6. Doppelstunde 18.11.2020 ESF-II/8 WS2020/21

3. Teil: Die Herstellung von Lebewesen I (Zusammenfassung der 5. DSt.)

1. Die Herstellung von Lebewesen aus einer lebenden diploiden Zelle.

1.1. Diploide (2n) Zellen

1.1.1.-3. PSCs→ Blastuloide/Gastruloide → Implantation in Placentoide.

1.1.4. PSCs → Primordial Germ cells (PGCs) Meiose notwendig!

1.1.5. PGCs → Gonadiode → Ovaroide / Testiculoide → Oocyten/Spermine

1.2.. Haploide (1n) Zellen

1.2.1. Partenogenetische PSCs

1.2.2. Androgenic PGC-like ahESCs

2. Primäre Keimzellen (Primordial gern cells (PGCs) – Einwandern der PCG in den Mesonephros

2.1.-3. Mehrere unterschiedliche epigenetische Änderungen während der Keinzellenentwicklung

2.4. Meiose in weiblichen und männlichen Keimzellen

3. Der weibliche Reproduktionszyklus in vivo (3.1) und ex vivo (3.2)

4. Der männliche Reproduktionszyklus in vivo (4.1) und ex vivo (4.2)

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2. BASICS OF THE MEIOTIC CELL CYCLE: ANOTHER LAYER OF COMPLEXITY (NACHTRAG ZU 2.4. MEIOSE)

Meiosis is restricted to germ line cells and has features of cell division that simply do not exist in somatic cells. One striking unique feature, for example, is that there are two metaphase segregation events that occur without an intervening round of DNA synthesis. Another aspect of meiosis that distinguishes it from mitosis is the behaviour of sister chromatids during the first meiotic division. That is, in mitosis, the sister chromatids separate in the single phase of chromosome segregation, while in meiosis, sister chromatids do not separate from one another until the second round of segregation—the first stage involves segregation of homologous chromosomes. In addition, it is critical that meiosis-unique processes, including the synapsis or pairing of homologous chromosome, recombination and formation of chiasmata, be strictly coordinated with cell cycle progression, as it would be catastrophic for the gamete cell to attempt to move into the cell cycle without these other processes having been completed.

Higher organisms are characterized by having sexually dimorphic gametes. While male and female germ cells have stages of cell cycle regulation in common, including a mitotic proliferative stage, entry into meiosis, completion of a reductive division and entry into a quiescent state prior to fertilization, the timing of these events and the stage of development at which they occur differ in the two sexes (reviewed in Wolgemuth *et al*. 1995, 2002; Handel & Eppig 1998). In the mouse model, the germ line is specified early in embryonic development, probably as early as embryonic day 6.0–6.5. The progenitor germ cells migrate from the proximal epiblast to the gonadal ridge, at which point sexual determination based upon genotype occurs. The germ cells then follow either a male pathway, in which the cells enter into a mitotic arrest, or alternatively, a female pathway, in which they enter into pre-meiotic DNA synthesis and meiotic prophase. Thus, germ cells of both sexes undergo mitotic divisions in the embryonic gonad, but the female germ cells enter meiosis during foetal development, whereas this is a postnatal event in the male. Once the male germ cell has entered meiosis, the process continues without interruption until the haploid sperm is produced. In contrast, the oocyte is arrested in the diplotene stage of meiotic prophase I, where it can remain for months or years depending on the species. Following a growth period, which begins at puberty, the oocyte resumes meiosis, but arrests a second time, at metaphase II. Fertilization then triggers the completion of meiosis and extrusion of the second polar body. Given these striking differences in the sequence of mitotic and meiotic events, it is almost given that the genetic programme underlying this regulation will be distinct between the male and female and will be reflected in a sexual dimorphism in the genes involved in regulating these processes (Handel 1998; Wolgemuth *et al*. 2002). See **Regulating mitosis and meiosis in the male germ line: critical functions for cyclins. 2012** D.J. Wolgemuth[1]and S.S. Roberts, Philos Trans R Soc Lond B Biol Sci. 365(1546): 1653–1662. doi: 10.1098/rstb.2009.0254

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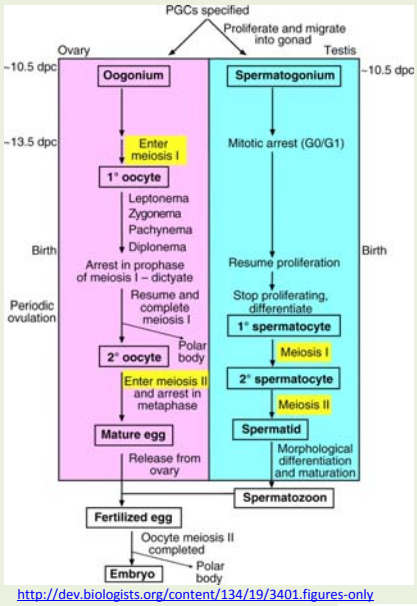
2

Vergleich der Meiose in Keimzellen beider Geschlechter (Kapitel: 2a, 3a und 4a)

Weibchen



http://dev.biologists.org/content/134/19/3401.figures-only

Männchen

6. Doppelstunde ESF II 2020

In vitro Reproduktion von Säugetieren inklusive Homo sapiens

3. Teil: Die Herstellung von Lebewesen II

3. Der weibliche Reproduktionszyklus 3.1. in vivo und 3.2. ex vivo

4. Der männliche Reproduktionszyklus 4.1.in vivo und 4.2. ex vivo

5. Herstellung von Zygoten

6. Ethische und juridische Überlegungen zur Herstellung von Lebewesen.

In vitro Reproduktion von Säugetieren inklusive Homo sapiens

Somatische Zelle → Keimbahnstammzellen (Primordial Germ Cells (PGCs)) →

→ Oozyten und Spermien → in vitro Fertilisation (iVF) oder Klonen→ künstlicher Uterus

und Plazenta oder Leihmutter → Fötalentwicklung → Neugeborenes Lebewesen.

Notwendige Voraussetzungen hierfür ist die Herstellung von Gameten bzw. Zygoten aus diploiden Zellen - gefolgt vom Einnisten des Blastocysten in einem künstlichen Uterus oder einer Leihmutter.

3.2. Der weibliche Reproduktionszyklus ex vivo

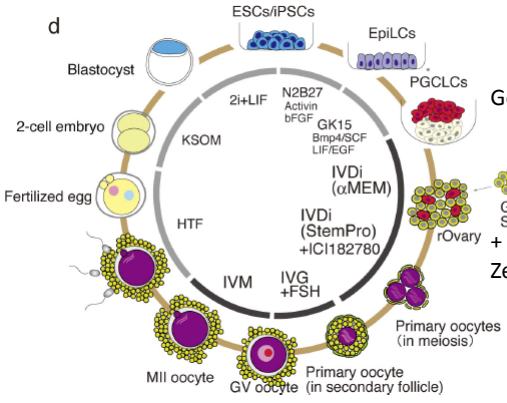
LETTER

doi:10.1038/nature20104

2016

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[2], Norio Hamada[3,4], So Shimamoto[3], Takuya Imamura[3], Kinichi Nakashima[1], Mitinori Saitou[5,6,7,8] & Katsuhiko Hayashi[1,9]*



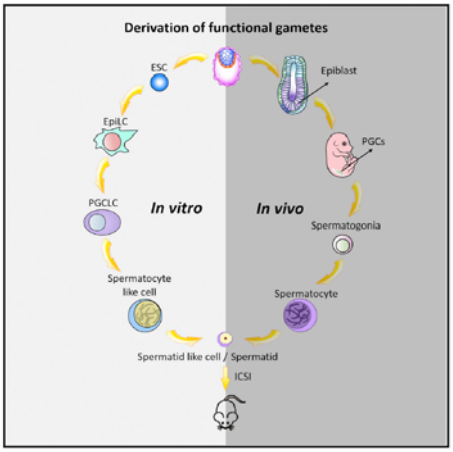
Genetische Selektion

+ Primäre somatische Zellen

4.2. Der männliche Reproduktionszyklus ex vivo

Complete Meiosis from Embryonic Stem Cell-Derived Germ Cells In Vitro. Zhou et al., 2016
Cell Stem Cell. 2016 Mar 3;18(3):330-40. doi: 10.1016/j.stem.2016.01.017. Epub **2016** Feb 25.

In Vitro Derivation and Propagation of Spermatogonial Stem Cell Activity from Mouse Pluripotent Stem Cells. Ishikura Y, Yabuta Y, Ohta H, **Hayashi K**, Nakamura T, Okamoto I, Yamamoto T, Kurimoto K, Shirane K, Sasaki H, Saitou M. Cell Rep. 2016 Dec 6;17(10):2789-2804. doi: 10.1016/j.celrep.2016.11.026



Link to paper

Are human oocytes from stem cells next?
Johan E J Smitz & Robert B Gilchrist
Nature Biotechnology **volume 34**, pages 1247–1248 (2016)

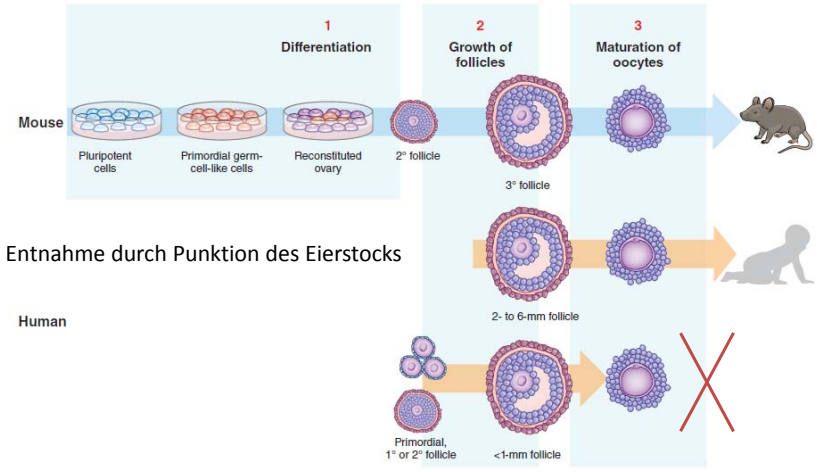


Figure 1 Hikabe *et al.*[1] succeeded in producing mouse oocytes entirely *in vitro* from stem cells. Making human oocytes *in vitro* is likely to be particularly challenging. To date, human pregnancies have been achieved after *in vitro* culture of nearly full-grown oocytes from 2- to 6-mm follicles, but progress in using earlier-stage oocytes has been limited.

Human induced pluripotent stem cells and male infertility: an overview of current progress and perspectives

Fang Fang [1], Zili Li [1, 2], Qian Zhao [1], Honggang Li [1], Chengliang Xiong Review. 2018 Feb 1;33(2):188-195. doi: 10.1093/humrep/dex369.

Abstract

Recently, significant progress has been made in ART for the treatment of male infertility. However, current ART has failed to help infertile patients with non-obstructive azoospermia, unless donor sperm is used. In fact, most couples wish to have their own genetically related child. Human induced pluripotent stem cells (hiPSCs) can be generated from patients' somatic cells and in vitro derivation of functional germ cells from patient-specific iPSCs may provide new therapeutic strategies for infertile couples. The overall developmental dynamics of human primordial germ cells are similar to that in mice, but accumulating evidence suggests that there are **crucial differences between human and mouse PGC specification**. Unlike mouse iPSCs (miPSCs) in naive state, hiPSCs exhibit a primed pluripotency which possess less potential for the germ cell fate. Based on research in mice, male germ cells at different stages have been derived from hiPSCs with different protocols, including spontaneous differentiation, overexpression of germ cell regulators, addition of cytokines, co-culture with gonadal cells in vitro and xeno-transplantation. The aim of this review is to summarize the current advances in derivation of male germ cells from hiPSCs and raise the perspectives of hiPSCs in medical application for male infertility, as well as in basic research for male germ cell development.

5. Herstellung von Zygoten

5.0. In vitro Fertilisation (IVF) seit 1970 möglich und in ca. 3% der Versuche erfolgreich.

5.1. Herstellen von bi-maternalen (parthenogenetischen) und bi-androgenetischen Zygoten und Mäusen (Zur Erforschung der Unterschiede zwischen weiblicher und männlicher epigenetischer Regulation der Keimzellenentwicklung)

Vorgeschichte: Herstellung von haploiden ESCs

5. 1.1. Partenogenetische PSCs → 1n ESCs[1] → PGCs/Keimzellen.

[1]Martin Leeb bzw. Ulrich Elling, 2011; hpESC (2016)

5.1.2. Androgenic PGC-like ahESCs aus Spermien und entkernten Oocyten (2012) →

Alle sind 19+X und nicht 19+Y !

Es stellte sich nach der Herstellung von haploiden PSCs heraus, dass sich im Laufe der Kultur derer das Methylierungsmuster der DNA in Richtung dessen, das in Gameten vorgefunden wird, verändert.

5.1. Bi- maternal und Bi- parternale Mäuse 2018

Generation of Bimaternal and Bipaternal Mice from Hypomethylated Haploid ESCs with Imprinting Region Deletions

Zhi-Kun Li [5]Le-Yun Wang [5]Li-Bin Wang [5]Wei LiQi Zhou [6]Bao-Yang Hu Show all authors Show footnotes

Published:October 11, 2018 DOI:https://doi.org/10.1016/j.stem.2018.09.004

← verpflichtend zu lesen!

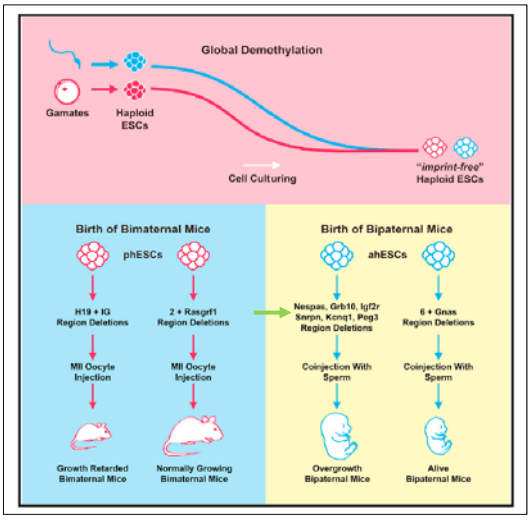
Highlights

- Haploid ESCs display PGC-like methylation profiles following *in vitro* cultivation
- Parthenogenetic and androgenetic haploid ESCs show different demethylation dynamics
- phESCs carrying 3 deleted imprinted regions support normal growth of bimaternal mice
- ahESCs carrying 7 deleted imprinted regions produce live full-term bipaternal mice

Summary

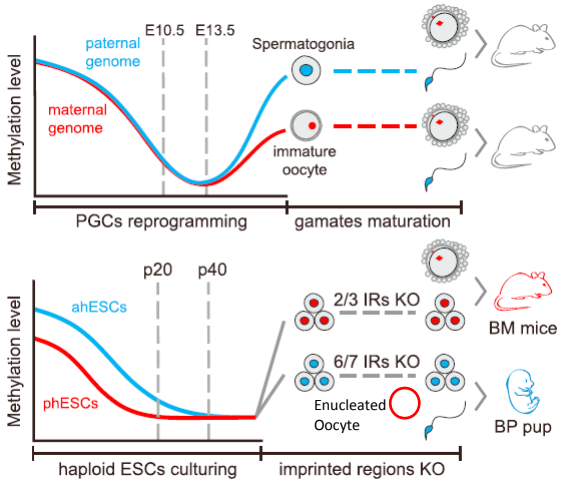
Unisexual reproduction is widespread among lower vertebrates, but not in mammals. Deletion of the H19/Igf2 imprinted region in immature oocytes produced bi-maternal mice with defective growth; however, bi-paternal reproduction has not been previously achieved in mammals. We found that cultured parthenogenetic and androgenetic haploid embryonic stem cells (haESCs) display DNA hypomethylation resembling that of primordial germ cells. Through MII oocyte injection or sperm coinjection with hypomethylated haploid ESCs carrying specific imprinted region deletions, we obtained live bi-maternal and bi-paternal mice. Deletion of 3 imprinted regions in parthenogenetic haploid ESCs restored normal growth of fertile bi-maternal mice, whereas deletion of 7 imprinted regions in androgenetic haploid ESCs enabled production of live bi-paternal mice that died shortly after birth. Phenotypic analyses of organ and body size of these mice support the **genetic conflict theory** of genomic imprinting. Taken together, our results highlight the factors necessary for crossing same-sex reproduction barriers in mammals.

5.1. Bi- maternal und Bi- parternale Mäuse 2018



→ Injektion eines Kerns einer phESCs in einem MII Oozyten bzw.
Koinjektion eines Kernes einer ahESC gemeinsam mit einem Spermium in einem entkernten MII Oozyten.

Gegenüberstellung: natürliche und unisexulelle Herstellung von Lebewesen



Imprinting und „Genetic conflict theory“

Was sind **imprinted genes**?

Regionen auf Genen (derzeit ca. 100 bekannt) die durch epigenetische Mechanismen (hier vor allen die Methylierung von CpG Islands) auf den maternalen oder paternalen Schwesterchromatiden alternativ stillgelegt werden. Vorallem 5mC aber auch 6mA, 4mC, etc.

z.B.: Das Igf2 Gen ist paternal aktiv und maternal inaktiviert. (Xist und H19 umgekehrt) - Dies verhindert eingeschlechtliche Fortpflanzung. - Wenn dennoch uniparentale Disomie auftritt (durch temporäre Trisomie in den Oocyten), kommt es zu Fehlbildungen.

Z.B.: Prader Willi Syndrom durch Verlust eines Teils des paternalen Chromosoms 15, d.h. wenn also in diesem Bereich maternale Disomie* vorliegt. Liegt in demselben Bereich paternale Disomie (oder maternale Mikrodeletionen) vor kommt es zum Angelman Syndrom (siehe auch Wikipedia und UBE-3A Gen in OMIM Datenbank).

*Bei einer **Uniparentale Disomie** stammen beide Chromosomen eines homologen Chromosomenpaares von einem Elternteil.

Imprinting und „Genetic conflict theroy“

Allgemein glaubt man folgende „Regel“ zu finden:

Aktive paternale Allele fördern das Wachstum des Embryos (negative Konsequenz: Embryo wird zu groß und schädigt die Mutter). zB Igf

Altive maternale Allele fördern beschützen die Mutter vor der Ausbeutung durch den Embryo und fördern so das Überleben der Mutter und das entstehen von mehreren Nachkommen. (negative Konsequenz: Embryonen werden zu klein und verkümmern.) z.B. H19

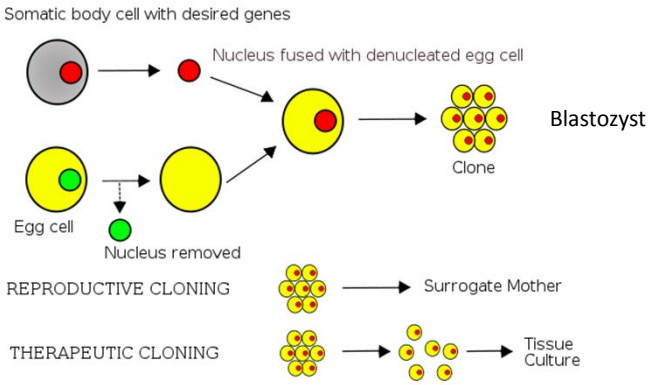
Diese Befunde führten zur „**Genetischen Konflikttheorie**“ zwischen den Geschlechtern.

5.2. Alternative zur Herstellung von Gamenten: Klonen (SCNT)

Herstellung von Zygoten

- von Schafen 1996 (gefolgt von vielen andere Huftiere → Landwirtschaft, Tierzucht und Arterhaltung)
- Klonen von Mäusen 1972 – 1999 (Igf2 locus imprinting entscheidend)
- Klonen von Menschen 2001 - (~~2005 Hwang~~) – 2013 Mitalipov und Co.
- Klonen von hESCs mit homozygoten human leukocyte antigen (HLA) Haplotypus 2020

Alternative zur Herstellung von Gamenten: Klonen (SCNT)



Siehe Wikipedia

Auch hier sind die korrekten epigenetischen Modifikationen des Genoms während der Beendigung der Meiose II und der Verschmelzung der maternalen und paternalen Pronuklei entscheidend und spezies-spezifisch.

- Inhibierung der Histondeacetylaseaktivität mittels 10nM **Trichostantin A** für 12 h ist eine unabdingbare Voraussetzung, dass MII Oocyten sich weiterentwickeln. Nur so kommt es zur Ausbildung eines Pronukleus.
- Ebenso waren Koffeingaben notwendig, weil wahrscheinlich Koffein. Die Phosphorylierung von cyclin B inhibiert und CDC2 + cyclin B, wenn phosphoryliert =„Maturation promoting factor“ → Koffein setzt so den „cell cycle checkpoint“ außer Kraft und fördert die Teilung der Zygote in zwei Blastomere.
- „… H3K27me3 functions as an epigenetic barrier and that KDM6A overexpression improves SCNT efficiency by facilitating transcriptional reprogramming.“-Zhou, C., Wang, Y., Zhang, J., Su, J., An, Q., Liu, X., Zhang, M., Wang, Y., Liu, J., Zhang, Y. H3K27me3 is an epigenetic barrier while KDM6A overexpression improves nuclear reprogramming efficiency. FASEB J. 2019 Mar;33(3):4638-4652. doi: 10.1096/fj.201801887R

PSC derivation efficiency ~9%

Stem Cell Reports
Article
ISSCR
OPEN ACCESS

Cryopreserved Human Oocytes and Cord Blood Cells Can Produce Somatic Cell Nuclear Transfer-Derived Pluripotent Stem Cells with a Homozygous HLA Type

Jeoung Eun Lee,[1,8] Ji Yoon Lee,[1,8] Chang-Hwan Park,[2,8] Jin Hee Eum,[3] Soo Kyung Jung,[1] A-Reum Han,[4] Dong-Won Seol,[4] Jin Saem Lee,[2] Hyun Soo Shin,[5] Jung Ho Im,[5] Taehoon Chun,[6] Kyungsoo Ha,[7] Deok Rim Heo,[7] Tae Ki Yoon,[4] and Dong Ryul Lee[1,4,*]

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