eshre

SCIENCE MOVING
PEOPLE
MOVING SCIENCE

Stem cells in reproductive medicine

Special Interest Group Stem Cells

8

27 June 2010
Rome, Italy

PRE-CONGRESS COURSE 8 – Table of contents

Stem cells in reproductive medicine

Organised by the Special Interest Group Stem Cells

ESHRE – European Society of Human Reproduction and Embryology

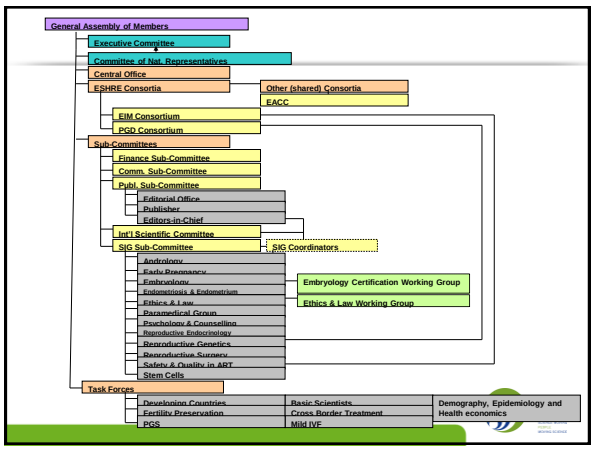
What is ESHRE?

ESHRE was founded in 1985 and its **Mission Statement** is to:

- promote interest in, and understanding of, reproductive science and medicine.
- facilitate research and dissemination of research findings in human reproduction and embryology to the general public, scientists, clinicians and patient associations.
- inform politicians and policy makers in Europe.
- promote improvements in clinical practice through educational activities
- develop and maintain data registries
- implement methods to improve safety and quality assurance

Executive Committee 2009/2011

Chairman	Luca Gianaroli	Italy
Chairman Elect	Anna Veiga	Spain
Past Chairman	Joep Geraedts	Netherlands
	Jean François Guérin	France
	Timur Gürgan	Turkey
	Ursula Eichenlaub-Ritter	Germany
	Antonis Makrigiannakis	Greece
	Miodrag Stojkovic	Serbia
	Anne-Maria Suikkari	Finland
	Carlos Plancha	Portugal
	Françoise Shenfield	United Kingdom
	Etienne Van den Abbeel	Belgium
	Heidi Van Ranst	Belgium
	Veljko Vlaisavljevic	Slovenia
	Søren Ziebe	Denmark



ESHRE Activities – Annual Meeting

- One of the most important events in reproductive science and medicine
- Steady increase in terms of attendance and of scientific recognition

Track record:

ESHRE 2008 – Barcelona: 7559 participants
ESHRE 2009 – Amsterdam: 8132 participants

Future meetings:

ESHRE 2010 – Rome, 27-30 June 2010
ESHRE 2011 – Stockholm, 3-6 July 2011

ESHRE Activities – Scientific Journals

Human Reproduction with impact factor 3.773

Human Reproduction Update with impact factor 7.590

Molecular Human Reproduction with impact factor 2.537

ESHRE Activities – Campus and Data Collection

- Educational Activities / Workshops
  - Meetings on dedicated topics are organised across Europe
  - Organised by the Special Interest Groups
  - Visit: www.eshre.eu under CALENDAR
- Data collection and monitoring
  - EIM data collection
  - PGD data collection
  - Cross border reproductive care survey

ESHRE Activities - Other

- Embryology Certification
- Guidelines & position papers
- News magazine “Focus on Reproduction”
- Web services:
  - RSS feeds for news in reproductive medicine / science
  - Find a member
  - ESHRE Community

ESHRE Membership (1/3)

- ESHRE represents over 5,300 members (infertility specialists, embryologists, geneticists, stem cell scientists, developmental biologists, technicians and nurses)
- Overall, the membership is distributed over 114 different countries, with 50% of members from Europe (EU). 11% come from the US, India and Australia.

ESHRE Membership (2/3)

	1 yr	3 yrs
Ordinary Member	€ 60	€ 180
Paramedical Member*	€ 30	€ 90
Student Member**	€ 30	N.A.

*Paramedical membership applies to support personnel working in a routine environment such as nurses and lab technicians.
**Student membership applies to undergraduate, graduate and medical students, residents and post-doctoral research trainees.

ESHRE Membership – Benefits (3/3)

1) Reduced registration fees for all ESHRE activities:

Annual Meeting	Ordinary	€ 480	(€ 720)
	Students/Paramedicals	€ 240	(€ 360)
Workshops	All members	€150	(€ 200)

2) Reduced subscription fees to all ESHRE journals – e.g. for Human Reproduction €191 (€ 573!)

3) ESHRE monthly e-newsletter

4) News Magazine "Focus on Reproduction" (3 issues p. a.)

5) Active participation in the Society's policy-making

Special Interest Groups (SIGs)

The SIGs reflect the scientific interests of the Society's membership and bring together members of the Society in sub-fields of common interest

- Andrology
- Early Pregnancy
- Embryology
- Endometriosis / Endometrium
- Ethics & Law
- Safety & Quality in ART
- Psychology & Counselling
- Reproductive Genetics
- Reproductive Surgery
- Stem Cells
- Reproductive Endocrinology

Task Forces

A task force is a unit established to work on a single defined task / activity

- Fertility Preservation in Severe Diseases
- Developing Countries and Infertility
- Cross Border Reproductive Care
- Reproduction and Society
- Basic Reproductive Science
- Fertility and Viral Diseases
- Management of Infertility Units
- PGS
- EU Tissues and Cells Directive

Annual Meeting

Rome, Italy 27 June to 30 June 2010

Pre-congress courses (27 June):

- PCC 1: Cross-border reproductive care: information and reflection
- PCC 2: From gametes to embryo: genetics and developmental biology
- PCC 3: New developments in the diagnosis and management of early pregnancy complications
- PCC 4: Basic course on environment and human male reproduction
- PCC 5: The lost art of ovulation induction
- PCC 6: Endometriosis: How new technologies may help
- PCC 7: NOTES and single access surgery
- PCC 8: Stem cells in reproductive medicine
- PCC 9: Current developments and their impact on counselling
- PCC 10: Patient-centred fertility care
- PCC 11: Fertility preservation in cancer disease
- PCC 12: ESHRE journals course for authors

Annual Meeting – Scientific Programme (1/2)

Rome, Italy 27 June to 30 June 2010

- Molecular timing in reproduction
- Rise and decline of the male
- Pluripotency
- Preventing maternal death
- Use and abuse of sperm in ART
- Live surgery
- Emerging technologies in the ART laboratory
- Debate: *Multiple natural cycle IVF versus single stimulated cycle and freezing*

Annual Meeting – Scientific Programme (2/2)

- Fertility preservation
- Congenital malformations
- ESHRE guidelines
- Data from the PGD Consortium
- European IVF Monitoring 2007
- Debate: *Selection of male/female gametes*
- Third party reproduction in the United States
- Debate: *Alternative Medicine, patients feeling in control?*
- Historical lecture: "Catholicism and human reproduction"

Certificate of attendance

1/ Please fill out the evaluation form during the campus

2/ After the campus you can retrieve your certificate of attendance at www.eshre.eu

3/ You need to enter the results of the evaluation form online

4/ Once the results are entered, you can print the certificate of attendance from the ESHRE website

5/ After the campus you will receive an email from ESHRE with the instructions

6/ You will have TWO WEEKS to print your certificate of attendance

Contact

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www.eshre.eu

PRE-CONGRESS COURSE 8 - Programme

Stem cells in reproductive medicine

Organised by the Special Interest Group Stem Cells

Course coordinators: Carlos Simon (Spain) and Ana Veiga (Spain)

Course description: Human embryonic stem cell lines are promising tools not only for regenerative medicine but also in reproductive medicine and for research in germ line formation and early human development. Provocative new data suggest that stem cell populations, in the gonads, endometrium and placenta, could have a major impact on our understanding of human reproduction in health and disease. The purpose of this course is to update clinicians and embryologists about recent advances in embryonic and somatic stem cell technology with particular emphasis in reproductive medicine. The course will provide insight into the possible generation of gametes from embryonic stem cells, as well as to explore the potential of the endometrial and placenta progenitor stem cell population. Participants will discuss the new clinical, ethical and legal implications of stem cells for reproductive medicine.

Target audience: Clinicians and Scientists working in the field of human reproduction. Clinicians and Scientists interested in getting updated information about progress made in the field of stem cells

Scientific programme:

09:00 – 09:10 Welcome - **Carlos Simon (Spain)**

Chairperson: Ana Veiga (Spain)

09:00 – 09:30 Making a firm decision: multifaceted regulation of cell fate in the early mouse embryo - **Magdalena Zernicka-Goetz (United Kingdom)**
09:30 – 09:45 Discussion
09:45 – 10:15 Differentiation of Oocytes and Sperm from ESC – **Renee Reijo Pera (USA)**
10:15 – 10:30 Discussion
10:30 – 11:00 Coffee break

Chairperson: Carlos Simon (Spain)

11:00 – 11:30 Stem cell niches and testicular development - **Ellen Goossens (Belgium)**
11:30 – 11:45 Discussion
11:45 – 12:15 Stem cell niches in the ovary? - Ji Wu (Canada)
12:15 – 12:30 Discussion
12:30 – 13:30 Lunch

Chairperson: Karen Sermon (Belgium)

13:30 – 14:00 Somatic Stem Cells in the Endometrium and its putative implication in endometriosis - **Carlos Simon (Spain)**
14:00 – 14:15 Discussion
14:15 – 14:45 Somatic Stem Cells in the Myometrium and its putative implication in myoma

Formation - **Tetsuo Maruyama (Japan)**
14:45 – 15:00 Discussion
15:00 – 15:30 Coffee break

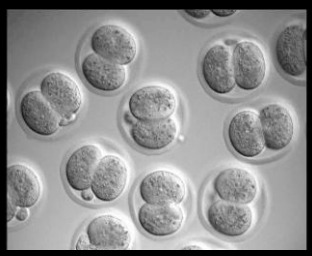
Chairperson: Anis Feki (CH)

15:30 – 16:00 Stem cells from the Extraembryonic Tissues – Placenta, Amniotic fluid and Fetal Membranes - **Susan Fisher (USA)**
16:00 – 16:15 Discussion
16:15 – 16:45 The ESHRE registry of hESC lines with monogenic defects - **Karen Sermon (Belgium)**
16:45 – 17:00 Discussion

Cell Fate Decisions in the Mouse Embryo

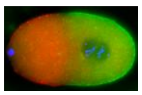
Magdalena Zernicka-Goetz
The Gurdon Institute
University of Cambridge

The separation of ICM from TE is the first key decision which is accomplish during the first 3 days of mammalian embryo life



How does it happen?

Flies, worms and frogs, in fact the embryos of most animals, have localised determinants that are key to development of pattern



What about our eggs and embryos?

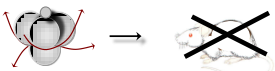
Such localised determinants often lead to invariant development which is refered to as determinative: If you ablate certain parts, it cannot reconfigure

Based on what was known about other organisms, a similar mechanism was originally proposed for the mammalian embryo

Early asymmetry
(Dalcq 1965)

But then the mammalian embryo was thought to be completely different as its development is quite flexible

Tarkowski, Nature, 1959



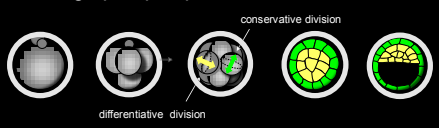
Tarkowski and Wroblewska, JEEM, 1967

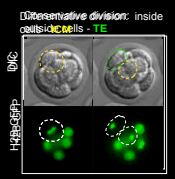
Regulative development:
If you ablate certain parts, it can reconfigure

Tarkowski, Nature, 1961
Mintz, Science, 1962

The First Cell Fate Decision:

setting apart pluripotent cells - ICM, from TE



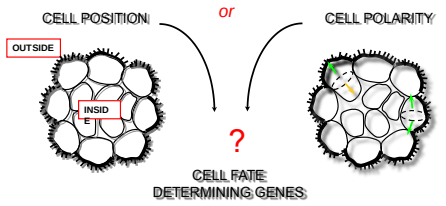


Questions

- How do inside and outside cells become different from each other?
  Is it just cell position or are asymmetric divisions truly asymmetric?

- How is the formation of inside and outside cells regulated?
  - What makes some cells divide conservatively and others differentiatively?
  - Is this regulated or does it occur at random?

Two alternative hypotheses for how inside and outside cells become different



Inside/Outside hypothesis
Andrzej Tarkowski 1960s

Polarisation hypothesis
Martin Johnson 1980s

But then Richard Gardner made tantalising observations that there are some regularities in early mouse development - the zygote animal-vegetal axis relates to the blastocyst axis!

Development 124, 289-301 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
DEV7597

The early blastocyst is bilaterally symmetrical and its axis of symmetry is aligned with the animal-vegetal axis of the zygote in the mouse

Could it be, after all, there some prepattern also in a mammalian egg?

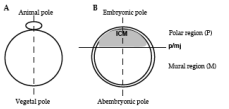
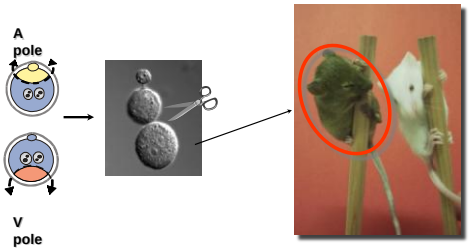


Fig. 1. Diagrams illustrating polarity of the zygote (A) and blastocyst (B) in which the animal-vegetal (AV) axis of the zygote and the embryonic-abembryonic (EmAb) axis of the blastocyst are indicated by dashed lines. The horizontal line in (B) represents the polar-mural junction (p/m), the boundary between the polar region of the trophectoderm overlying the inner cell mass (ICM) and the mural region embracing the blastocoelic cavity.

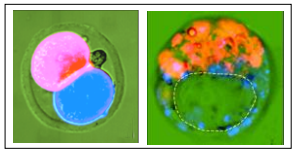
Cut away poles

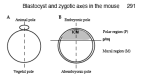


No essential determinants at either A or V pole

Zernicka-Goetz, Dev, 1998

Lineage tracing reveals the majority of embryos develops with some pattern: their cells are not distributed at random along the embryonic-abembryonic axis



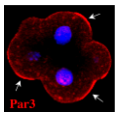


Gardner, Dev 1997

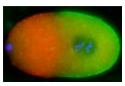
Zernicka-Goetz et al., Dev 1997
Piotrowska and Zernicka-Goetz, Nature 2001
Piotrowska et al, Dev 2001
Gardner, Dev 2001
Fujimori et al., Dev 2003

But it is so so unexpected that generates lots and lots of attack!

Conserved polarity molecules, such as Par3, Par6 and aPKC, that establish polarity of all other model organisms, are also present in mouse embryos and ..... their distribution is highly polarised

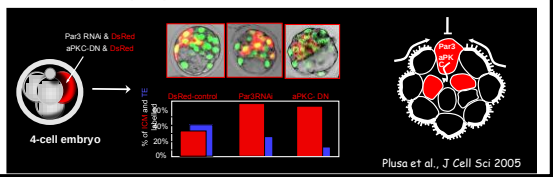


8-cell mouse embryo



Par proteins in C.elegans embryo

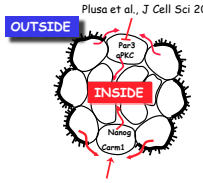
Decreasing cell polarity, by down-regulating either Par3 or aPKC, drives cells to become pluripotent ICM



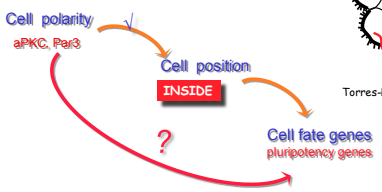
Plusa et al., *J Cell Sci* 2005

Developmental consequences of cell polarisation

Plusa et al., *J Cell Sci* 2005

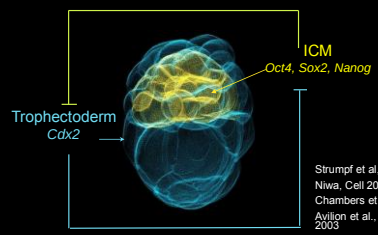


Torres-Padilla et al., Nature, 2007



Does cell polarisation affect expression of genes involved in cell fate determination?

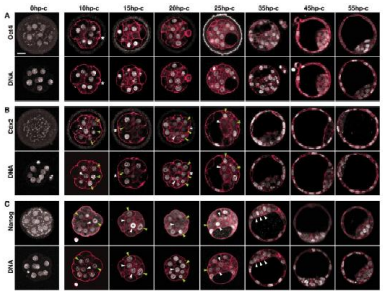
Cdx2 is a key player in TE specification



Strumpf et al, Dev 2005
Niwa, Cell 2005
Chambers et al, Cell 2003
Avilion et al., Genes Dev 2003

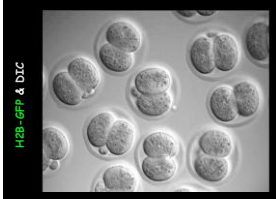
So how does this exclusive pattern of gene expression become set up?

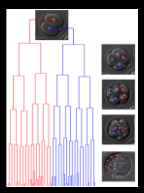
Spatial Variability in the Expression of Pluripotency Genes between Inside and Outside cells, but Less so in Cdx2



Dietrich and Hiiragi, Dev 2008

Can we follow how different cell types arise and relate these to molecular differences between cells?





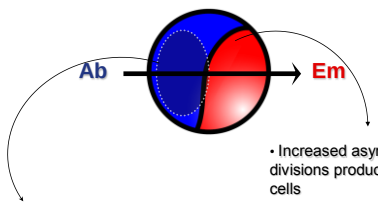
- position of cells
- mitoses
- cell cycle lengths
- division orientation
- generation of ICM or TE

Approach - combination of:

- Following origins, behavior and fate of each and all cells for the first 3 days of development
- Gene expression profiling of individual cells of known origin and fate
- Modulating gene expression in individual cells

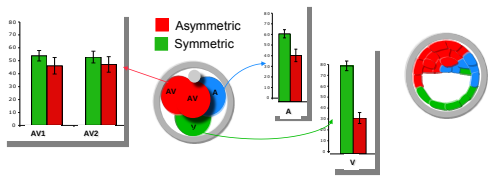
How does the distribution of symmetric and asymmetric divisions, that form TE and ICM cells, relate to the blastocyst cavity formation?

Spatial Distribution of Symmetric vs Asymmetric Divisions Defines the Orientation of the Embryonic-Abembryonic Axis



- Increased asymmetric divisions produce more ICM cells
- Increased symmetric divisions produce predominantly trophectoderm adjacent to blastocyst cavity

Symmetric and Asymmetric Divisions and ICM and TE formation

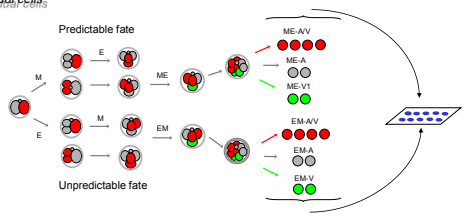


- blastocyst cavity forms in the vicinity of symmetric divisions
- some cells undertake greater proportion of symmetric divisions that others
- identity of such cells is predictable according to their position and history, and this history can be recognised in about half of embryos

Bischoff et al., Dev 2008

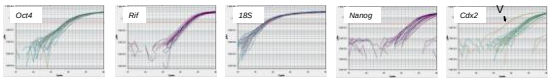
What is the molecular signature of cells that take more symmetric divisions?

Screen:
Monitor division orientations in respect to AV axis & biopsy at the 8-cell stage followed by QPCR in individual cells



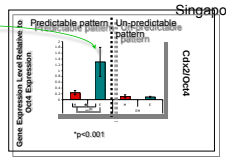
Cells that take more symmetric divisions and contribute preferentially to trophectoderm, have highest Cdx2 levels

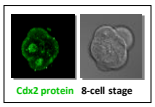
- Expression of 35 genes examined and quantitated by rt-PCR on LDAs in individual cells at the 8-cell stage



- Real-time PCR in individual cells at the 8-cell stage

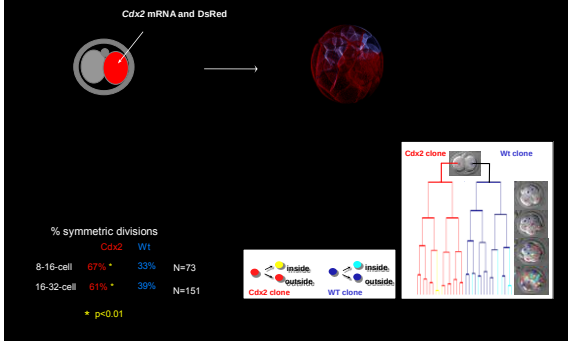
Paul Robson, Genome Center, Singapore



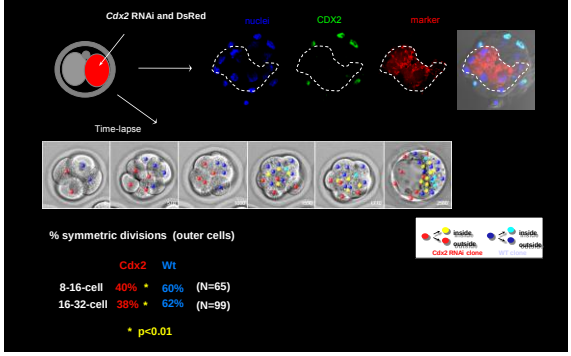


Jedrusik et al, Genes Dev, 2008

Elevation of Cdx2 in half of the embryo directs most of its progeny to trophectoderm by promoting symmetric divisions



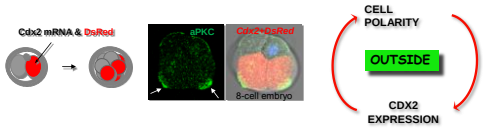
Down-regulation of Cdx2 in half of the embryo directs most of its progeny to ICM by promoting asymmetric divisions



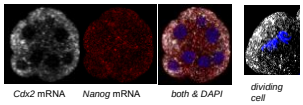
How Cdx2 can affect division orientation?

Cdx2 and Cell Polarity

- Cdx2 influences cell polarity and cell polarity affects division orientation (Plusa et al., JCS, 2005)



- Cellular polarity affects *Cdx2* mRNA localisation

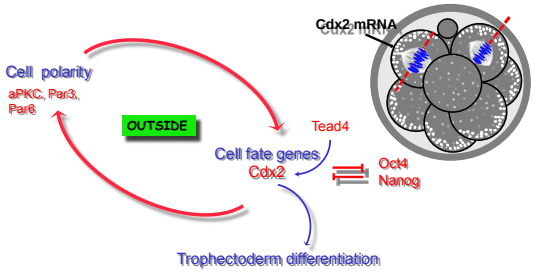


Cdx2 mRNA | *Nanog* mRNA | *both & DAPI* | *dividing cell*

8-cell embryo

Jedrusik et al, Genes Dev, 2008

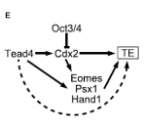
Model for how inside-outside symmetry is broken: Effect of Cell Polarity

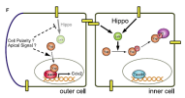


Jedrusik et al, Genes Dev, 2008

Model for how inside-outside symmetry is broken: Effect of Cell Position

- **TEAD/TEF family transcription factor Tead4 is essential for TE development and zygotic Cdx2expression prior to the blastocyst stage**
- **TEAD-mediated transcription is regulated by the Ser/Thr kinase Hippo in Drosophila**
- **Yap1 = Yes associated protein 1 is a co-activator of Tead4**
- **Lats phosphorylates Yap and excludes it from the nucleus**
- **The degree of cell contact could influence Lats- and/or Hippo-mediated cell signalling**



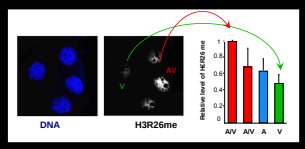


Nishioka et al., Dev Cell, 2009

Mouse embryo cells differ at the 8-cell stage

How does this happen?

Epigenetic asymmetry at the 4-cell stage



ean in

Torres-Padilla et al., Nature, 2007; Wu et al, Stem Cells, 2009

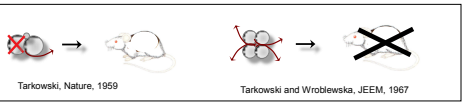
Mouse embryo cells differ at the 8-cell stage

How does this happen?

4-cell stage progenitor of cells that express Cdx2 stronger differ in specific chromatin modifications

What does this mean in development?

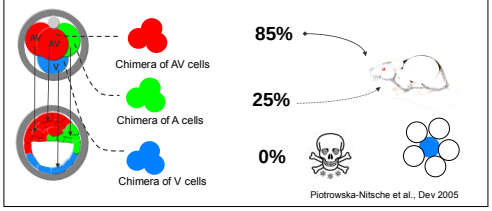
Developmental consequences of early heterogeneity



Tarkowski, Nature, 1959

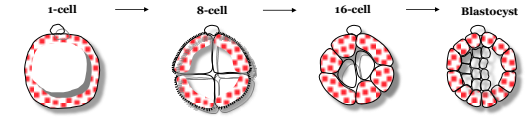
Tarkowski and Wroblewska, JEEM, 1967

making chimeras of like cells



Piotrowska-Nitsche et al., Dev 2005

Three hypotheses for how cells become different from each other



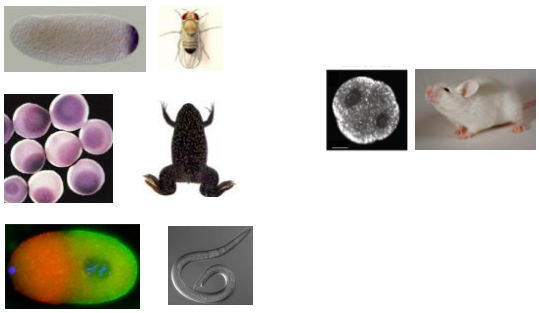
Early asymmetry
(Dalcq 1965; Gardner 1997)

Polarisation
(Johnson & Ziomek 1982)

Inside-Outside
(Tarkowski & Wroblewska 1968)

Our results integrate these three hypotheses

Mouse has its polarised embryo!



Differentiation of Oocytes and Sperm from ESC

Renee A Reijo Pera, PhD
Professor
Director Center for Human Embryonic Stem Cell Research and Education
Institute for Stem Cell Biology & Regenerative Medicine
Director of Basic and Translational Research
Women's Health @ Stanford
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Department of Obstetrics and Gynecology
Stanford University School of Medicine

Commercial interests: None

Outline

I. Introduction: Human embryo and germ cell development

II. Differentiation of hESCs and iPSCs to the germ cell lineage

III. Conclusions and challenges for basic studies and clinical applications for restoration of fertility and/or treatment of infertility

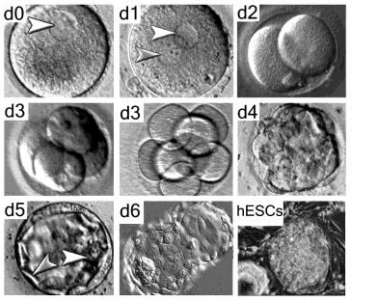
Learning Objectives

I. To examine human embryo and germ cell development from a basic science perspective

II. To describe the use of hESCs and iPSCs for differentiating sperm and oocytes

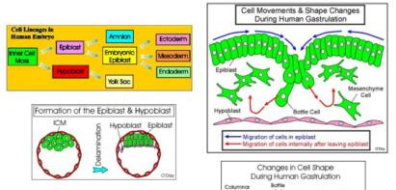
III. To discuss limitations to the current technology

I. Introduction: Human Embryo and Germ Cell Development



R. Reijo Pera, unpublished

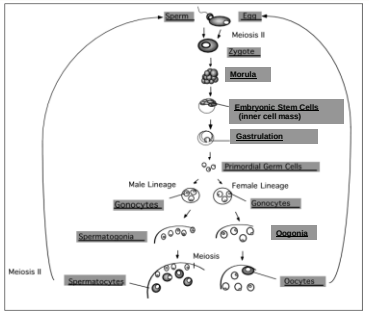
Moving Forward: The Germ Cell Versus Somatic Cell Lineages



*Not all of these morphogenetic movements have been detailed in humans but they all have been shown to occur in other animals.

From: http://www.erin.utoronto.ca/~w3bio380

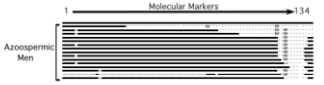
Development of the Human Germ Cell Lineage



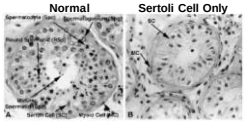
R. Reijo Pera, unpublished

Men with *DAZ* Deletions Make Few or No Sperm

The *DAZ* genes were identified in a screen for genes on the Y chromosome that cause azoospermia. They are deleted in 10-15% of men with few or no sperm.

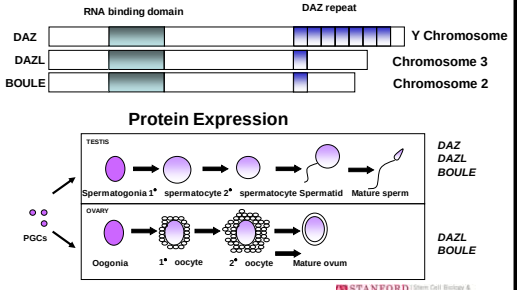


The most common phenotype associated with deletions is SCO.



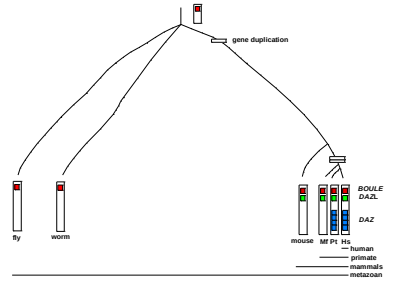
Reijo et al., 1995, 1996, 2000

Genes of the *DAZ* Family Encode RNA-Binding Proteins



Reijo et al., 1995, 1996, 2000; Saxena, et al., 1997; Xu et al., 2001

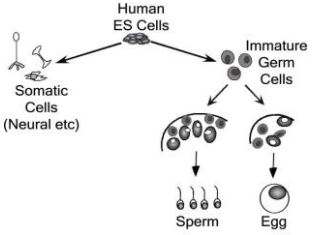
Distinct Genetic Differences in Human Germ Cell Development



GCD in men and women has unique genetic requirements and pathologies relative to other organisms

Xu et al., 2001

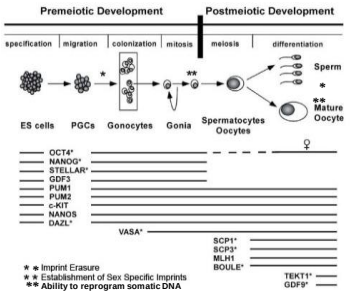
II. Differentiation of hESCs and iPSCs to the Germ Cell Lineage



Challenges: Diagnosis, isolation, and functional assessment

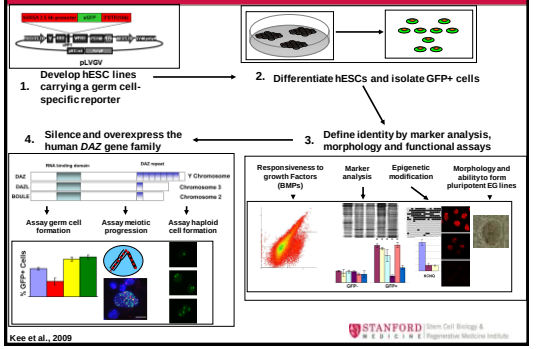
R. Reijo Pera, unpublished

Diagnosis Of Differentiation Of Germ Cells



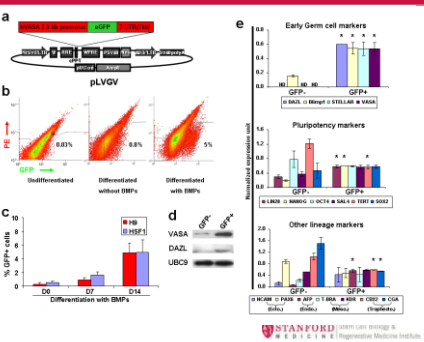
Moore et al., 2003; Clark et al., 2004B; Kee et al., 2006; Nicholas et al., 2008; Haston et al., 2009

General Experimental Design to Accomplish Differentiation of Human Germ Cells *In Vitro*



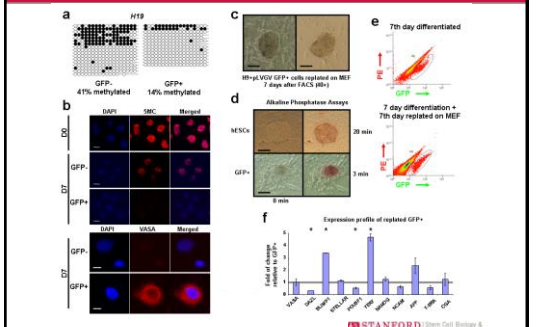
Kee et al., 2009

Isolation of VASA-GFP+ Cells



Kee et al., 2009

Functional Assessment: VASA-GFP+ Cells Undergo Imprint Erasure and Form Pluripotent EG Lines



Kee et al., 2009

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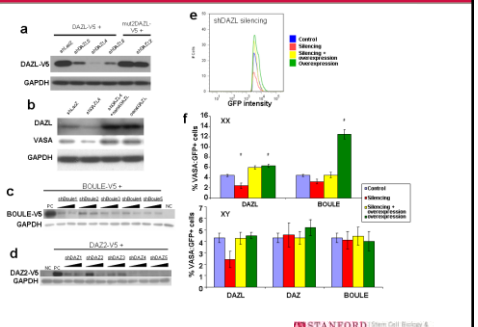
Silencing of *DAZ* Gene Family Members and VASA-GFP+ Cells



Kee et al., 2009

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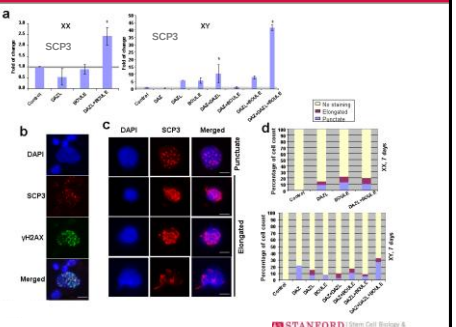
Meiotic Progression of VASA-GFP+ Cells



Kee et al., 2009

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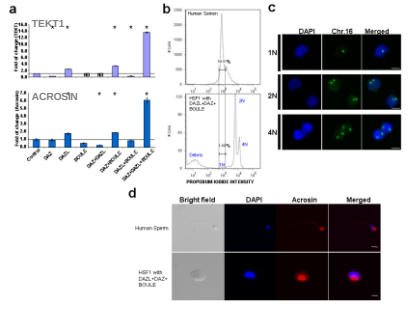
Production of Haploid Cells



Kee et al., 2009

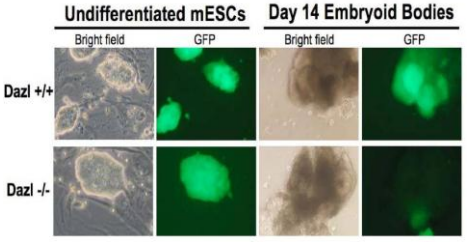
Summary of Results to This Point

- Constructed a VASA-GFP reporter to isolate cells
- GC number increased by addition of BMPs
- Gene and protein expression indicative of GCs
- Erasure of genome-wide methylation marks as well as those at imprinted loci
- Can propagate EG lines from VASA:GFP+ cells
- Genetic dissection indicates divergence of function of *DAZ* family members
- Production of haploid cells
- System is suitable for studies of environmental toxins

Next? Direct comparison of genetic requirements *in vitro* & *in vivo* & promote oocyte differentiation via transplantation

Kee et al., 2006; Fox et al., 2007; Kee et al 2009

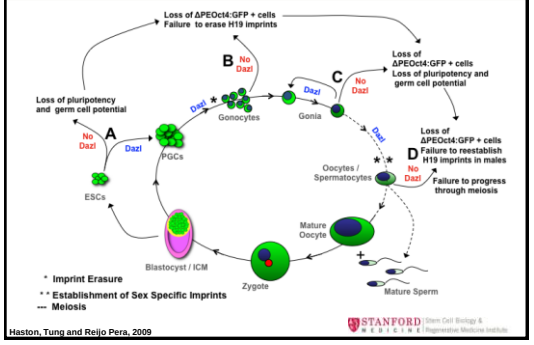
Differentiation of mESCs Derived from Mice with Disruption of *Dazl*



Expression from *Oct4*ΔPE promoter in mESC lines becomes restricted during *in vitro* differentiation.

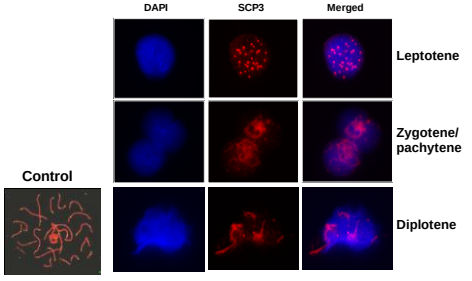
Haston, Tung and Reijo Pera, 2009

Loss of Function of *Dazl* Results in Quantitative and Qualitative Defects in Germ Cell Development *In Vivo* and *In Vitro*



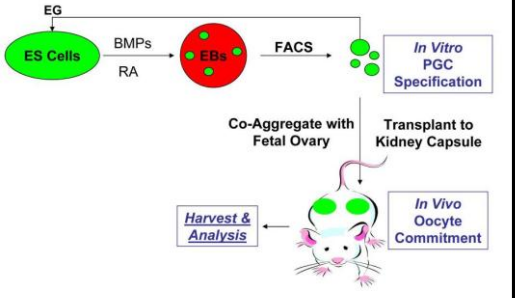
Haston, Tung and Reijo Pera, 2009

Differentiated hESCs Can Be Driven to Different Stages of Prophase I By Overexpression of DAZ Family Members



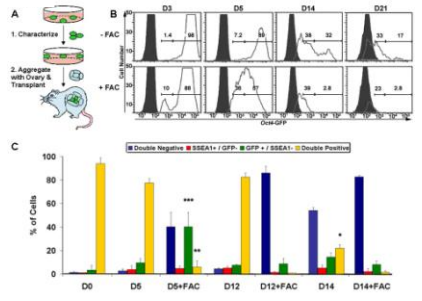
Kee et al., 2009

Complete Maturation Strategy: Transplantation



Nicholas et al., 2009

Complete Maturation Strategy: Isolation Strategy



Nicholas et al., 2009

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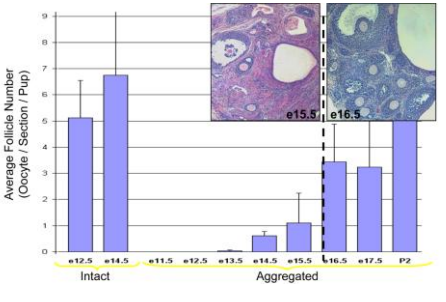
Follicular Maturation after Ovary Aggregation and Transplantation for 3 weeks



Nicholas et al., 2009

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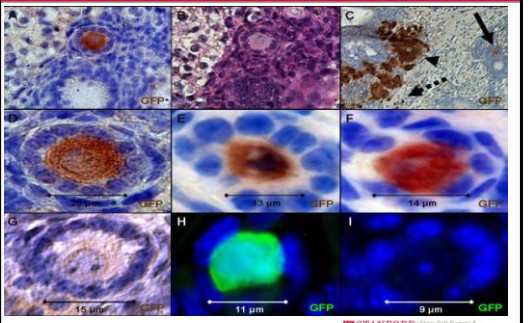
Transplantation of Coaggregates of Day 21 Oct4:GFP+ Cells with P2 Gonad



Nicholas et al., 2009

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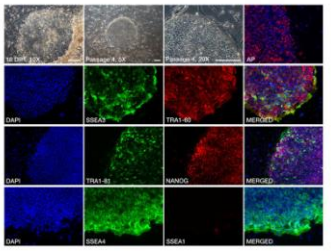
iPSCs and Germ Cell Development



Nguyen et al, unpublished

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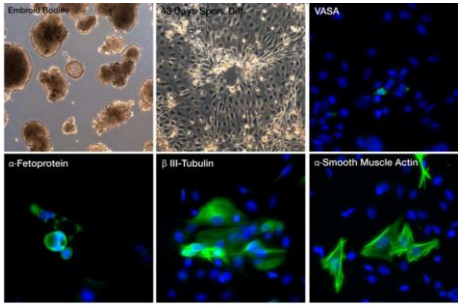
iPSCs Differentiate to Both Somatic and Germ Cell Lineages *In Vitro*



Nguyen et al, unpublished

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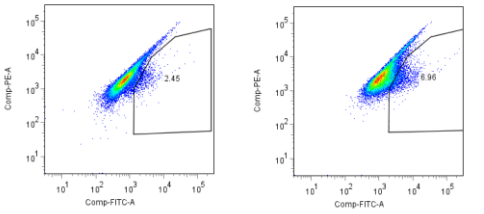
Comparable Quantitative Differentiation of hESCs and iPSCs to the Germ Cell Lineage



HSF1+pLVGV
After 7 day BMPs diff.
2.45%VASA:GFP+

Huf2iPs+pLVGV
After 7 day BMPs diff.
6.96% VASA:GFP+

Panula et al, unpublished

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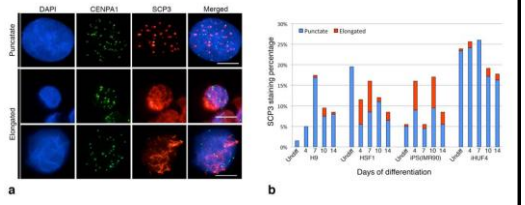
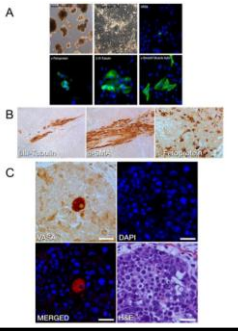
Meiotic Progression of iPSC-Derived Germ Cells



Panula et al, unpublished

iPSCs Differentiate to Both Somatic and Germ Cell Lineages *In Vivo*



Panula, et al.; unpublished

III. Overall Summary

1. Functional and molecular landmark events in GC development occur in differentiation of both hESCs and iPSCs providing a valuable system for human germ cell development
2. Genetic dependence of differentiation and/or maintenance of the pluripotent GCs *in vivo* and *in vitro* is shared (at least in part)
3. ESCs with GC-specific genetic mutations result in reduction (or even absence of "Residual OCT4-positive" cells (these are the putative "teratoma-forming" cells remaining after somatic differentiation); somatic/germ line differentiation is balanced *in vitro*
4. Human germ cell development is modulated by members of the *DAZ* gene family with Y chromosome *DAZ* implicated in meiotic progress and autosomal genes implicated in PGC formation/maintenance
5. Transplantation difficult but successful with mESC-derived PGCs (human?) and may allow completion of meiosis
6. Parallel studies are currently underway to promote oocyte development in human germ cell development
7. Demonstrated utility in assessing response to environmental toxins (as in polycyclic aromatic hydrocarbons)
8. Efforts are aimed at developing novel models for basic studies and clinical applications of diagnosis and preservation/restoration of fertility and/or treatment of infertility

R Reijo Pera, unpublished

Major Challenges

1) **Directing cell decisions**
   (optimized cell surfaces, molecular signals, cell interactions)

2) **Analysis of single cells**
   (gene expression, protein expression, epigenetic status, cell cycle length, morphology)

3) **Diagnostics of fate**
   (progenitor differentiation, tumorigenesis)

4) **Recapitulation of disease parameters in a dish**

5) **Complete maturation and functional tests of germ cells**

R.Reijo Pera, unpublished

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No place like home

Stem cell niches and testicular development

Ellen Goossens, PhD

Biology of the Testis (BITE)
Vrije Universiteit Brussel
Belgium

There is no conflict of interest with the material contained within the presentation

Learning objectives

At the end of the course the participants should be able to:

- comprehend the definition and function of stem cells and their niche
- summarize the different steps that occur during testicular development
- understand the differences between non-primate and primate mammels concerning spermatogonial stem cell proliferation and differentiation
- value the importance of spermatogonial stem cell transplantation

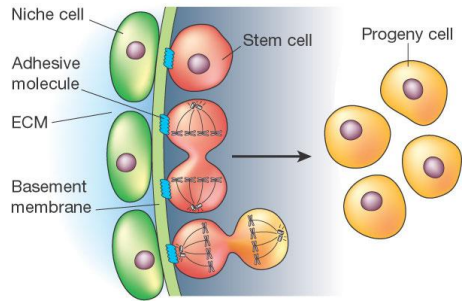
Stem cell niches

Theory

- Stem cells are characterized by the ability to renew themselves through mitotic cell division and differentiating into a diverse range of specialized cell types *(Wikipedia)*
- Niche: the microenvironment in which stem cells are found, which interacts with stem cells to regulate stem cell fate *(Wikipedia)*

In general

- The niche determines what cell will be a stem cell, not the cell itself
- one stem cell per niche
- When a cell moves out of the niche → differentiation starts
- Invariable number of niches
- Cell loss → self-renewal of stem cells to fill up empty niches



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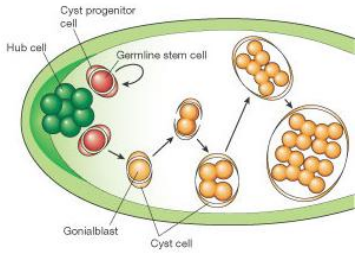
Spermatogonial stem cell niche

Drosophila



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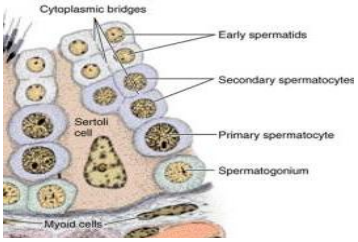
Spermatogonial stem cell niche

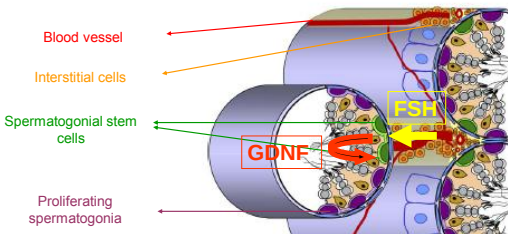
Mammels



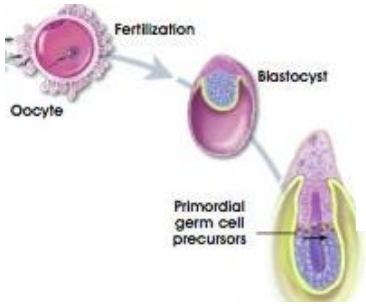
Adapted from **Junqueira and Carneiro**, Basic Histology, a text and atlas, p. 433, Figure 22-4.

Spermatogonial stem cell niche

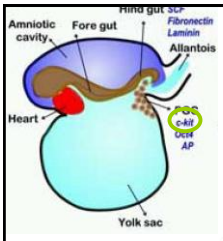
Mammels



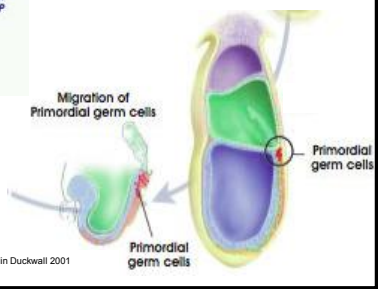
Adapted from Shetty et al., 2007.



Adapted from Terese Winslow, Caitlin Duckwall 2001



Adapted from http://www.endotext.org/pediatrics/pediatrics7/pediatrics7.html



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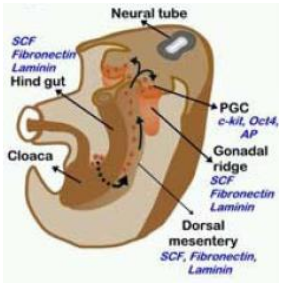
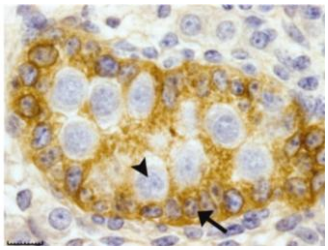


Adapted from http://www.endotext.org/pediatrics/pediatrics7/pediatrics7.html



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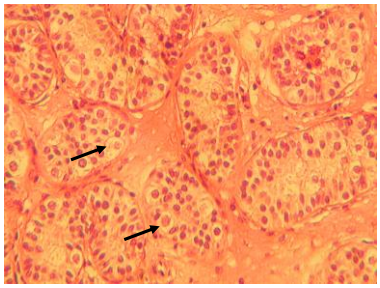
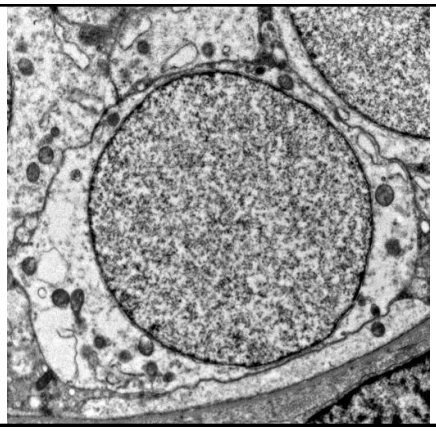
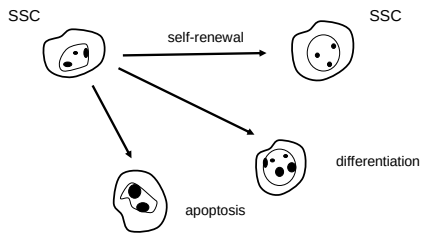
SSCs





SSC proliferation



2 proliferation schemes in rodents

- A_s-scheme

$$A_s \rightarrow A_{pr} \rightarrow A_{al} \rightarrow A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_4 \rightarrow In \rightarrow B$$

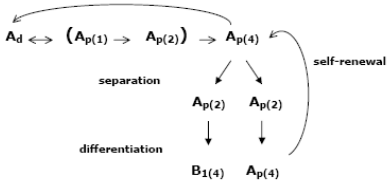
- A_0/A_1-scheme

$$A_0 \xrightarrow{\text{after injury}} A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_4 \rightarrow In \rightarrow B$$

Usual way

SSC proliferation human

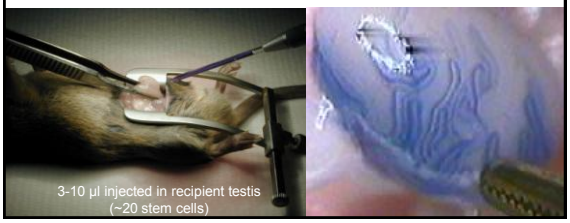
- A_d/A_p-scheme



SSCT

Spermatogonial stem cell transplantation:
injection of SSCs from a fertile donor into the seminiferous tubules of a sterile recipient

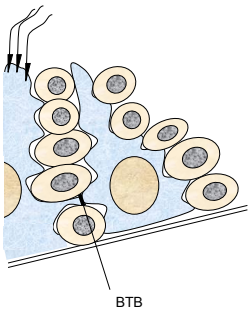
→ transplantation of cells into empty niches



Homing of SSCs after SSCT

SSCs have to cross the blood-testis barrier (BTB)

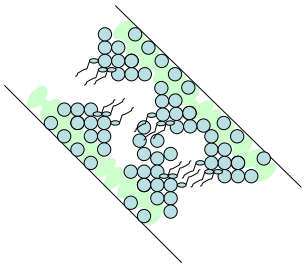
Relocation to the basal membrane by β-integrin



Kanatsu-Shinohara et al. 2008

Colonisation of tubules after SSCT

A) One day after transplantation, blue donor cells are scattered in the lumen of the tubule.
B) One week after transplantation, some donor cells have reached the basement membrane.
C) Two weeks after transplantation, donor cells on the basement membrane of the tubule are forming chains of spermatogonia.
D) One month after transplantation, many blue cells are found on the basement membrane.
E) Two months after transplantation, spermatogenesis has been established in the central areas of colonies.
F) Three months after transplantation, spermatogenesis is well organized.



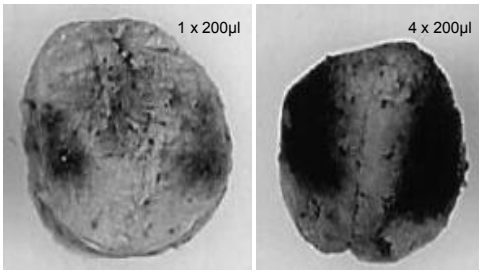
Nagano et al., 1999

SSCT with SSCs from other species

Donor cells obtained from	Colonisation efficiency
Rat	Spermatozoa
Hamster	Spermatozoa (abnormal, lower number)
Rabbit / Dog	Chains of spermatogonia
Primate	Colonization, no proliferation
Human	Colonization, spermatogonia survive for 6 months

➔ Phylogenetic distance between donor and acceptor plays a major role in the efficiency of SSC transplantation

Human-to-human SSCT



Adapted from Brook et al., 2001

Clinical study in the human

Studies on autologous SSCT in humans are very scarce.

Only one by Radford et al. in 1999

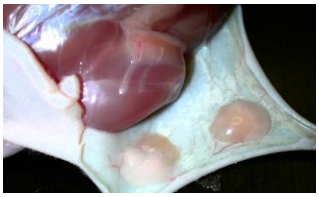
- Offered as alternative to sperm banking
- No news at this moment.
- How to evaluate?

Grafting

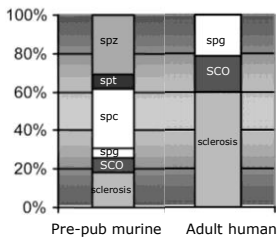
= transplantation of testicular tissue

= transplantation of SSCs together with their niches

= transplantation of SSCs in their natural environment

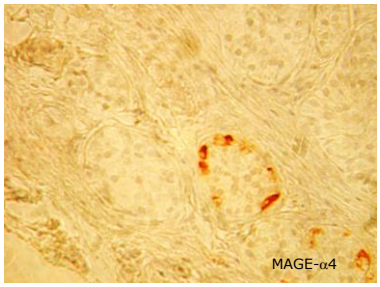


(Xeno)grafting under the back skin



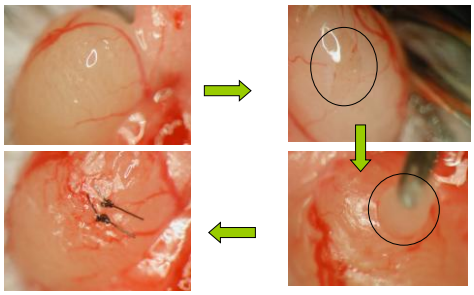
Adapted from Geens et al., 2006

Prepubertal human to mouse



Goossens et al., 2008

Intratesticular grafting



Van Saen et al., 2009

Mouse-to-mouse	Transplantations (n)	Testes with donor spermatogenesis [n (%)]	Total colony lenght/testis (mm)
SSCT	9	**5 (55)**	**41.3**
Intratesticular grafting	16	**16 (100)**	**125.3**

Van Saen et al., 2009

Spermatogenesis in vitro

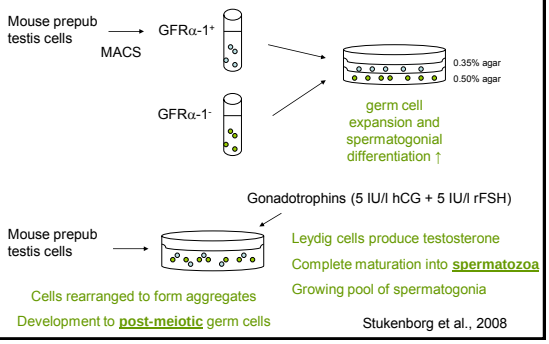
A little bit of history

1915: Goldschmidt: testis tissue in culture
→ progression of spermatogonia into meiosis, but no full spermatogenesis
→ long-term organ culture: ischaemia

1999: Cremades: conventional culture of round or elongating spermatids
→ develop in mature gametes
→ not possible when starting from spermatogonia

2008: Stukenborg : spatial arrangement of germ cells and somatic cells is important for the regulation and completion of germ cell maturation
→ cultures should provide a microenvironment that resembles the spermatogonial niche
→ 3D cultures support contacts between spermatogonia and Sertoli cells

Spermatogenesis in vitro

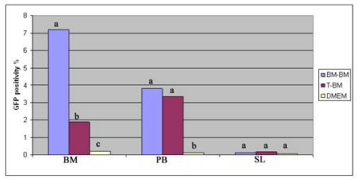


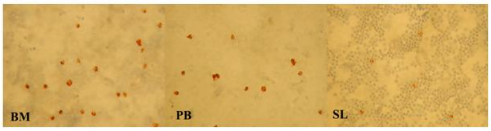
Stukenborg et al., 2008

Transdifferentiation

- "the conversion of a differentiated cell of one developmental commitment into a differentiated cell of another lineage without first reverting to a more primitive stem cell or progenitor, with concomitant loss of tissue-specific markers and function of the original cell type, and acquisition of markers and function of the transdifferentiated cell type" (Wagers and Weissman, 2004)
- Conversion only possible when under influence of specific environmental factors
- Transplantation of SSCs to another niche (eg. to bone marrow stem cell niche) → Will SSCs transdifferentiate to HSCs?

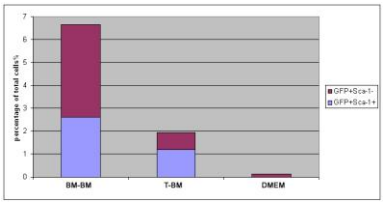
GFP+ donor cells are present in recipient's BM, PB and SL



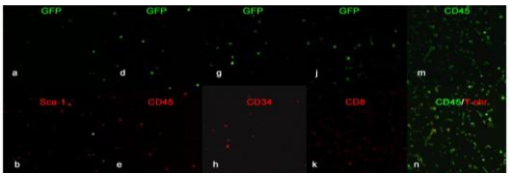


Ning et al., 2010

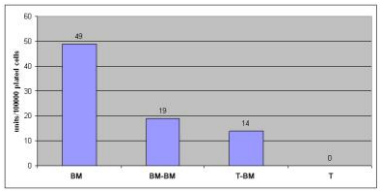
GFP+ donor cells express markers for HSCs



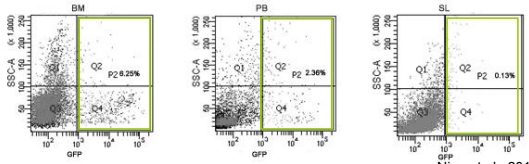
Ning et al., 2010



Functionality test in-vitro: GFP$^+$Sca1$^+$H2kb$^+$ → CFU



Functionality test in-vivo: GFP$^+$Sca1$^+$H2kb$^+$ → transplanted to BM



Ning et al., 2010

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Female Germline Stem Cells in Adult Mammal

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Professor, Shanghai Jiao Tong University

Conflict of Interest Statement:
The authors declare no conflict of interest.

Learning objective

- To achieve an advanced understanding of the scientific and clinical importance of stem cells, including the relationship between stem cells and disease.
- To identify and locate female germline stem cell (FGSC) in vivo
- To isolation and culture of FGSCs
- To characterize FGSCs
- To understand application of FGSCs

Overview

I. What is stem cell? And its characteristics?

II. Germline stem cell

Including:

Spermatogonial stem cells (SSC) (Wu et al., 2008; Yuan et al., 2009)

Female germline stem cells (FGSC) or Oogonia (Zou et al. 2009)

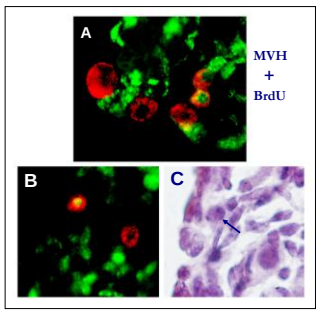
Characteristics

Capable of self-renewal

Capable of differentiation

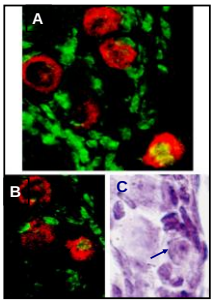
Passing genetic information to the next generation (Orwig et al., 2002)

III. Morphology and location of FGSCs in vivo



Female germline stem cells (FGSCs) in day 5 ovaries.

Mvh
+
BrdU



FGSCs in adult mouse ovaries.

IV. Isolation and culture of FGSCs

Collect and Dissect
Ovaries from Neonate or
Adult Mice

↓

Enzymatic (Collagenase and Trypsin)
Digestions

↓

Single Cell
Suspension

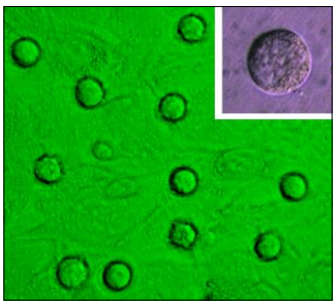
↓

Immunomagnetic Isolation

↓

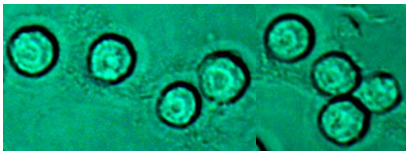
Plate On Inactivated STO Cell Feeders

Comparison of Morphology of FGSCs and SSCs



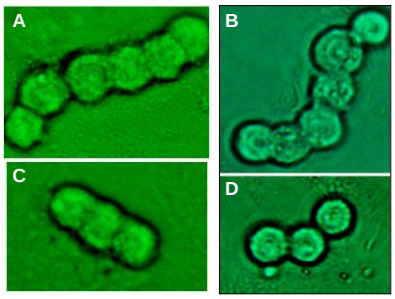
FGSCs

Morphology of FGSCs cultured on mitomycin C-treated STO cells for 24 h after purification by immunomagnetic isolation of MVH+ cells.

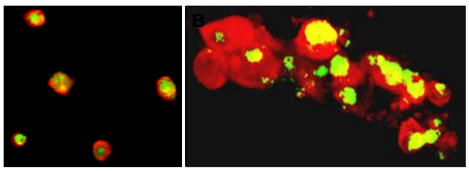


Morphology of SSCs cultured on mitomycin C-treated STO cells for 24 h

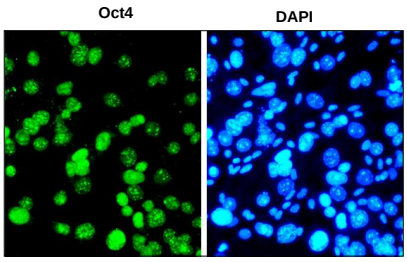
Comparison of growth pattern of FGSCs and SSCs



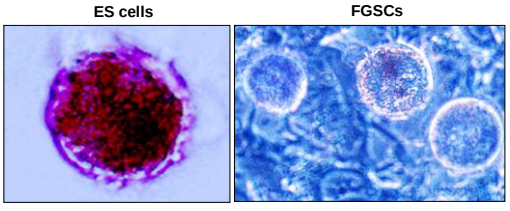
V. Characteristics of FGSCs



Dual immunofluorescence for MVH (red) and BrdU (green):
FGSCs are positive for MVH and BrdU expresssion

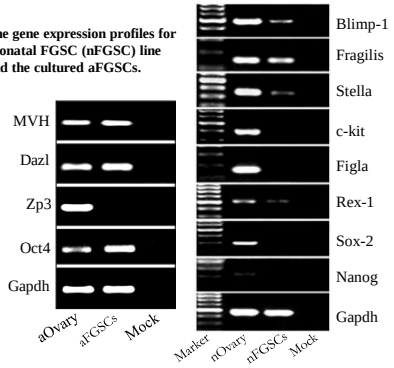


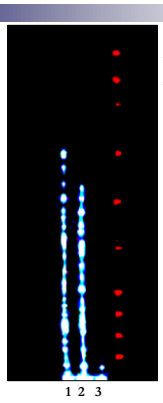
Oct4 expression in FGSCs



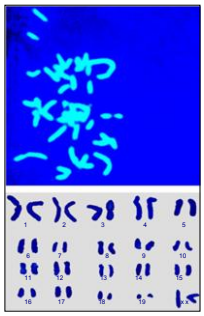
FGSCs are weakly positive for alkaline phosphatase.

The gene expression profiles for neonatal FGSC (nFGSC) line and the cultured aFGSCs.



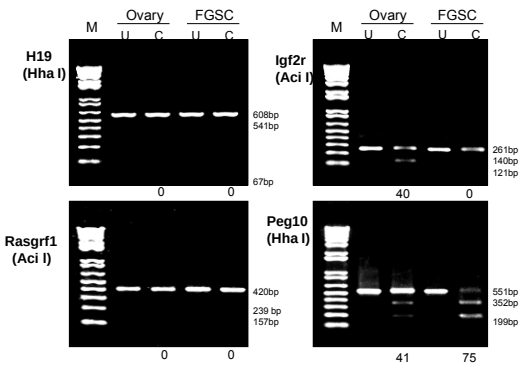


The FGSCs had high telomerase activity.

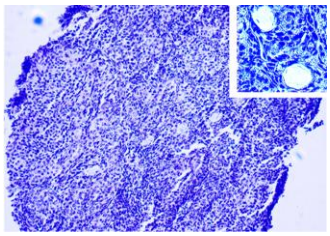


The FGSCs had a normal karyotype (40, XX).

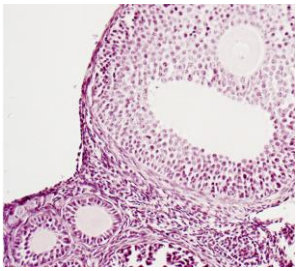
The imprinting pattern of the FGSCs



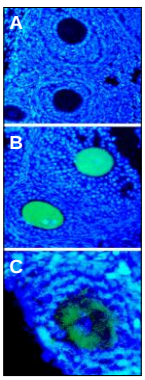
Differentiation in vivo



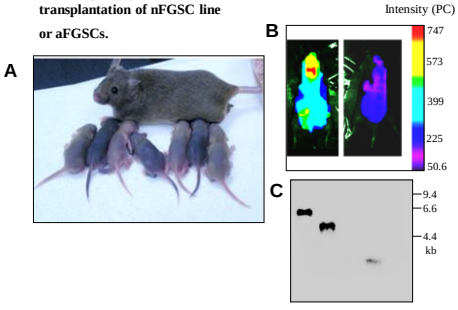
Differentiation in vivo



Differentiation in vivo



GFP offspring generated from the transplantation of nFGSC line or aFGSCs.



VI. Application

FGSCs have implications for clinical and animal biotechnological applications related to the generation of new oocytes. They are also crucial to the future use of stem cells in regenerative medicine (Zou et al., 2009).

VII.References

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Zou K, Yuan Z, Yang Z, Luo H, Sun K, Zhou L, Xiang J, Shi L, Yu Q, Zhang Y, Hou R, Wu J. Production of offspring from a germline stem cell line derived from ovaries. Nature Cell Biology 2009; 11: 631-636.

Acknowledgements

Grants

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Contributions

Kang Zou, Huacheng Luo, Zhe Yuan, Zhaojuan Yang, Kejing Sun, Li Zhou, Jie Xiang, Lingjun Shi,Qingsheng Yu, Yong Zhang, Ruoyu Hou

SOMATIC STEM CELLS IN THE ENDOMETRIUM AND ITS PUTATIVE IMPLICATION IN ENDOMETRIOSIS

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Professor Obs/Gyn, University of Valencia.
Scientific Director, Fundación IVI.
Scientific Director, Centro de Investigación Príncipe Felipe

The author declares no conflict of interest.

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

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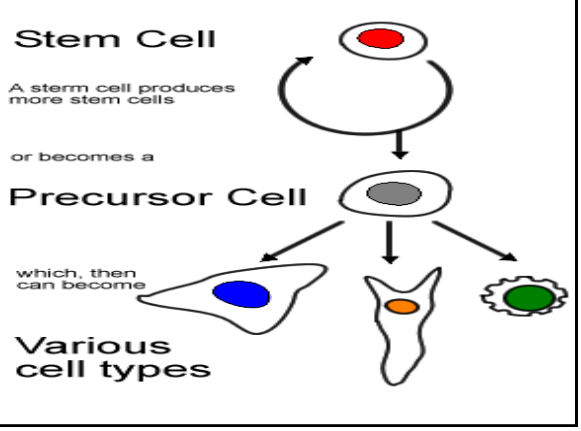
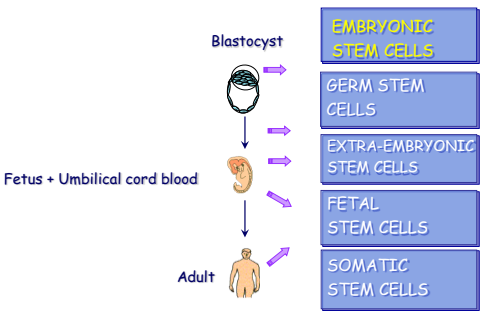
LEARNING OBJECTIVES

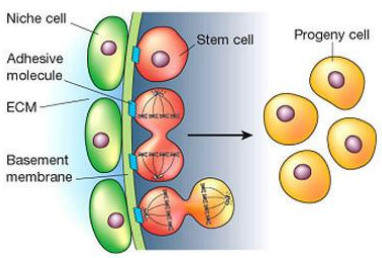
- To acquire new concepts concerning the biology and origin of somatic stem cells, and their niche.
- To learn more about the existence of somatic stem cells (SSC) in murine and human endometrium.



STEM CELLS. SOURCES AND TYPES

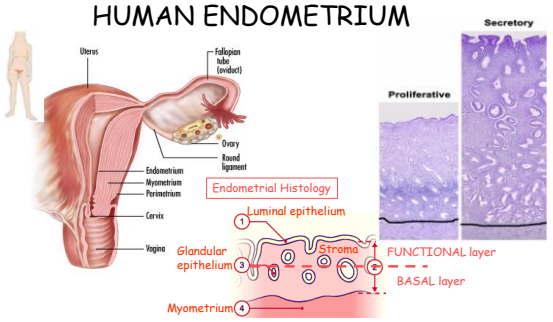


THE NICHE



The role of the niche is the support of the SSC population in an organ that suffers very frequently shedding of the functional layer.

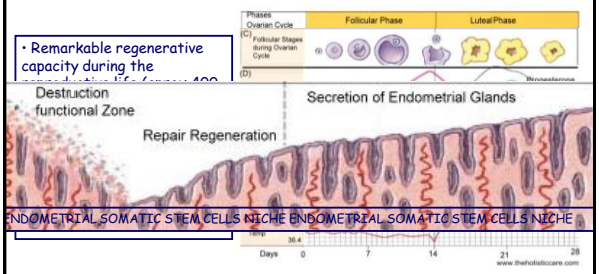
HUMAN ENDOMETRIUM



The endometrium is composed of luminal epithelium and epithelial-lined glands surrounded by a supportive stroma. It can be divided into a functional (removed layer) and basal (regenerating layer) compartments.

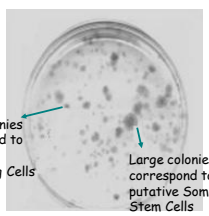
HUMAN ENDOMETRIUM

• Remarkable regenerative capacity during the



INDIRECT EVIDENCE FOR THE EXISTENCE OF ENDOMETRIAL SSC (ESSC)

CELL-CLONING STUDIES
Capacity of forming colonies, one of the typical characteristics of stem cells.



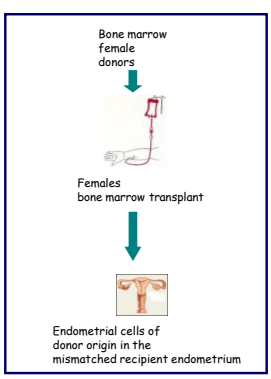
Clones	Clonogenicity (%)	
	Epithelial	Stromal
Large	0.08 ± 0.03	0.02 ± 0.01**
Small	0.14 ± 0.04	1.23 ± 0.18**
Total	0.22 ± 0.07*	1.25 ± 0.18*

The cloning efficiency does not vary significantly across the menstrual cycle; or between cycling and inactive endometrium

(Schwab et al.,Fertil Steril 2005).

Chan et al., Biol Reprod 2004.

IMPLICATION OF BONE MARROW

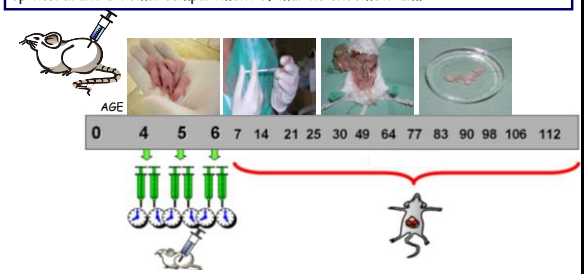


• Percentage of donor-derived cells in endometrium increased with time:
- 0.25% at 24 months,
- 4% at 35 months,
- 10.5% at 129 months,
- 50% at 147 months.

Taylor., JAMA 2004.

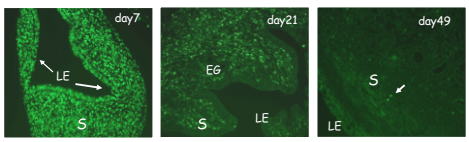
ESSC IN MURINE ENDOMETRIUM

METHOD OF 5-BROME-2-DEOXYURIDINE (BrdU)
BrdU is incorporated into genomic DNA during the replication phase of the mitotic cycle labelling new cells. Identification of label retaining cells (LRC) in epithelial and stromal compartment of murine endometrium.



Chan and Gargett., Stem cells 2006.
Cervelló et al., Human Reprod 2007.

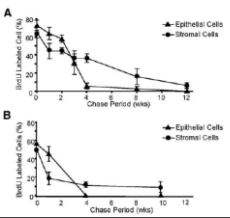
ESSC IN MURINE ENDOMETRIUM



The BrdU retained cells in mice endometrium decreases with age.

Cervelló et al., Human Reprod 2007.

After labelling, the BrdU signal is progressively decreased in each division. The retention of the labelling in some populations means no division or very low rate of division, one of the main characteristic of somatic/progenitor stem cells.



Chan and Gargett., Stem Cells 2006.

PUTATIVE ENDOMETRIAL STEM CELL MARKERS

	Stem Cell Marker	Endometrial localization	Reference
POU5F1	Embryonic stem cell	In humans, it co-localise with Vimentin and Cytokeratin. In murine populations, co-localization of BrdU- retaining cells.	Matthai *et al*,2006 Cervelló *et al*.,2007
CD90	Cultured Mesenchymal stem cell	In humans, it differentiates the expression in the basalis and functionalis stroma.	Schwab and Gargett, 2008
CD146	Endothelial cell, perivascular cell and Mesenchymal stem cell	In humans, it co-expresses with PDGF-Rβ.	Schwab and Gargett, 2007,2008
c-Kit	Hematopoietic stem cell and mast stem cells	In humans, mainly in the stroma. In murine samples, co-localization of BrdU- retaining cells.	Cho *et al*.,2004 Cervelló *et al*,2007 Goodell *et al*,2008
CD34	Hematopoietic stem cell and endothelial cells	In humans, mainly in the stroma.	Cho *et al*.,2004
STRO-1	Mesenchymal Stem cells	In humans, is located on the perivascular regions of the endometrium	Schwab et al., 2008.

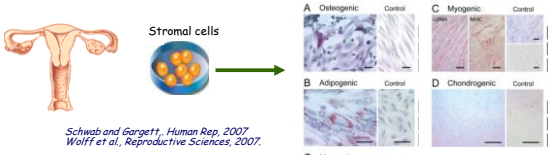
Cervelló et al., Expert Reviews 2009.

• Existence of SSC in murine endometrium was demonstrated by BrdU method.
(Chan and Gargett., Stem cells, 2006; Cervelló et al., Human Rep., 2007)

• Recent studies demonstrate that human endometrium contains a rare MSC-like population.
(Chan et al., Biol Rep., 2004; Schwab and Gargett,. Human Rep, 2007; Wolff et al., Reprod.Sci., 2007.; Gargett et al., Biol Rep., 2009)

• In the menstrual blood the existence of a stem cell-like population has been demonstrated.
(Meng et al., J Transl Med, 2007)

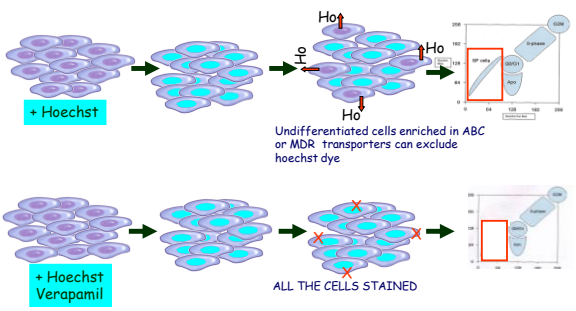
• SSC have differentiated into mesoderm-derived lineages *in vitro*.



Schwab and Gargett,. Human Rep, 2007
Wolff et al., Reproductive Sciences, 2007.

ESSC IN HUMAN ENDOMETRIUM

Side Population: Hoechst Method and Cell Sorting



SIDE POPULATION METHOD

• Side Population (SP) method was described for SSC isolation in bone marrow based on the ability to efflux Hoechst33342-fluorescence dye.
(Goodell et al., J Exp Med. , 1996)

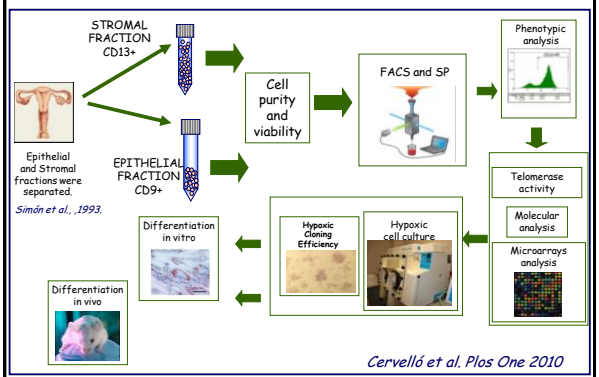
• This property is present in cells enriched in ABC transporters and has been documented in the detection of SSC in human myometrium, lung and dental pulp.
(Ono et al., PNAS, 2007; Martin et al., Cytotherapy, 2008; Iohara et al., Stem Cells, 2008)

• It has also been proposed recently in the human endometrium although not functionally demonstrated yet.
(Kato et al., Human Rep., 2007; Tsuji et al.,Fertil Steril., 2008)

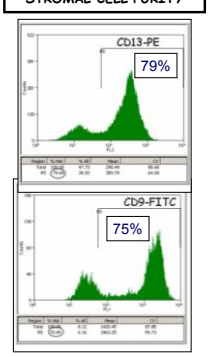
HYPOTHESIS

COULD THE SP REPRESENT THE SOMATIC STEM CELL POPULATION IN THE HUMAN ENDOMETRIUM?

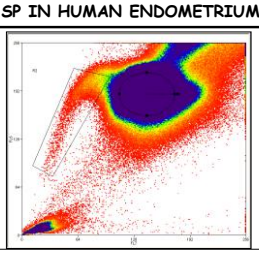
STUDY DESIGN



EPITHELIAL AND STROMAL CELL PURITY

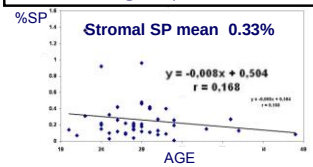


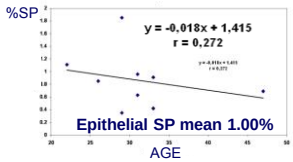
SP IN HUMAN ENDOMETRIUM



Epithelial SP 1.0 ± 0.8% (n=60)
Stromal SP 0.3 ± 0.3% (n=90)

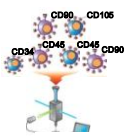
% SP during Reproductive Life





PHENOTYPIC ANALYSIS INTRODUCTION

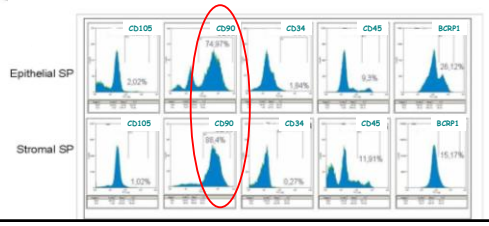
✓ Phenotypic analysis of Side Population cells:



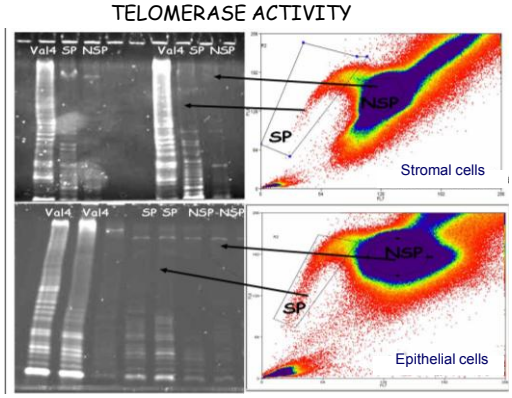
The SP cells were labeled with:

- markers associated with Hematopoietic progenitor cells like CD45-FITC and CD34-PE.

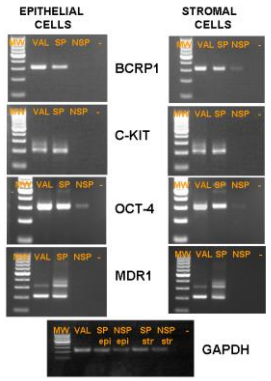
- Mesenchymal stem cells markers like CD90-PE and CD105-FITC.



TELOMERASE ACTIVITY

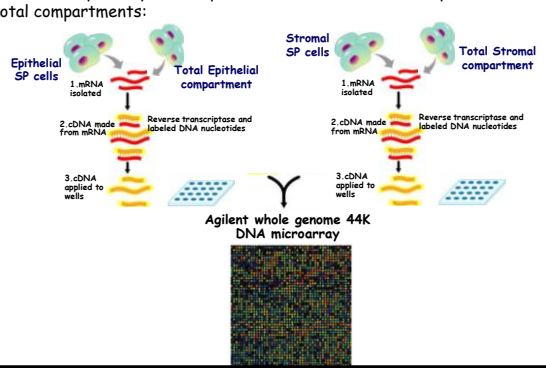


SP MOLECULAR ANALYSIS



MICROARRAYS ANALYSIS

✓ Microarrays analysis of Epithelial and Stromal Side Population versus total compartments:



MICROARRAYS ANALYSIS

TOP TEN MOLECULES SP stromal cells

Molecules	Exp. Value
MMP3	↑6.532
RND3	↑6.412
SERPINB2	↑6.275
SLC4A1	↑6.026
ANGPTL4	↑5.820
INHBA	↑5.674
IER3	↑5.578
KRT34	↑5.502
GDF15	↑5.367
ADM	↑5.324

Molecules	Exp. Value
VWF	↓-4.331
SCGB1D2	↓-4.321
SERPINA5*	↓-4.185
ASRGL1*	↓-3.589
SOX17	↓-3.567
SCGB2A1	↓-3.169
HGD	↓-3.105
ACSL5	↓-3.105
TPD52L1	↓-3.085
ST6GALNAC1	↓-3.083

SP stromal cells vs Stromal cells:
121 genes up-regulated
74 genes down-regulated

Molecular and cellular Functions:
-Cell signalling and interaction
-Cellular growth and proliferation
-Cellular movement
-Cell death
-Cell cycle

TOP TEN MOLECULES SP epithelial cells

Molecules	Exp. Value
IL1B	↑19.823
CXCL1	↑19.069
HSPA6	↑16.887
TUBA4A	↑13.661
CCL4	↑13.402
POLR2J2	↑13.321
CACNG5	↑13.281
GDF15	↑12.487
CD69	↑11.971
RGS1	↑11.604

Molecules	Exp. Value
FOXE1	↓-12.402
PCSK1N	↓-11.151
POU3F3	↓-9.126
NEUROG1	↓-8.531
SYNPO	↓-7.969
CACNA1E	↓-7.794
CRHR2	↓-7.654
IER5*	↓-7.654
NEUROG3	↓-7.593
SYN1	↓-7.002

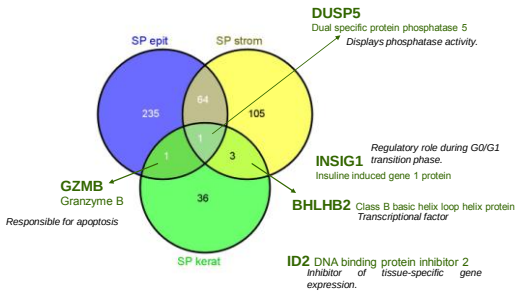
SP epit cells vs Epithelial cells:
196 genes up-regulated
116 genes down-regulated

Molecular and cellular Functions:
-Cell signalling and interaction
-Cellular growth and proliferation
-Cellular movement
-Cell death

MICROARRAYS ANALYSIS

✓**Microarrays comparison with keratynocites SP:**

Larderet et al., Keratynocites. Stem cells. 2006.

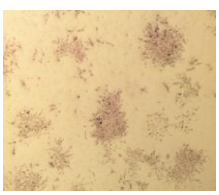


CLONING OF SP CELLS IN HYPOXIC CONDITION

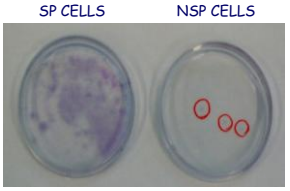
✓ Cell culture conditions: 2% O_2, 37°C, 5% CO_2, 90 % humidity:

300-500 cells/cm² were seeded into 60-mm Petri dishes coated with gelatine 0.1%, cultured in serum medium, incubated for **15 days** and stained with 0.5%Toluidine Blue for 5 min.

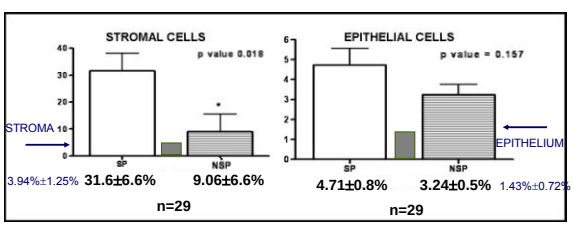
CLONING EFFICIENCY CE (%) = (Number of colonies/number of cells seeded) x 100.



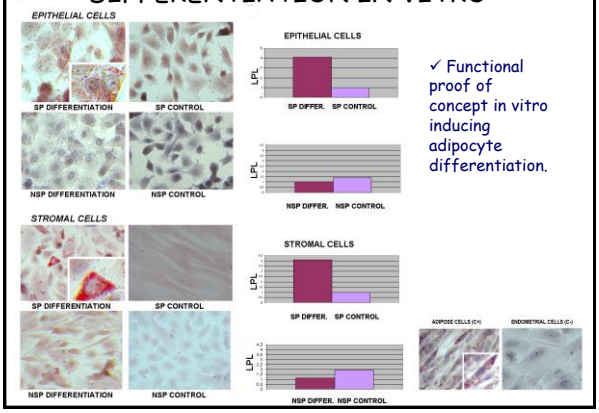
SP CELLS NSP CELLS



CLONING EFFICIENCY

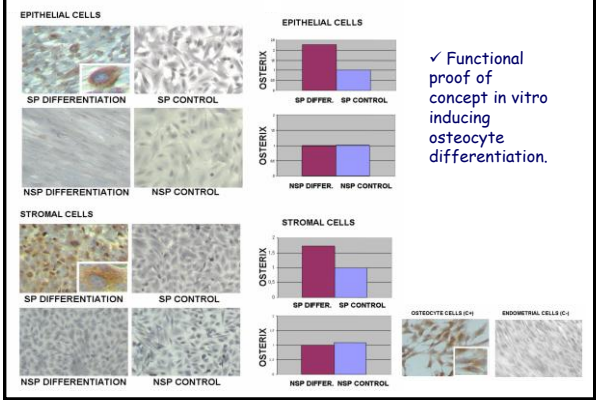


DIFFERENTIATION IN VITRO



✓ Functional proof of concept in vitro inducing adipocyte differentiation.

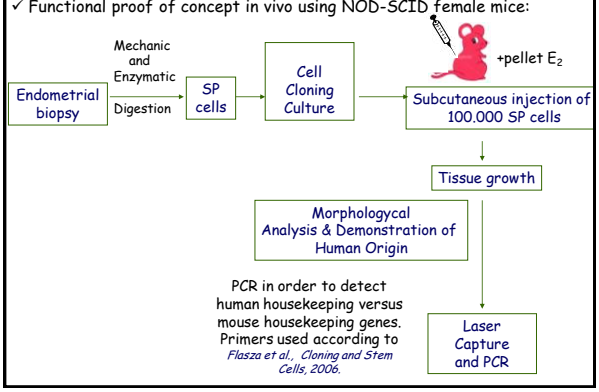
DIFFERENTIATION IN VITRO



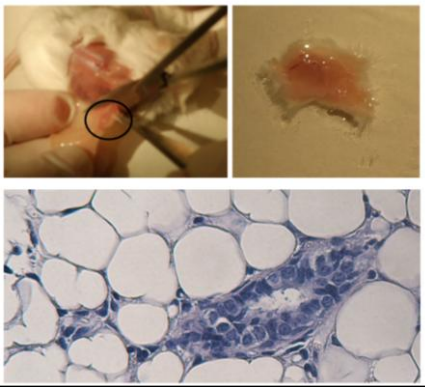
✓ Functional proof of concept in vitro inducing osteocyte differentiation.

IN VIVO DIFFERENTIATION

✓ Functional proof of concept in vivo using NOD-SCID female mice:



IN VIVO DIFFERENTIATION



IN VIVO DIFFERENTIATION

- **mRNA human (79 pb)**

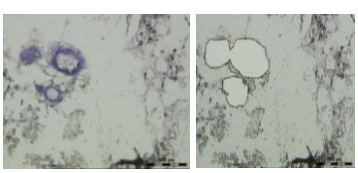
CTCTCTGCTCCTCCTGTTCGACAGTCAGCCGCATCTTCTTTTGCGTCGCCAGCCGAGCCACATCGCTCAGACACCATGGGGAAGGTGAAGGTCG
GAGTCAACGGATTTGGTCGTATTGGGCGCCTGGTCACCAGGGCTGCTTTTAACTCTGGTAAAGTGGATATTGTTGCCATCAATGACCCCTTCATTG
ACCTCAACTACATGGTTTACATGTTCCAATATGATTCCACCCATGGCAAATTCCATGGCACCGTCAAGGCTGAGAACGGGAAGCTTGTCATCAATG
GAAATCCCATCACCATCTTCCAGGAGCGAGATCCCTCCAAAATCAAGTGGGGCGATGCTGGCGCTGAGTACGTCGTGGAGTCCACTGGCGTCTT
CACCACCATGGAGAAGGCTGGGGCTCATTTGCAGGGGGGAGCCAAAGGGTCATCATCTCTGCCCCCTCTGCTGATGCCCCATGTTCGTCATG
GGTGTGAACCATGAGAAGTATGACAACAGCCTCAAGATCATCAGCAATGCCTCCTGCACCACCAACTGCTTAGCACCCCTGGCCAAGGTCATCCA
TGACAACTTTGGTATCGTGGAAGGACTCATGACCACAGTCCATGCCATCACTGCCACCCAGAAGACTGTGGATGGCCCCTCCGGGAAACTGTGG
CGTGATGGCCGCGGGGCTCTCCAGAACATCATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAG
CTCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCTAGAAAAACCTGCCAATATGATGACATCAA
GAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTCAAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACAC
CCACTCCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTGGTATGACAACGAATTTGGCTACAGCA
ACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGAC
CCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACACAGTGGATCTCCCCTCCTCACAGTTGCCATGTAGACCCCTTGAAGAGGGGAG
GGGCCTAGCGAGCCGCACCTTGTCATGTACCATCAATAAAGTACCCTGTGCTCAACC

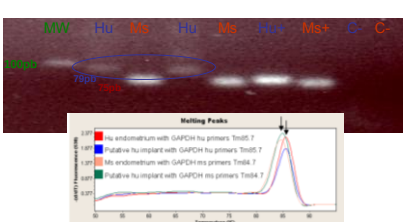
- **mRNA mice (75 pb)**

AGAGACGGCCGCATCTTCTTGTGCAGTGCCAGCCTCGTCCCGTAGACAAAATGGTGAAGGTCGGTGTGAACGGATTTGGCCGTATTGGGCGCCTGGT
CACCAGGGCTGCCATTTGCAGTGGCAAAGTGGAGATTGTTGCCATCAACGACCCCTTCATTGACCTCAACTACATGGTCTACATGTTCCAGTATGACTC
CACTCACGGCAAATTCAACGGCACAGTCAAGGCCGAGAATGGGAAGCTTGTCATCAACGGGAAGCCCATCACCATCTTCCAGGAGCGAGACCCCACTA
ACATCAAATGGGGTGAGGCCGGTGCTGAGTATGTCGTGGAGTCTACTGGTGTCTTCACCACCATGGAGAAGGCCGGGGCCCACTTGAAGGGTGGAGC
CAAAGGGTCATCATCTCCGCCCCTTGTCCGATGCCCCATGTTTGTGATGGGTGTGAACCACGAGAAATATGACAACTCACTCAAGATTGTCAGCAA
TGCATCCTGCACCACCAACTGCTTAGCCCCCCTGGCCAAGGTCATCCATGACAACTTTGGCATTGTGGAAGGGCTCATGACCACAGTCCATGCCATCA
CTGCCACCCAGAAGACTGTGGATGGCCCCTCTGGAAAGCTGTGGCGTGATGGCCGTGGGGCTGCCCAGAACATCATCCCTGCATCCACTGGTGCTGC
CAAGGCTGTGGGCAAGGTCATCCCAGAGCTGAACGGGAAGCTCACTGGCATGGCCTTCCGTGTTCCTACCCCCAATGTGTCCGTCGTGGATCTGACG
TGCCGCCTGGAGAAACCTGCCAAGTATGATGACATCAAGAAGGTGGTGAAGCAGGCATCTGAGGGCCCACTGAAGGGCATCTTGGGCTACACTGAGG
ACCAGGTTGTCTCCTGCGACTTCAACAGCAACTCCCACTCTTCCACCTTCGATGCCGGGGCTGGCATTGCTCTCAATGACAACTTTGTCAAGCTCATTT
CCTGGTATGACAATGAATACGGCTACAGCAACAGGGTGGTGGACCTCATGGCCTACATGGCCTCCAAGGAGTAAGAAACCCTGGACCACCCACCCA
GCAAGGACACTGAGCAAGAGAGGCCCTATCCCAACTCGGGCCCCACACTGAGGATCTGCCTCACAATTTCCATCCCAGACCCCCATAATAACAGGAG
GGGCCTAGCGAGCCCTCCCTACTCTCTTGAATACCATCAATAAAGTTCGCTGCACCCACAAAAAAAAAAAAAAAAAAAA

Flasza et al., Cloning and Stem Cells, 2006.

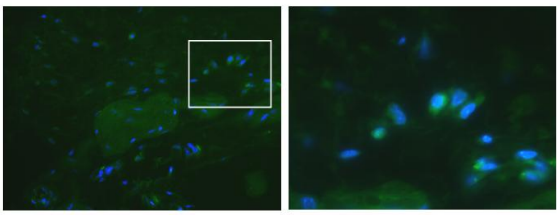
IN VIVO DIFFERENTIATION





Hu: putative human glands
Ms: mice glands.
Hu+: human endometrium
Ms+: mice endomtrium
C-: water

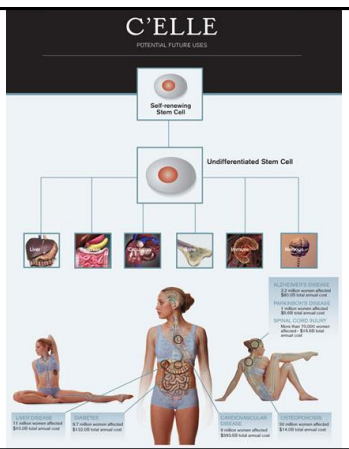
IN VIVO DIFFERENTIATION



Immunohistochemical analysis for Human Progesterone Receptor in endometrial like structures in mice subcutaneous tissue after stroma SP injection (40X). Right, Detail of green fluorescent signal due to Hu-PR co-localized with DAPI

CONCLUSIONS

- SP account for 0.3% and 1% of the stromal and epithelial compartment respectively, remaining constant during reproductive life.
- Phenotype of SP suggest a mesenchymal origin and they display an intermediate pattern of telomerase activity, being positive for c-Kit, Oct-4 and BCRP-1
- Wide genome analysis demonstrated a differential gene expression profile of SP compared to its endometrial fraction. A common SP signature is suggested.
- SP cells do not growth in normoxic conditions. In hypoxic conditions, SP cells display high cloning efficiency compared to NSP and total fraction.
- Stromal and epithelial SP have been differentiated in vitro to adipocytes and osteocytes.
- The functional proof of concept is given by the ability of SP cells to reconstruct the human endometrium in an animal model.



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6. Cervelló, I., Martinez-Conejero, JA., Horcajadas, et al. (2007) Identification, characterization and co-localization of label-retaining cell population in mouse endometrium with typical undifferentiated markers. Human Reproduction 22: 45-51.
7. Cervelló, I., Gil-Sanchis C, Mas A and Simón C. (2009) Current understanding of endometrial stem cells. Expert Review of Obstetrics & Gynecology Vol. 4, No. 3.
8. Cervelló I, Simón C.(2009) Somatic stem cells in the endometrium. Reprod Sci.;16(2):200-5. Review.
9. Cervelló I, Gil-Sanchis C, Mas A, Delgado-Rosas F, Martínez-Conejero JA, Galán A, Martínez-Romero A, Martínez S, Navarro I, Ferro J, Horcajadas JA, Esteban FJ, O´Connor JE, Pellicer A and Simón C. (2010) Human endometrial side population cells exhibit genotypic, phenotypic and functional features of somatic stem cells. Plos One (accepted).

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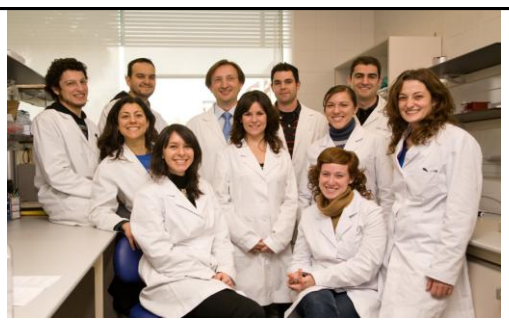
Biol Reprod.;80(6):1136-45.



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PRINCIPE FELIPE
CENTRO DE INVESTIGACION

Somatic Stem Cells in the Myometrium and its Putative Implication in Myoma Formation*

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Department of Obstetrics and Gynecology
School of Medicine, Keio University

* I have declared that no competing interests exist.

Learning Objectives

#1. Somatic stem cell

#2. Side population (SP) cells

#3. Candidates for myometrial stem cells
   a. Myometrial SP cells
   b. Myometrial Lin-/CD34+/CD49f cells

#4. Tumor/cancer stem cells

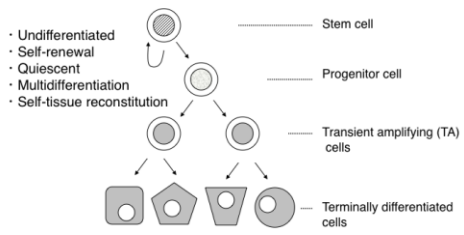
#5. Leiomyoma formation and myometrial stem cells

Somatic stem cells / adult stem cells / tissue-specific stem cells

- Undifferentiated cells, found throughout the body after embryonic development
- Self-renewal through indefinite and/or asymmetric cell division
- Generation of cells committed to differentiation

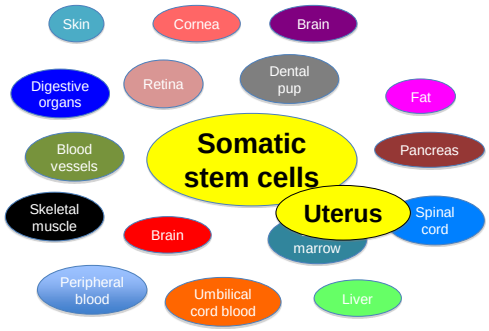
Replenishment of dying cells and regeneration of damaged tissues, thereby leading to growth and maintenance of the organs and tissues

Hierarchy of somatic stem cells



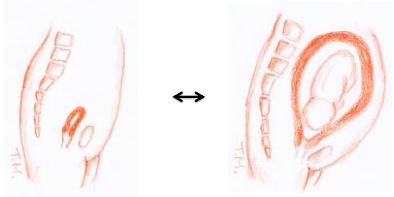
Maruyama T, Reprod Med Biol, 2010

Where are somatic stem cells found?



Gargett CE. Uterine stem cells: what is the evidence? Hum Reprod Update, 2007.

Multiple cycles of pregnancy-induced uterine enlargement and regression after parturition



	Non-pregnant		Pregnant at term
Weight:	≈ 70 g	↔	≈ 1,100 g
Cavity:	≈ 10 mL		≈ 5 – 20 L

Hypertrophy ≥ Stretching ≥ ECM production >> Hyperplasia

Cunningham F, et al. Williams Obstetrics 23rd edition, 2010.

Learning Objectives

#1. Somatic/tissue-specific stem cells

#2. Side population (SP) cells

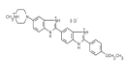
#3. Myometrial stem cells
   a. Myometrial SP cells
   b. Myometrial Lin-/CD34+/CD49f cells

#4. Tumor/cancer stem cells

#5. Leiomyoma formation and myometrial stem cells

Side Population (SP)

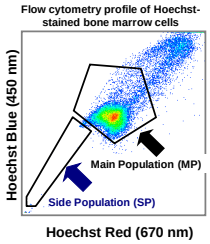
- **Hoechst 33342: DNA dye**



$C_{27}H_{37}Cl_3N_6O_4$ MW=616

- **Pumping out of DNA dye by ABCG2 transporter**

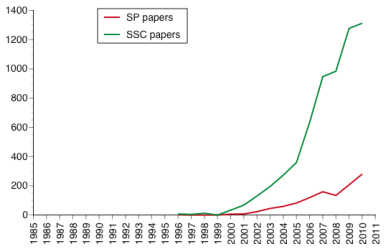
SP cells = understained cells



Hematopoietic stem cells are enriched in SP fraction

Goodell et al., J Exp Med, 1996

Increase of "SP" and "somatic stem cell (SSC)" papers during the past 15 years*



* A Medline-based literature search (1985–2010) was conducted using the keywords "somatic stem cells," "adult stem cells," "tissue-specific stem cells," and "side population."

Learning Objectives

#1. Somatic/tissue-specific stem cells

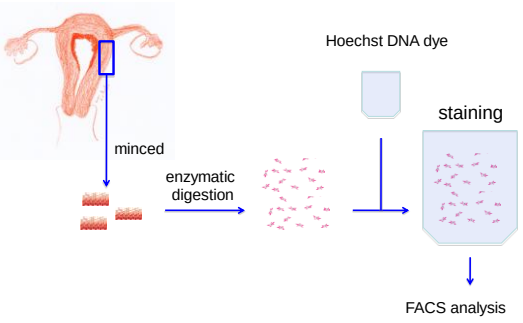
#2. Side population (SP) cells

#3. Myometrial stem cells
   a. Myometrial SP cells
   b. Myometrial Lin-/CD34+/CD49f cells

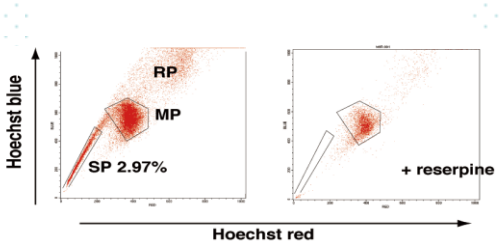
#4. Tumor/cancer stem cells

#5. Leiomyoma formation and myometrial stem cells

Procedures for isolation of myometrial SP cells

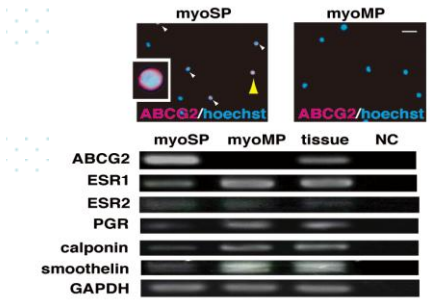


SP and MP cells in human myometrium (myoSP and myoMP)



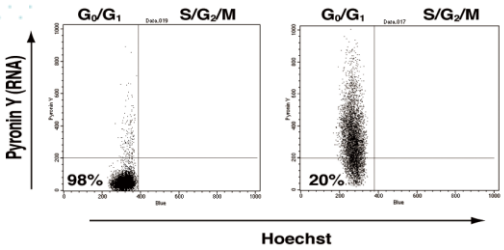
Ono M, et al. PNAS, 2007

Undifferentiated status of myoSP, not myoMP



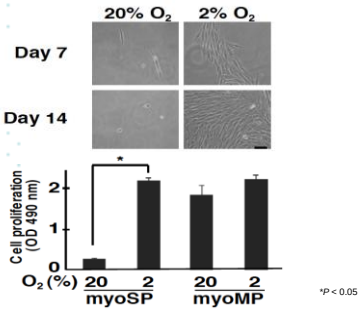
Ono M, et al. PNAS, 2007

Quiescence (G0 status) of myoSP, but not myoMP



Ono M, et al. PNAS, 2007

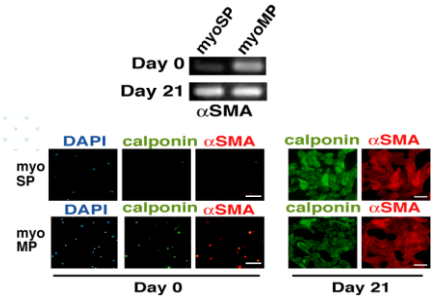
Preferential proliferation of myoSP under hypoxic condition



*P < 0.05

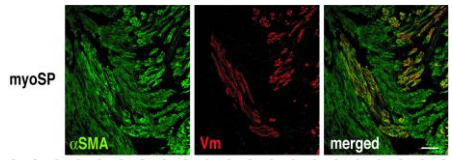
Ono M, et al. PNAS, 2007

Spontaneous myodifferentiation of myoSP



Ono M, et al. PNAS, 2007

Reconstitution of human smooth muscle tissues in NOG mouse uteri transplanted with myoSP, but not myoMP



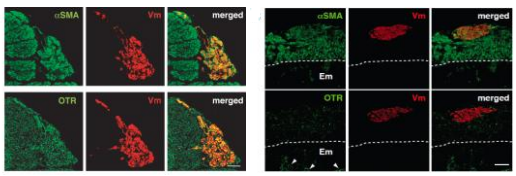
Bar = 50 μm

	Reconstitution rate*
myoSP	10/16
myoMP	0/16

* Rate of presence of tissues/cells doubly positive for αSMA and Vm

Ono M, et al. PNAS, 2007

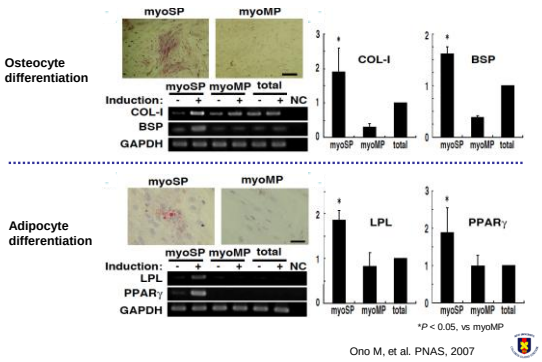
Pregnancy-specific induction of oxytocin receptor in human myometrial tissues reconstituted from myoSP



Pregnant 7.5 dpc **Non-pregnant**

Ono M, et al. PNAS, 2007

Multidifferentiation of myoSP, but not myoMP



Ono M, et al. PNAS, 2007

Myometrial SP cells possess ···

- undifferentiated phenotype
- quiescent cell cycle status
- self-renewal potential under hypoxic condition
- ability of self-tissue regeneration (self organization)
- potentials to give rise to pregnant myometrium
- multi-differentiation capabilities

Myometrial stem/progenitor cells are highly enriched in myoSP

Learning Objectives

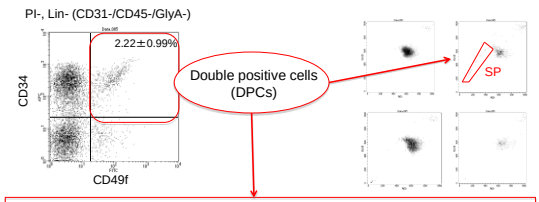
#1. Somatic/tissue-specific stem cells

#2. Side population (SP) cells

#3. Myometrial stem cells
   a. Myometrial SP cells
   b. Myometrial Lin-/CD34+/CD49f cells

#4. Tumor/cancer stem cells

#5. Leiomyoma formation and myometrial stem cells



DPCs exhibit the following properties:

#1. up-regulation of ABCG2

#2. preferential proliferation under hypoxic condition

#3. reconstitution of human myometrium in the mouse uterus

#4. preferential expansion in the mouse pregnant uterus

#5. multi-differentiation into adipocytes, chondrodytes, and osteocytes

Ono M and Maruyama T, unpublished

Learning Objectives

#1. Somatic/tissue-specific stem cells

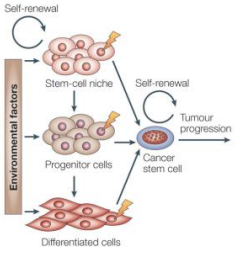
#2. Side population (SP) cells

#3. Myometrial stem cells
   a. Myometrial SP cells
   b. Myometrial Lin-/CD34+/CD49f cells

#4. Tumor/cancer stem cells

#5. Leiomyoma formation and myometrial stem cells

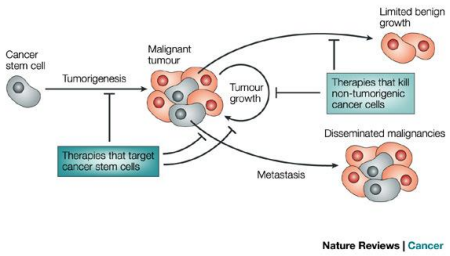
Tumor/cancer stem cells



Mutations in stem cells and/or progenitor cells might give rise to tumor/cancer stem cells

Bjerkvig R, et al., Nat Rev Cancer. 2005

Therapeutic implications of cancer stem cells



Pardal R, et al., Nat Rev Cancer. 2003

Learning Objectives

#1. Somatic/tissue-specific stem cells

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#3. Myometrial stem cells
   a. Myometrial SP cells
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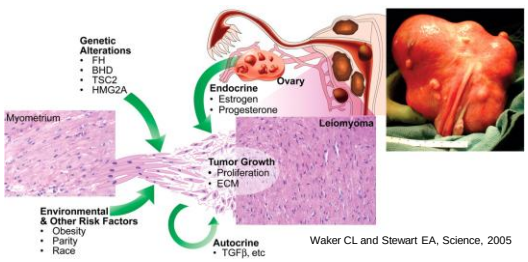
#5. Leiomyoma formation and myometrial stem cells

Myoma / leiomyoma/ fibroid

A benign, monoclonal tumor of the smooth muscle cells of the myometrium

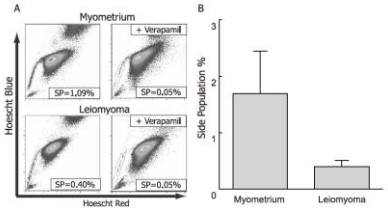


Waker CL and Stewart EA, Science, 2005

"Monoclonal" means that all of the muscle cells in a leiomyoma are descendents of one cell that has reproduced itself extensively.

Uterine Leiomyomas Exhibit Fewer Stem/Progenitor Cell Characteristics When Compared With Corresponding Normal Myometrium

Henry L. Chang, MD, Tharanga N. Senaratne, AB, LiHua Zhang, BS, Paul P. Szotek, MD, Ethan Stewart, BA, David Dombkowski, MS, Frederic Preffer, PhD, Patricia K. Donahoe, MD, and Jose Teixeira, PhD

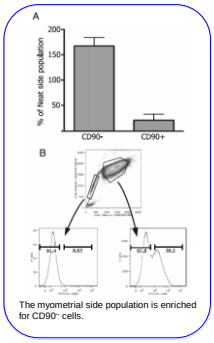


Reproductive Sciences Vol. 17 No. 2 February 2010 158-167

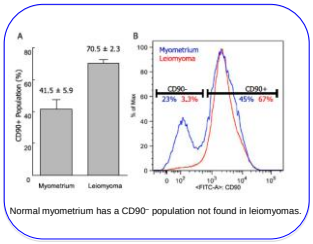
CD90/Thy-1: a GPI anchored cell surface protein/stem cell marker

CD90- myometrial cells ➔ lipofibroblasts

CD90+ myometrial cells ➔ myofibroblasts (Koumas, AJP, 2003)

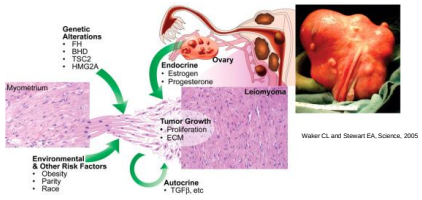


The myometrial side population is enriched for CD90⁻ cells.



Normal myometrium has a CD90⁻ population not found in leiomyomas.

Chang HL, et al. Reprod Sci, 2010

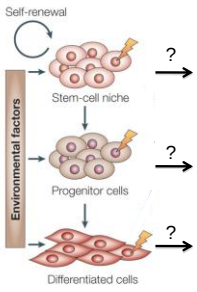


Walker CL and Stewart EA, Science, 2005

Myometrium	vs	Leiomyoma
myoSP:	>>	differentiated
CD90/Thy-1:	<	more myofibroblastic
	↓	
Myodifferentiation:	<<	more myodifferenieated

Chang HL, et al. Reprod Sci, 2010

Hypothetical pathogenesis of uterine tumors



Malignant Mixed Müllerian tumor (MMMT):
- *Epithelial components*
  squamous cell carcinoma, adenocarcinoma, adeno-squamous carcinoma etc.
- *Mesenchymal components*
  fibroblasts, smooth muscle, cartilage, striated muscle, bone etc.

Leiomyoma

or

Leiomyosarcoma, if is severe?

Bjerkvig R, et al., Nat Rev Cancer. 2005

References

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- Pardal R, et al., Applying the principles of stem-cell biology to cancer. Nat Rev Cancer. 2003.
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- Chang HL, et al. Uterine leiomyomas exhibit fewer stem/progenitor cell characteristics when compared with corresponding normal myometrium. Reprod Sci, 2010.

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  Hideyuki Okano, MD, PhD

- **Central Institute for Experimental Animals**

Human Trophoblast Progenitor Cells (hTPCs)

Susan Jane Fisher, Ph.D. and
Olga Krtolica Genbacev Ph.D.

Declaration of Conflicts of Interests

None to declare

Learning Objectives:

- To understand the cellular composition and anatomical relationships of the amnion, chorion, and placenta during early gestation.
- To understand the basic properties of human trophoblast progenitors.
- To understand the transcriptional profiles of human trophoblast progenitors in relationship to cytotrophoblasts that populate chorionic villi.

Olga Genbacev

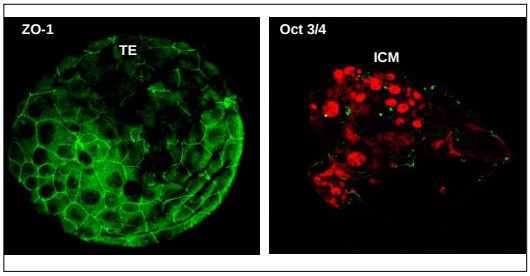
We tested the hypothesis that:

There are 2 sources of trophoblast progenitors:

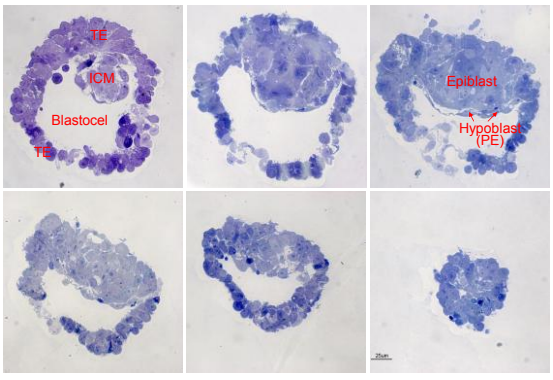
- Trophectoderm of the blastocyst
- Chorionic membranes

To locate these cells we used insights gained from our work with human embryos.

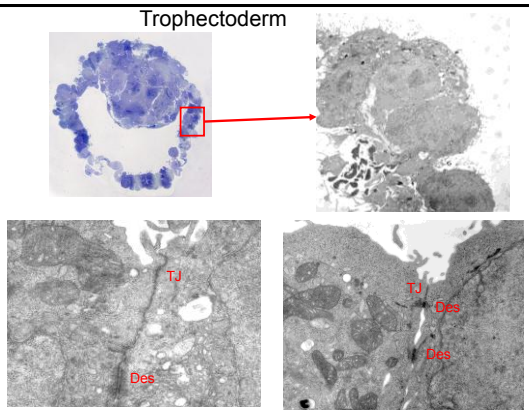
In blastocysts, TE is polarized, ICM is not.



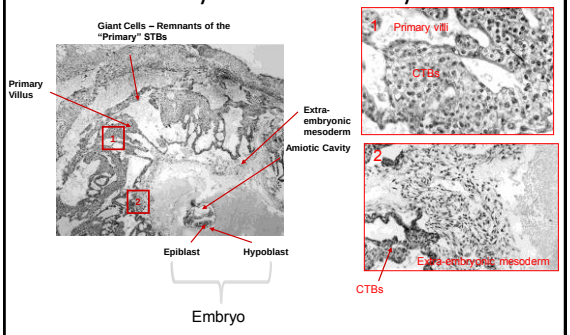
Semi-fine sections of a human blastocyst



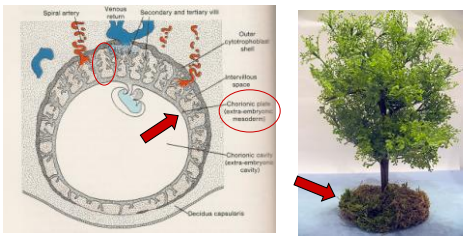
Trophectoderm

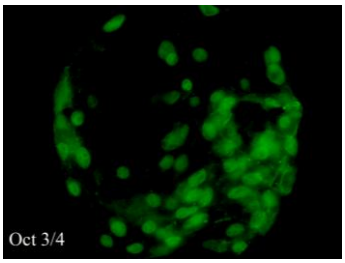


Formation of Placenta - Fully Implanted Day 10 Human Embryo



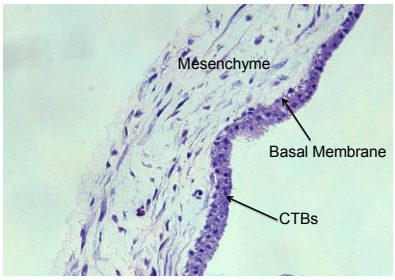
Formation of placenta from 2nd week to term



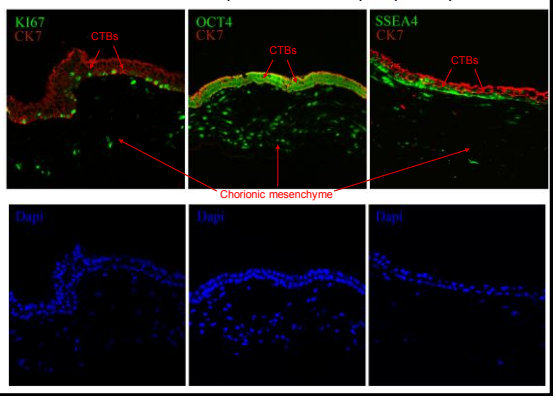


Human trophectoderm stains for Oct4..

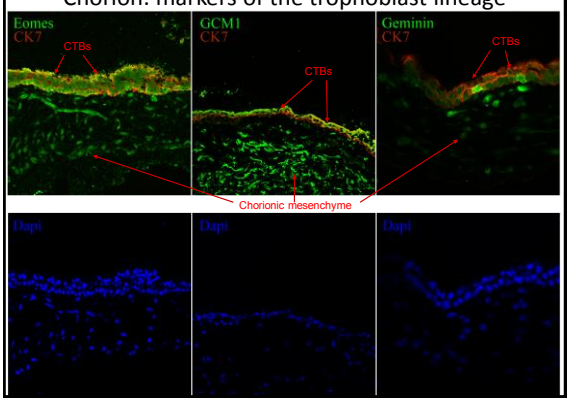
Histology of 7.5 wk human chorion



Chorion: markers of proliferation and pluripotency



Chorion: markers of the trophoblast lineage



How to isolate human trophoblast progenitors from the chorion?

"Combinatorial Signals of Activin/Nodal and Bone Morphogenic Protein Regulate the Early Lineage Segregation of Human Embryonic Stem Cells"

By

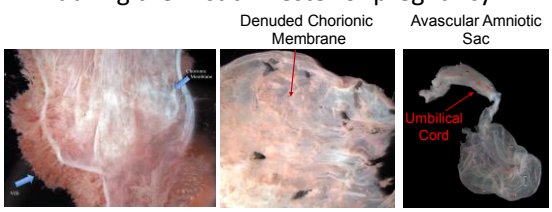
Wu *et al.*, 2008

Experimental Design

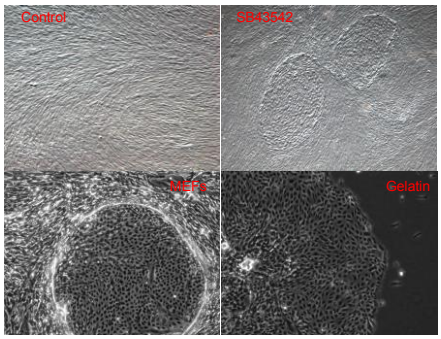
Rationale:

- As activin, inhibin and follistatin are produced by the placenta, we hypothesized that the activin/nodal pathway may control hTPC self-renewal. We used chorion-derived cells and treated them with SB431542 (activin/nodal inhibitor) or follistatin and FGF2.

The chorionic membrane is completely **separated** from the amniotic membrane during the first trimester of pregnancy.

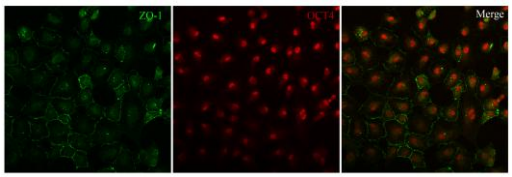


Initial derivation of hTPC colonies from cells of the chorionic membranes

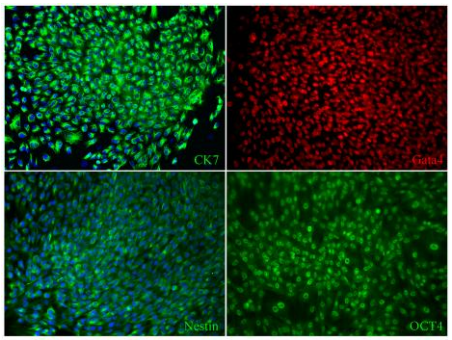


Characteristics of Undifferentiated hTPC Colonies

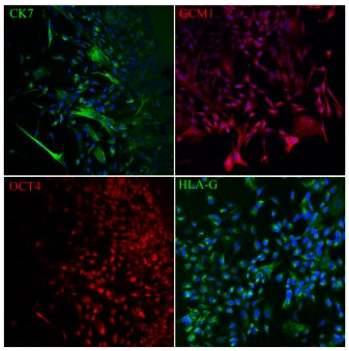
Cells in colonies are polarized and stain for OCT4.



Undifferentiated Colonies

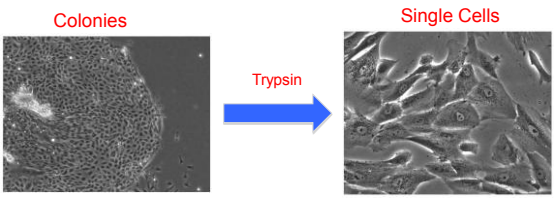


Colonies differentiate into cells that express trophoblast markers.

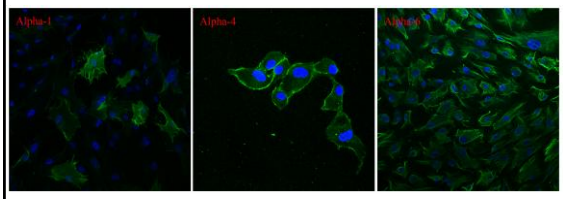


Differentiation Media- Keratinocyte Media + Pituitary Extract + 10% FBS

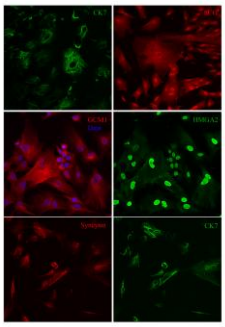
Trypsinization of colonies yields self-renewing hTPCs.



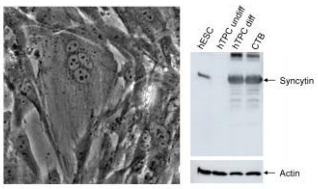
hTPC integrin expression patterns



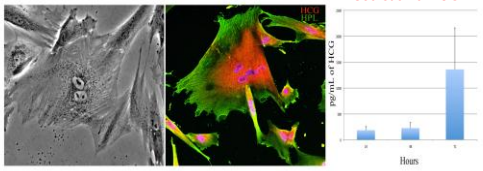
In differentiation medium, human trophoblast progenitor cells express trophoblast markers.



In differentiation medium, human trophoblast progenitor cells fuse and upregulate syncytin expression.

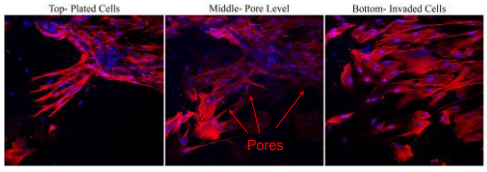


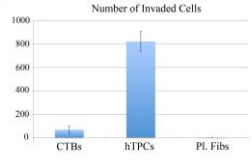
Differentiation of hTPCs into fused syncytiotrophoblasts

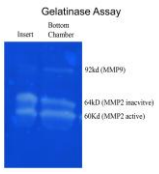


Secretion of hCG

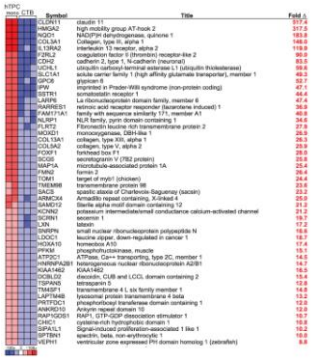
hTPCs differentiate into invasive cytotrophoblasts when they are cultured on Matrigel-coated filters.



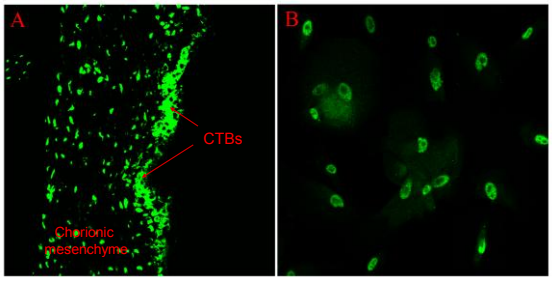




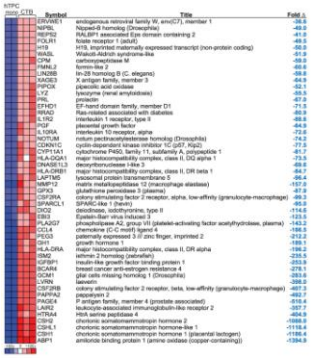
hTPC^{monolayer} vs. CTB^{2TM} - Up Regulated



Expression of HMGA2 in the chorion (A) and by hTPCs (B)



hTPC^{monolayer} vs. CTB^{2TM} - Down Regulated



Summary

- We derived hTPCs from the human chorion, in the presence of FGF-2, by inhibition of the activin/nodal, TGFβ pathway.
- We developed a feeder free/defined medium protocol for propagation of these cells.
- We tested their ability to differentiate into invasive CTBs and to fuse/produce hCG.
- We carried out a microarray analysis (hTPCs vs. freshly isolated CTBs).

Reference

- Wu, Z., Zhang, W., Chen, G., Cheng, L., Liao, J., Jia, N., Gao, Y., Dai, H., Yuan, J., Cheng, L., Xiao, L. Combinatorial Signals of Activin/Nodal and Bone Morphogenic Protein Regulate the Early Lineage Segregation of Human Embryonic Stem Cells (2008) J. Biol. Chem. 283, No. 36, pp. 24991–25002.

The ESHRE registry of hESC lines with monogenic defects

Karen Sermon, MD, PhD
Department of Embryology and
Genetics of the Vrije Universiteit Brussel
Vrije Universiteit Brussel

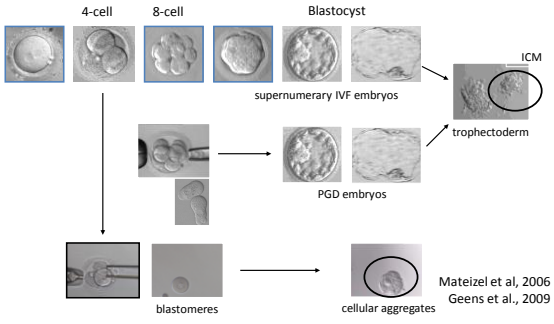
Conflict of interest

- I declare to have no conflict of interest

Learning objectives

- To learn how hESC with monogenic diseases are obtained
- To know where to find information on and how to obtain hESC lines with monogenic diseases (hESC-MD)
- To learn about the possible uses of hESC-MD for research and therapy development
- To learn about alternatives to hESC

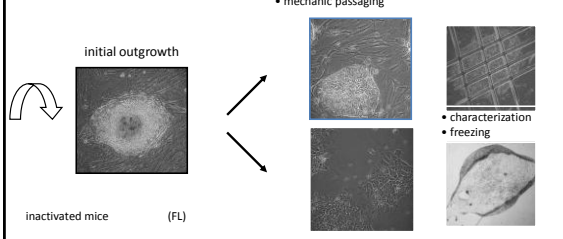
Isolation of hESC lines



Mateizel et al, 2006
Geens et al., 2009

Culture conditions for hESC

- mice/human FL
- hESC medium
- mechanic passaging



- characterization
- freezing

- hESC medium: KO-DMEM, KO-SR, NEAA, L-Glutamine, βME, bFGF
- feeder free
- hESC medium conditioned by FL
- enzymatic passaging

Mateizel et al, 2006

Where to find (information on) hESC-MD

- Scientific literature – see Sermon et al., HR, 2009
- Banks
  - UKSCB: http://www.ukstemcellbank.org.uk/
  - Umass Human Stem Cell Bank and Registry: http://www.umassmed.edu/MHSCB/index.aspx
  - Spanish National Stem Cell Bank – 3 nodes: http://www.isciii.es/htdocs/terapia/terapia_bancocel u lar. jsp
- Registries: give information only, give links to banks: http://www.hescreg.eu/

EU hESC line Registry (hESCReg)

- Primary objective: provide information about all hESC lines available in Europe
- Specific Support Action funded by VI FP European Commission (1.048.000 €, 2007 - 2010)
- Coordinated by:
  - Joeri Borstlap (BCRT - Technical Coord.)
  - Anna Veiga (CRMB - Scientific Coord.)

Borstlap et al., 2009

hESCreg

- hESC information is collected through national contacts
- The registry aims to provide
  - Contact and availability information about cell lines
  - Biological data: source, characterisation, genetic mutation,…
  - Data about providers, working groups and research projects

Borstlap et al., 2009

 Borstlap et al., 2009

Borstlap et al., 2009

hESCreg

- Collaboration with International Stem Cell Initiative (ISCI - http://www.stemcellforum.org/isci_project.cfm)
  - To characterise as many hESC lines as possible
  - To set a standard for characterisation
- Collaboration with ESHRE – SIG Reproductive Genetics – SIG Stem cells for hESC with monogenic diseases
  - To disseminate knowledge about these lines
  - To further research into monogenic diseases using hESC as a tool

Sermon et al., 2009

ESHRE registry for hESC-MD

- 01/12/2008: 527 hESC lines in hESCreg
  48 hESC-MD
- 56 lines in the paper:
  - 34 from literature
  - 21 from hESCreg only
  - 30 from internet web sites
  - Only 14 fully characterised

Sermon et al., 2009

Uses of hESC-MD

- Currently available models for monogenic disease study
  - Primary cell cultures from patients:
    - Not always available (neurons)
    - Limited life span unless transformed (cancerous)
    - Short developmental window
  - Transgenic mice
    - Often divergent fenotype
    - No rodent counterpart (eg Fragile X syndrome)
    - No fenotype due to different pathways (eg Lesh-Nyhan)

Ben-Yosef et al., 2008

Uses of hESC-MD

- Especially when no good model available
- Study of the abnormal fenotype in an autonomous cell system
- Control and manipulate cells in vitro
  - Differentiate large amounts of eg neurons
  - Study pathogenesis
- Study of early lethal fenotypes
- Study of cancer – eg hESC with cancer predisposition mutations
- Development of therapies

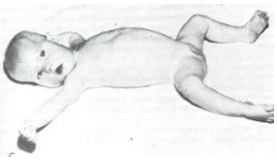
Ben-Yosef et al., 2008

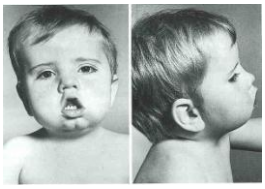
Example: myotonic dystrophy type 1

- Caused by a CTG expansion in the 3' end of the DMPK gene
  - Normal individuals: 5-37 repeats
  - Mildly affected individuals: > 50
  - Severely affected individuals: > 500
  - Congenital form: several thousands
- Clinical features
  - Muscle weakness, Myotonia
  - Sudden death through heart rhythm disturbances
  - Cataracts, male infertility
  - Congenital form: floppy infant, tented upper lip, breathing difficulties, early death
  - Anticipation: worsening of the symptoms over generation

Unpublished results

Clinical features of DM1





Example: myotonic dystrophy type 1

- Instability of the repeat in the germ line and in somatic tissues
- Instability in oocytes > instability in sperm
- Somatic instability causes degeneration
- In mouse models somatic expansions are dependent on DNA repair

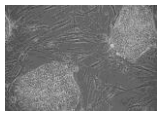
Unpublished results

Mismatch repair machinery (MMR)

- MSH2
  - Msh2 is involved in the expansion of the CAG/CTG
  - Forms heterodimers with Msh3 and Msh6
- MSH3
  - Msh3-/+ mice => decreased expansions of CTG
- PMS2
  - Involved in the process of excision and resynthesis after Msh2/Msh3 recognition
  - Forms an heterodimer with Mlh3

Unpublished results

Experimental design



hESC

Differentiation

- Collagenase
- 24 days diff. media
- Trypsinization
- P1

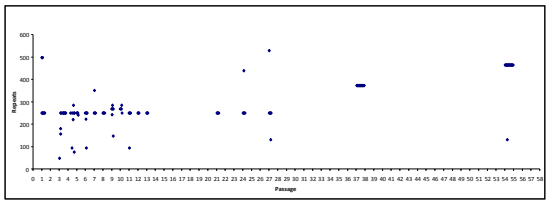
(Mateizel et al., 2007)

OPLs

hESC		OPLs	
RNA	DNA	RNA	DNA
Gene expression	Number of repeats	Gene expression	Number of repeats
RT-PCR	Small pool PCR (SP-PCR)	RT-PCR	Small pool PCR (SP-PCR)
	Southern Blot		Southern Blot

DM1 repeat is unstable in hESC

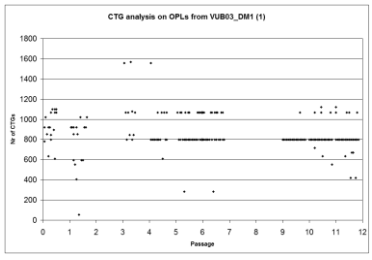
VUB19_DM1 repeat



Small pool PCR results from *DMPK1* CTG expansion in hESC

Unpublished results

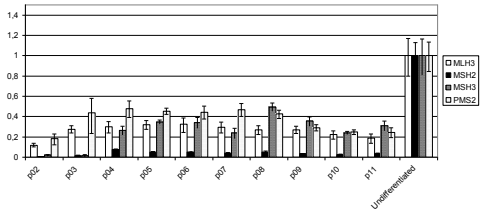
CTG repeat stabilizes upon differentiation



Small pool PCR results from *DMPK1* CTG expansion in OPLs

Unpublished results

MMR genes are down-regulated in OPLs



Relative quantification of the MMR genes expression by Real-Time PCR

Unpublished results

Example: myotonic dystrophy type 1

- Gene expression of MMR is down-regulated in OPLs
- The repeat is unstable in hESC and stabilizes in OPL
- The stabilization is simultaneous with down-regulation of MMR
- Undifferentiated cells ≈ the germ line: unstable CTG
- OPL differentiation is a model for the study of MMR and CTG instability

Unpublished results

hESC-MD in pharmacology

- Preclinical efficacy and toxicity testing:
  - In large animals
  - To validate mechanism of action and predict adverse effects
  - Animal models are only 50% efficient in prediction of liver, heart and during development
  - In primary cultures: disadvantages

Cezar, 2007

hESC-MD in pharmacology

- In vitro models for target validation
  - Example: genetically modified cells differentiated to striatal neurons as a model for Parkinson
  - Example: differentiation to insulin-producing cells model diabetes
- High throughput screening of small compounds,
- Signalomics to uncover new targets

Cezar, 2007

Example: differentiation into lung tissue and cystic fibrosis

- Generation of lung epithelial tissue
- As a first step towards an in vitro model for cystic fibrosis
- Differentiation driven by physical means: air-liquid interface (ALI)

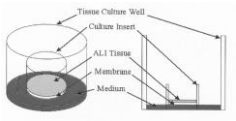
Van Haute et al., 2009

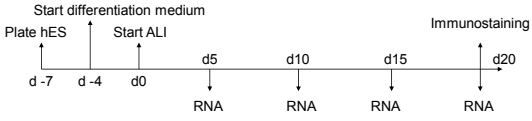
Methods: differentiation: Air – Liquid Interface (ALI)

⇒ hESC were plated on culture inserts

⇒ Differentiation medium for 4 days

⇒ ALI for 20 days



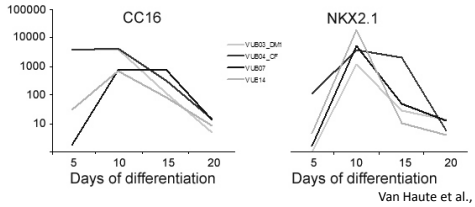


⇒ Samples were taken for RNA, Immunostaining and ELISA

Van Haute et al., 2009

Results

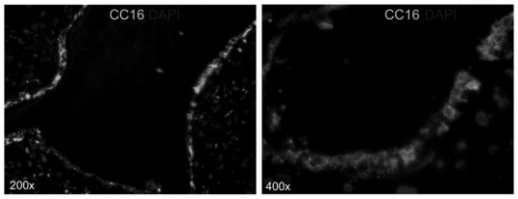
- Quantitative real-time RT-PCR:
  - CC16 and NKX2.1: peak around day 10



Van Haute et al., 2009

Results

- Immunohistochemistry: CC16

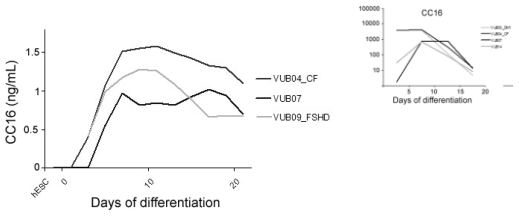


VUB03_DM1

Van Haute et al., 2009

Results

- ELISA (CC16 secretion)
  - Peak around day 8-10 → comparable with RT-PCR



Van Haute et al., 2009

Alternatives: induced pluripotent stem cells from patients

- Reprogramming of somatic cells from patients with monogenic disease through overexpression of stemness genes or alternative methods
- ALL diseases are accessible – not dependent on PGD
- Equivalence with hESC needs to be shown

Park et al., 2008

Why are so few hESC-MD used in genetic research?

- Immaturity of the model – efficient differentiation protocols are needed!
- Insufficient knowledge with geneticists – dissemination of information is necessary
  - Effort by ESHRE and hESCreg
- Genetic diseases = orphan diseases – no interest with large pharma companies

References

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- Van Haute et al., "Generation of lung epithelial-like tissue from human embryonic stem cells" 2009. Respir Res, 10, 105

Mark your calendar for the upcoming ESHRE campus workshops!

- **Basic Genetics for ART Practitioners**
  organised by the SIG Reproductive Genetics
  16 April 2010 - Porto, Portugal

- **Array technologies to apprehend developmental competence and endometrial receptivity: limits and possibilities**
  organised by the Task Force Basic Science in Reproduction
  22 April 2010 - Brussels, Belgium

- **The management of infertility – training workshop for junior doctors, paramedicals and embryologists**
  organised by the SIG Reproductive Endocrinology, SIG Embryology and the Paramedical Group
  26-27 May 2010 - Kiev, Ukraine

- **Preimplantation genetic diagnosis: a celebration of 20 years**
  organised by the SIG Reproductive Genetics
  1 July 2010 - Rome, Italy

- **EIM 10 years' celebration meeting**
  organised by the European IVF Monitoring Consortium
  11 September 2010 - Munich, Germany

- **The determinants of a successful pregnancy**
  organised by the SIGS Reproductive Surgery, Early Pregnancy and Reproductive Endocrinology
  24-25 September 2010 - Dubrovnik, Croatia

- **Basic training workshop for paramedics working in reproductive health**
  organised by the Paramedical Group
  6-8 October 2010 - Valencia, Spain

- **Forgotten knowledge about gamete physiology and its impact on embryo quality**
  organised by the SIG Embryology
  9-10 October 2010 - Lisbon, Portugal

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(see "Calendar")

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Keep an eye on our calendar section for more information on

Upcoming events

- **Female and male surgery in human reproductive medicine**
  8-9 October 2010 - Treviso, Italy
- **Promoting excellence in clinical research: from idea to publication**
  5-6 November 2010 - Thessaloniki, Greece
- **"Update on pluripotent stem cells (hESC and iPS)" and hands on course on "Derivation and culture of pluripotent stem cells"**
  8-12 November 2010 - Valencia, Spain
- **Women's health aspects of PCOS (excluding infertility)**
  18 November 2010 - Amsterdam, The Netherlands
- **Endoscopy in reproductive medicine**
  24-26 November 2010 - Leuven, Belgium
- **Fertility and Cancer**
  25-26 November 2010 - Bologna, Italy
- **The maternal-embryonic interface**
  2-3 December 2010 - Valencia, Spain
- **GnHR agonist for triggering of final oocyte maturation – time for a paradigm shift**
  3 December 2010 - Madrid, Spain
- **Raising competence in psychosocial care**
  3-4 December 2010 - Amsterdam, The Netherlands

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