生殖細胞の発生機構の解明とその試験管内再構成

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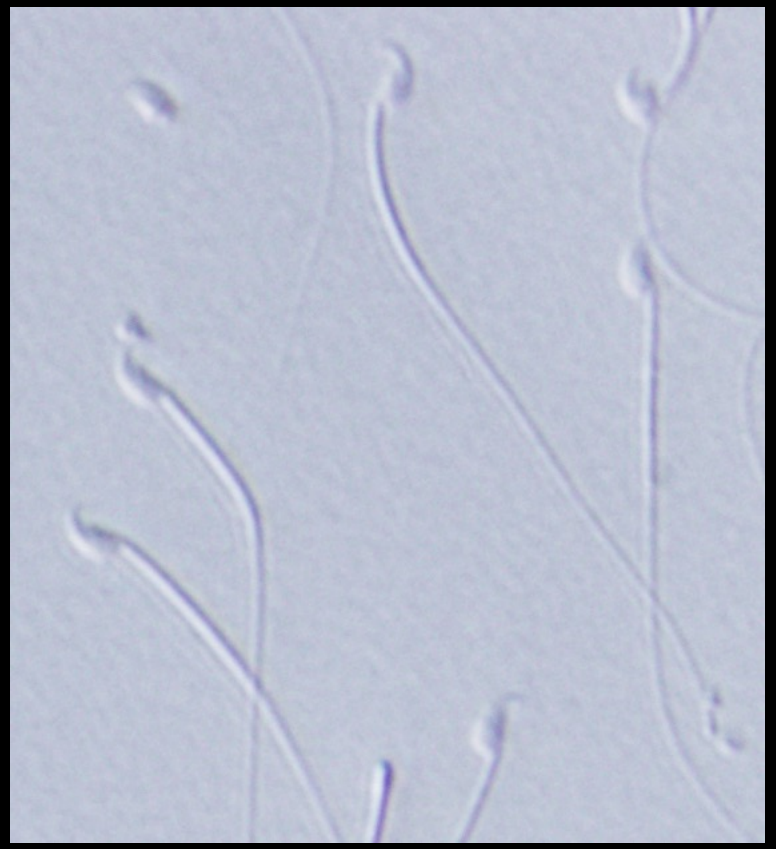
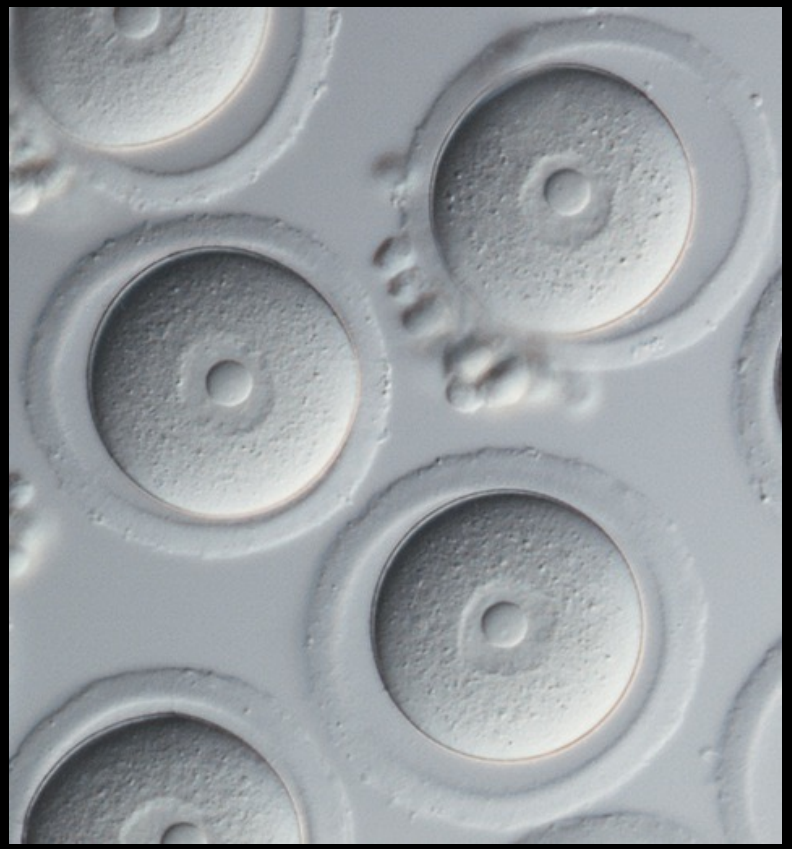
京都大学iPS細胞研究所

生殖細胞とはどのような細胞か？

精子



卵母細胞



精子や卵子、さらにはそれらの起源となる細胞のことを生殖細胞と呼びます。

生殖細胞は、１） 受精により 全能性 を獲得する、２） それにより 遺伝情報と Epigenetic 情報を次世代に伝達する、　能力を有します。

世界初の体外受精児の誕生, Edwards and Steptoe, 1978

図３　世界初の体外受精児の誕生（向って左）（朝日新聞　1978 年 7 月 26 日），わたしはルイーズよ　体外受精胚（右）（朝日新聞　1978 年 7 月 27 日）

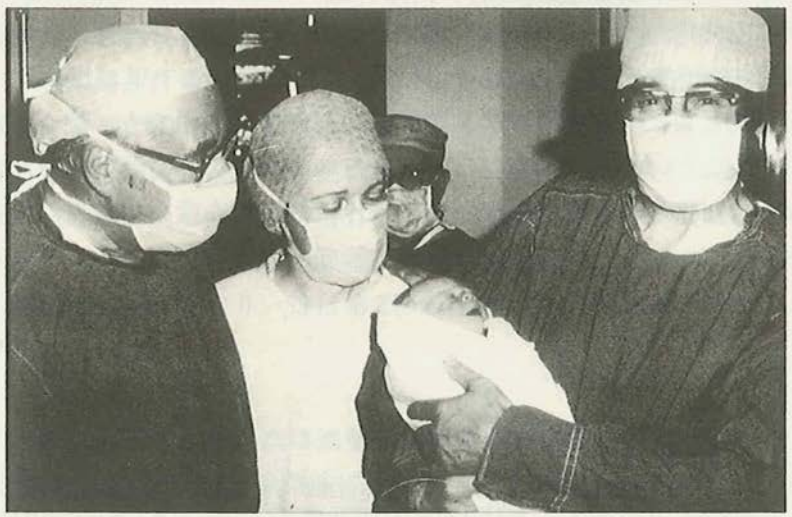


図４　世界初の体外受精児の帝王切開出産直後の写真
July 25, 1978: Patrick Steptoe, Jean Purdie, and Louise Brown.. in the arms of Bob Edwards（ウィーン大学 W Feichtinger 教授提供）

体外受精：精子と卵子を培養ディッシュ上で受精させ、得られた胚を培養し、母体に戻し産仔を得る技術。

Bob Edwards 2010年 ノーベル賞受賞

現在日本を含む先進国において、~3-5 %の新生児が体外受精によって生まれている。
(2022年の日本では10人に1人がARTにより出生)

一方で、体外受精はインプリント異常を含むエピゲノム異常をより高頻度に誘発するとの報告もあり、正確な研究が必要である。

生殖細胞研究の社会へのインパクト

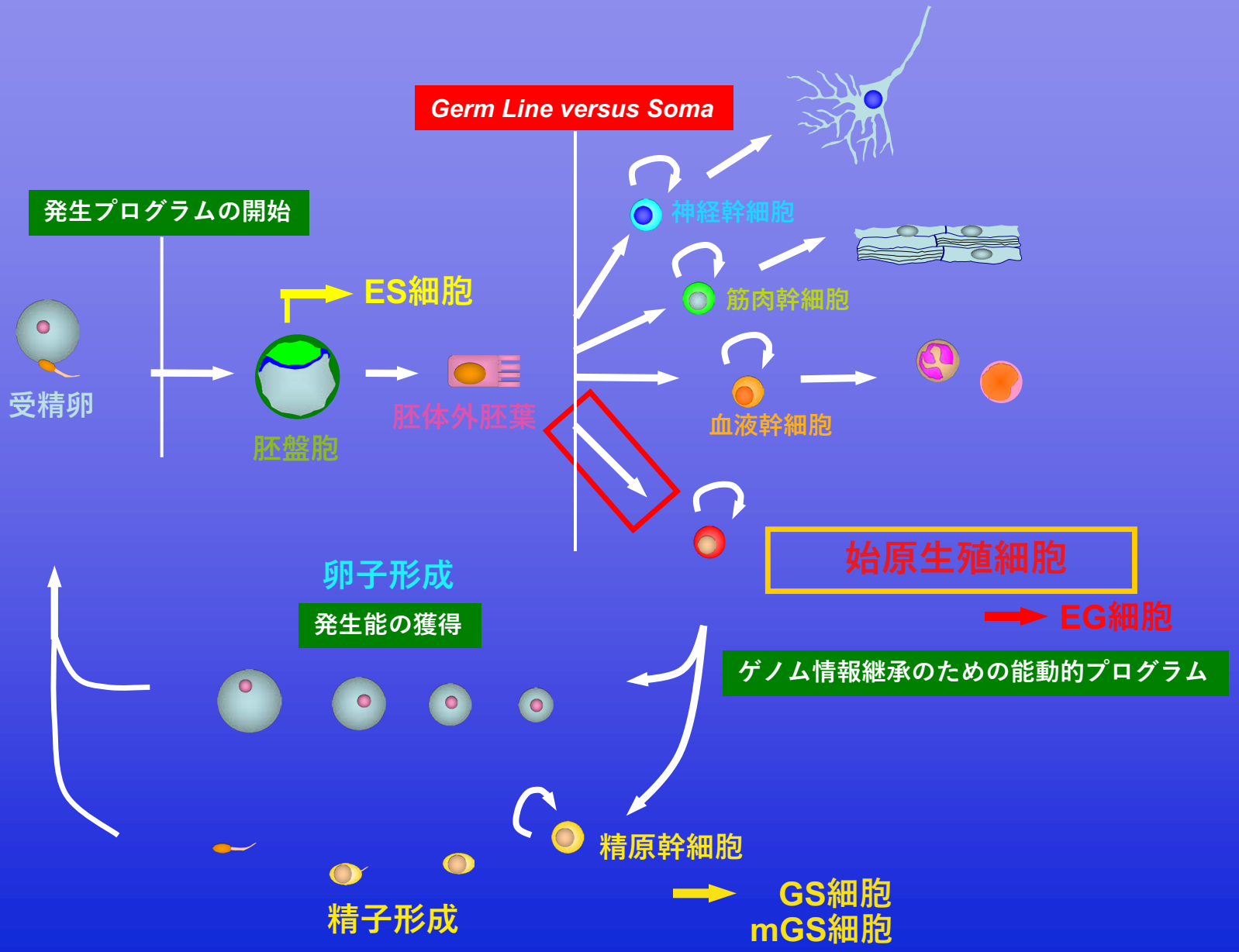
生殖細胞は、精子及び卵子に分化し、それらが融合することで新しい個体をつくり、新しい世代に遺伝情報を伝える細胞です。

哺乳類の生殖細胞研究は、20世紀半ばの齧歯類を用いた初期胚培養系・試験管内授精法の確立により発展し、その技術がヒトに応用され、1978年イギリスで初の試験管ベビーが誕生しました。現在、先進国では、～20－30人に1人（2022年の日本では10人に1人）が生殖補助医療により誕生しています。

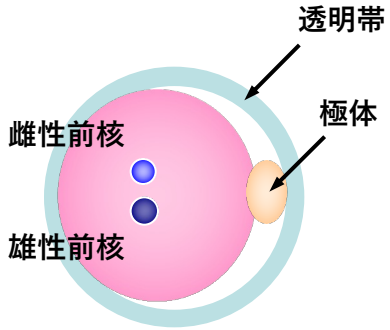
さらに、生殖細胞研究は、ES細胞の樹立・体細胞クローンの作出・ゲノムインプリントの発見等をもたらし、転写因子により体細胞をリプログラムするiPS細胞の樹立につながりました。

生殖細胞研究は、医学、医療、さらには生命哲学に大きな影響を与えています。

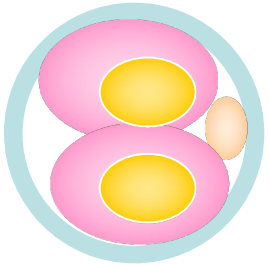
生殖細胞は生命の連続性を保証する



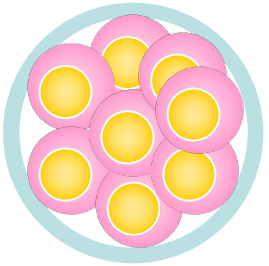
マウスの初期発生と新たな生殖細胞系列の発生



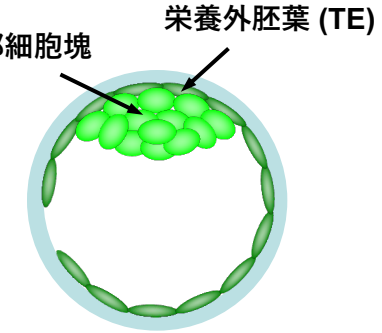
受精卵



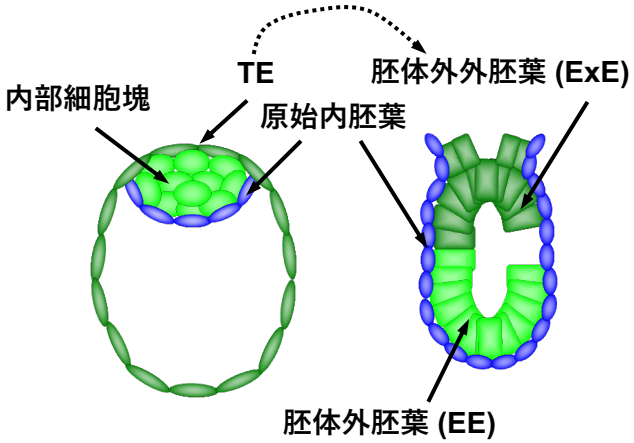
2細胞胚



8細胞胚

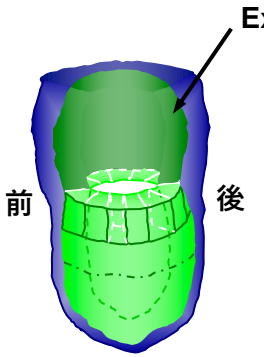


胚盤胞 (発生3.5日目: E3.5)

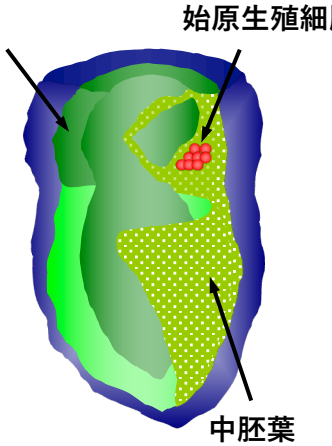


胚盤胞 (E4.5)

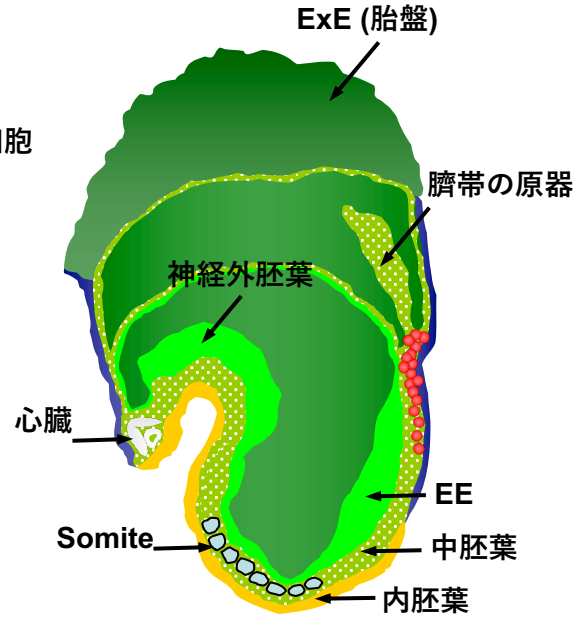
E5.5



E6.25

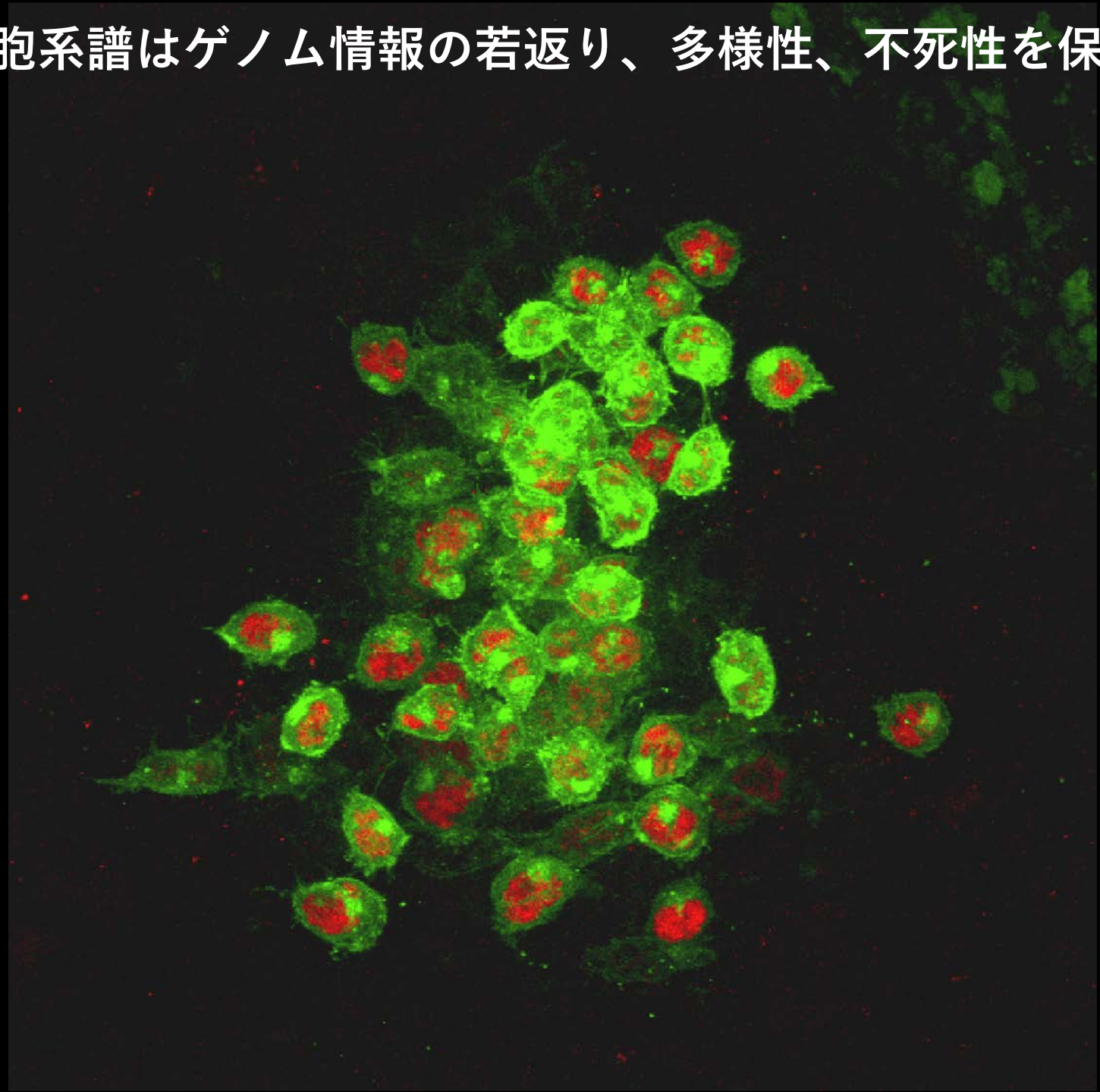


E7.0

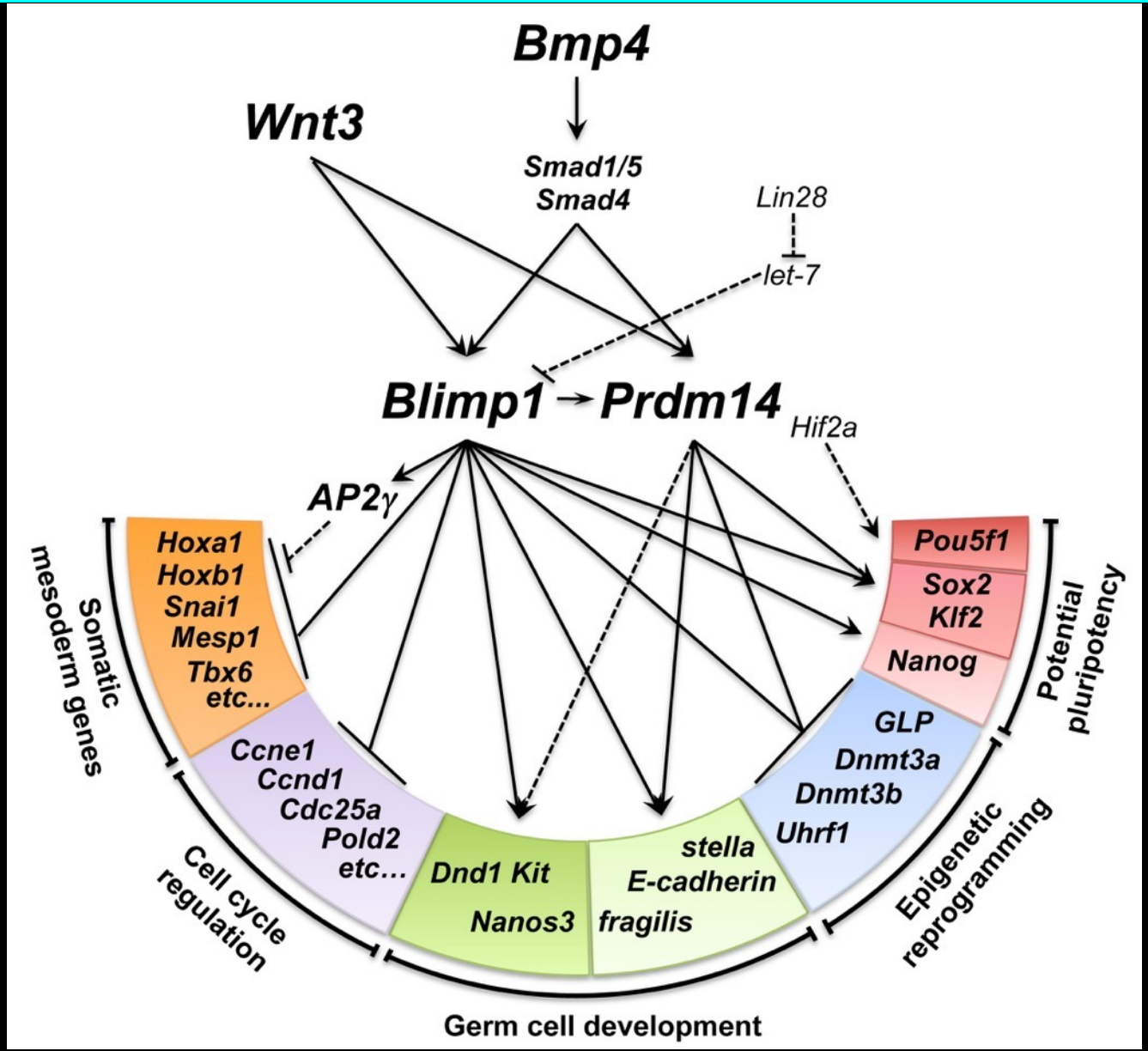


E8.25

生殖細胞系譜はゲノム情報の若返り、多様性、不死性を保証する



マウス生殖細胞形成機構の解明

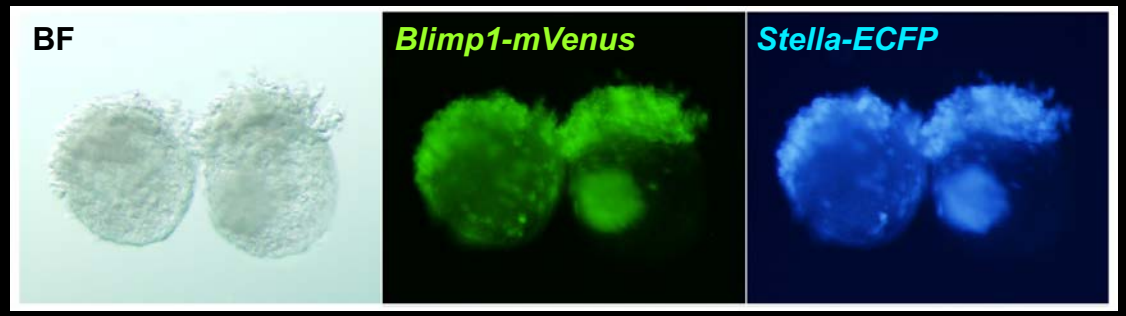


(Kurimoto et al., Genes Dev., 2008; Yamaji et al., Nat. Genet., 2008; Ohinata et al., Nature, 2005; Cell, 2009; Seki et al., Development, 2007)

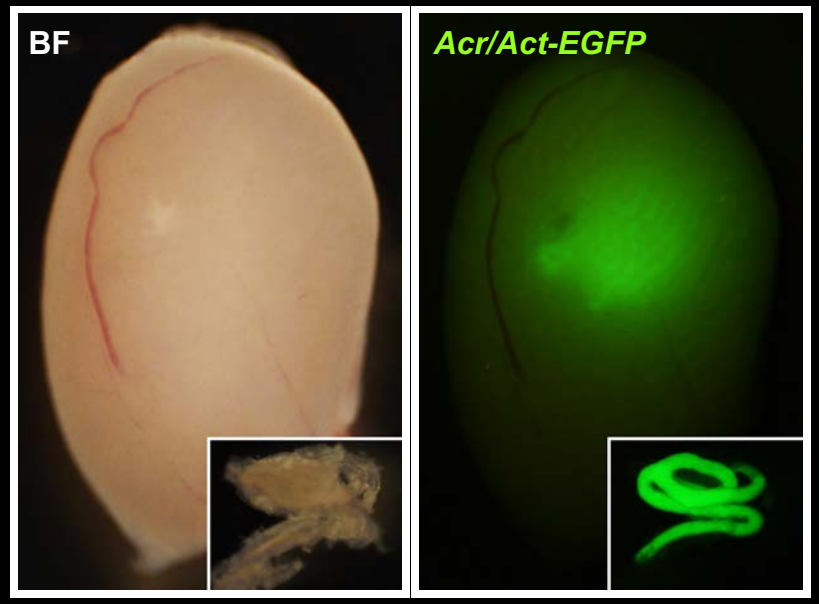
マウス生殖細胞形成を制御するシグナル機構の解明

(Ohinata et al., Cell, 2009)

PGC-like cells induced from epiblasts by cytokines including BMP4 *ex vivo*

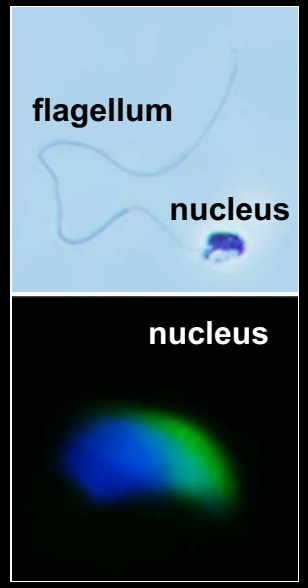


Ex vivo PGCs contribute to spermatogenesis upon transplantation into testes



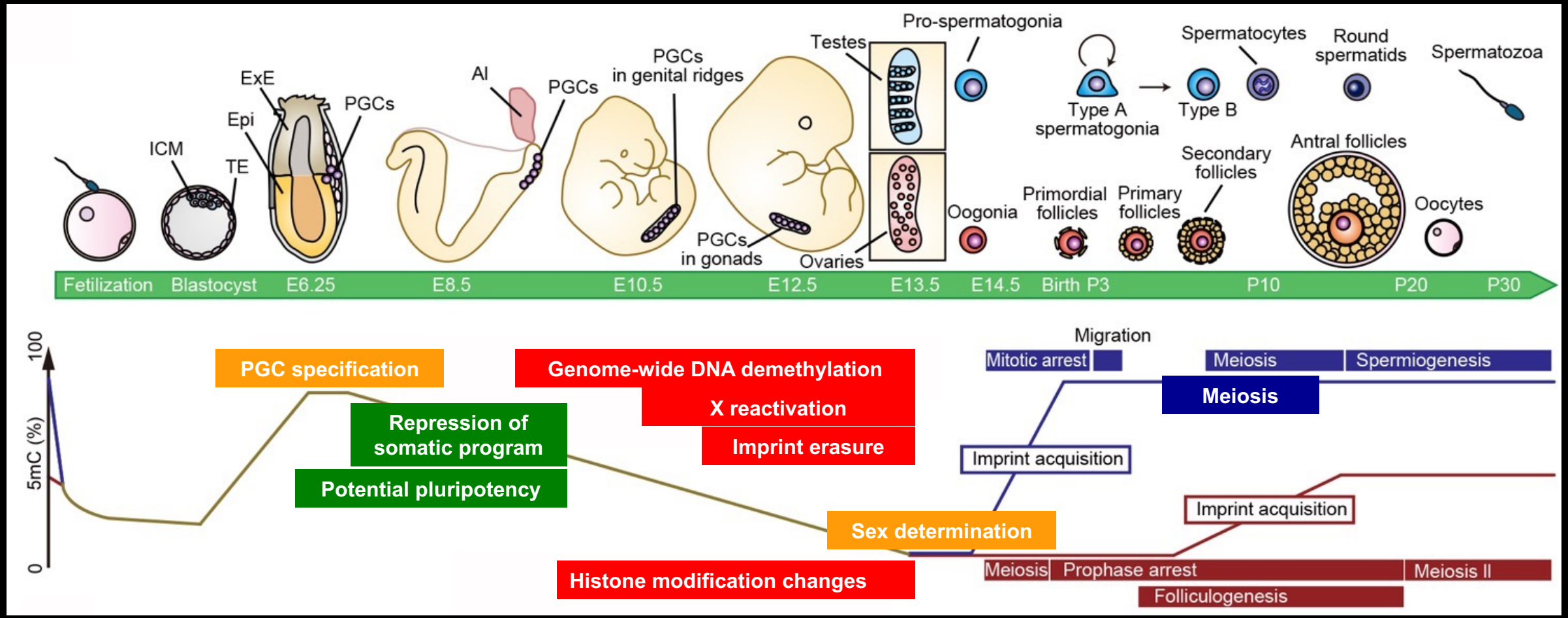
Seminiferous tubule contributed by *ex vivo* PGCs

Spermatozoon from *ex vivo* PGCs



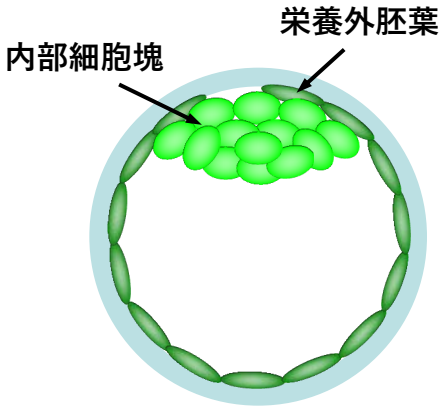
Such spermatozoa contribute to fertile offspring

マウス生殖細胞発生過程の概要



(Saitou and Miyauchi, Cell Stem Cell, 2016)

ES細胞とiPS細胞：多能性幹細胞



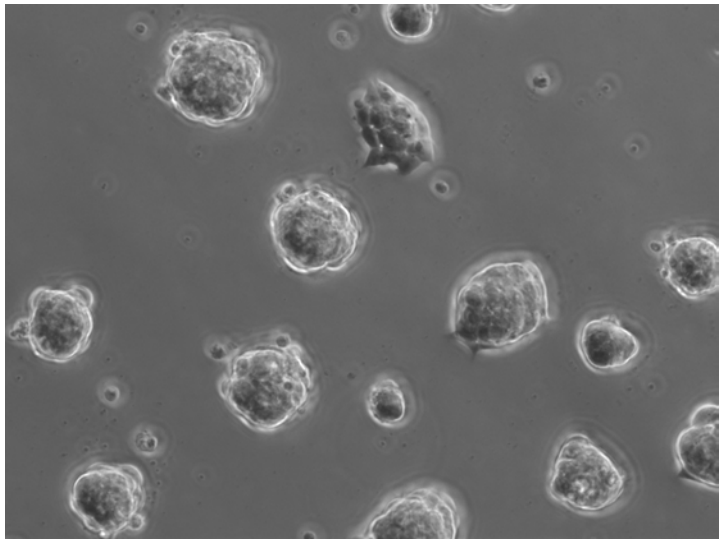
基本培地+血清+LIF

基本培地+血清代替物+2i+LIF

Evans and Kaufman, 1981

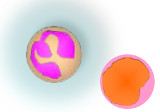
Ying and Smith et al., 2007

ES細胞: 2004年ノーベル賞




上皮細胞


線維芽細胞


血液細胞

４つの転写制御因子

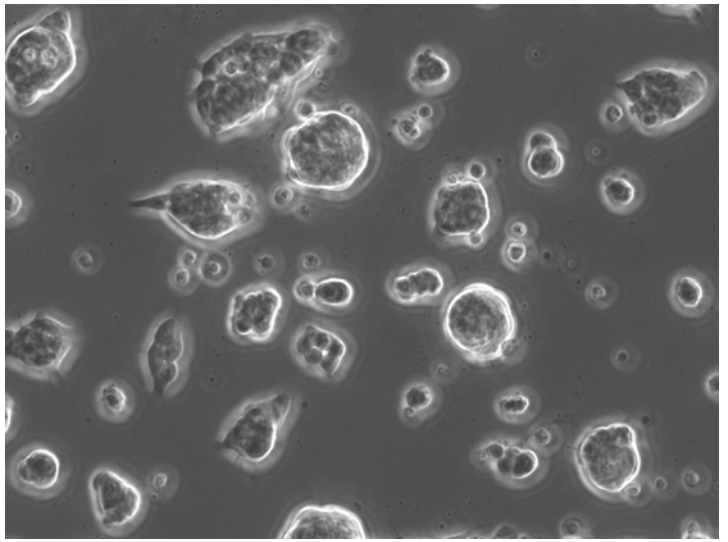
OCT4, SOX2, KLF4, MYC

基本培地+血清+LIF

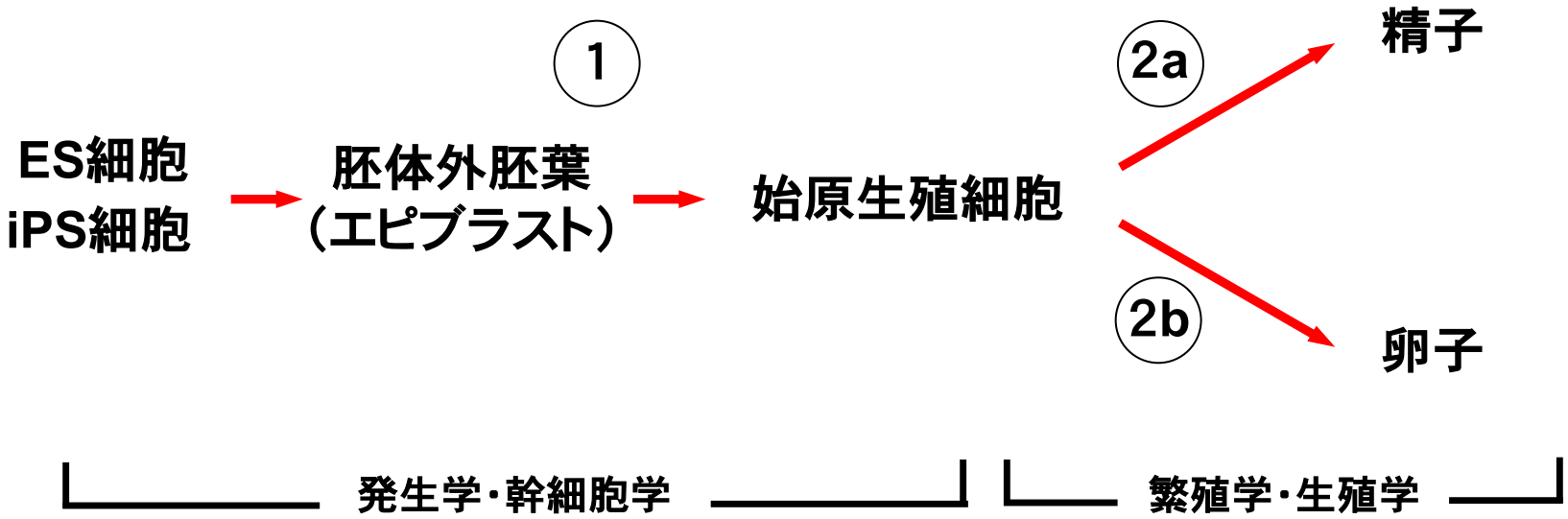
基本培地+血清代替物+2i+LIF

Takahashi and Yamanaka, 2006

iPS細胞: 2012年ノーベル賞



多能性幹細胞（ES or iPS細胞）からの生殖細胞作成方法

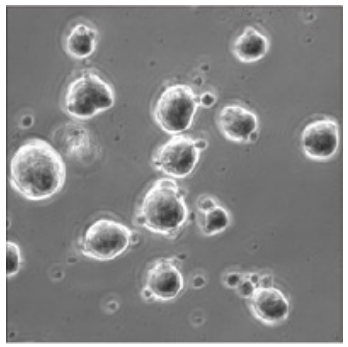


1. ES/iPS細胞から配偶子を作成する場合も必ず始原生殖細胞（あるいはそれに類似した細胞）を経由する。

2. よってES/iPS細胞から生殖細胞を作成する研究は、大きく始原生殖細胞の前後、①と②に分けて整理できる。

マウス生殖細胞形成過程の試験管内再構成

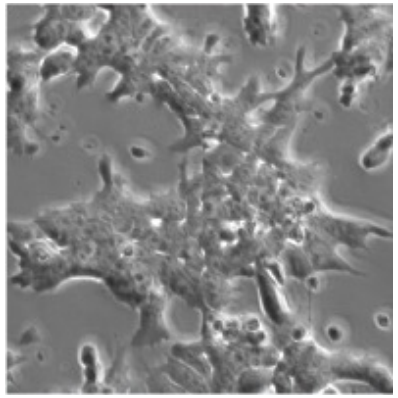
(Hayashi et al., Cell, 2011; Science, 2012)



mESCs/miPSCs
(XY or XX)

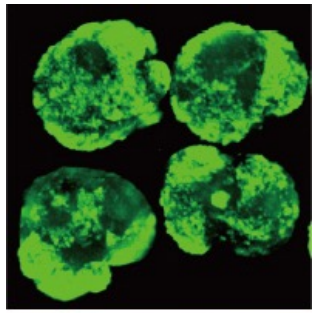
Blimp1-mVenus:
Stella-ECFP
(BVSC)

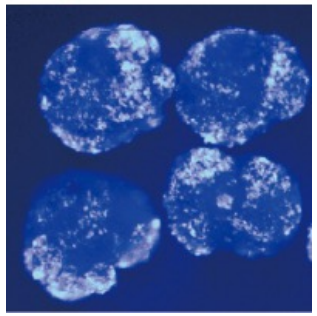
ActA
bFGF
KSR



EpiLCs
(epiblast-like cells)

Floating aggregate
culture
BMP4
LIF
SCF
EGF

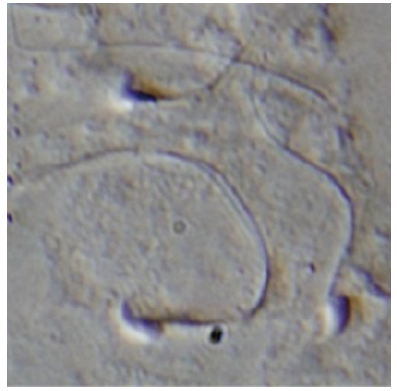




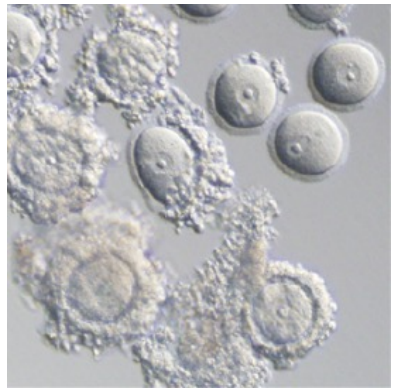
mPGCLCs
(mPGC-like cells)

Transplantation
Into neonatal testes

Reconstituted ovary
Transplantation into
ovary



Spermatozoa



Oocytes

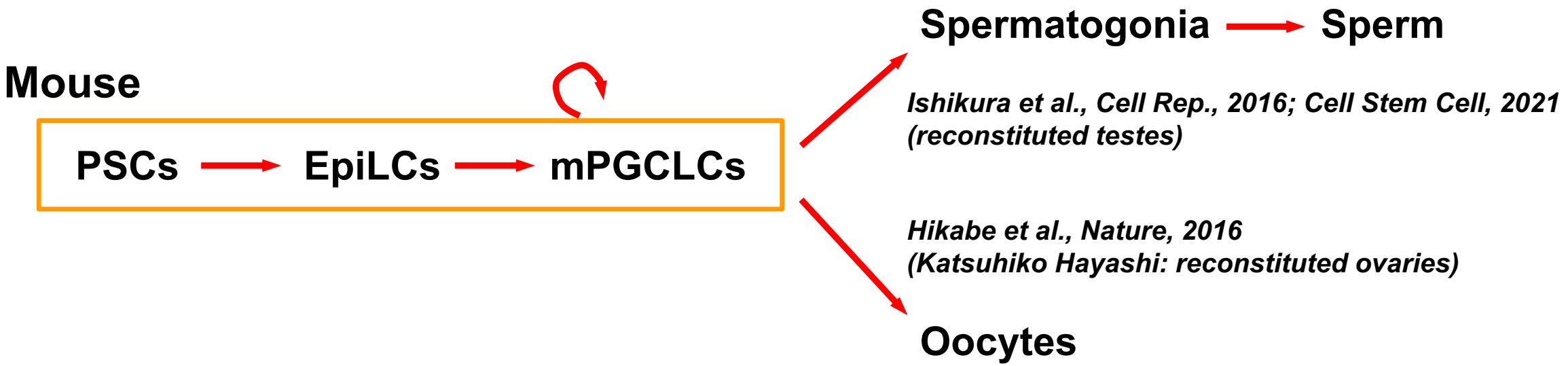
ICSI

IVM
IVF

Fertile offspring

Fertile offspring

マウス生殖細胞発生過程の試験管内再構成



Transcriptional/Signaling mechanism of PGC specification:

Nakaki et al., Nature, 2013; Yamaji et al., Cell Stem Cell, 2013; Aramaki et al., Dev. Cell, 2013

Chromatin/Nucleome programming during *in vitro* germ-cell development:

Kurimoro et al., Cell Stem Cell, 2015; Shirane et al., Dev. Cell, 2016; Nagano et al., EMBO J, 2022; NSMB, 2025

Fertile offspring from sterile sex chromosome trisomic mice:

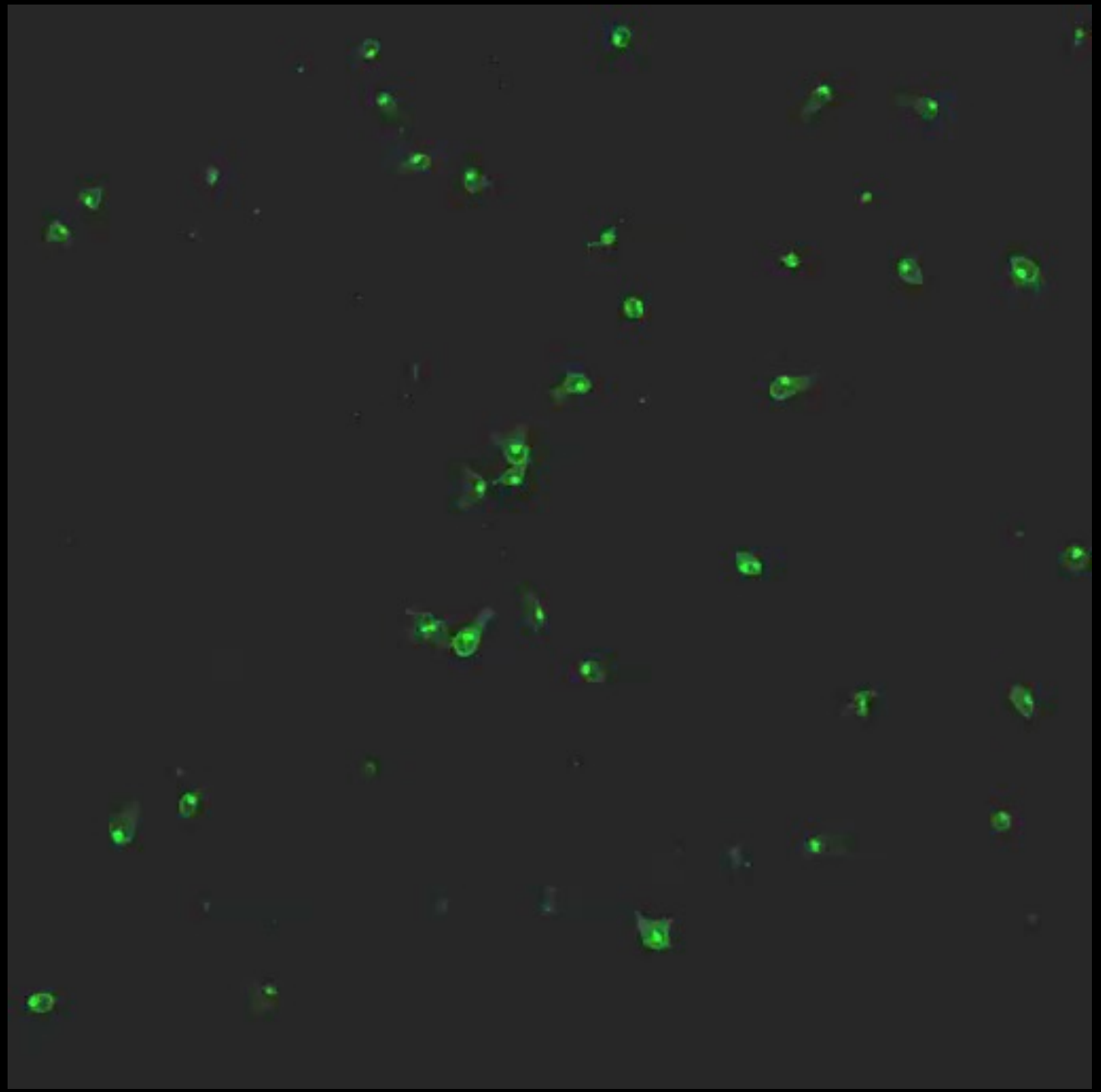
Hirota et al., Science, 2017: with James Turner

***In vitro* propagation of PGC(LC)s/Mechanism for female germ-cell sex determination:**

Ohta et al., EMBO J, 2017; Biol. Reprod., 2020; Miyauchi et al., EMBO J, 2017; Nagaoka et al., Science, 2020; Nosaka et al., Dev. Cell, 2025

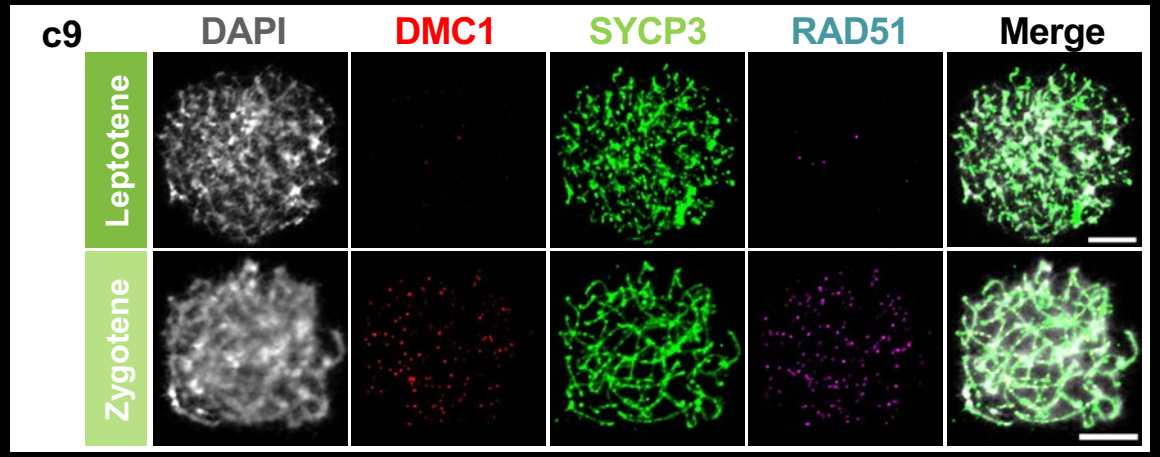
Culture of mPGCLCs (SCF, forskolin/rolipram on m220 feeders)

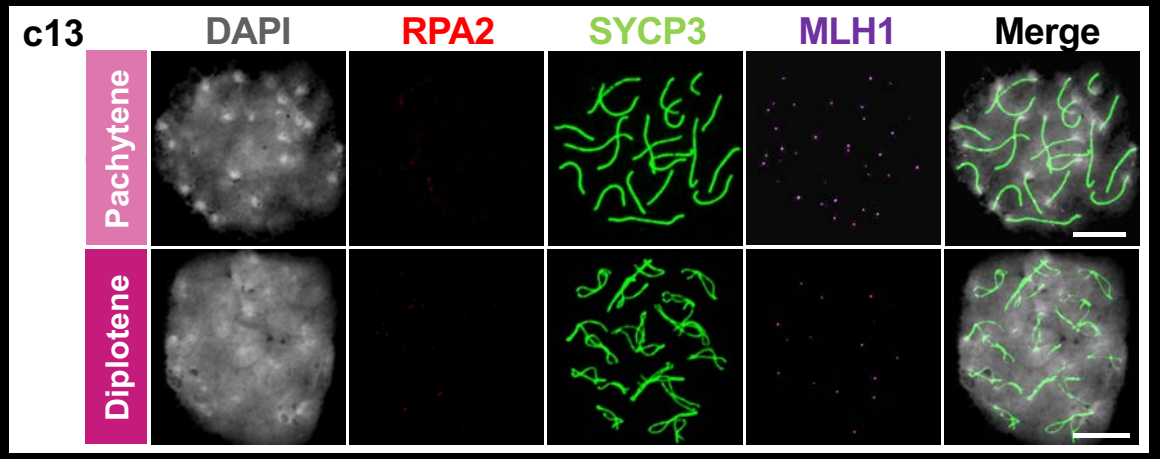
An initial phase of propagation (12 h)

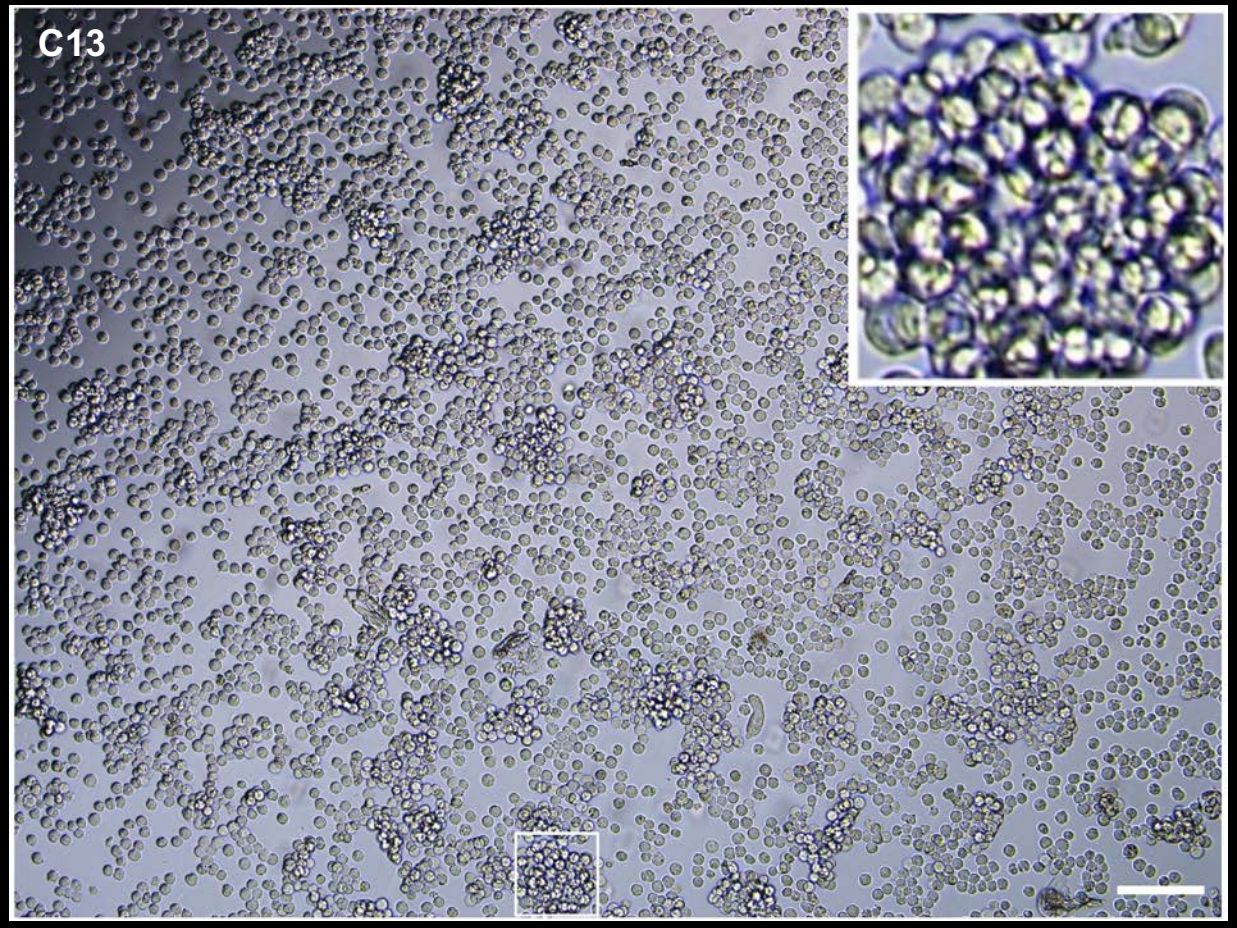


卵巣体細胞を用いない卵母細胞発生過程の試験管内再構成

RA
BMP2
mPGCLC induction
mPGCLC expansion, epigenetic reprogramming, female germ-cell development
(SCF, forskolin/rolipram on m220 feeders)
d4/c0 mPGCLCs
c3 cells
c9 cells
c13 cells







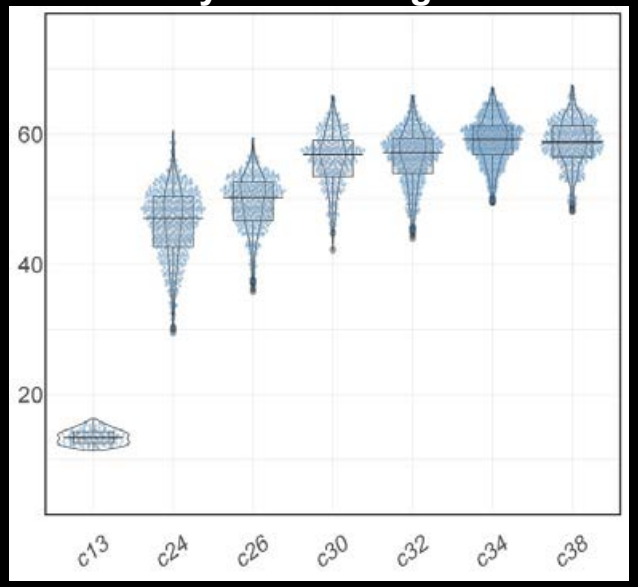
(>10^5 diplotene oocytes/experiment)

卵巣体細胞を用いない卵母細胞発生過程の試験管内再構成

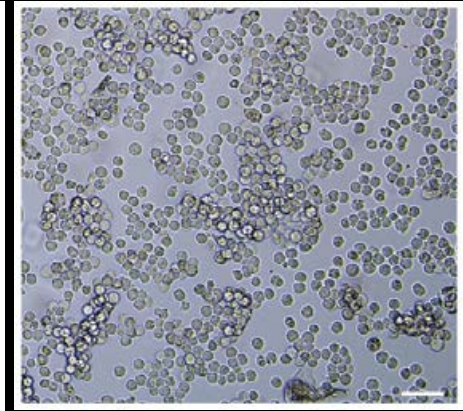
Oocyte growth
(SCF, other cytokines, mTOR stimulators, antioxidants, forskolin/rolipram on m220 feeders)

c13 cells → c24 cells → c34 cells

Oocyte-like cell growth

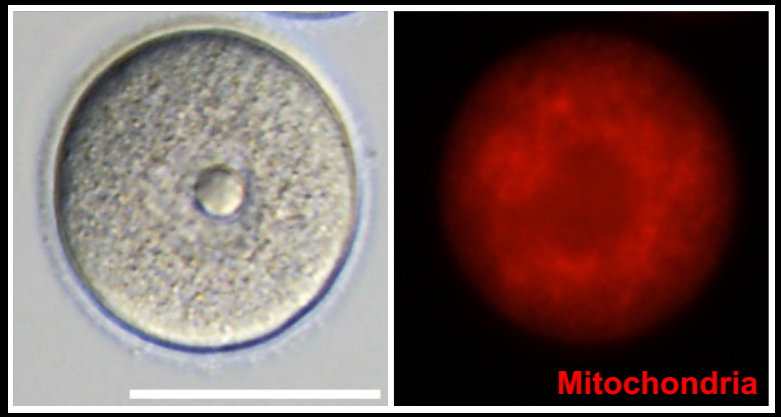


c13 cells

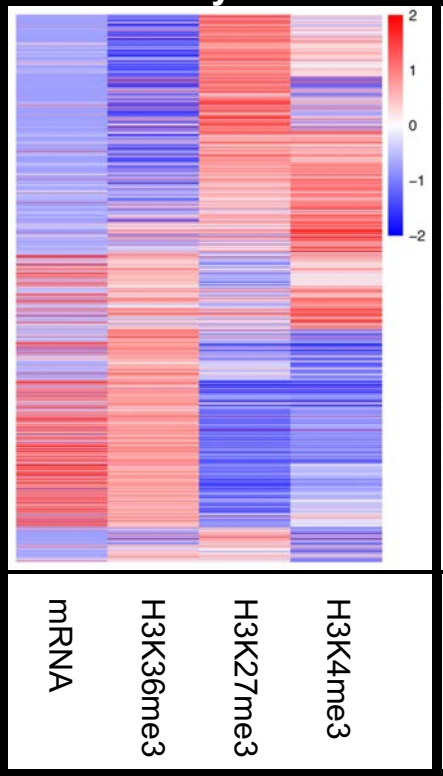


c34 cells

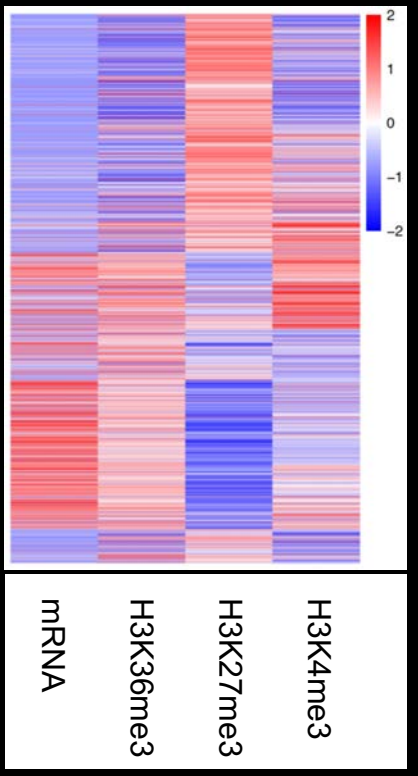
c34 cells



Antral oocytes *in vivo*

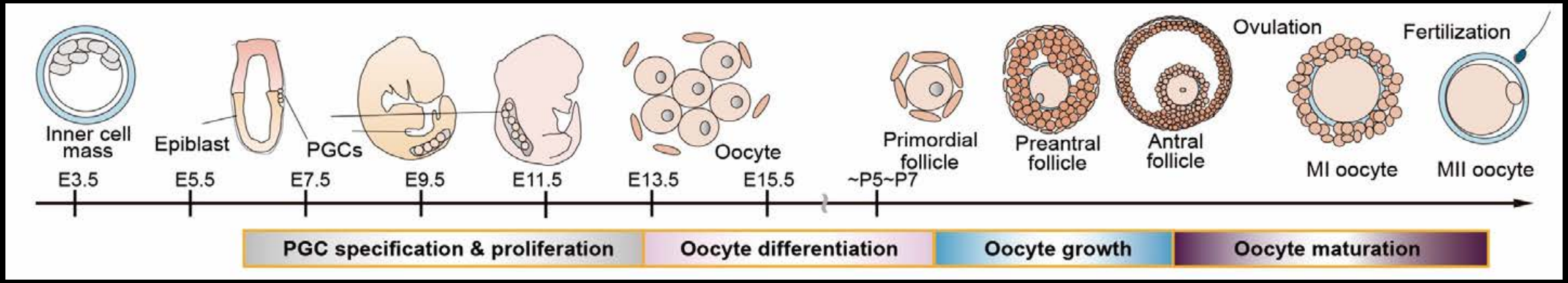


c34 cells

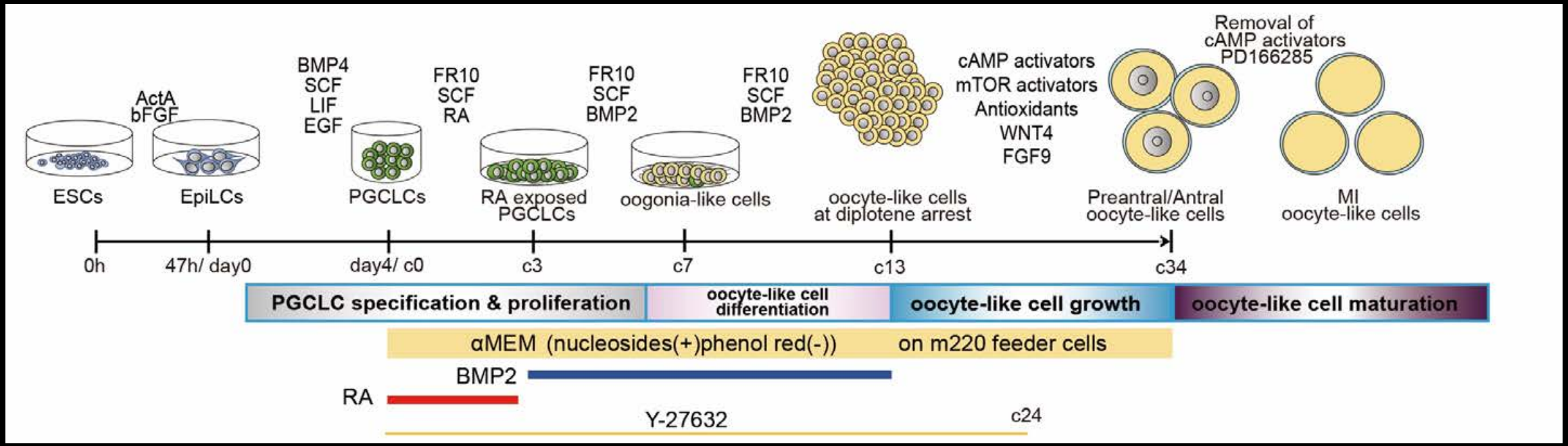


卵巣体細胞を用いない卵母細胞発生過程の試験管内再構成

In vivo



In vitro



(Nosaka et al., Dev. Cell, 2025)

Cell PRESS

Developmental Cell
Forum

ヒトとマウスは違う！

The Impact of Developmental Biology on Pluripotent Stem Cell Research: Successes and Challenges

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[1]Program in Developmental and Stem Cell Biology, Hospital for Sick Children
[2]Department of Molecular Genetics
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DOI 10.1016/j.devcel.2011.06.010

Research on developmental pathways in model organisms provides key information on how to isolate, maintain, and differentiate human pluripotent stem cells. However, details of developmental pathways differ even across mammalian species. Full realization of the potential of stem cells will require more direct studies of human or primate developmental biology.

ヒト多能性幹細胞を基盤とした医療の実現には
ヒトもしくは霊長類の発生生物学的研究が重要。

In the past decade, there have been major advances in our understanding of the basic biology of stem cell self-renewal and differentiation in many different systems. One of the major drivers for this growth has been the excitement about the potential of stem cells for understanding and treating human disease. Pluripotent stem cell research is the fastest rising area. This rise was fueled initially opmen cell res ahead.

Understanding Pluripotency: Mouse versus Human

Our understanding of the regulatory networks that underlie the initiation and maintenance of the pluripotent state has expanded considerably based on both

the naive state under certain conditions (Hanna et al., 2010a). EG cells are derived from culturing early primordial germ cells and show many of the same properties as ES cells in terms of differentiation and chimera formation. EG cells have been

全能性エピゲノムの制御と再構成

マウス

ES/iPS細胞から始原生殖細胞を経て
精子・卵子へ

霊長類（カニクイザル）

着床前／初期着床後発生機構の解明

始原生殖細胞の特性解析

生殖細胞発生過程の再構成

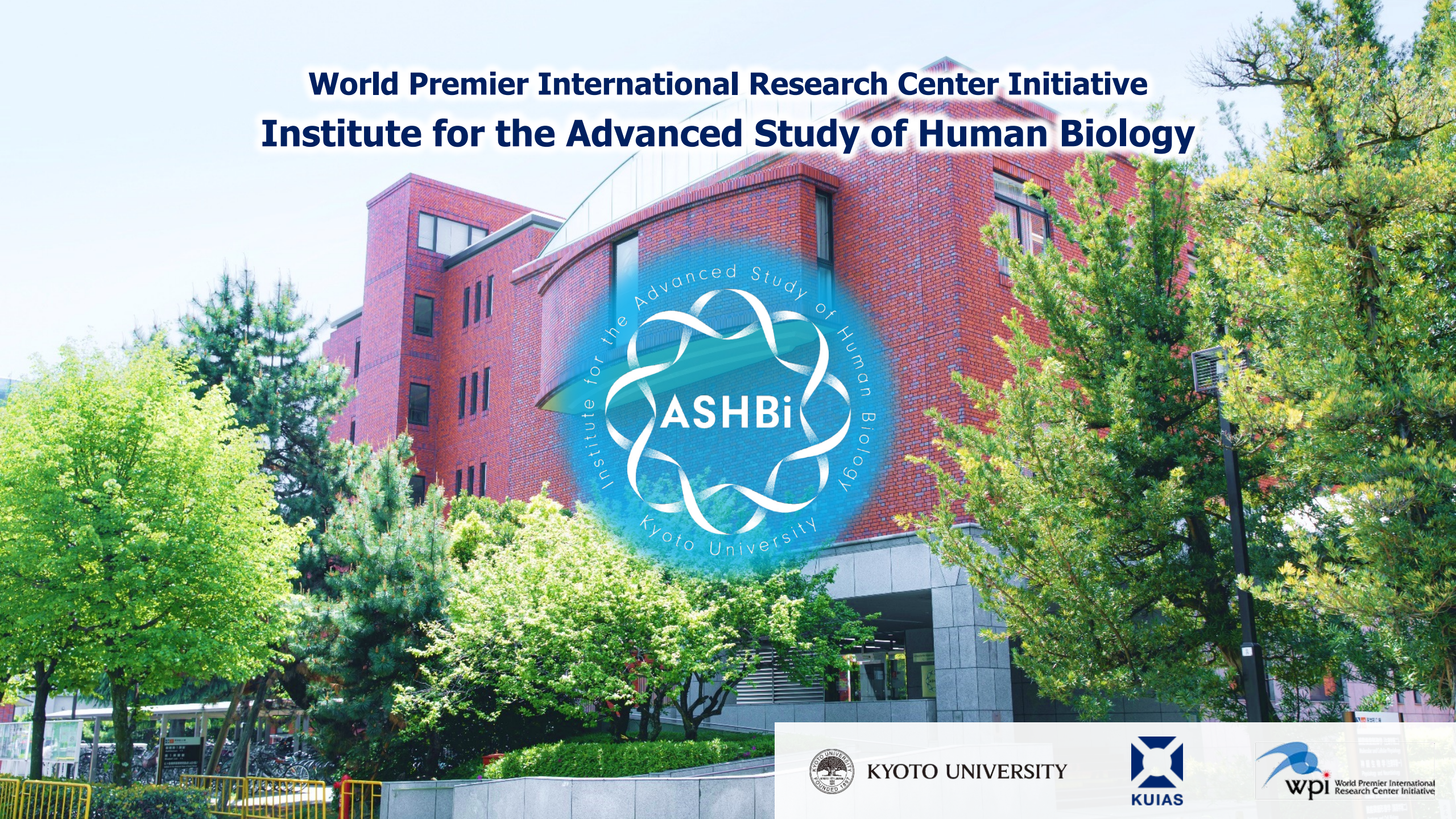
エピゲノムリプログラミング

分子機構解明とその再構成

微量エピゲノム解析法の樹立

ヒト生殖細胞発生過程の再構成

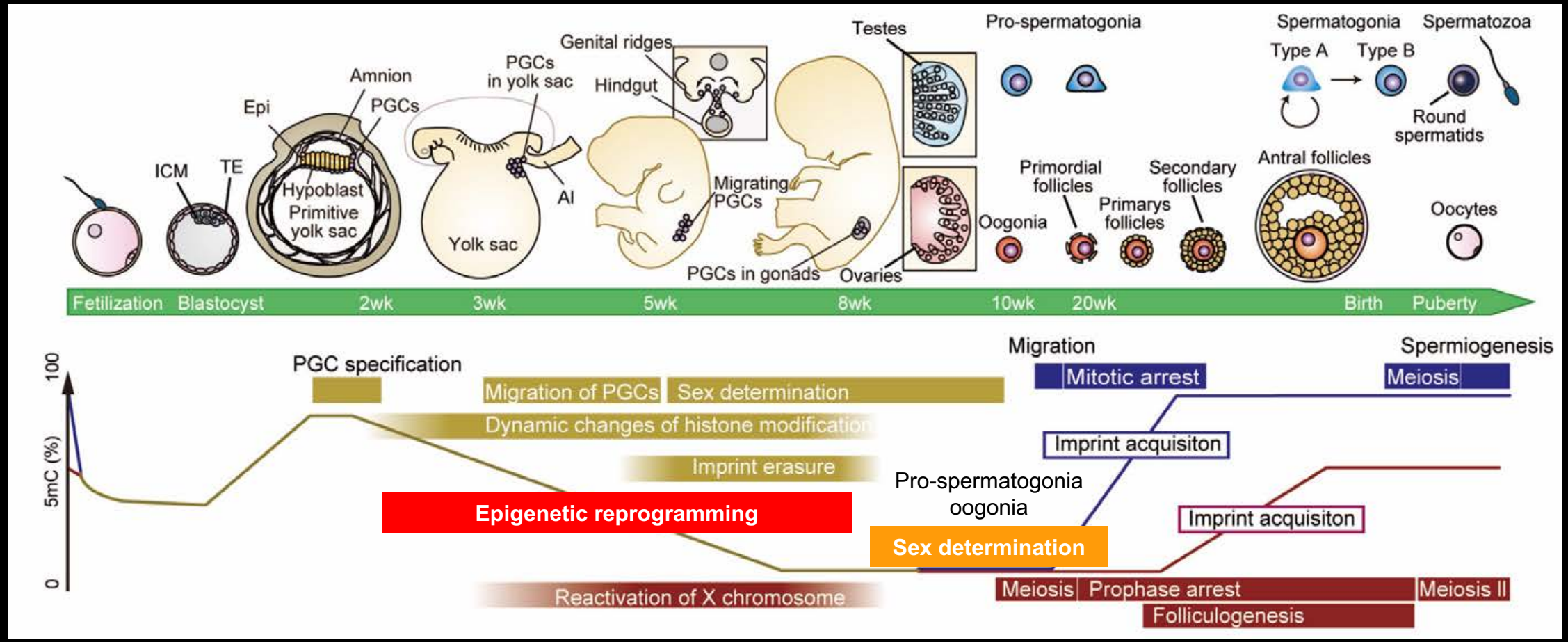
細胞のエピゲノム状態制御戦略の開発
（幹細胞／前駆細胞の試験管内増殖）


World Premier International Research Center Initiative
Institute for the Advanced Study of Human Biology
Institute for the Advanced Study of Human Biology
ASHBi
Kyoto University
KYOTO UNIVERSITY
KUIAS
wpi
World Premier International
Research Center Initiative

ヒト生殖細胞発生過程の概要

Key differences from mice:

Much longer time, asynchronous developmental transitions
divergent signaling, transcriptional and metabolic regulation

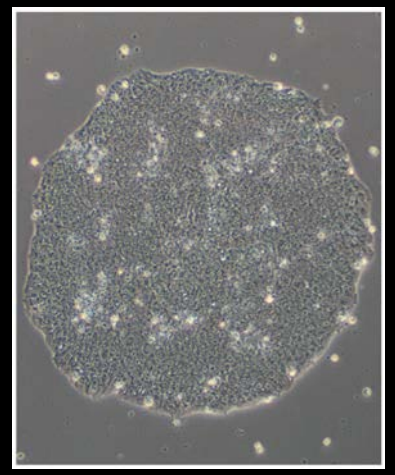


(Saitou and Miyauchi, Cell Stem Cell, 2016)

Mechanism of human germ-cell development remains largely unknown.

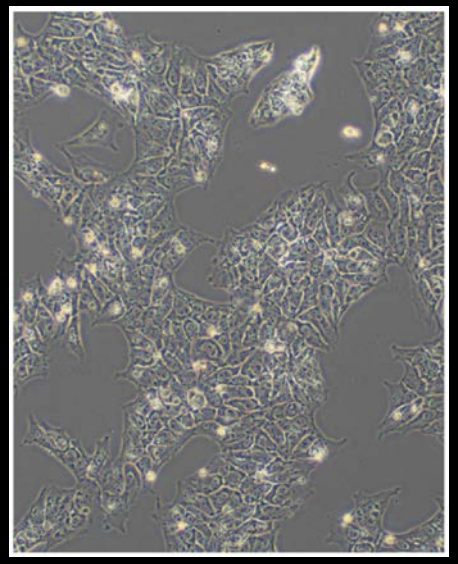
ヒト生殖細胞発生過程の試験管内再構成

(Sasaki, Yokobayashi et al., Cell Stem Cell, 2015; Yamashiro et al., Science, 2018; Murase et al., EMBO J., 2020; Murase, Yokogawa et al., Nature., 2024)



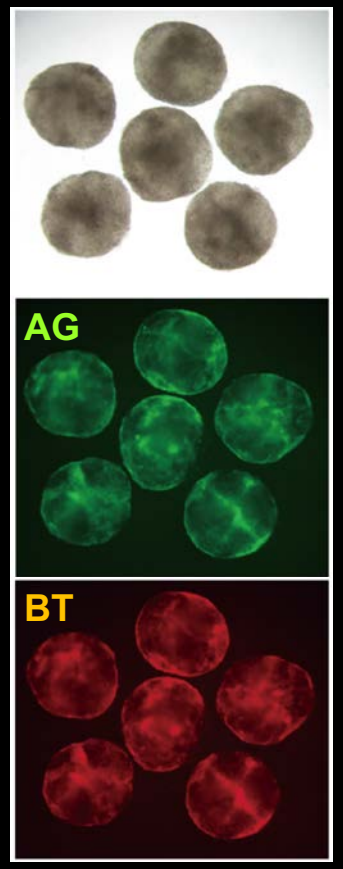
hiPSCs (XY or XX)

ActA
CHIR



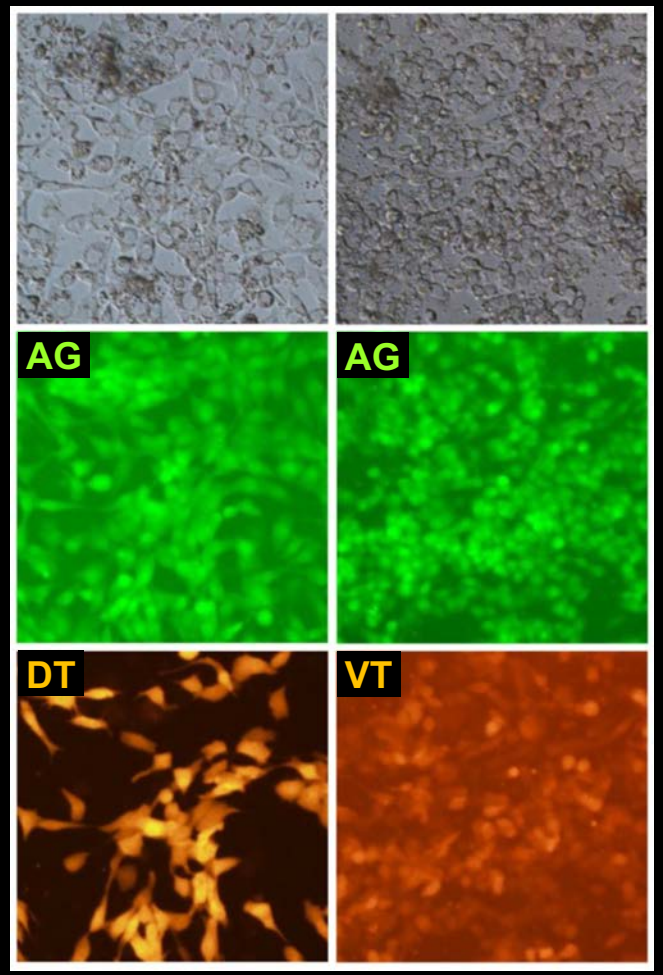
iMeLCs
(incipient mesoderm-like cells)

Floating aggregates
BMP4
LIF
SCF
EGF



hPGCLCs

BMP-driven
hPGCLC differentiation



Mitotic
pro-spermatogonia

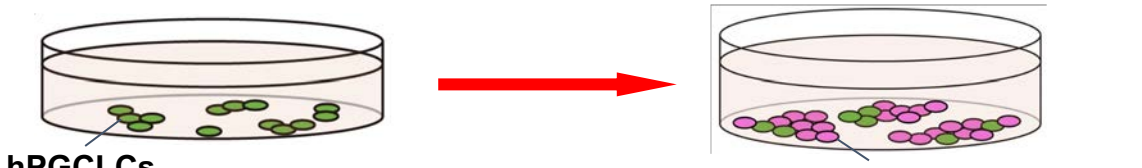
Oogonia

XY: *BLIMP1-tdTomato;TFAP2C-EGFP* (BTAG)

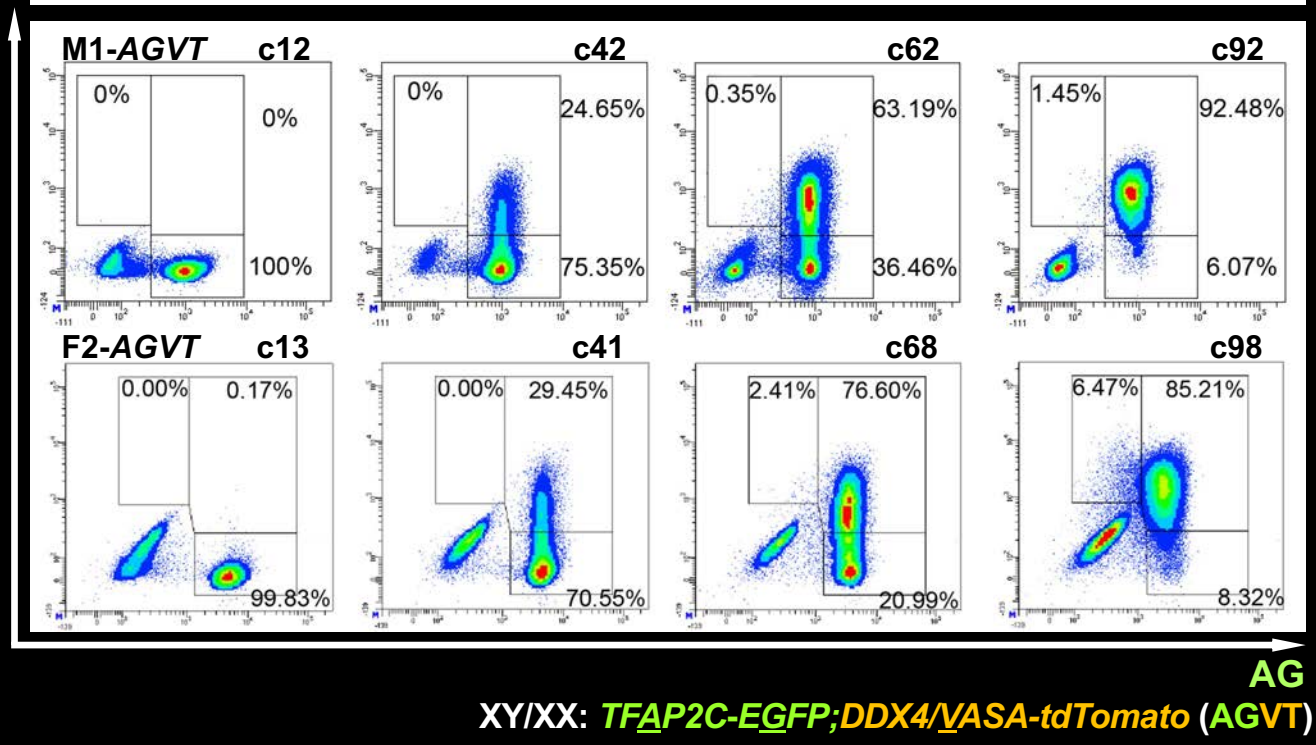
XY: *TFAP2C-EGFP;DAZL-tdTomato* (AGDT)
XY/XX: *TFAP2C-EGFP;DDX4/VASA-tdTomato* (AGVT)

BMPによるヒトPGCLCsの前精原細胞及び卵原細胞への分化

BMP-driven hPGCLC differentiation



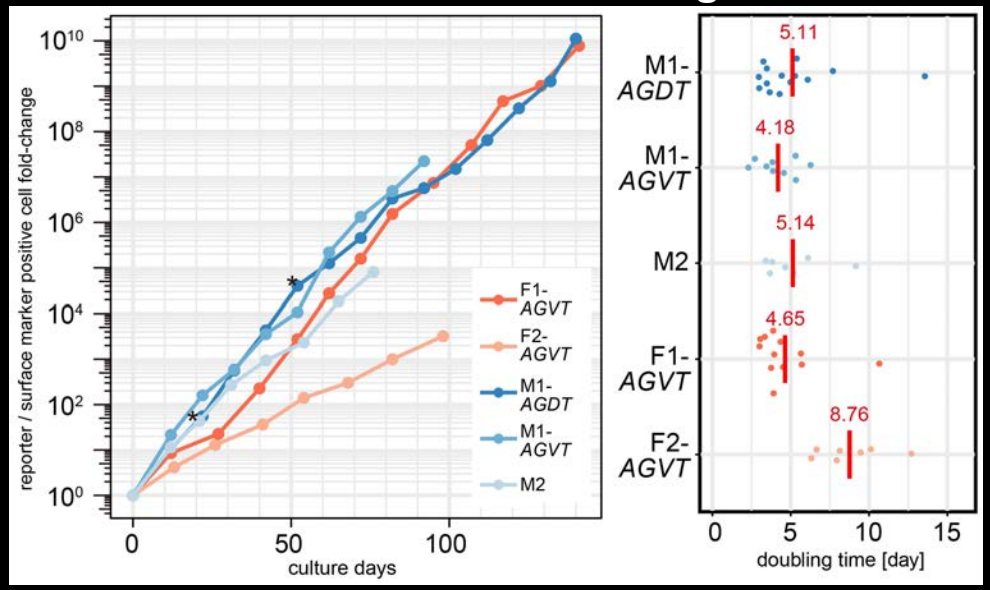
（adRPMI, 15%KSR, 2.5%FBS, SCF/forskolin, BMP2, bFGF , IWR1 on m220-5 feeders）



XY/XX: *TFAP2C-EGFP;DDX4/VASA-tdTomato* (AGVT)

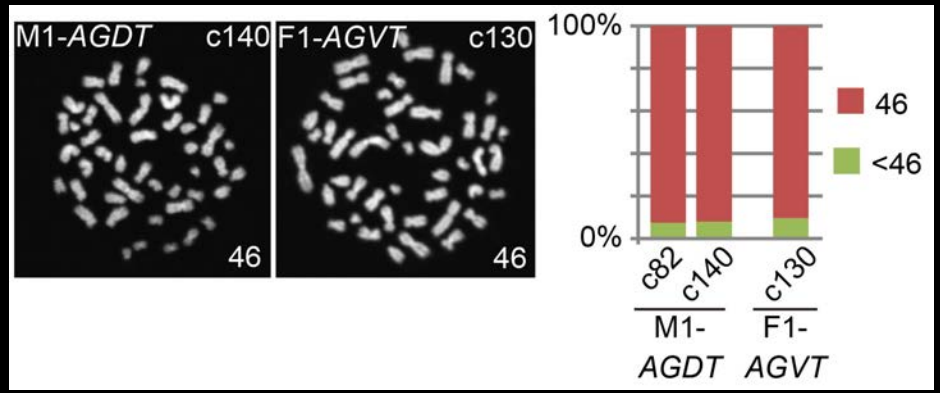
DDX4/VASA: one of the genes up-regulated upon their promoter demethylation in mitotic pro-spermatogonia/oogonia (“germline genes”)

Growth curve and doubling time



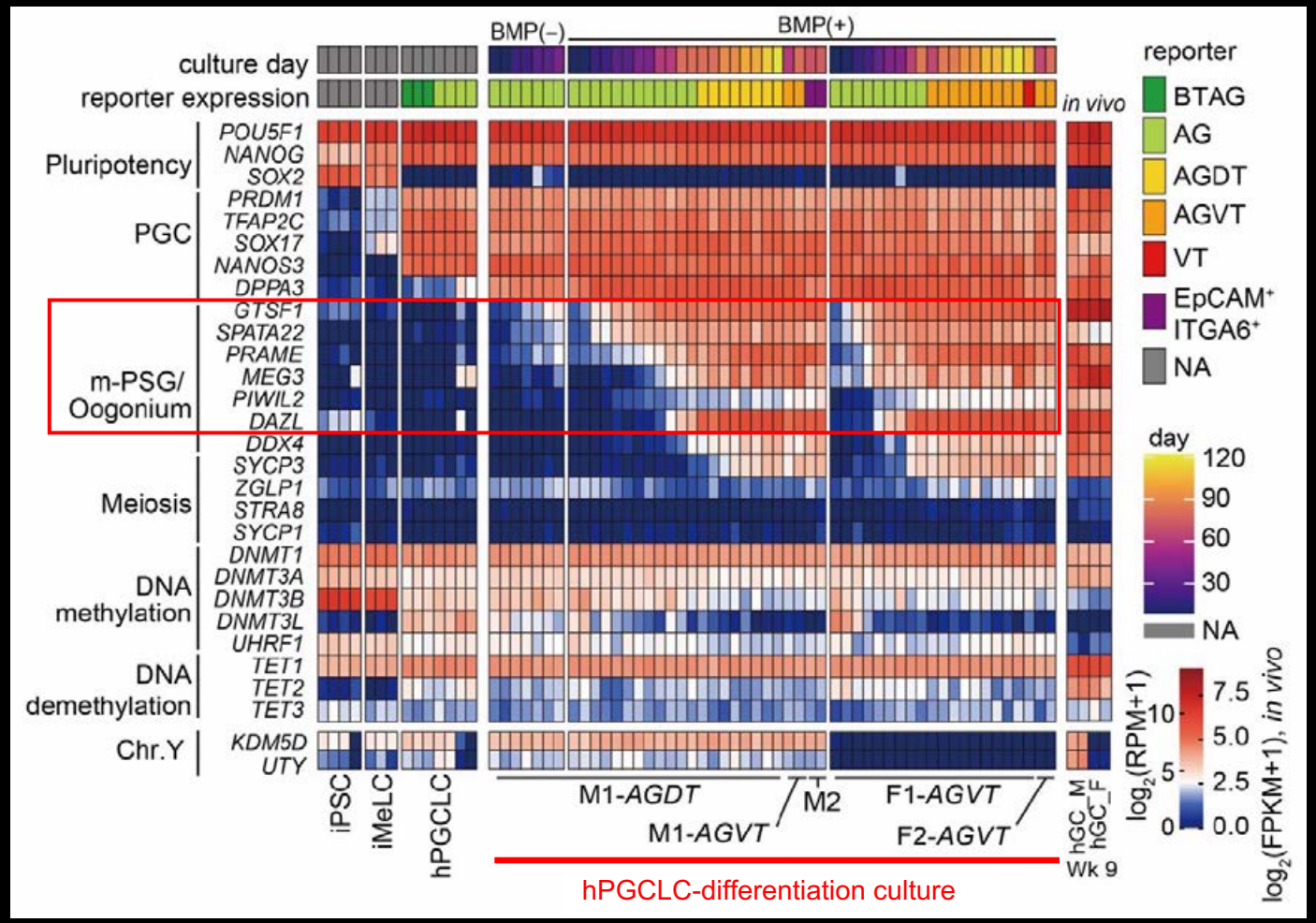
> Billions-fold amplification

Karyotype

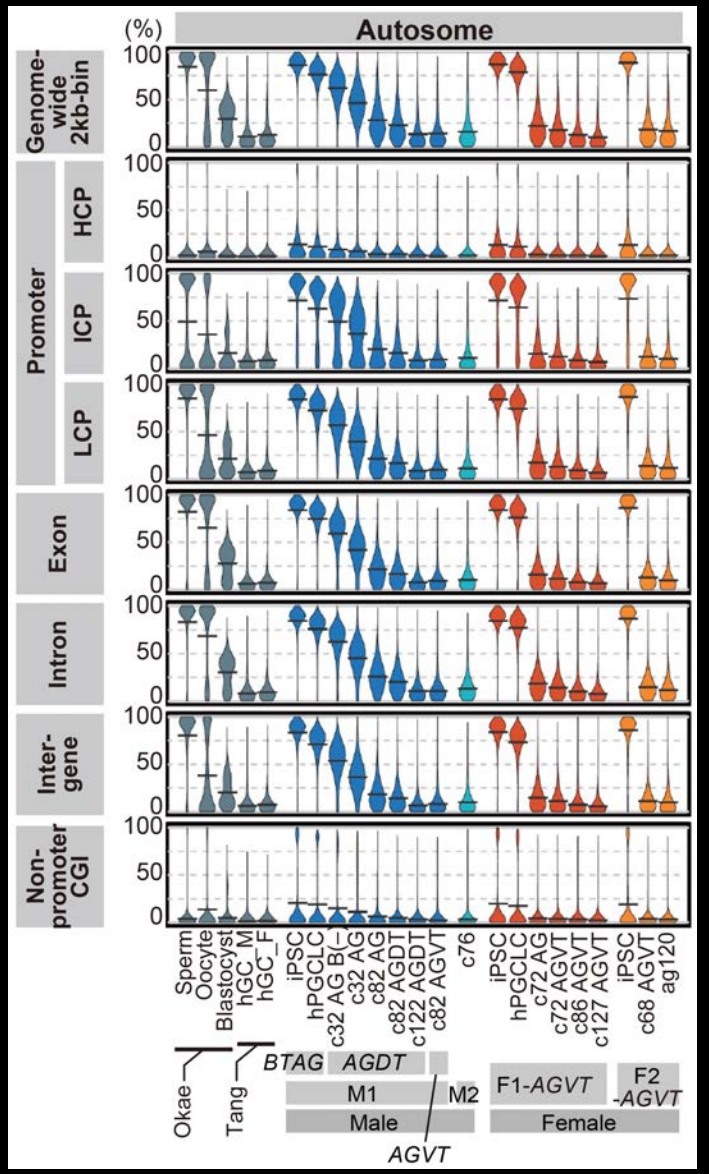


BMPによるヒトPGCLCsの前精原細胞及び卵原細胞への分化

遺伝子発現



DNAメチル化

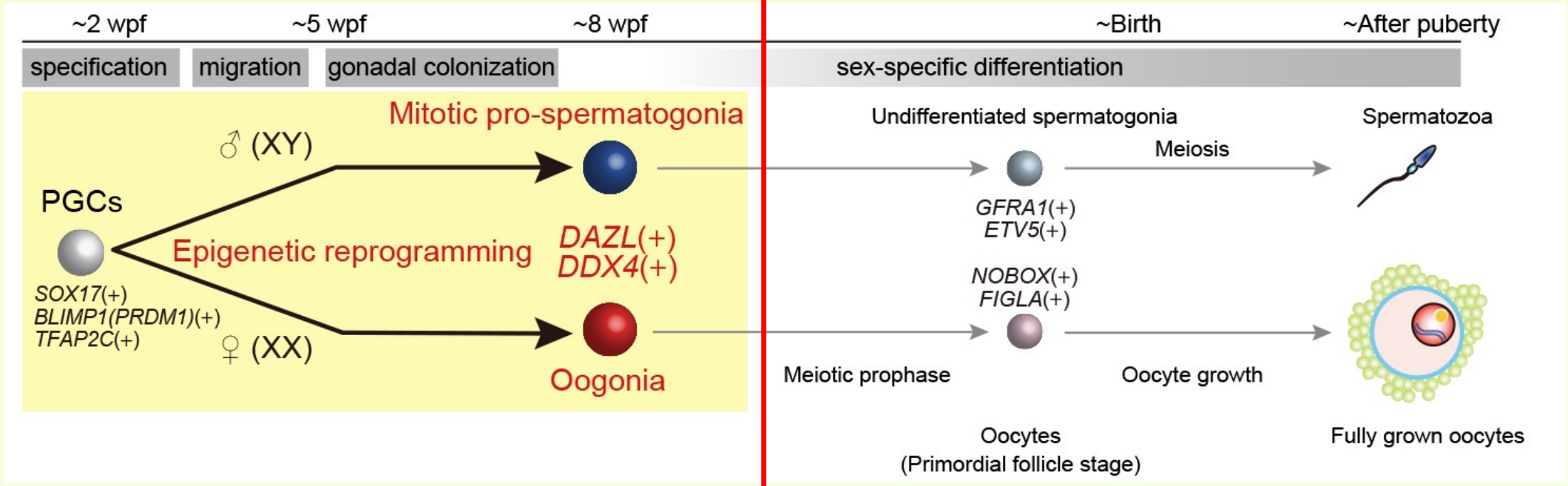


(Murase, Yokogawa et al., Nature, 2024)

ヒト生殖細胞発生過程の試験管内再構成研究：*Human in vitro gametogenesis*

Robustly reconstituted

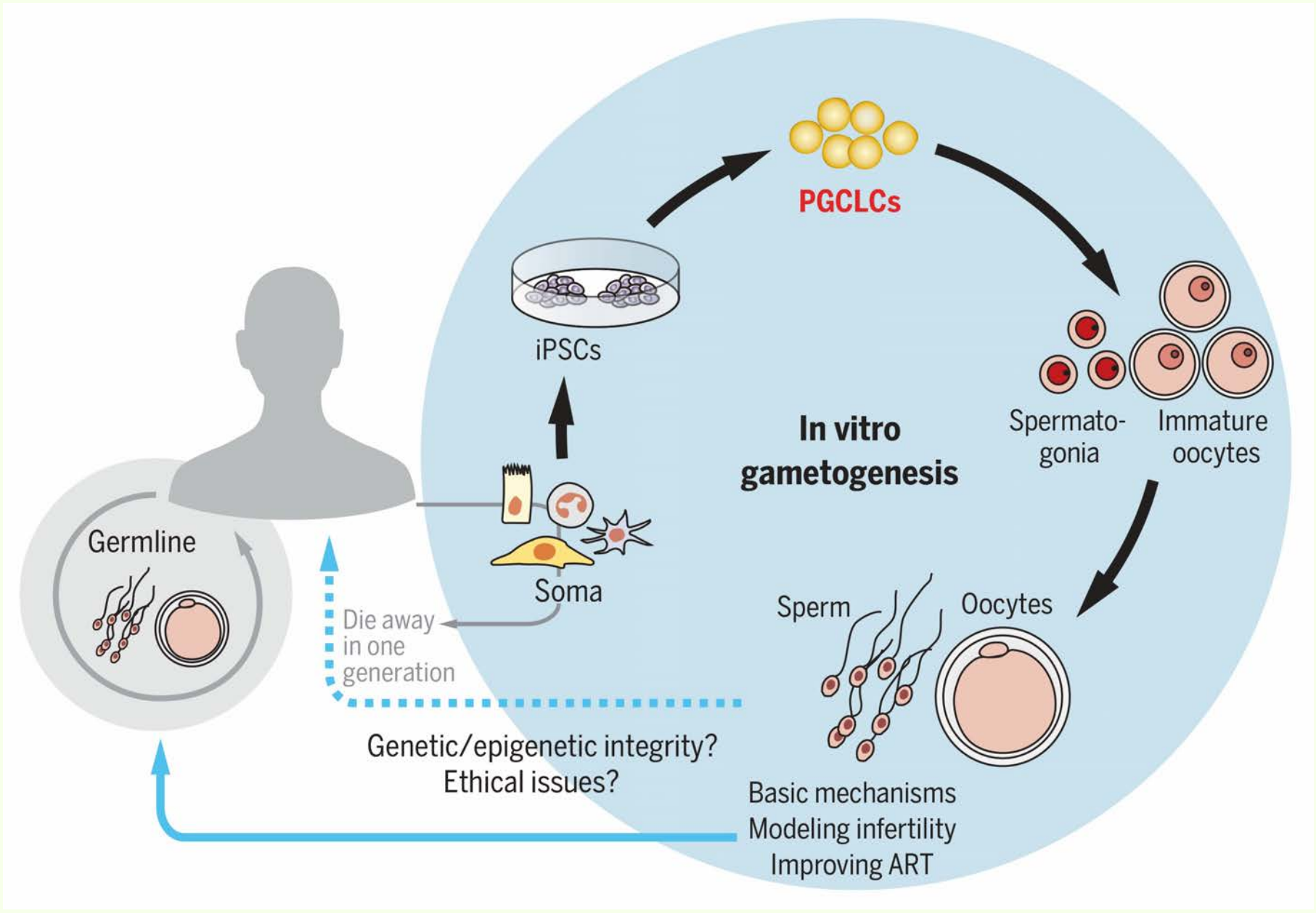
Phase 1: PGC specification	Phase 2: M pro-spermatogonia differentiation (Epigenetic Reprogramming)	Phase 3: Spermatogonia differentiation (Epigenetic Programming)	Phase 4: Spermatogenesis (Meiosis)



Phase 1: PGC specification	Phase 2: Oogonia differentiation (Epigenetic Reprogramming)	Phase 3: Oocyte differentiation (Meiotic prophase)	Phase 4: Oocyte growth

Human in vitro gametogenesis

(Saitou and Hayashi, Science, 2021)



マウスES細胞は個体を形成出来る

Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 8424–8428, September 1993
Developmental Biology

Derivation of completely cell culture-derived mice from early-passage embryonic stem cells

(pluripotency/tetraploid embryos/chimeras)

ANDRÀS NAGY*†‡, JANET ROSSANT*§, RÈKA NAGY¶, WANDA ABRAMOW-NEWERLY¶, AND JOHN C. RODER§¶

*Division of Molecular and Developmental Biology and ¶Division of Neuro- and Immunobiology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, ON, Canada M5G 1X5; †Department of Biochemistry, Loránd Eötvös University, Budapest, Hungary; and §Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada M5S 1A8

Table 1. Developmental potentiality of ES cell lines at early-passage numbers (passages 6–8) tested by ES cell–tetraploid embryo aggregates

Cell line	Tetraploid aggregates transferred, no.	Resorptions, no.	Midgestation dead embryos,* no.	Newborns, no.	Recovered newborns, no.
R1	53	21	6	9	3†
R2	31	7	7	0	0
R6	40‡				
R13	37	17	5	2	0

*Resorptions above 4 mm in diameter at term were considered in this category.
†Two of the three recovered newborns survived and reached adulthood.
‡Uteruses with obvious signs of early abortion were recovered from these recipients at term.

Table 3. Prenatal developmental potential of R1 tested by R1–tetraploid embryo aggregates as a function of *in vitro* culture time (passage number)

No. of passages	Tetraploid aggregates transferred, no.	Implantations, no.	Resorptions, no.	Midgestation dead embryos, no.	Newborns, no.	Recovered newborns, no.	Survivors, no.
6–14	130	87 (67)	45 (35)	22 (17)	20 (15)	9	5
16–24	162	109 (67)	51 (31)	46 (28)	12 (7)	1	0
	χ^2	0.002	0.22	4.10	4.19	8.37	6.23
	P (df = 1)	≫0.2	≫0.2	<0.05	<0.05	<0.01	<0.02

The numbers in parentheses are percentages of aggregates transferred.

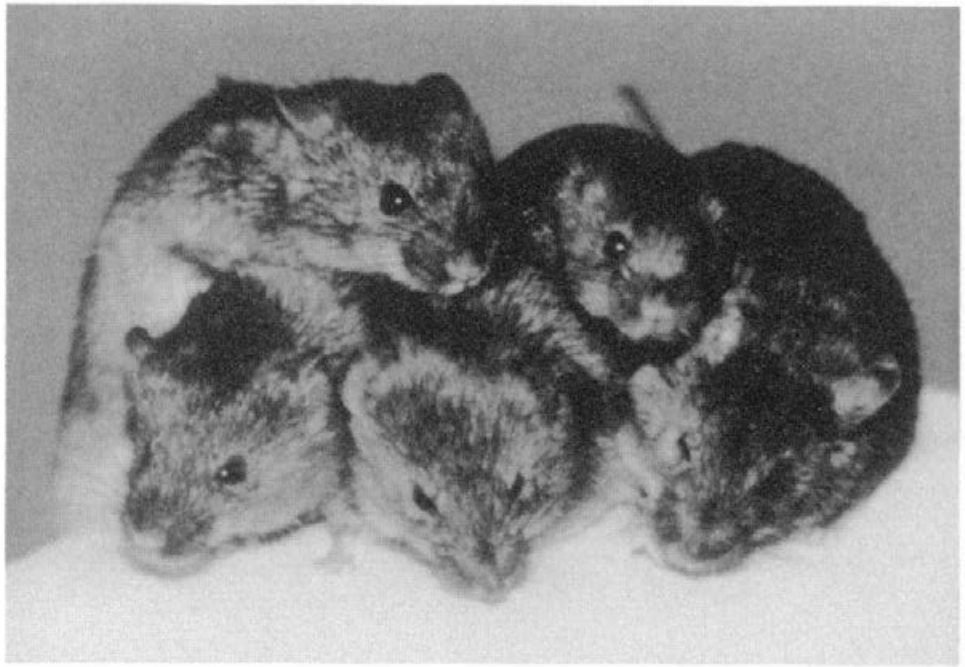


FIG. 3. Group of five R1-derived animals produced by R1–tetraploid embryo aggregates.

マウスES細胞は個体を形成出来る

ARTICLES

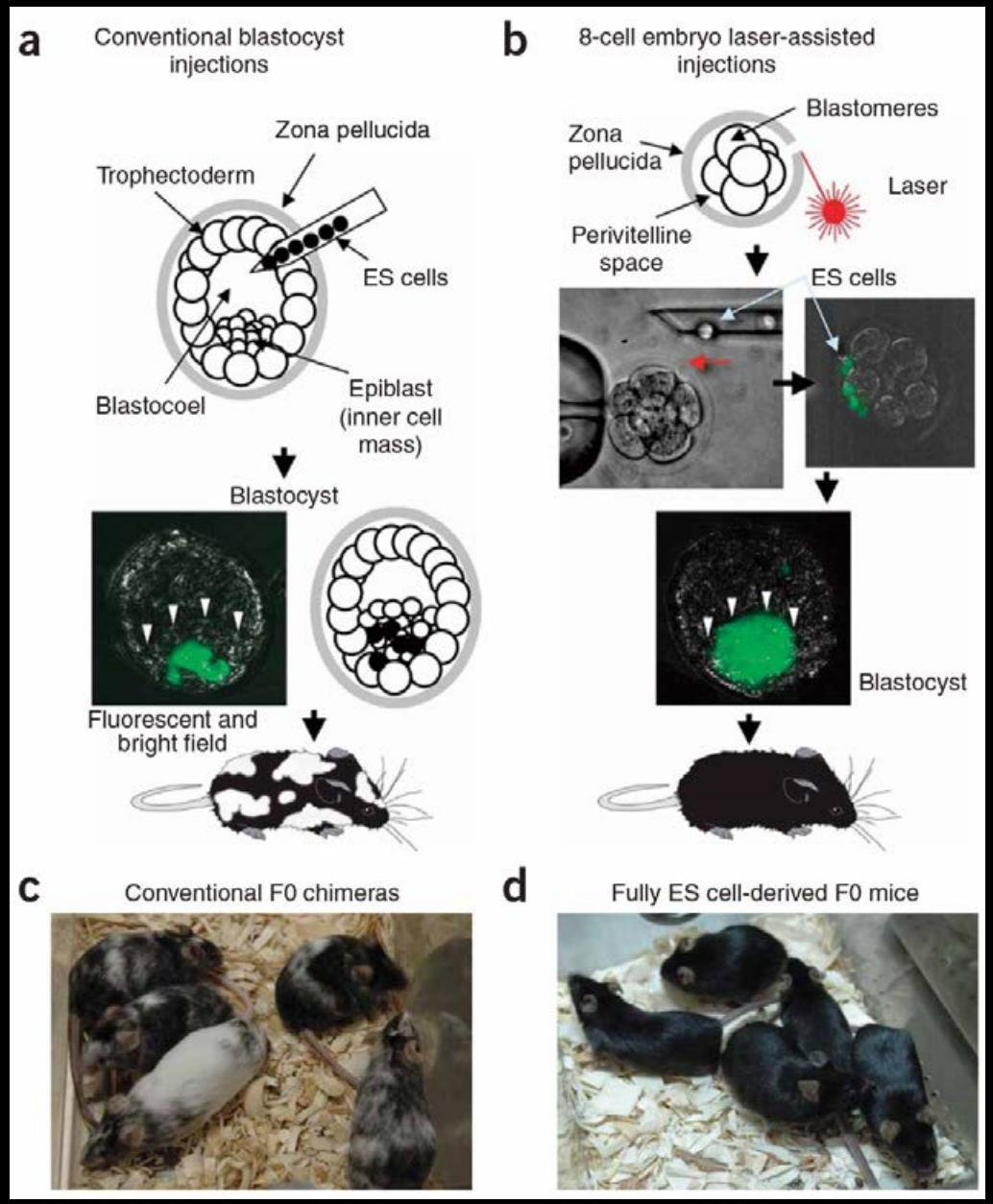
nature biotechnology

F0 generation mice fully derived from gene-targeted embryonic stem cells allowing immediate phenotypic analyses

William T Poueymirou[1], Wojtek Auerbach[1], David Frendewey[1], Joseph F Hickey[1], Jennifer M Escaravage[1], Lakeisha Esau[1], Anthony T Doré[1], Sean Stevens[1], Niels C Adams[1,2], Melissa G Dominguez[1], Nicholas W Gale[1], George D Yancopoulos[1], Thomas M DeChiara[1] & David M Valenzuela[1]

A useful approach for exploring gene function involves generating mutant mice from genetically modified embryonic stem (ES) cells. Recent advances in genetic engineering of ES cells have shifted the bottleneck in this process to the generation of mice. Conventional injections of ES cells into blastocyst hosts produce F0 generation chimeras that are only partially derived from ES cells, requiring additional breeding to obtain mutant mice that can be phenotyped. The tetraploid complementation approach directly yields mice that are almost entirely derived from ES cells, but it is inefficient, works only with certain hybrid ES cell lines and suffers from nonspecific lethality and abnormalities, complicating phenotypic analyses. Here we show that laser-assisted injection of either inbred or hybrid ES cells into eight cell–stage embryos efficiently yields F0 generation mice that are fully ES cell–derived and healthy, exhibit 100% germline transmission and allow immediate phenotypic analysis, greatly accelerating gene function assignment.

(*Nat. Biotech.*, 25, 91-99, 2007)



霊長類ES細胞の個体形成能は未証明

CellPress

Cell

Article

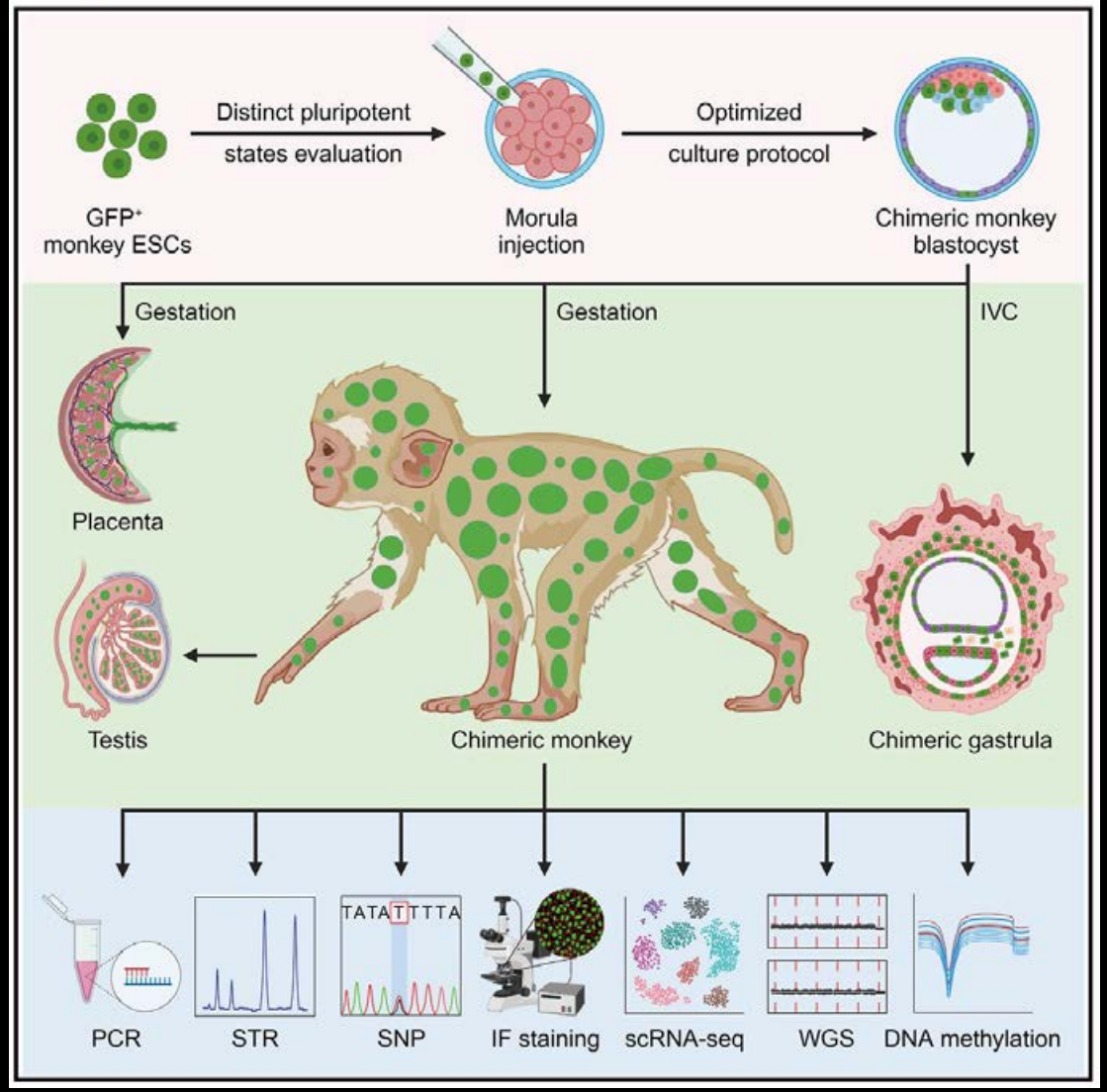
Live birth of chimeric monkey with high contribution from embryonic stem cells

Jing Cao,[1,5,13] Wenjuan Li,[2,13] Jie Li,[1,13] Md. Abdul Mazid,[2,13] Chunyang Li,[1,13] Yu Jiang,[9,13] Wenqi Jia,[2,4,13] Liang Wu,[2] Zhaodi Liao,[1,4] Shiyu Sun,[1,4] Weixiang Song,[1] Jiqiang Fu,[1] Yan Wang,[1] Yong Lu,[1] Yuting Xu,[1] Yanhong Nie,[1] Xinyan Bian,[1] Changshan Gao,[1] Xiaotong Zhang,[1] Liansheng Zhang,[1] Shenshen Shang,[1] Yunpan Li,[2] Lixin Fu,[2] Hao Liu,[2] Junjian Lai,[2] Yang Wang,[6] Yue Yuan,[6] Xin Jin,[7,10] Yan Li,[7] Chuanyu Liu,[7] Yiwei Lai,[6] Xuyang Shi,[6] Patrick H. Maxwell,[11] Xun Xu,[6,7,12] Longqi Liu,[6] Muming Poo,[1,3,4] Xiaolong Wang,[5] Qiang Sun,[1,3,4,*] Miguel A. Esteban,[6,8,9,*] and Zhen Liu[1,3,4,14,*]

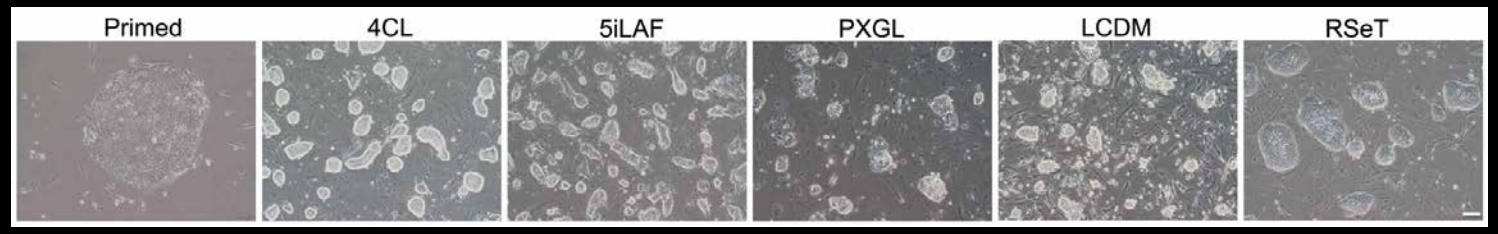
[1]Institute of Neuroscience, CAS Center for Excellence in Brain Science and Intelligence Technology, CAS Key Laboratory of Primate Neurobiology, State Key Laboratory of Neuroscience, Chinese Academy of Sciences, Shanghai 200031, China

(*Cell*, 186, 4996-5014)

Graphical abstract



霊長類ES細胞の個体形成能は未証明



ID of pregnant surrogates	Transferred blastocysts	Injected ESC lines	Fetuses	Gender	Outcome
892	1	cyES-1	1	Female	Early abortion at day 116 (#1)
650	2	cyES-1	1	Female	Early abortion at day 78 (#2)
0314	2	cyES-1	1	Female	Early abortion at day 103 (#3)
525	2	cyES-1	2	/	Early abortion without fetus
957	2	cyES-2	2	Female	Caesarean section at day 145 (#4)
626	2	cyES-2	1	Female	Normal delivery at day 168 (#5)
846	2	cyES-2	1	Female	Normal delivery at day 156 (#6)
542	2	cyES-2	1	/	Early abortion without fetus
588	1	cyES-3	1	Female	Normal delivery at day 153 (#7)
957	2	cyES-2	2	Male	Caesarean section at day 145 (#8)
669	2	cyES-1	1	Male	Early abortion at day 90 (#9)
874	2	cyES-2	1	Male	Normal delivery at day 159 (#10)

A total of 91 blastocysts were obtained from 206 injected morulae. Of these, 74 blastocysts with clear GFP signals were transferred into 40 surrogates, yielding 12 surrogates with confirmed pregnancy. Among the 12 pregnancies, 4 aborted fetuses and 6 full-term live offspring were obtained.

The GFP sequence was detected by PCR analysis in one aborted male fetus (#9) and one live-birth male (#10)

After surviving for 10 days, the health state of chimeric monkey #10 deteriorated with respiratory failure and hypothermia, and it was euthanized by a veterinarian for detailed analysis.

多能性細胞（ES細胞、iPS細胞）からいのちを生み出せるのか？

マウス多能性幹細胞が生殖細胞や全個体を “生み出す” 能力を有することは、多くの発生工学的研究により証明されてきた。

マウス多能性幹細胞を用いた生殖細胞発生過程の試験管内再構成研究（マウス IVG 研究）は、そうした先行する科学的知見に裏打ちされた研究と言える。

一方、ヒトや非ヒト科霊長類の多能性幹細胞が、機能的な生殖細胞や全個体を “生み出す” 能力を有することを証明した発生工学的研究は存在しない。

ヒト多能性幹細胞を用いた生殖細胞発生過程の試験管内再構成研究（ヒト IVG 研究）は、世界的に活発に推進されており、そうした証明がなされる以前に、ヒト精子様細胞・ヒト卵子様細胞が作成される可能性がある。

ヒト多能性幹細胞の遺伝学的変異、エピジェネティックな変異は多く報告されている。それに由来する生殖細胞様細胞の遺伝学的変異、エピジェネティックな変異の研究はヒト IVG 研究の重要なテーマとなる。

ヒト IVG 研究は、ゲノム科学研究、エピジェネティックス研究と合わせ、拙速を排し、丁寧に推進することが肝要となる。