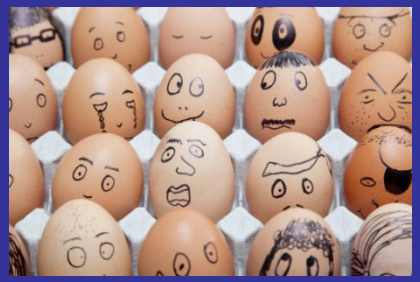
In Vitro Gametogenesis

Producing Eggs and Sperm from Buccal Smears



Eli Y. Adashi, MD, MS, MA (ad eundem)

Dr. Adashi has no conflicts of interest to disclose

Expected Learning Outcomes

1. To discuss In Vitro Gametogenesis
2. To explain the relevance to In Vitro Fertilization
3. To describe the limitations of the technology

COMMENTARY

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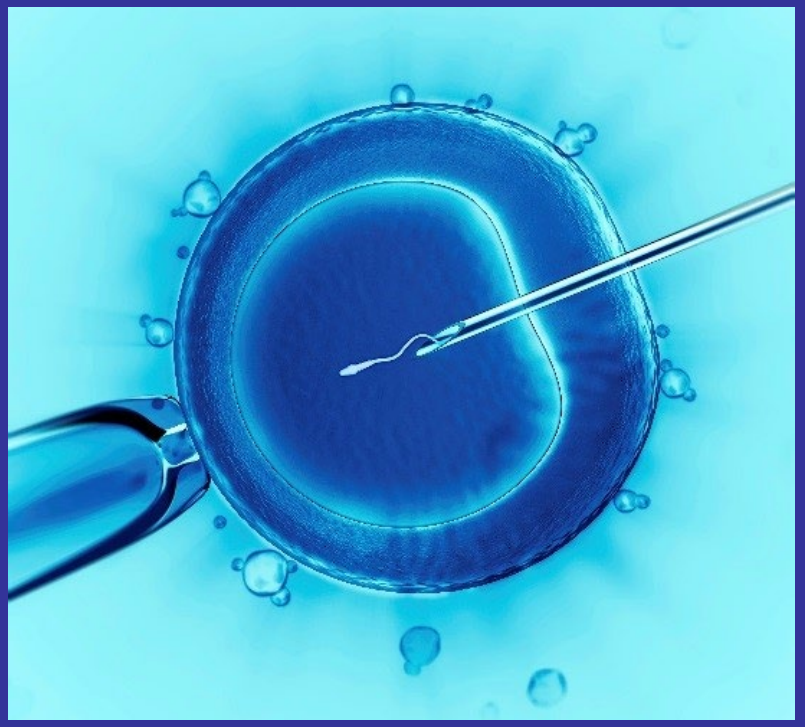
The Demise of In-Person Grand Rounds: The Triumph of Virtuality

In-person grand rounds have been nothing short of a fixture for decades in both academic and non-academic contexts.[1] As noted by Sir William Osler, in-person grand rounds constituted an element of the "natural method of teaching the subject of medicine."[1] A staple of the 20th and 21st centuries, in-person grand rounds served a multiplicity of purposes. At their core, in-person grand rounds subserved a leading educational mission widely viewed as indispensable to the maintenance of the currency of medical know-how. Apart and distinct from its core educational mission, in-person grand rounds played a key role in the maintenance of a sense of departmental identity and belonging. Moreover, in-person grand rounds afforded faculty, residents-in-training, and medical students an otherwise rare opportunity for in-person interaction with each other and with academic notables at the faculty ranks. The above notwithstanding, this time-honored grand rounds tradition came to an abrupt halt in early 2020 due to the rapid worldwide spread of coronavirus disease 2019 (COVID-19).[2] It was at that time that the in-person grand rounds format gave way to a virtual Zoom counterpart, a proprietary videotelephony software program available since 2012.[2] In this Commentary we compare and contrast the in-person and virtual options of grand rounds and call for the retention of the otherwise irreplaceable in-person experience.

Prior to the onset of the COVID-19 pandemic, the notion of virtual grand rounds was hardly entertained even though the enabling software was becoming increasingly available. Indeed, pre-COVID, in-person grand rounds were the dominant modus operandi of clinical departments and the parent hospitals thereof. Though highly variable in scope and character, in-person grand rounds were notable for assembling all of the relevant parties involved at the parent institution. The home institution, for its part, frequently saw to the provision of refreshment offerings and the prospect of social interpersonal interaction. Moreover, the podium-centered presentation afforded one with the opportunity to introduce and welcome a carefully selected local or guest speaker of some renown. Viewed in this light, in-person grand rounds constituted a recurring, time-honored event that, in the eyes of many, were tantamount to an iterative "academic celebration."

It was the COVID-19 pandemic that rendered the traditional in-person grand rounds all but a relic. Given that the outright cessation of grand rounds was not an option, a virtual solution had to be crafted. By then, a proprietary videotelephony software program—the product of Zoom Video Communications—made it possible for a substantial number of concurrent participants to partake in a virtual version of grand rounds. What was once an in-person functionality became a virtual one, during which participants were either home- or office-bound. The casualties associated with this inevitable transition were the all-important interpersonal in situ communications and the intangible atmospheric corollaries of an audience-filled auditorium or of a classroom.

Proponents of the virtual option extolled the element of convenience, that is, the feasibility of partaking in grand rounds while at home or in the office. Increased virtual attendance may thus be the rule. Note is also made of the reality that the virtual paradigm makes it possible to invite speakers from far-flung locales, including from abroad, thereby increasing the geographic diversity thereof.[3] Attendant budgetary savings related to travel- and room and board-associated expenses have not gone unnoticed either.[4] Although the aforementioned benefits cannot, indeed must not, be denied, the lost aura of a lecture hall replete with faculty, residents in training, and medical students cannot be denied or refurnished. In this light, the virtual option could be viewed as a temporary COVID-19 pandemic-related solution that could



Robert G. Edwards PhD **IVF---1978**

IVF

2010

In Vitro Gametogenesis

The Beginning

A Signaling Principle for the Specification of the Germ Cell Lineage in Mice

Yasuhide Ohinata,[1] Hiroshi Ohta,[2] Mayo Shigeta,[1] Kaori Yamanaka,[1] Teruhiko Wakayama,[2] and Mitinori Saitou[1]

Cell 137(3):571-584, 2009

"Germ cell fate in the epiblast is a direct consequence of Bmp4 signaling from the extraembryonic ectoderm…"

Mitinori Saitou, MD, PhD
Kyoto University

Katushiko Hayashi, PhD
Kyushu University

Experimental Paradigms

1. The Rodent

2. The Human

Pluripotent Stem Cells

“Embryonic”

Or

“Induced”

Pluripotent Stem Cell Lineages

- **Amacrine Neurons (Retina)**
- **Dopaminergic Neurons (Striatum)**
- **Motoneurons**

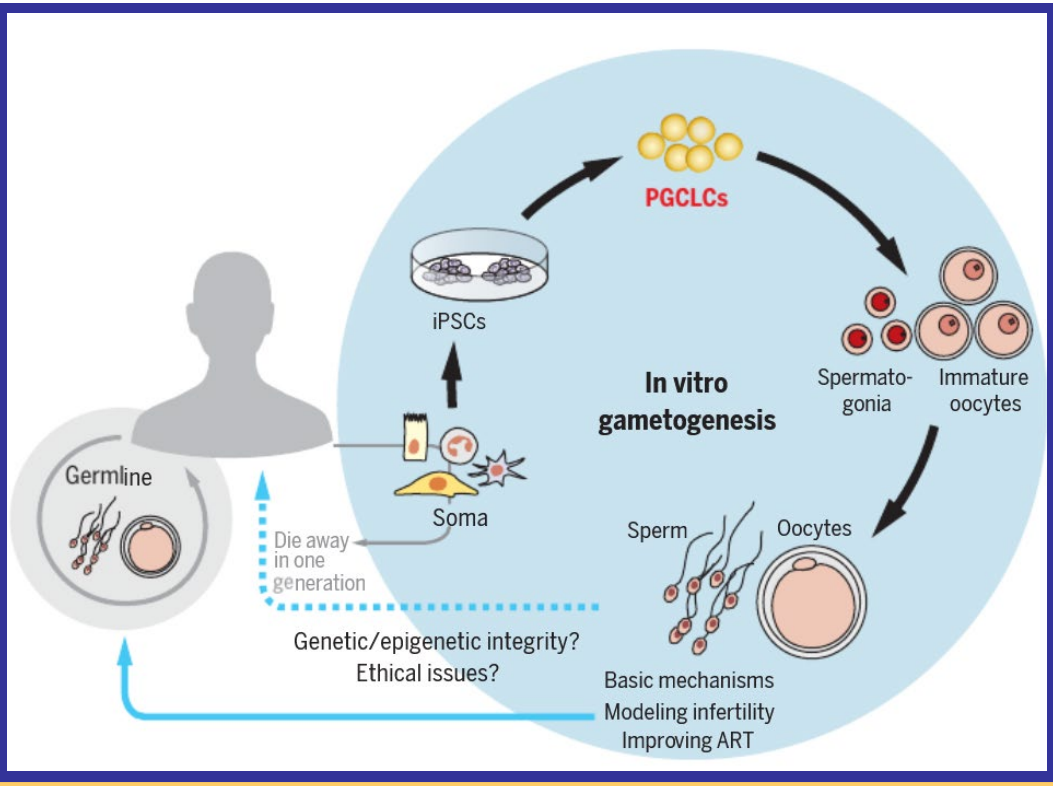
Stem Cell-Derived Gametes

REVIEW

GAMETOGENESIS

Mammalian in vitro gametogenesis

Mitinori Saitou[1,2,3]* and Katsuhiko Hayashi[4,5]*



Science 374:1-9, 2021

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Oocytes from Stem Cells

Mary Herbert, Ph.D., and Azim Surani, Ph.D.

N Engl J Med 386(2):188-190, 2022

NATIONAL ACADEMY of MEDICINE

Convened A Workshop In 2023

Consensus Committee Report May Follow

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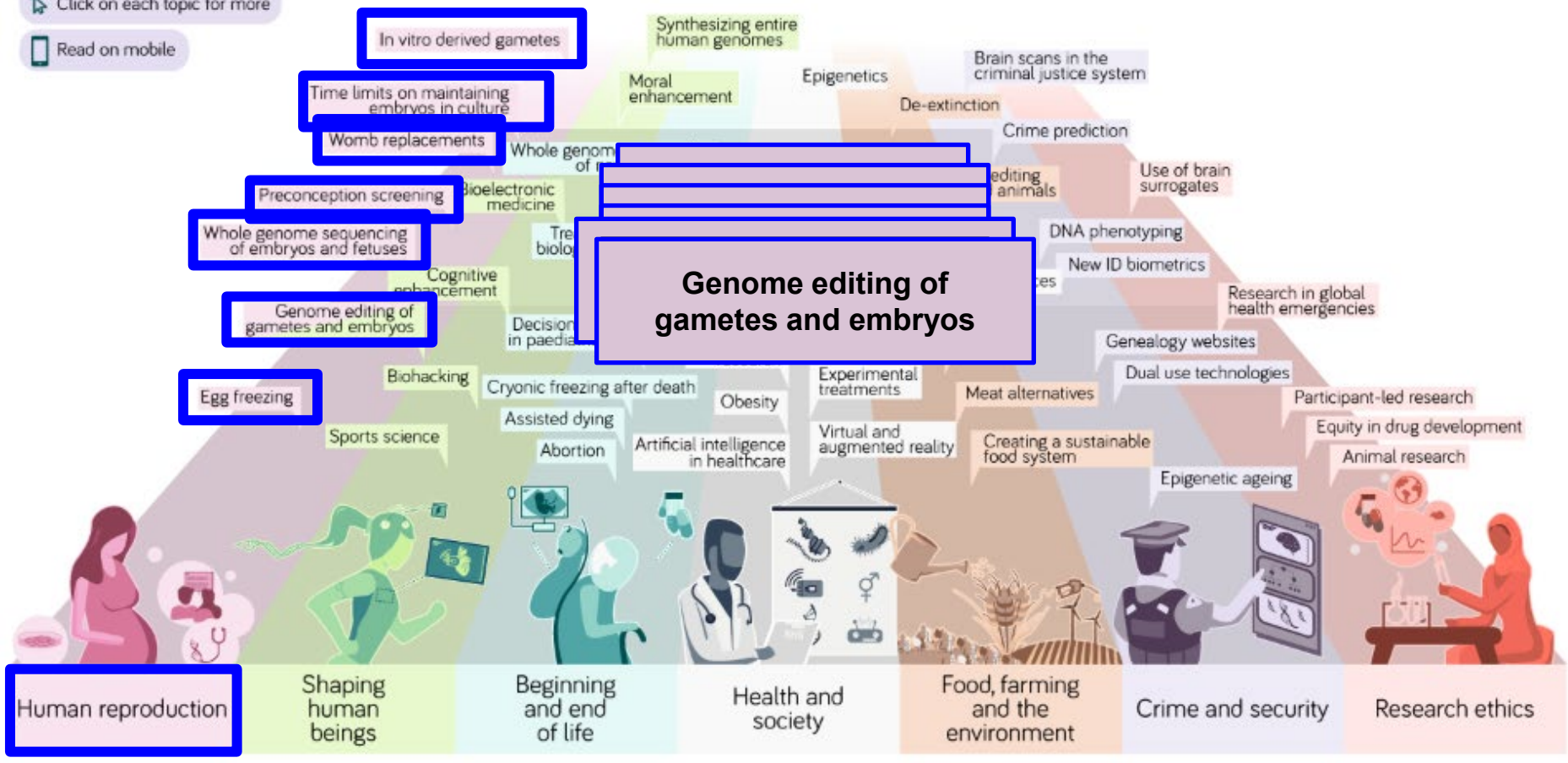
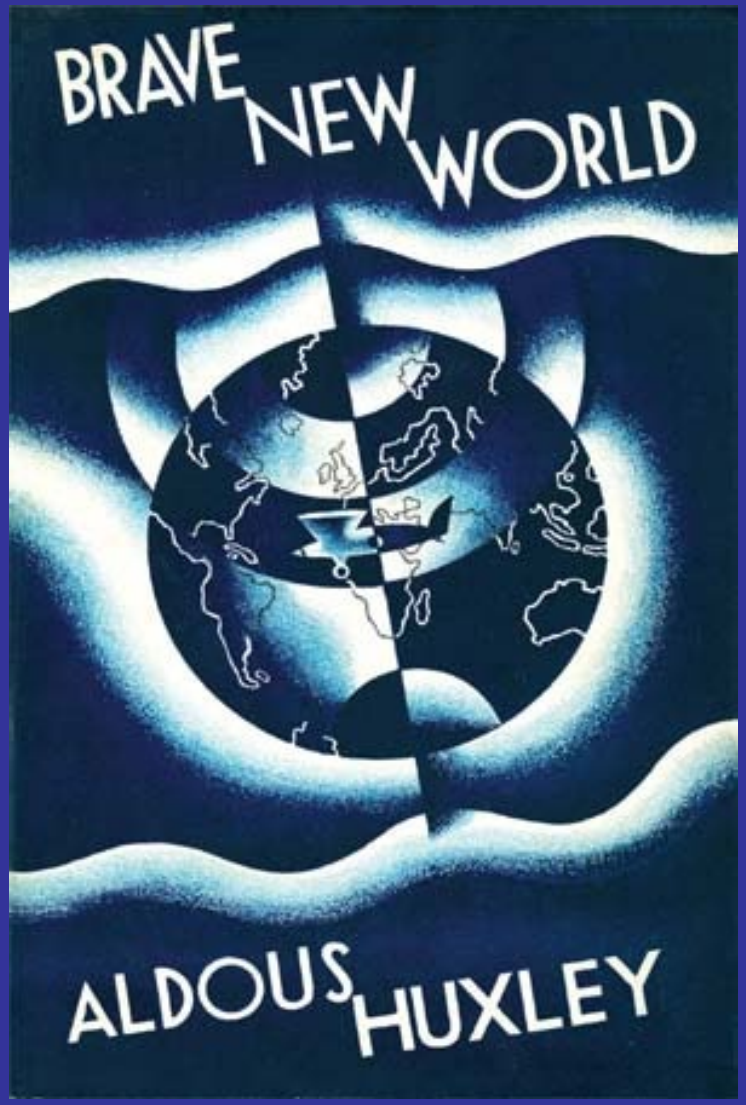


In-Vitro Gametogenesis

- **Disruptive technology**
- **Achievable in the human?**
- **Likely Timeline?**

Aldous L. Huxley

1894-1963



1932

Predictions

- Eggs via Ex-Vivo Ovarian Cultures
- Embryos via In Vitro Fertilization
- Newborns via Ex-Vivo Ectogenesis

Chapter One
(Brave New World)

"One egg, one embryo, one adult. Normality…Making ninety-six human beings grow where only one grew before. Progress."

Director of Hatcheries & Conditioning

In-Vitro Gametogenesis

Early Obstacles

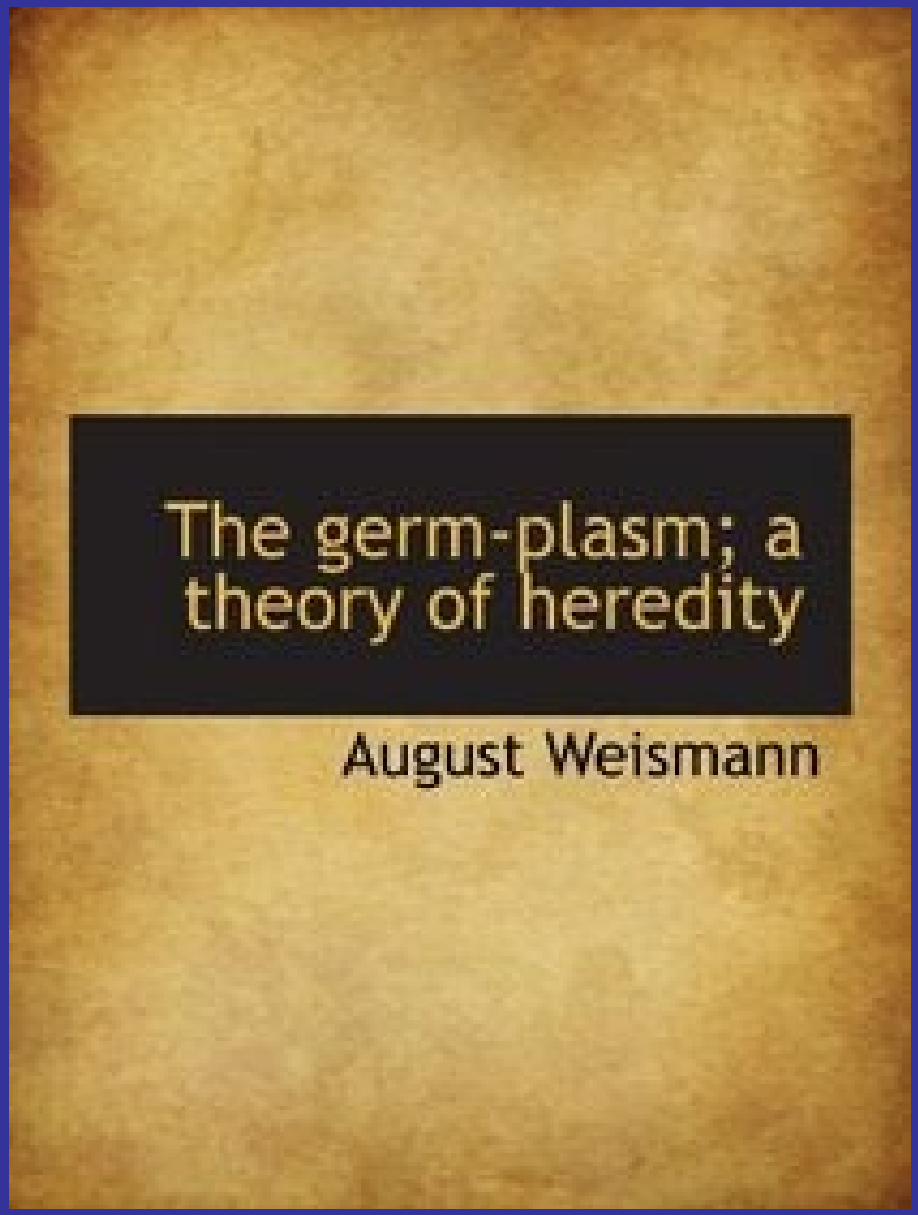
Soma-Germline Barrier Hypothesis

August F.L. Weismann, MD

1834-1914

Professor of Zoology

University of Freiburg



1893

The Soma-Germline Barrier Hypothesis

Germline

Gives rise to the Soma

Is immortal

Soma

Never gives rise to the Germline

Is mortal

Pluripotent

Stem Cell Formation

Technologis

Somatic Cell Nuclear Transfer (SCNT)

SCNT

(Somatic Cell Nuclear Transfer)

1958

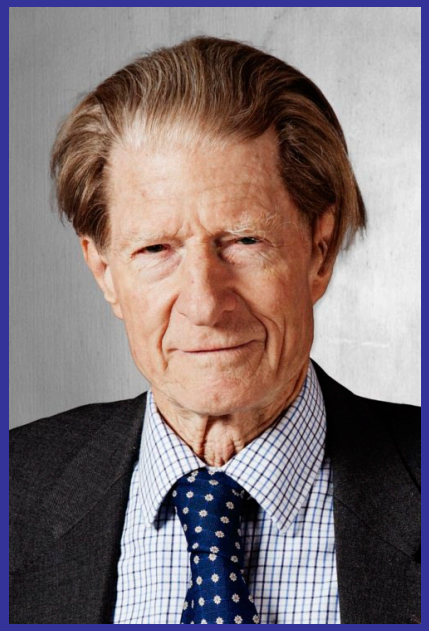
Somatic Karyoplast

+

Oocytic Cytoplast

"Zygote"→Blastocyst→Inner Cell Mass

ESCs



John B. Gurdon, PhD

Sexually Mature Individuals of *Xenopus laevis* from the Transplantation of Single Somatic Nuclei

Gurdon JB, Elsdale TR, Fischberg M

Nature 182(4627):64-5, 1958

Induction 2006

Somatic Cell

+

[Select Transcription Factors]

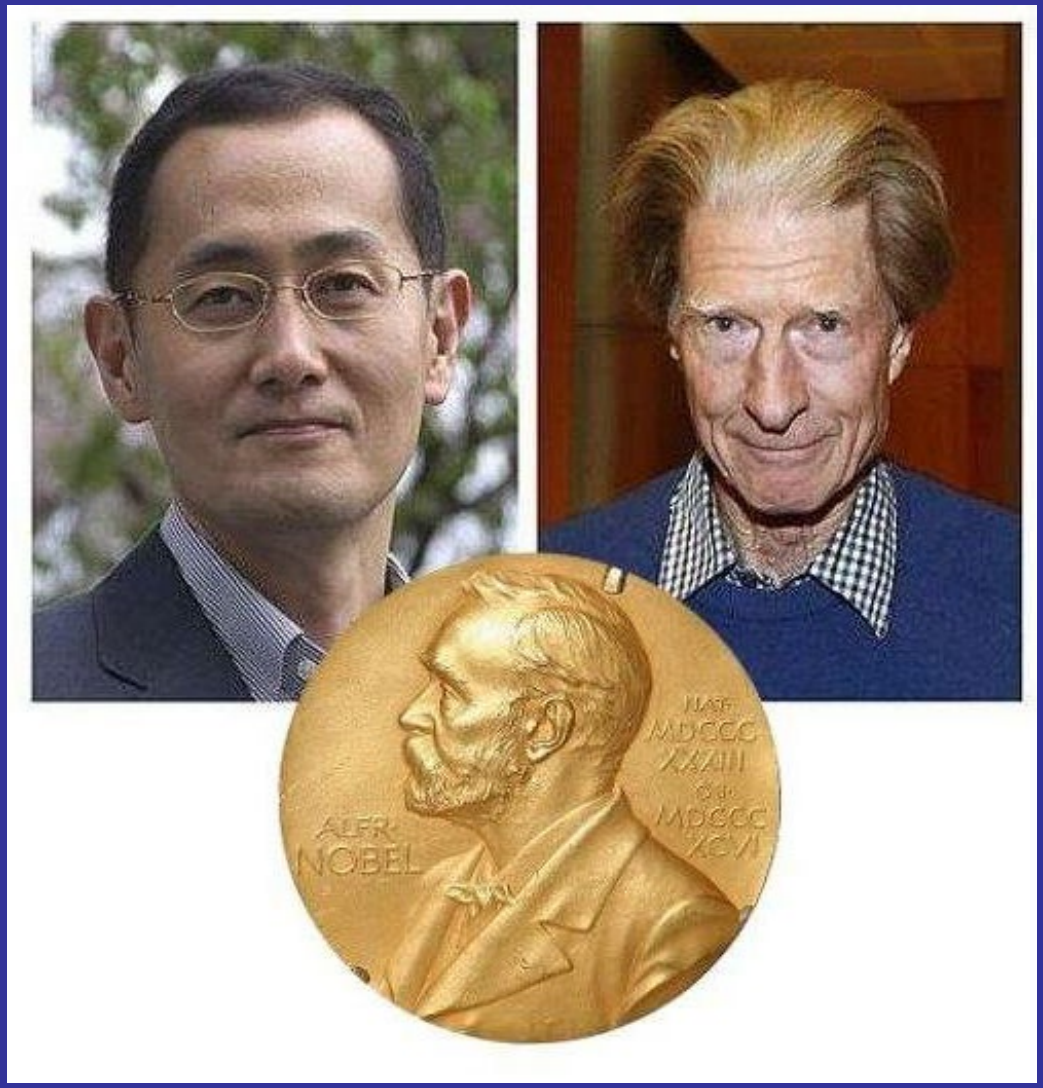
iPSCs

Shinya Yamanaka, MD, PhD

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Takahashi K, Yamanaka S
Cell 126(4):663-76, 2006

The 2012 Nobel Prize in Physiology & Medicine



Shinya Yamanaka, MD | **John B. Gurdon, PhD**

In Vitro Gametogenesis

Somatic Cell

e/iPSC

Gamete

Prerequisites?

Germ Cell Specification Pathway

1. Cues
2. Sequence
3. Timing
4. Niche

The Beginning?

RESEARCH ARTICLES

Derivation of Oocytes from Mouse Embryonic Stem Cells

Karin Hübner,[1] Guy Fuhrmann,[3] Lane K. Christenson,[4] James Kehler,[1] Rolland Reinbold,[1] Rabindranath De La Fuente,[2] Jennifer Wood,[4] Jerome F. Strauss III,[4] Michele Boiani,[1] Hans R. Schöler[1]*

Continuation of mammalian species requires the formation and development of the sexually dimorphic germ cells. Cultured embryonic stem cells are generally considered pluripotent rather than totipotent because of the failure to detect germline cells under differentiating conditions. Here we show that mouse embryonic stem cells in culture can develop into oogonia that enter meiosis, recruit adjacent cells to form follicle-like structures, and later develop into blastocysts. Oogenesis in culture should contribute to various areas, including nuclear transfer and manipulation of the germ line, and advance studies on fertility treatment and germ and somatic cell interaction and differentiation.

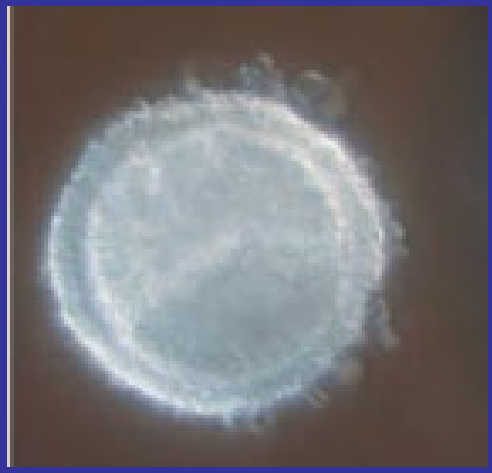
onic stem (ES) cells to generate all lineages of the embryo in vivo has been widely reported in the literature, in striking contrast to the lack of data describing the derivation of germ cells from ES cells in vitro. We attributed the inability to demonstrate the derivation of germ cells from ES cells in culture to the lack of a suitable reporter system for the noninvasive visualization of germ cell formation.

Induction of germ cells in culture. Elucidation of the various known regulatory elements within the germline-specific gene

[1]Germline Development Group, [2]Female Germ Cell Biology Group, Center for Animal Transgenesis and Germ Cell Research, School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, 382 West Street Road, Kennett Square, PA 19348, USA. [3]Centre de Neurochimie, Laboratoire de Neurobiologie du Développement et de la Régénération, FRE 2373 CNRS, 5 Rue Blaise Pascal, 67084 Strasbourg

Science 300:1251-6, 2003

Hans R. Schöler, PhD
Max Planck Institute
for
Molecular Biomedicine



Embryonic stem cells can form germ cells *in vitro*

Yayoi Toyooka, Naoki Tsunekawa, Ryuko Akasu, an Toshiaki Noce

Mitsubishi Kagaku Institute of Life Sciences, 11 Minamiooya Machida-shi, Tokyo 194-8511, Japan

Edited by David L. Garbers, University of Texas Southwestern Medical Center, Dallas, TX, and approved July 17, 2003 (received for review May 14, 2003)

Knock-in embryonic stem (ES) cells, in which *GFP* or *lacZ* was expressed from the endogenous mouse vasa homolog (*Mvh*), which is specifically expressed in differentiating germ cells, were used to visualize germ cell production during *in vitro* differentiation. The appearance of MVH-positive germ cells depended on embryoid body formation and was greatly enhanced by the inductive effects of bone morphogenic protein 4-producing cells. The ES-derived MVH-positive cells could participate in spermatogenesis when transplanted into reconstituted testicular tubules, demon-

differentiating germ cells from the late migration stage to the postmeiotic stage (9–11). Loss of *Mvh* function causes a deficiency in the proliferation and differentiation of male germ cells (12). We have therefore used a knock-in at the *Mvh* locus to detect the emergence of PGCs from ES cells *in vitro*. The results demonstrate the generation of PGCs from ES cells in culture and show that these ES-derived germ cells have the capacity to form sperm.

PNAS 100:11457-62, 2003

Derivation of embryonic germ cells and male gametes from embryonic stem cells

Niels Geijsen[1,2], Melissa Horoschak[1,3], Kitai Kim[1,3], Joost Gribnau[1], Kevin Eggan[4], George Q. Daley[1,3]

[1]*Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA*
[2]*Center for Regenerative Medicine and Technology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA*
[3]*Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, and Division of Pediatric Hematology/Oncology, The Children's Hospital and Dana Farber Cancer Institute, Boston, Massachusetts 02115, USA*
[4]*Department of Molecular and Cellular Biology, Harvard University, 7 Divinity Avenue, Cambridge, Massachusetts 02138, USA*

Nature 427:148-54, 2004

Spontaneous differentiation of germ cells from human embryonic stem cells *in vitro*

Amander T. Clark[1,2,3], Megan S. Bodnar[1,3], Mark Fox[1,2,3], Ryan T. Rodriquez[1,3], Michael J. Abeyta[1,2,3], Meri T. Firpo[1,3] an Renee A. Reijo Pera[1],[3,*]

[1]Center for Reproductive Sciences, Department of Obstetrics, Gynecology and Reproductive Sciences, [2]Departments of Physiology and Urology, and Programs in Human Genetics, Cancer Genetics and [3]Development and Stem Cell Biology, University of California at San Francisco, San Francisco, CA 94143-0556, USA

Received December 3, 2003; Revised January 16, 2004; Accepted February 2, 2004

Little is known of molecular requirements for specification of human germ cells. However, it is likely that they are specified through the action of sequentially expressed genes just as in model organisms. We sought to determine whether human embryonic stem (ES) cell lines, like those of mice, might be capable of forming

Hum Mol Genet 13:727-39, 2004

Germ Cell Specification Pathway: Rodent

articles

A molecular programme for the specification of germ cell fate in mice

Mitinori Saitou, Sheila C. Barton & M. Azim Surani

Wellcome Trust/Cancer Research UK Institute of Cancer and Developmental Biology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QR, UK

Nature 418:293-300, 2002

Blimp1 is a critical determinant of the germ cell lineage in mice

Yasuhide Ohinata[1]*, Bernhard Payer[2]*, Dónal O'Carroll[3]*, Katia Ancelin[2], Yukiko Ono[1], Mitsue Sano[1], Sheila C. Barton[2], Tetyana Obukhanych[4], Michel Nussenzweig[4], Alexander Tarakhovsky[3], Mitinori Saitou[1,5,6] & M. Azim Surani[2]

Nature 436:207-13, 2005

Germ Cells

REVIEW

Germ Cell Specification in Mice

Katsuhiko Hayashi, Susana M. Chuva de Sousa Lopes, M. Azim Surani*

Science 316:394-6, 2007

A Signaling Principle for the Specification of the Germ Cell Lineage in Mice

Yasuhide Ohinata,[1] Hiroshi Ohta,[2] Mayo Shigeta,[1] Kaori Yamanaka,[1] Teruhiko Wakayama,[2] and Mitinori Saitou[1,3,4,*]

Cell 137:571-84, 2009

Germ Cell Specification Pathway: Human

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

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

Nature 462:222-5, 2009

SOX17 Is a Critical Specifier of Human Primordial Germ Cell Fate

Naoko Irie,[1,2,3,5] Leehee Weinberger,[4,5] Walfred W.C. Tang,[1,2,3,5] Toshihiro Kobayashi,[1,2,3] Sergey Viukov,[4] Yair S. Manor,[4] Sabine Dietmann,[3] Jacob H. Hanna,[4,6,*] and M. Azim Surani[1,2,3,6,*]

Cell 160:1-16, 2015

Human primordial germ cell commitment *in vitro* associates with a unique PRDM14 expression profile

Fumihiro Sugawa[1,†], Marcos J Araúzo-Bravo[1,2,3,†], Juyong Yoon[1,†], Kee-Pyo Kim[1], Shinya Aramaki[1], Guangming Wu[1], Martin Stehling[1], Olympia E Psathaki[1], Karin Hübner[1] & Hans R Schöler[1,4,*]

EMBO J 34:1009-24, 2015

Robust In Vitro Induction of Human Germ Cell Fate from Pluripotent Stem Cells

Kotaro Sasaki,[1,2,12] Shihori Yokobayashi,[1,2,3,12] Tomonori Nakamura,[1,2] Ikuhiro Okamoto,[1,2] Yukihiro Yabuta,[1,2] Kazuki Kurimoto,[1,2] Hiroshi Ohta,[1,2] Yoshinobu Moritoki,[1,2,4] Chizuru Iwatani,[5] Hideaki Tsuchiya,[5] Shinichiro Nakamura,[5] Kiyotoshi Sekiguchi,[6] Tetsushi Sakuma,[7] Takashi Yamamoto,[7] Takahide Mori,[8] Knut Woltjen,[3,9] Masato Nakagawa,[3] Takuya Yamamoto,[3,10,11] Kazutoshi Takahashi,[3] Shinya Yamanaka,[3] and Mitinori Saitou[1,2,3,10,*]

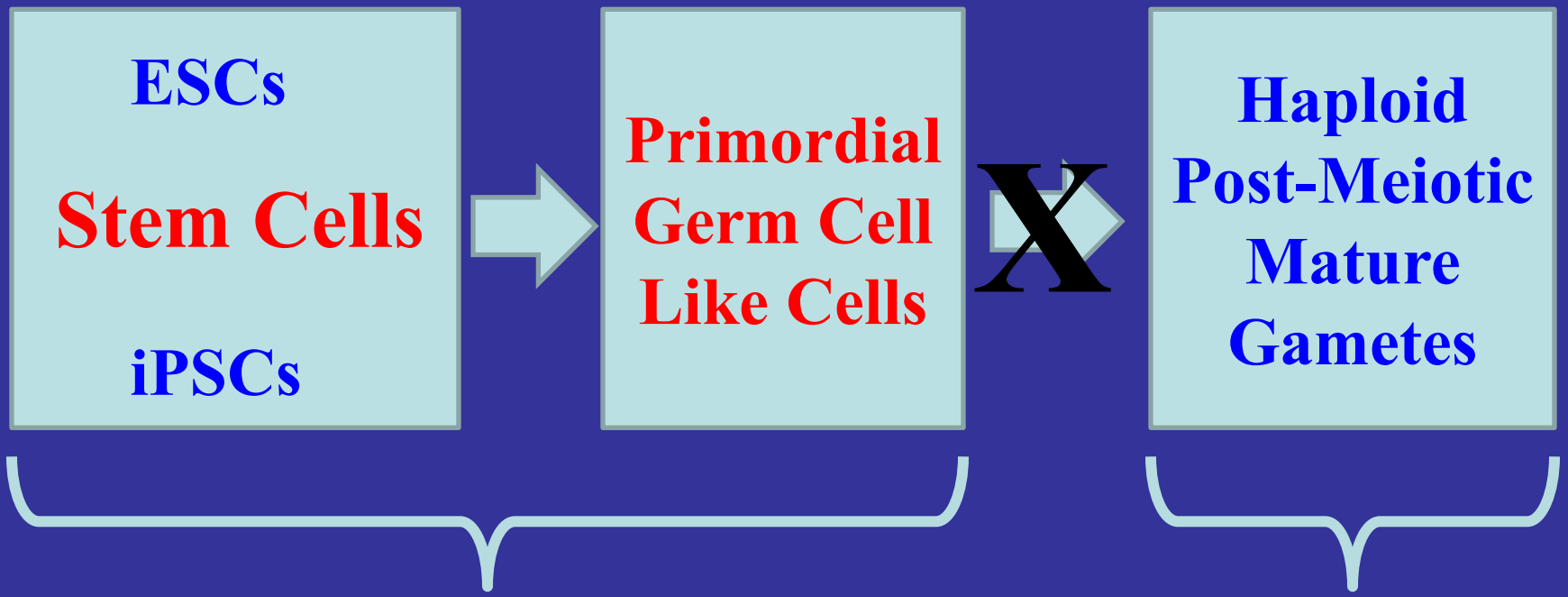
Cell Stem Cell 17:178-94, 2015

State of the Art in 2015

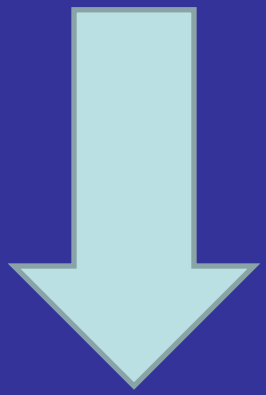
1. Germ cell specification pathway is not conserved between the rodent and the human.

2. The genetic networks involved are quite distinct as well

State of the Art in 2015



The “Final” Frontier



In Vitro Gametogenesis

“All The Way”

LETTER

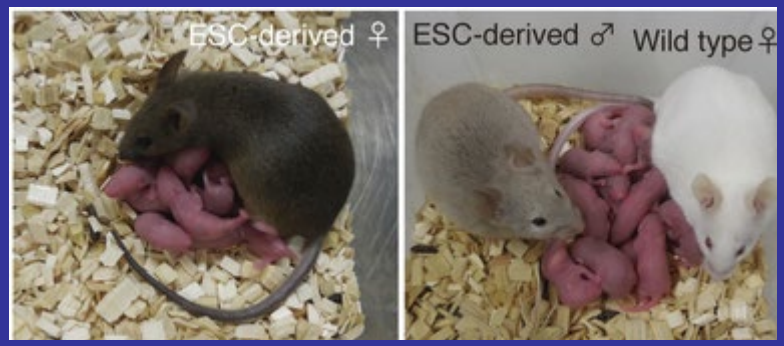
doi:10.103

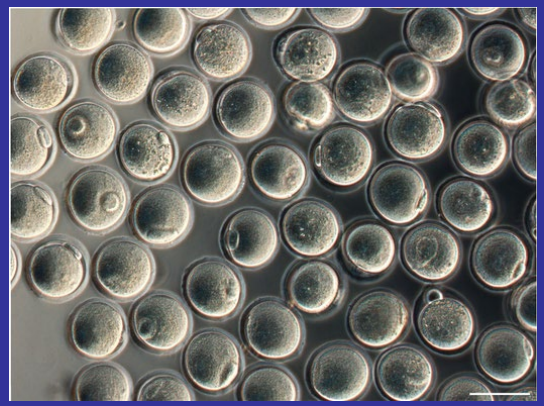
Reconstitution *in vitro* of the entire cycle of the mouse female germ line

Orie Hikabe[1]*, Nobuhiko Hamazaki[1], Go Nagamatsu[1], Yayoi Obata[2], Yuji Hirao[3], Norio Hamada[1,4], So Shimamoto[1], Takuya Imamura[1], Kinichi Nakashima[1], Mitinori Saitou[5,6,7,8] & Katsuhiko Hayashi[1,9]*

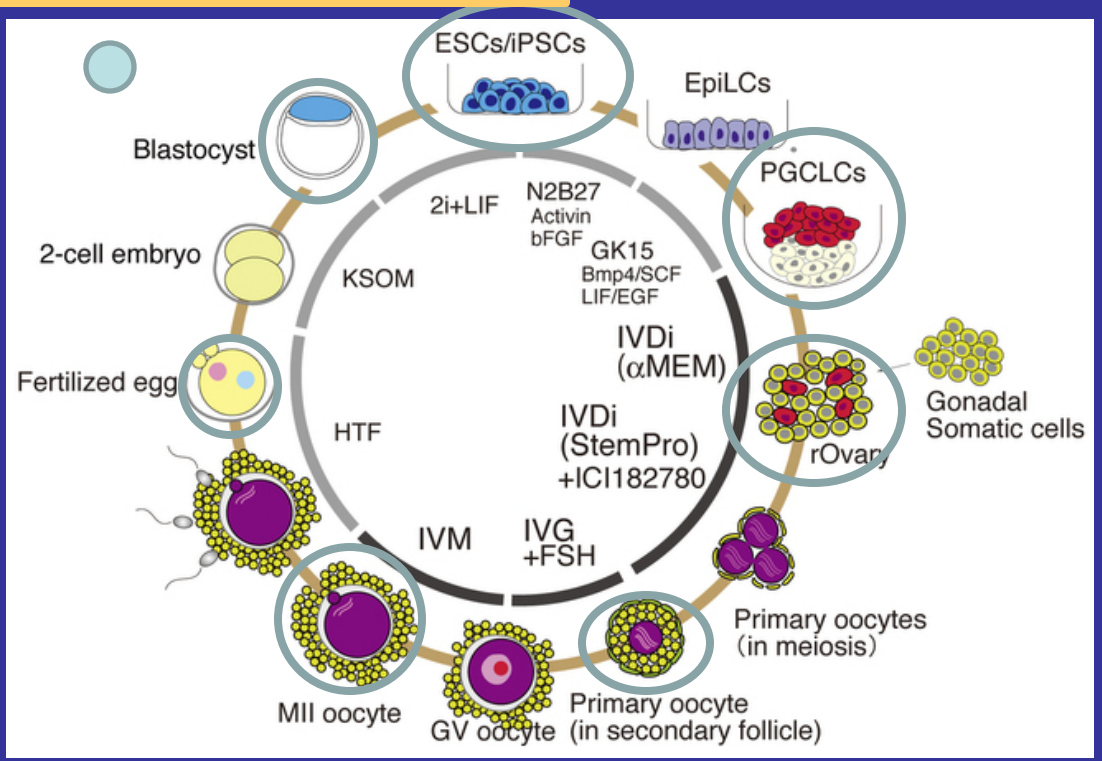
Katushiko Hayashi, PhD
Kyushu University

Nature 539(7628):299-303, 2016





MII Oocytes



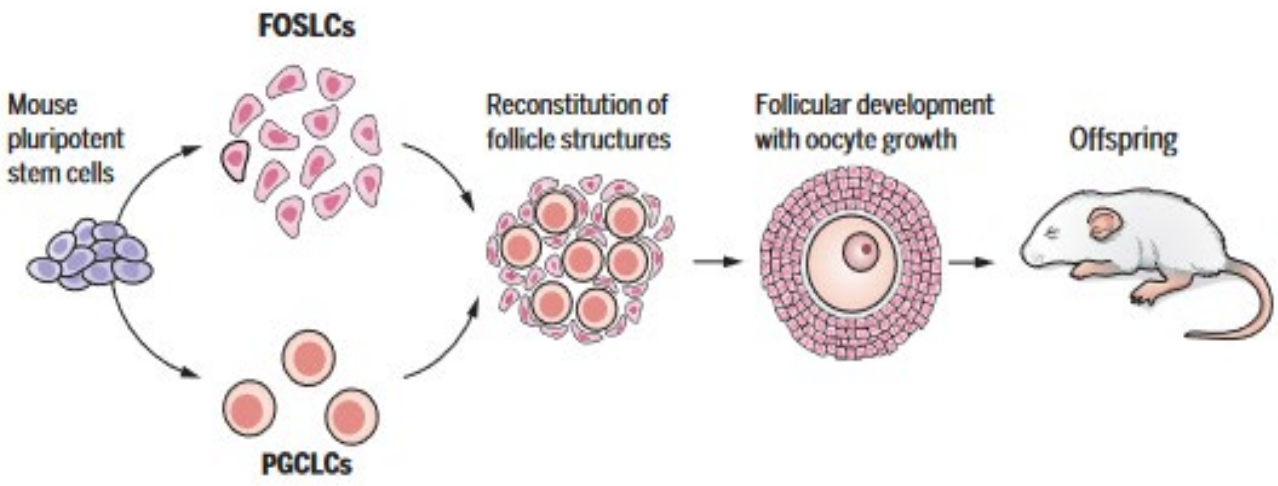
RESEARCH ARTICLE SUMMARY

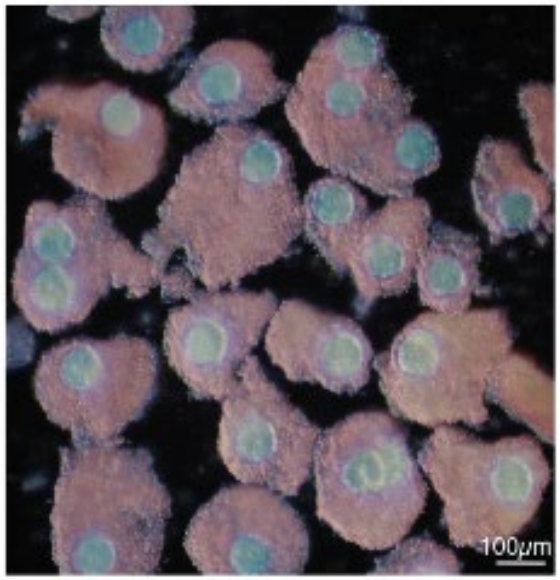
DEVELOPMENTAL BIOLOGY

Generation of ovarian follicles from mouse pluripotent stem cells

Takashi Yoshino, Takahiro Suzuki, Go Nagamatsu, Haruka Yabukami, Mika Ikegaya, Mami Kishima, Haruka Kita, Takuya Imamura, Kinichi Nakashima, Ryuichi Nishinakamura, Makoto Tachibana, Miki Inoue, Yuichi Shima, Ken-ichirou Morohashi, Katsuhiko Hayashi*

Science 373 (6552):299-306, 2021

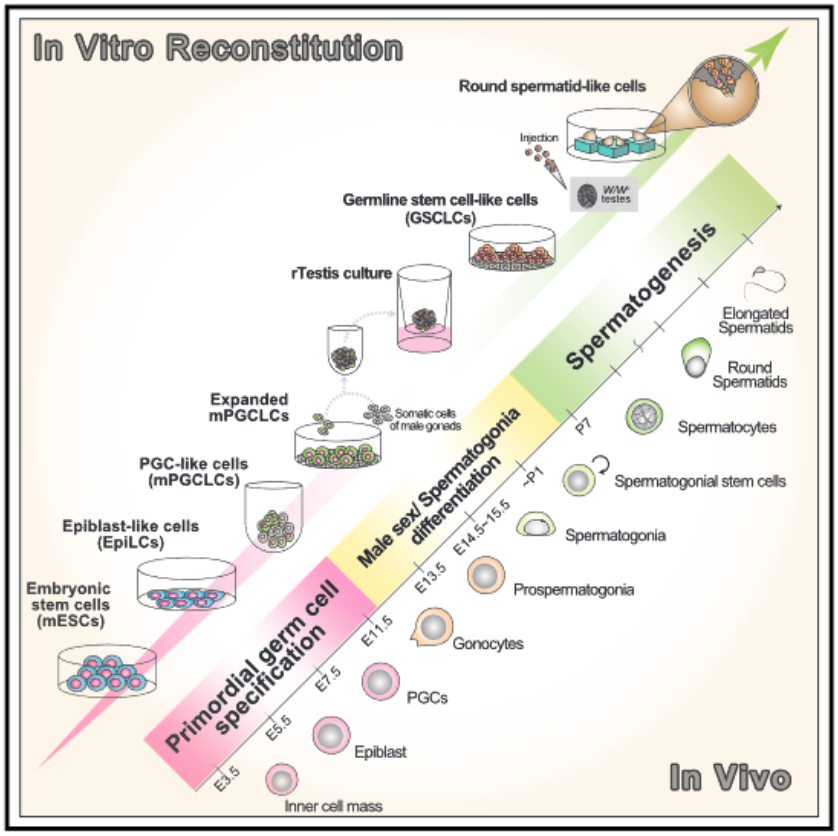




Cell Stem Cell

In vitro reconstitution of the whole male germ-cell development from mouse pluripotent stem cells

Graphical abstract



Authors

Yukiko Ishikura, Hiroshi Ohta, Takuya Sato, ..., Takuya Yamamoto, Takehiko Ogawa, Mitinori Saitou

Correspondence

saitou@anat2.med.kyoto-u.ac.jp

In brief

Male germ-cell development is a complex process that leads to the generation of haploid male gametes, the spermatozoa. Using the mouse as a model, Ishikura and colleagues establish a strategy to create functional spermatozoa from pluripotent stem cells *in vitro* by reconstituting entire male germ-cell development in a stepwise manner.

Cell Stem Cell 28(12):2167-2179, 2021

The Human Paradigm

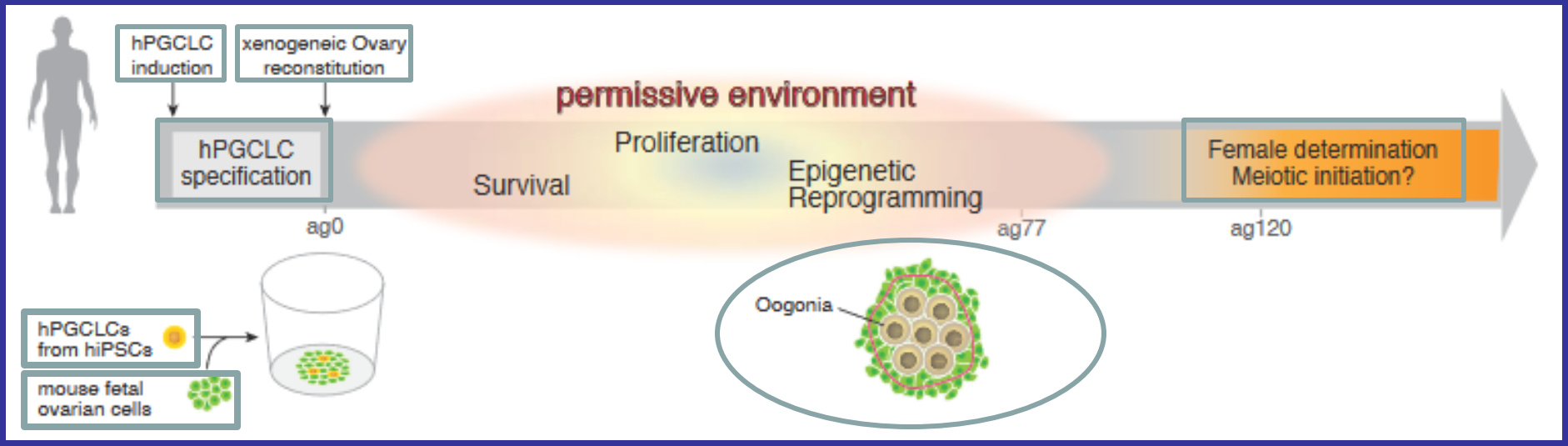
GERM CELL DEVELOPMENT

Generation of human oogonia from induced pluripotent stem cells in vitro

Chika Yamashiro[1,2], Kotaro Sasaki[1,2], Yukihiro Yabuta[1,2], Yoji Kojima[1,2,3,4], Tomonori Nakamura[1,2], Ikuhiro Okamoto[1,2], Shihori Yokobayashi[1,2,4], Yusuke Murase[1,2], Yukiko Ishikura[1,2], Kenjiro Shirane[5,6], Hiroyuki Sasaki[5,6], Takuya Yamamoto[3,4,7], Mitinori Saitou[1,2,3,4]*

Mitinori Saitou, MD, PhD
Kyoto University

Science 362(6412):356-360, 2018



nature COMMUNICATIONS

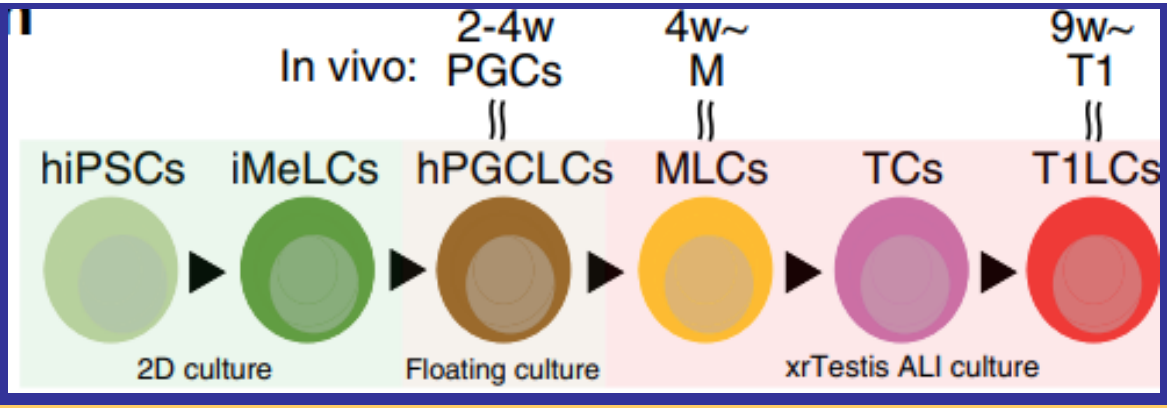
ARTICLE

https://doi.org/10.1038/s41467-020-19350-3 OPEN

Check for updates

Reconstitution of prospermatogonial specification in vitro from human induced pluripotent stem cells

Young Sun Hwang [1,5], Shinnosuke Suzuki[2,5], Yasunari Seita[1,3,5], Jumpei Ito [4], Yuka Sakata[1], Hirofumi Aso[4], Kei Sato [4], Brian P. Hermann [2] & Kotaro Sasaki [1✉]



Nat Commun 11(1):5656, 2020

Human In-Vitro Gametogenesis

MII Oocytes?

Spermatozoa?

The Scientific & Translational Promise

- **Advancement of science**
  - An inexhaustible supply of germ cells
  - Functional (null mutant) analysis
  - Study of Germ-Somatic cell interactions

- **Reversal of germ cell failure**
  - Autologous gamete replacement
  - Gene-edited gamete replacement
  - Same-sex parenthood

Impact of IVG on IVF

- **Eliminate need in stimulation/egg retrieval**
- **Do away with gamete donation (age-dependent?)**
- **Convert IVF to a laboratory procedure**
- **Require endometrial preparation & transfer**
- **Improve affordability and thus access?**

Regulation

- **FDA jurisdiction**
- **CBER (Center for Biologics Evaluation & Research)**
- **HCT/Ps (Human Cells, Tissues, and Products)**

WHAT'S ON THE HORIZON FOR BIOETHICS?

NUFFIELD COUNCIL ON BIOETHICS

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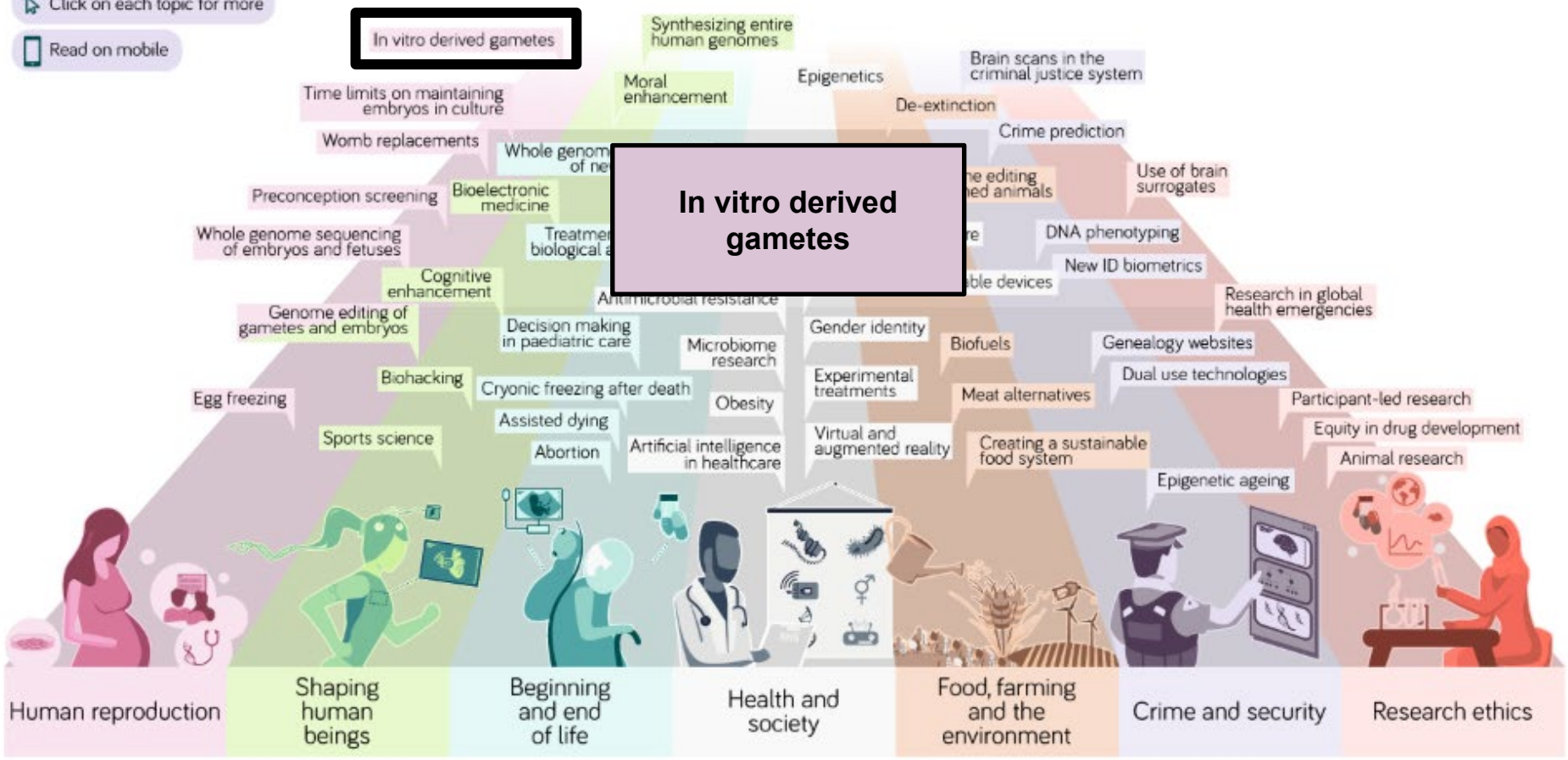


In vitro derived gametes

Gametes to Order

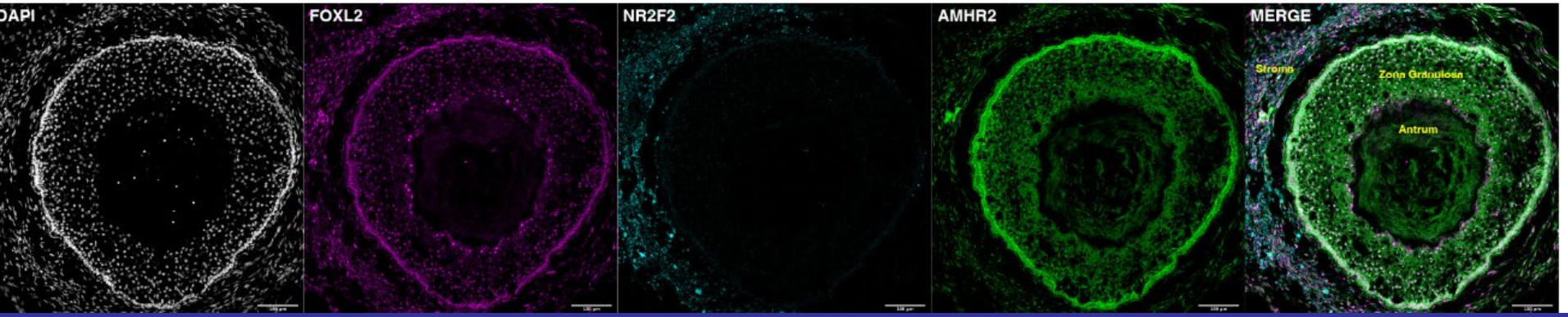
Stay Tuned

eLife

RESEARCH ARTICLE

Directed differentiation of human iPSCs to functional ovarian granulosa-like cells via transcription factor overexpression

Merrick D Pierson Smela[1,2†], Christian C Kramme[1,2†], Patrick RJ Fortuna[1,2], Jessica L Adams[1,2], Rui Su[1,2], Edward Dong[1,2], Mutsumi Kobayashi[3], Garyk Brixi[1,2,4,5], Venkata Srikar Kavirayuni[4,5], Emma Tysinger[4,5], Richie E Kohman[1,2], Toshi Shioda[3], Pranam Chatterjee[1,2,4,5], George M Church[1,2*]



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Human In-Vitro Gametogenesis

Granulosa Cells?

Theca Cells?

Sertoli Cells?