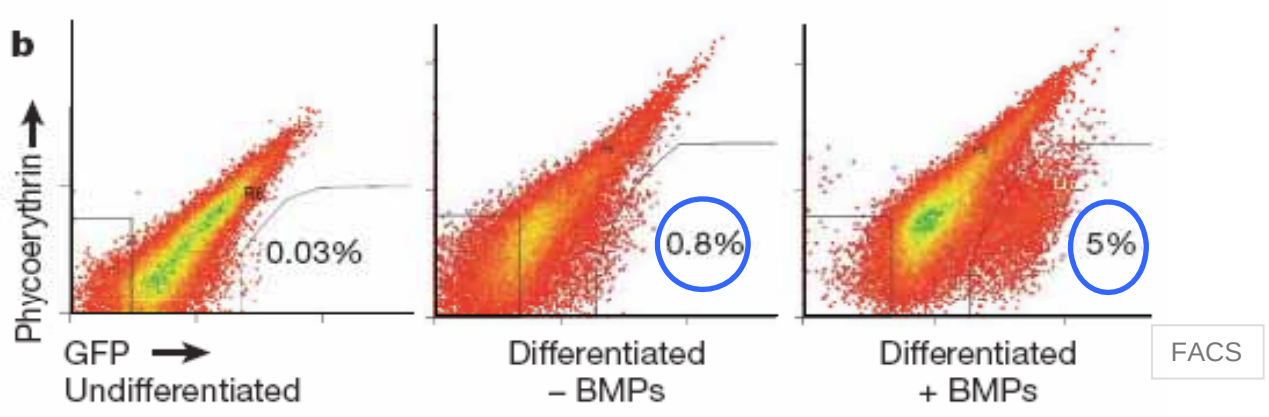
Kee et al., 2009 - Nature 462, 222-225

Haploid gametes from hESCs

PGCs and premeiotic stages. Impaired meiosis

- VASA + cells after 7d and 14d spontaneous EBs differentiation → **0.8%**
- VASA + cells after 7d and 14d EBs differentiation + **BMPs** addition → **5%**

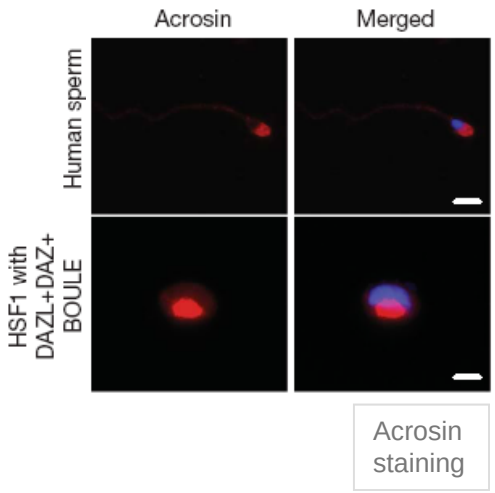


Kee et al., 2009 - Nature 462, 222-225

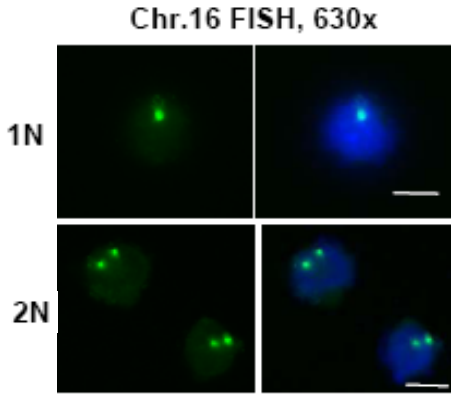
Haploid gametes from hESCs

Formation of haploid gametes after overexpression of the DAZ gene family:

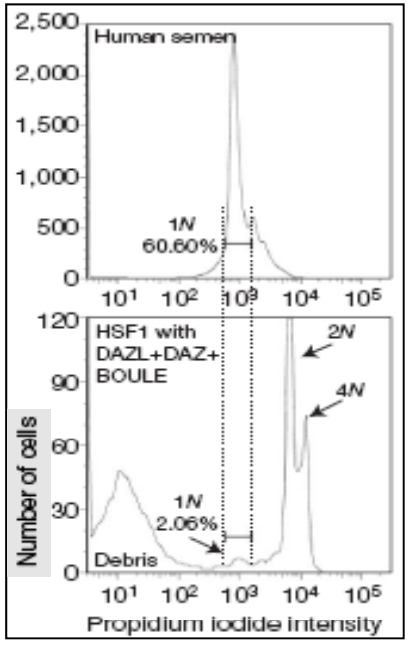
- Expression of TEKT1 and ACR (mature sperm markers).
- About 2% cells are 1N. Most 1N sorted cells expressed ACR (not in 2N).
- FISH for chromosome 16 shows only a copy.



Acrosin staining



FISH



FACS

Kee et al., 2009 - Nature 462, 222-225

Haploid gametes from iPSCs and hESCs

Human germ cell differentiation from fetal- and adult-derived induced pluripotent stem cells

Sarita Panula[1,2], Jose V. Medrano[1,3], Kehkooi Kee[1], Rosita Bergström[2], Ha Nam Nguyen[1], Blake Byers[1,4], Kitchener D. Wilson[5], Joseph C. Wu[5], Carlos Simon[3], Outi Hovatta[2], & Renee A. Reijo Pera[1]

[1]Institute for Stem Cell Biology & Regenerative Medicine, Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford University, Palo Alto, CA 94305, USA; [2]Department of Clinical Science, Intervention and Technology; Karolinska Institute, Karolinska University Hospital Huddinge; SE-171 86 Stockholm, Sweden; [3]Centro de Investigación Principe Felipe & Instituto Valenciano de Infertilidad (IVI) Valencia University; Avda. Autopista del Saler 16-3, Valencia, Spain; [4]Departmentof Bioengineering, Stanford University, Palo Alto, CA 94305, USA; [5]Departments of Medicine and Radiology; Stanford University School of Medicine, Stanford University, Palo Alto, CA 94305, USA.

Panula et al., Human Molecular Genetics, in press

Haploid gametes from iPSCs and hESCs

Cell lines

hESC:
- H9 (46, XX)
- HSF1 (46, XY)

iPSC:
- iPS IMR90 (46, XX) from fetal somatic cells
- iHUF4 (46, XY) from adult somatic cells

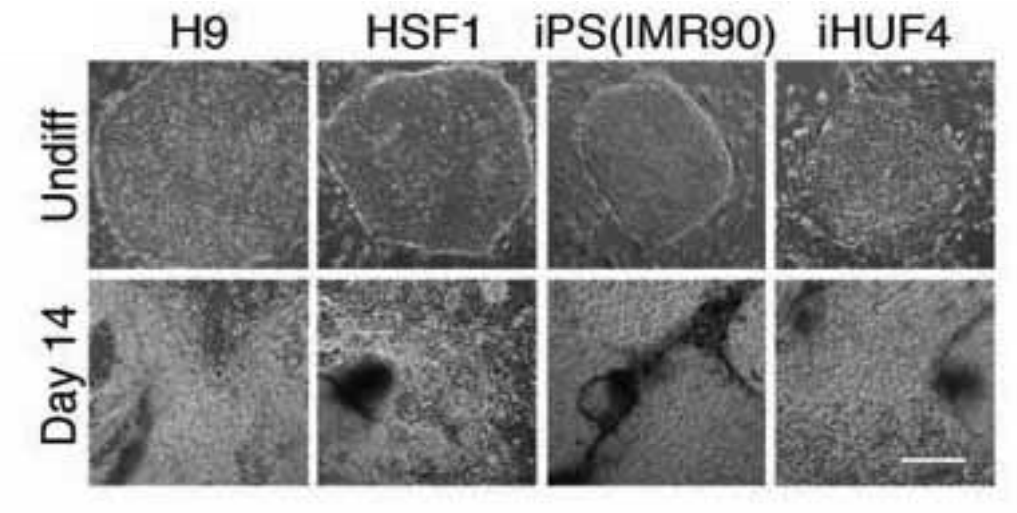
Differentiation process

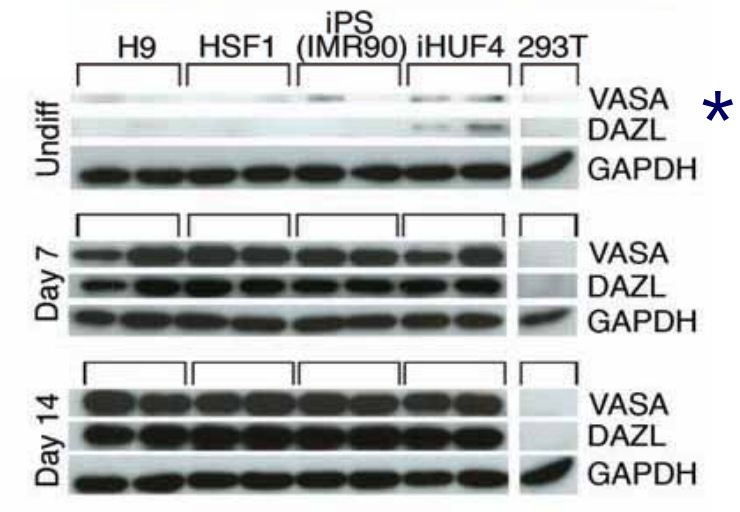
Spontaneous:
- Feeder-free: Matrigel+MC
- Addition of BMP-4, -7 y -8b

Induced:
- Overexpression DAZ gene family: DAZL, DAZ y BOULE

Panula et al., Human Molecular Genetics, in press

BMPs induced differentiation from iPSCs





7 - 14 days differentiation:

-Feeder-free

-Addition of BMP-4, -7, -8b

Premeiotic stages

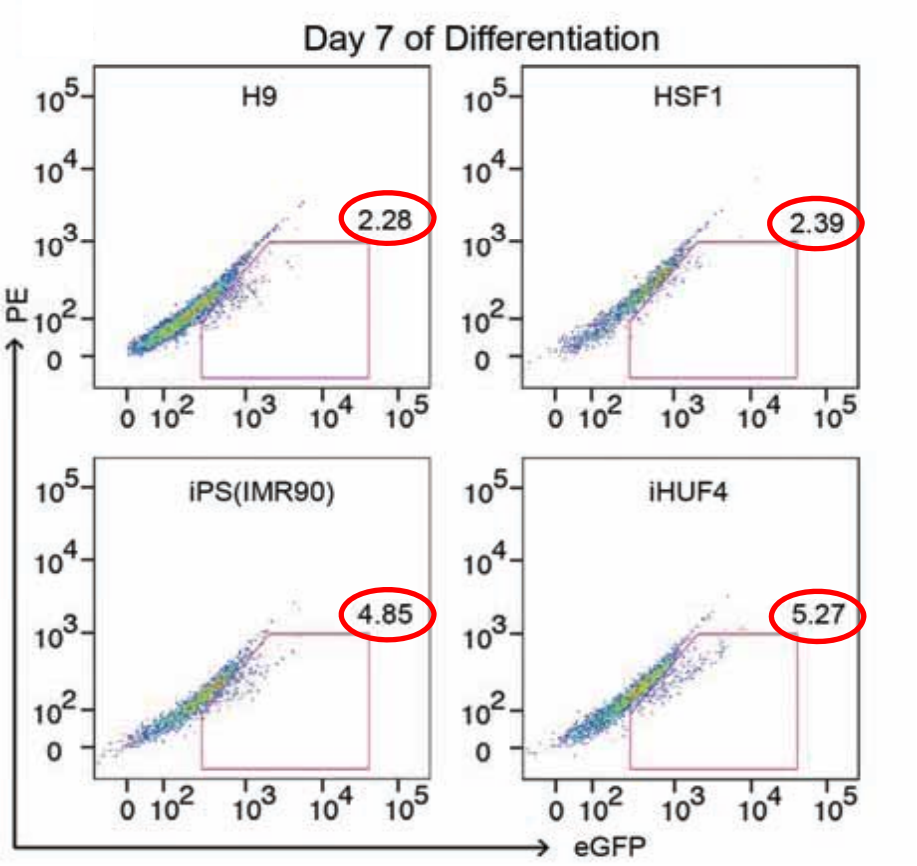
Similar VASA and DAZL protein levels in all the different cell lines.

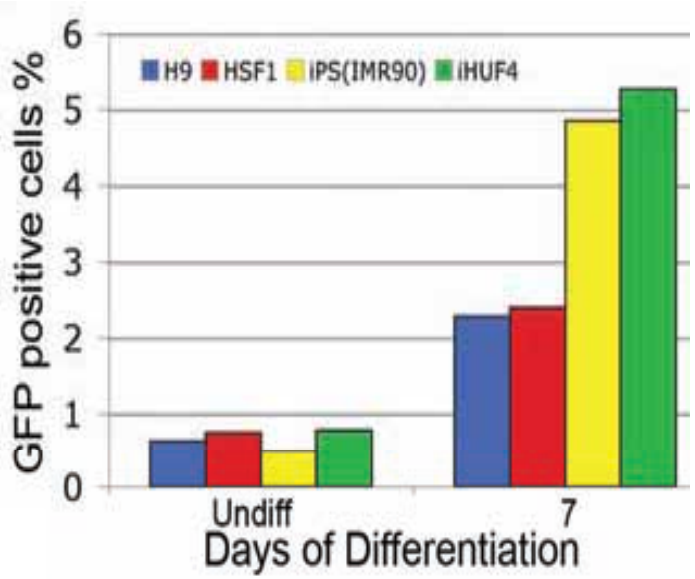
Panula et al., Human Molecular Genetics, in press

Detection of VASA-GFP positive cells

-Cell lines transduction with VASA-GFP reporter system and 7d differentiation

-FACS analysis: isolation of VASA + and VASA - populations





Panula et al., Human Molecular Genetics, in press

Differentiation overexpressing DAZ gene family

Differentiation process

Spontaneous:
- Feeder-free: Matrigel + CM
- Addition of BMP-4, -7 and -8b

Obtention of PGCs and premeiotic stages

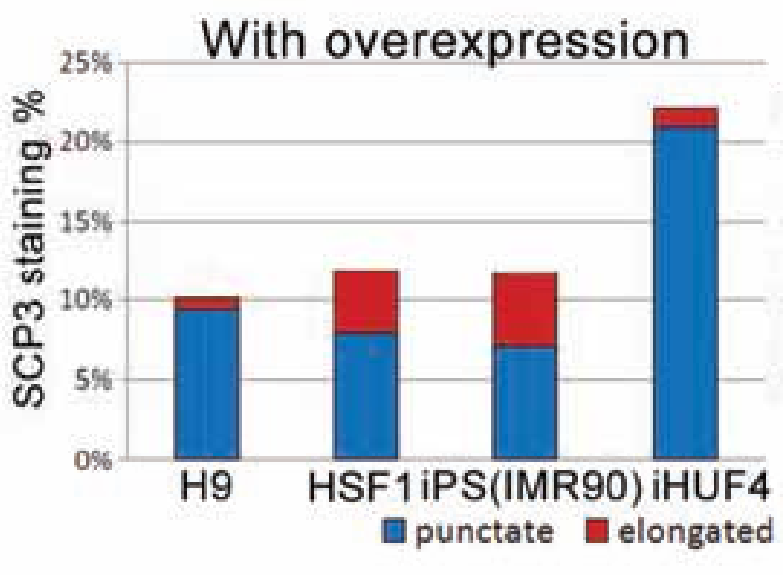
Induced:
- Overexpression of DAZ gene family: DAZL, DAZ y BOULE

Obtention of meiotic and postmeiotic cells

Panula et al., Human Molecular Genetics, in press

Differentiation overexpressing DAZ gene family

Ability to initiate meiosis after overexpression of DAZ, DAZL and BOULE



SCP3 detection

Synaptonemal complex protein:

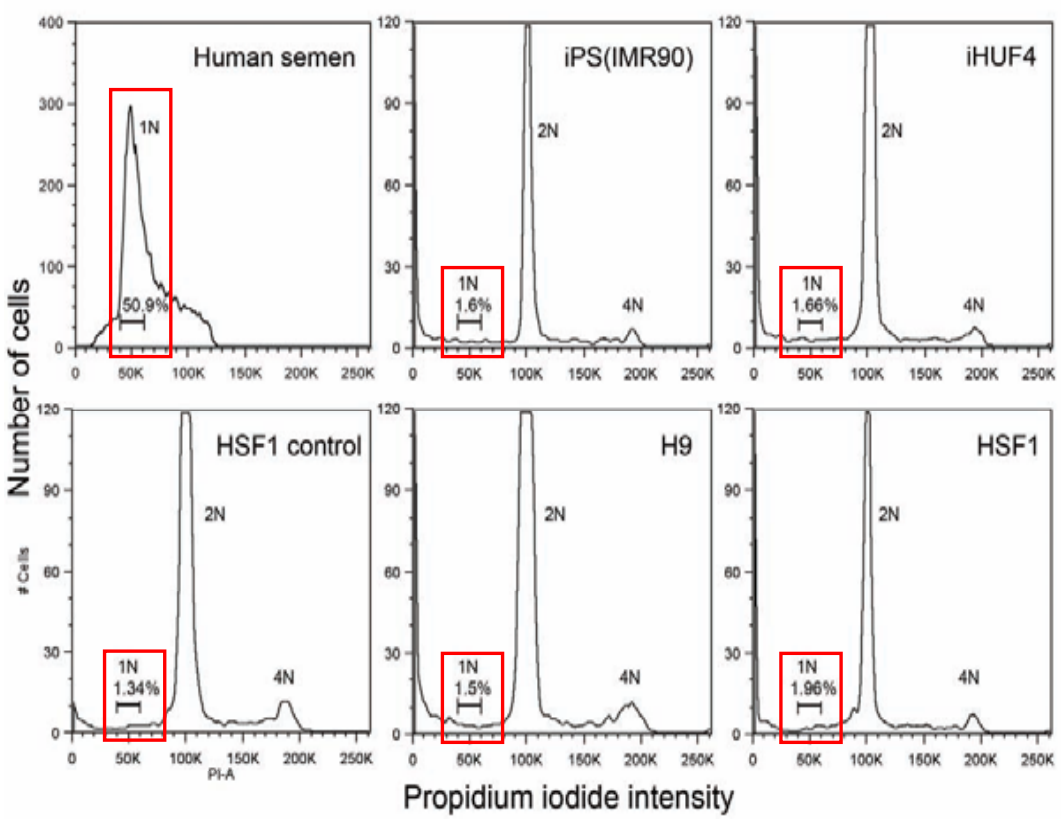
-Punctated staining: early Prophase I

-Elongated staining: Zy/Pa/Di

Panula et al., Human Molecular Genetics, in press

Differentiation overexpressing DAZ gene family

Completion of the meiotic process



<u>Haploid cells detection</u>:

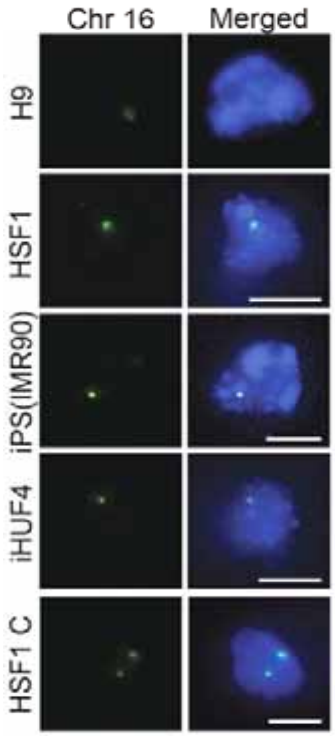
FACS sorting by DNA content after 14 days differentiation

-Haploid cells in all the cell lines with similar percentages

-Isolation of DNA 1N cells for FISH of Chr 16

Panula et al., Human Molecular Genetics, in press

Differentiation overexpressing DAZ gene family

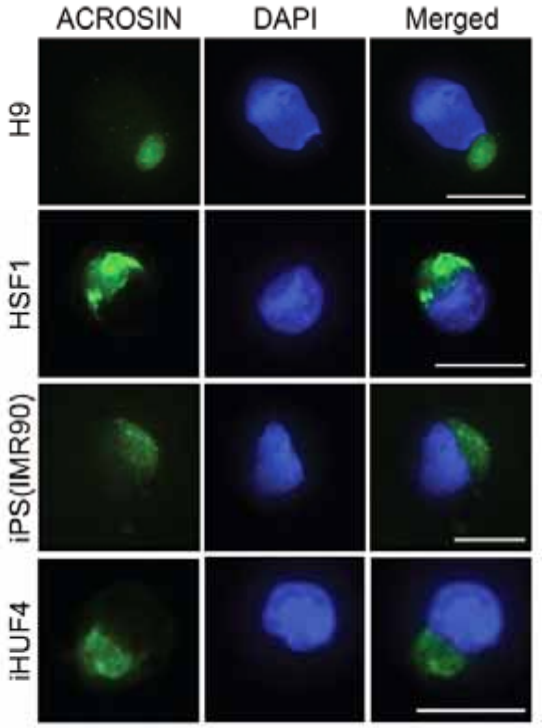


FISH Chr 16

Haploid cells

-13-21% cells with 1 chromosome 16

-Acrosin pattern similar to human spermatids: 35-72% Acrosin + cells



Acrosin ICQ

	1 Chr 16	Total	Percentage
H9 1N	19	102	19%
HSF1 1N	24	112	21%
iPS(IMR90) 1N	12	87	14%
iHUF4 1N	12	96	13%

	Positive	Total	Percentage
H9 1N	36	103	35%
HSF1 1N	36	50	72%
iPS(IMR90) 1N	32	52	62%
iHUF4 1N	53	100	53%

Panula et al., Human Molecular Genetics, in press

SUMMARY AND PERSPECTIVES

- Human iPSCs lines can differentiate *in vitro* into PGCs similarly to hESCs.
- When DAZ gene family is overexpressed germ cells differentiated from hESCs and iPSCs initiate and can complete meiosis leading to haploid cells.
- hESCs and iPSCs can be a useful tool to study human germ cell development and for infertility studies.
- Development of new strategies for proper meiosis completion without virus and epigenetic pattern establishment in culture.
- Potential use of obtained gametes in regenerative medicine and ART.