2017年5月29日

発生学(3):第2章

医学系研究科附属創生応用医学研究センター長
脳神経科学コアセンター長
発生発達神経科学分野教授
大隅典子

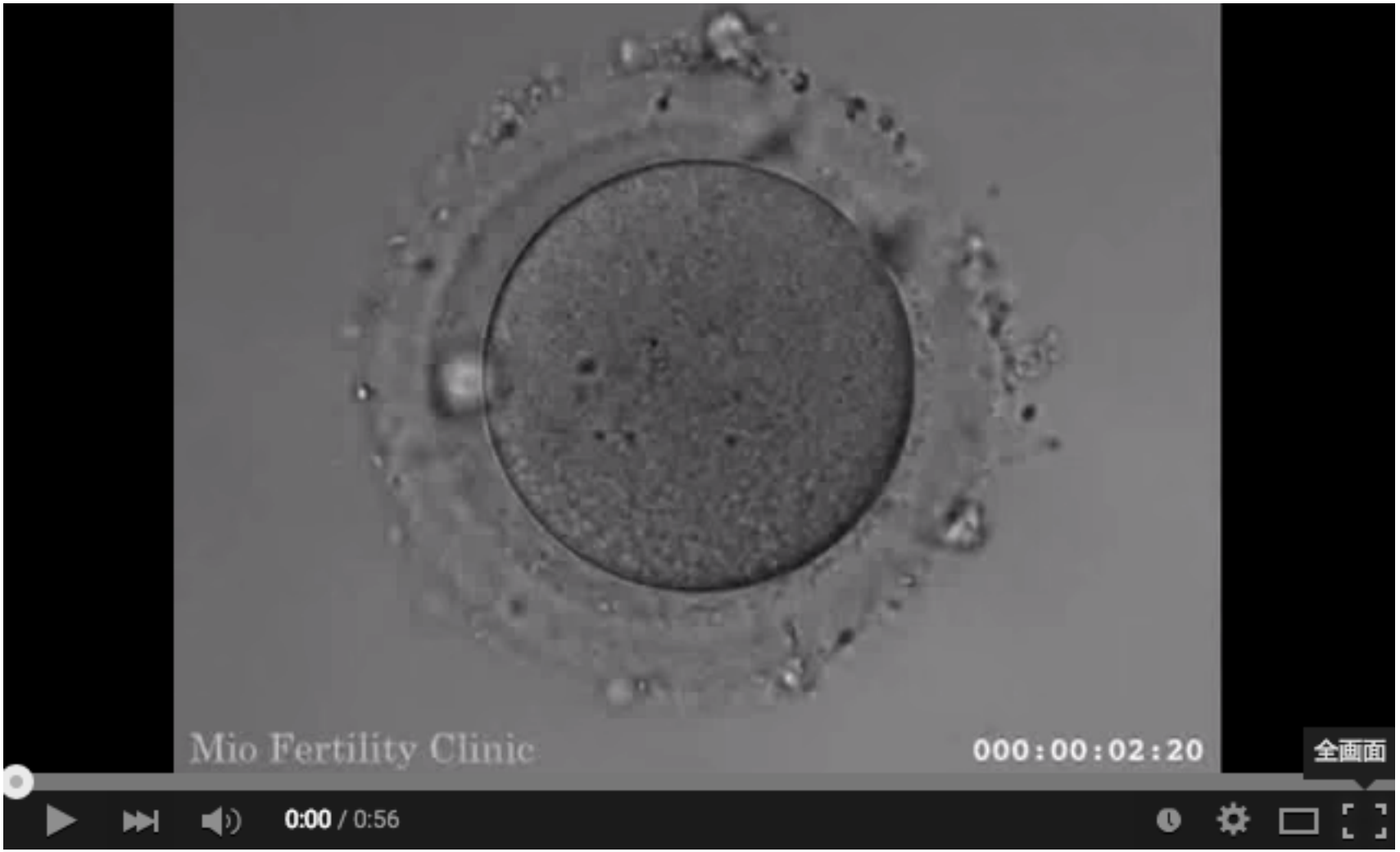
Center for
Neuroscience,
ART

発生第２週：二胚葉期

- 内部細胞塊が二層性に変わる
  - 上胚盤葉 epiblast
  - 下胚盤葉 hypoblast
  - 羊膜腔 amniotic cavity
  - 胚盤胞腔 blastocyst cavity
- 卵黄嚢 yolk sac 形成
  - 下胚盤葉から胚盤胞腔への細胞移動による一次卵黄嚢と二次卵黄嚢
- 胚体外中胚葉 extraembryonic mesoderm
  - 胚体外体腔 extraenbryonic coelom
- 絨毛膜 chorion
- 栄養膜から胎盤 placenta 形成へ
  - 栄養膜細胞層 cytotrophoblast
  - 栄養膜合胞体層 syncytiotrophoblast

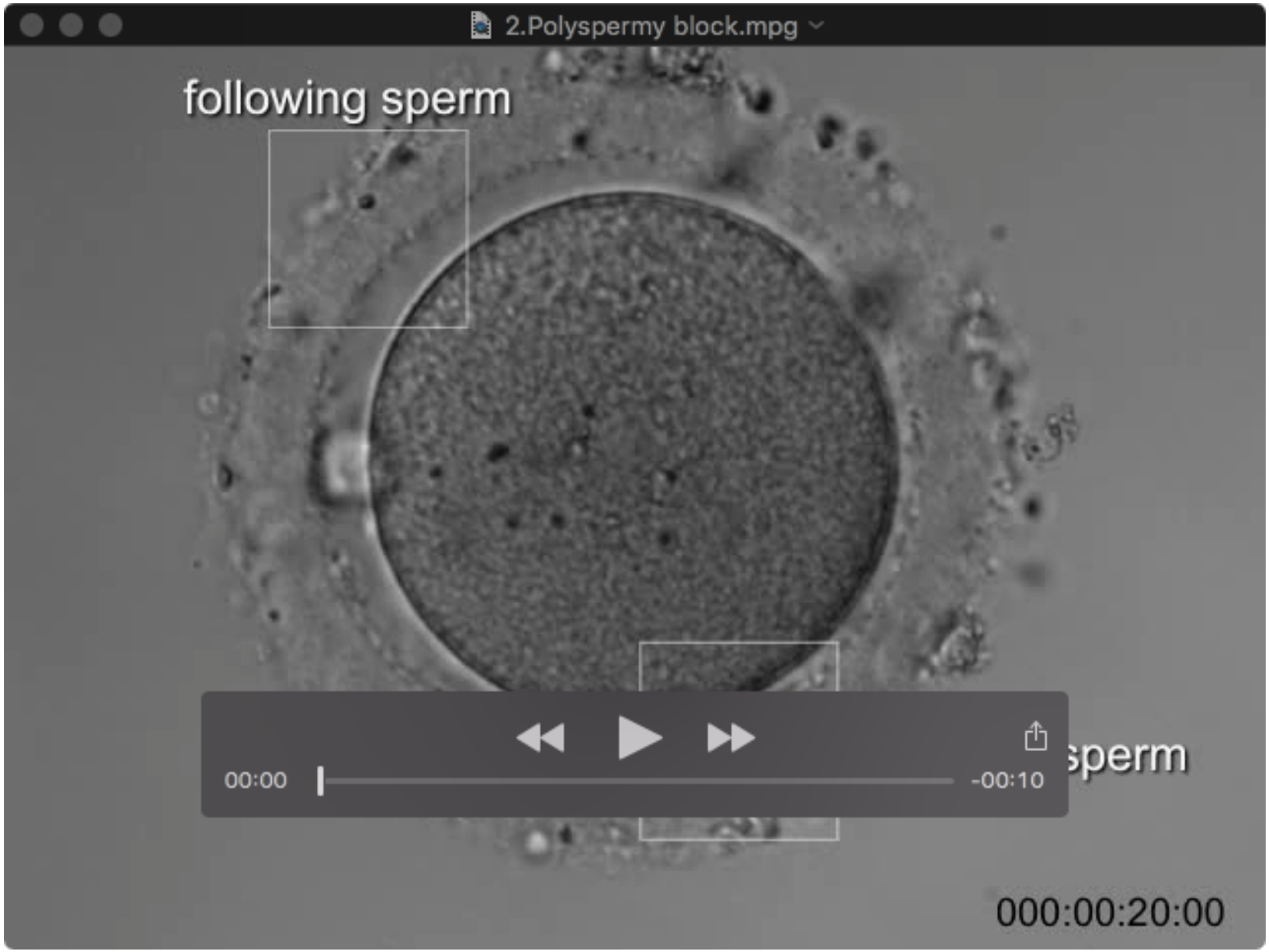
見尾先生よりご供与

受精から卵割の開始

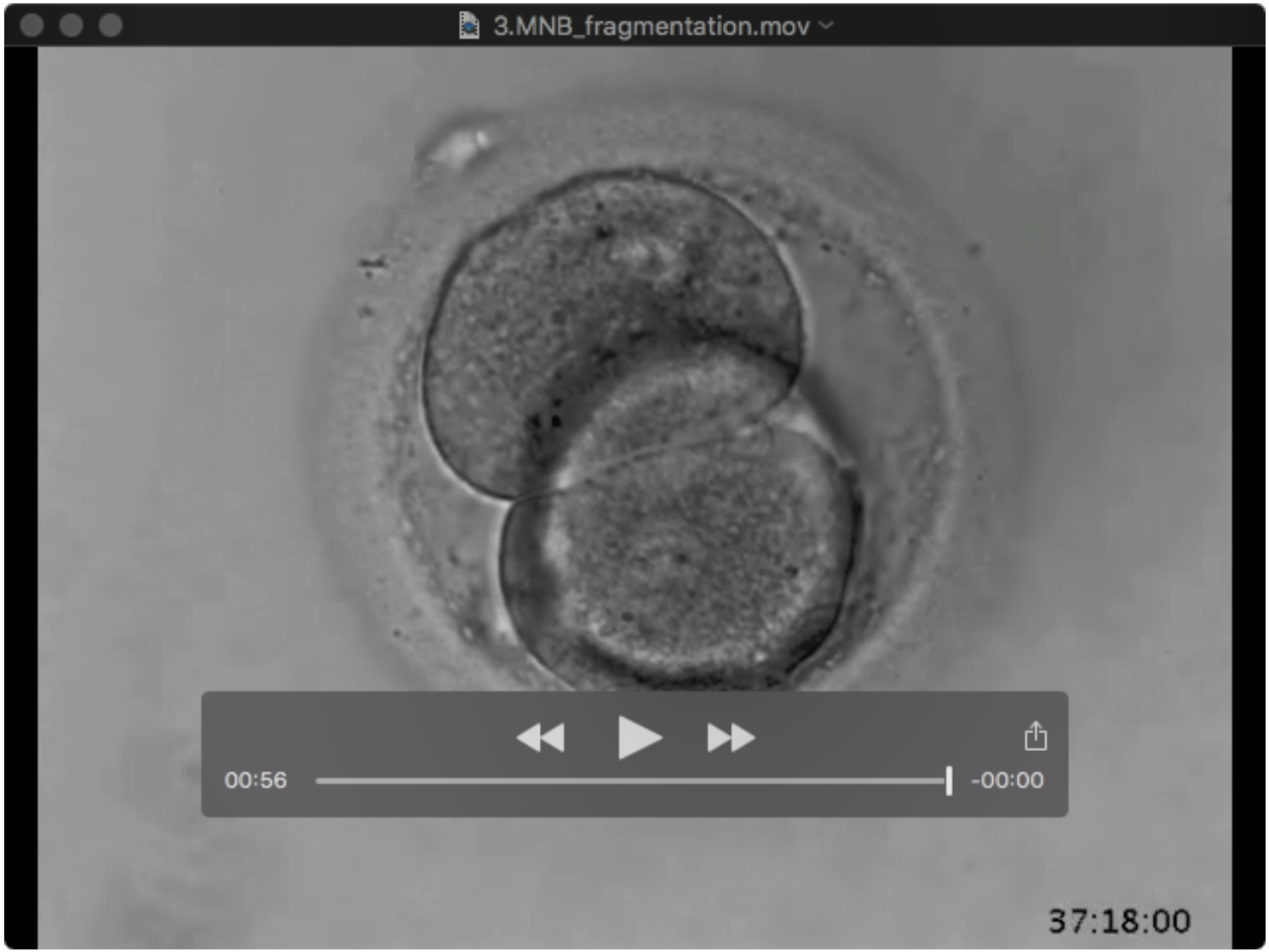


わたしがはじまる瞬間（YouTube）に動画あり

多精子受精が起こらない仕組み

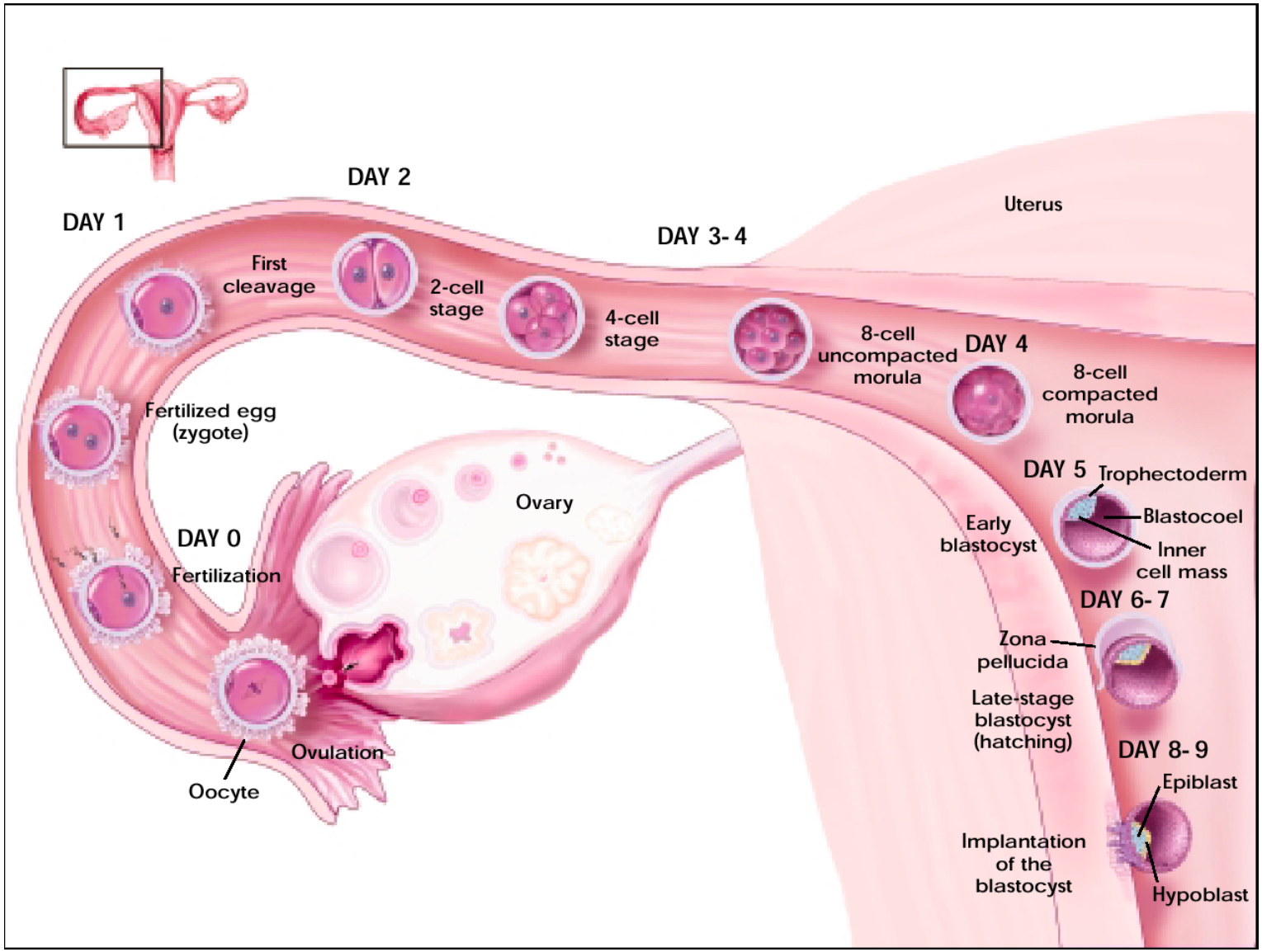


卵割の異常



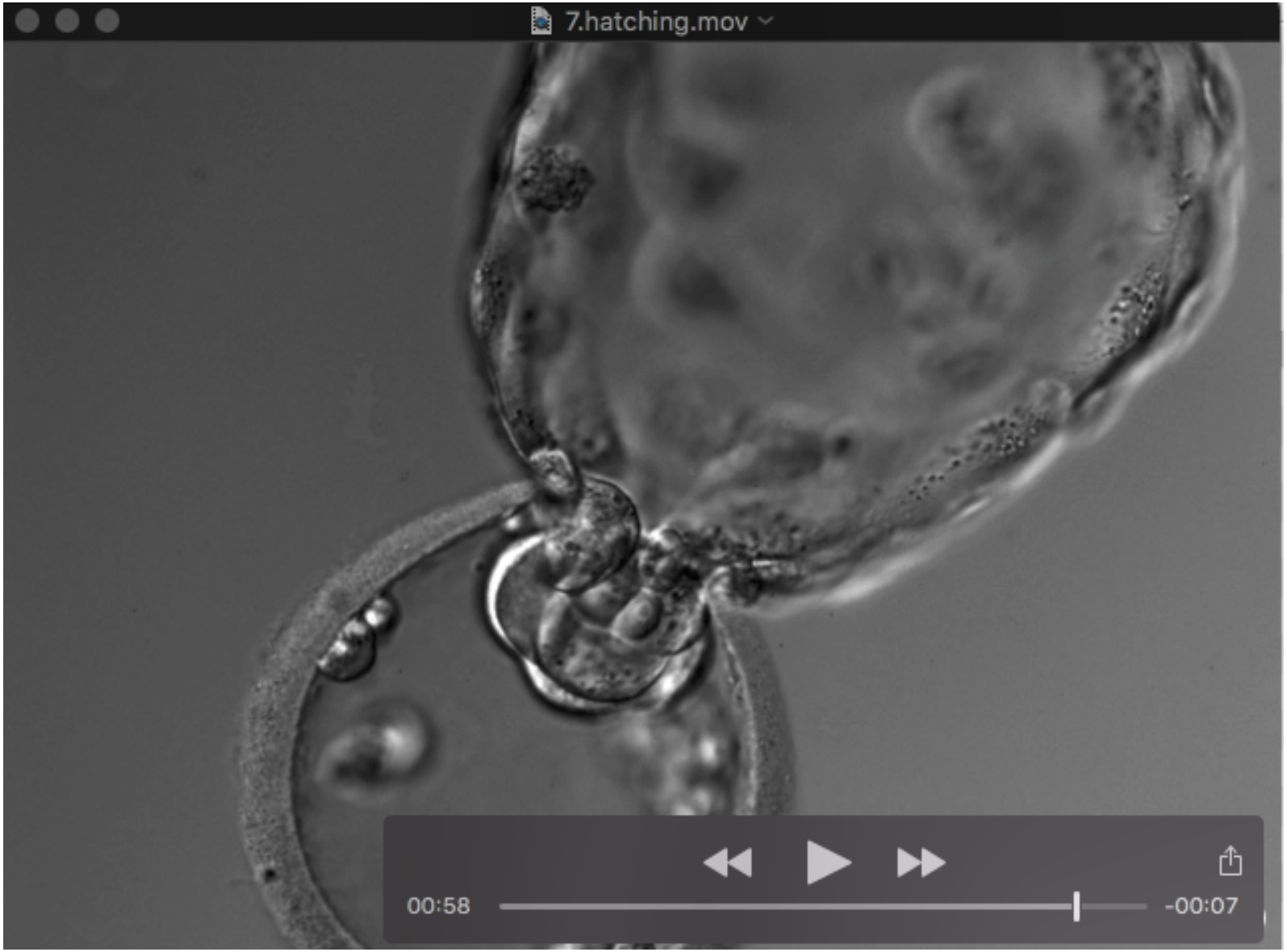
卵割異常は母側・父側両方からの影響
どちらの加齢も卵割異常を引き起こす！

ヒトの受精～着床まで



NIH Stem Cell Reportより

孵化（透明帯からの離脱）



７日目：着床直後

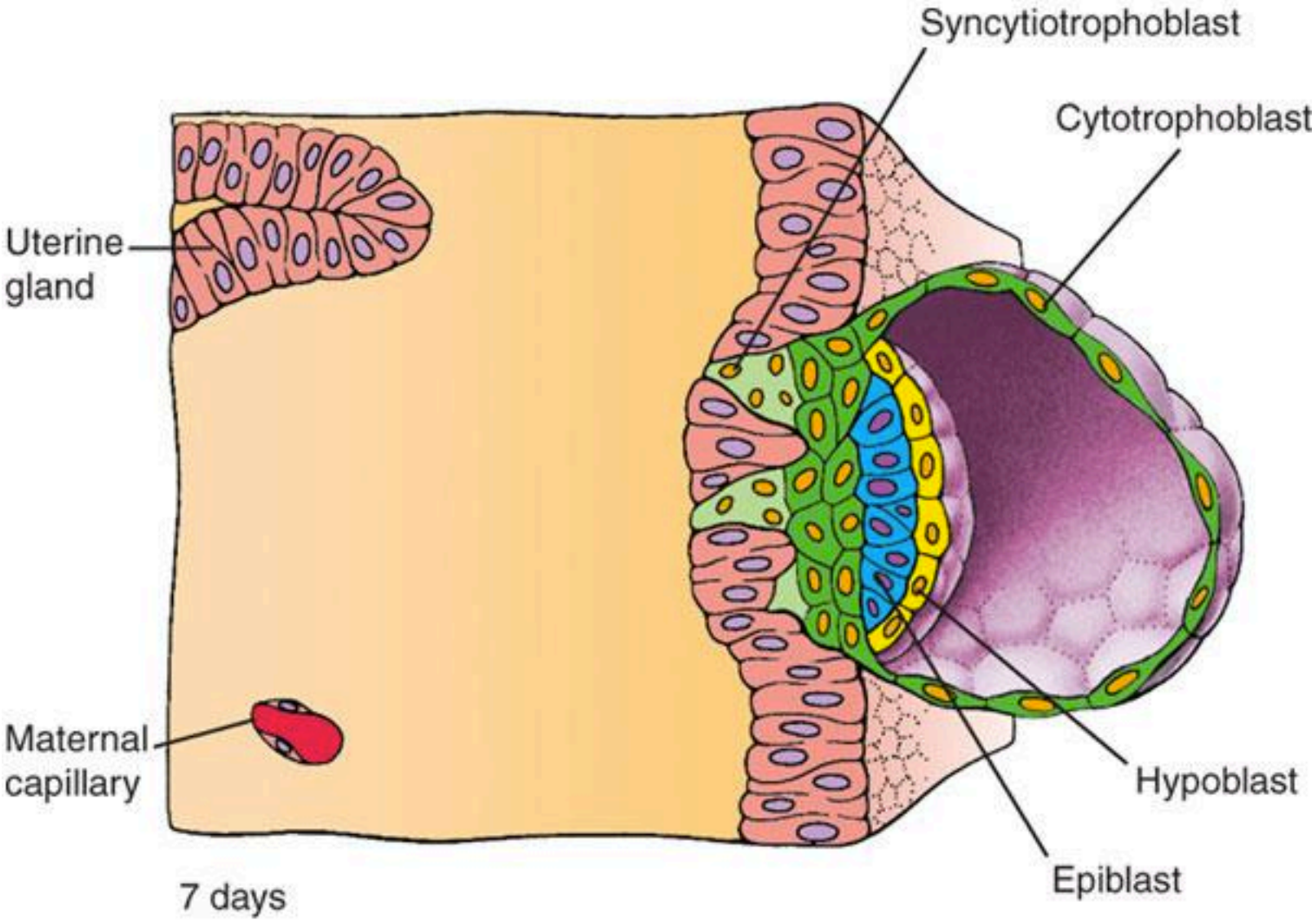


図2-1に相当する原図

9日目：栄養膜形成

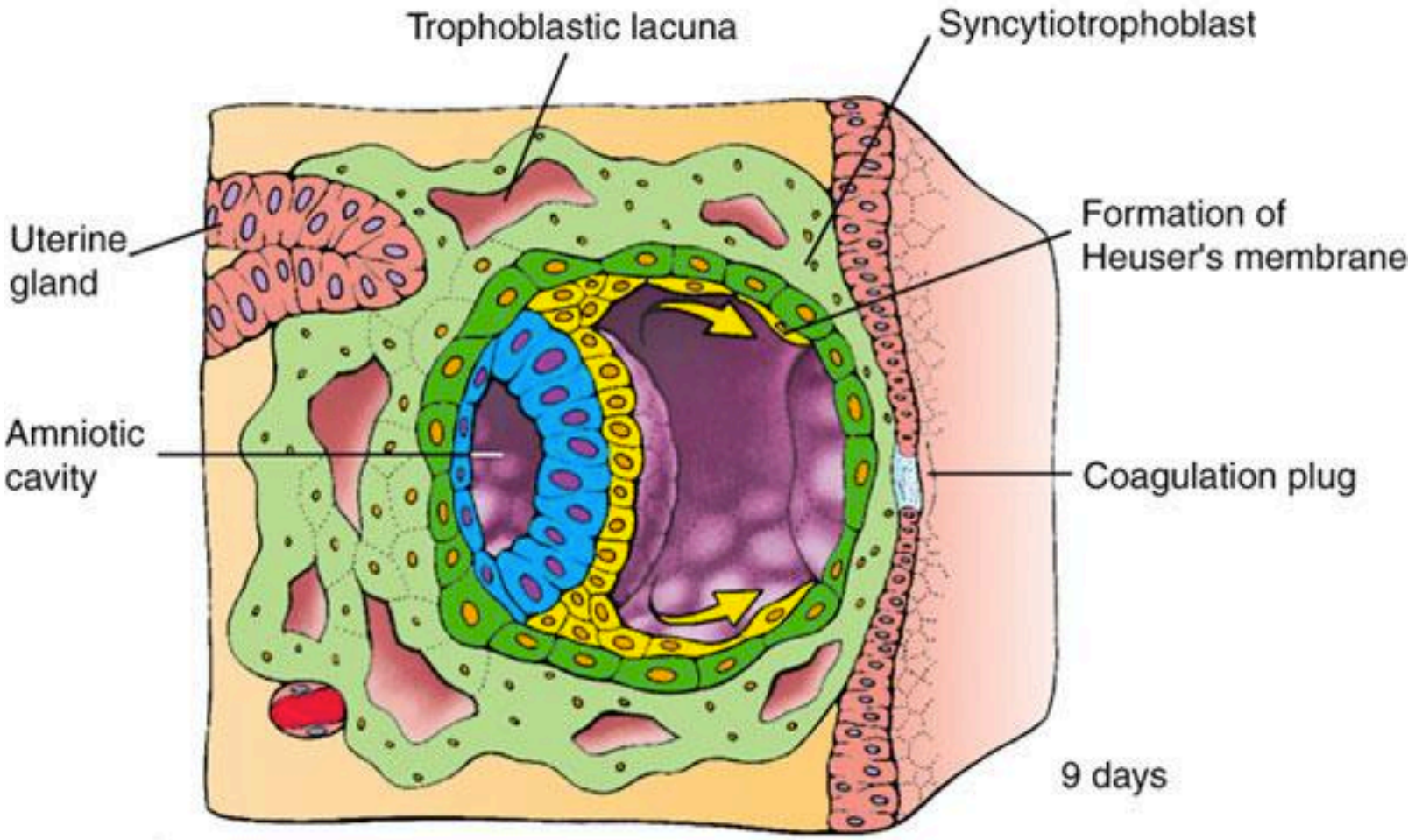


図2-3に相当する原図

10-11日目：絨毛膜形成

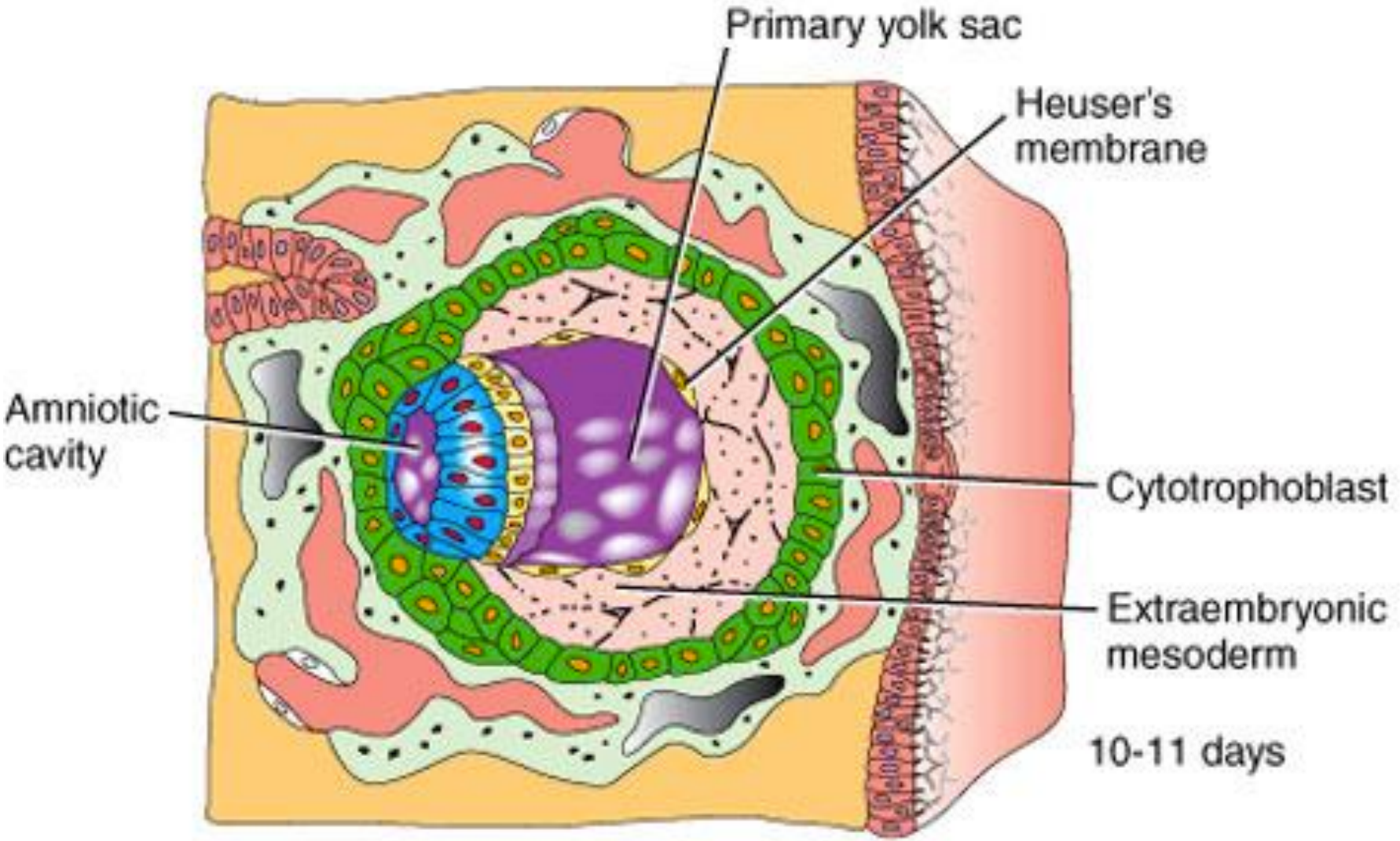


図2-4Aに相当する原図

11-13日：胚外中胚葉形成

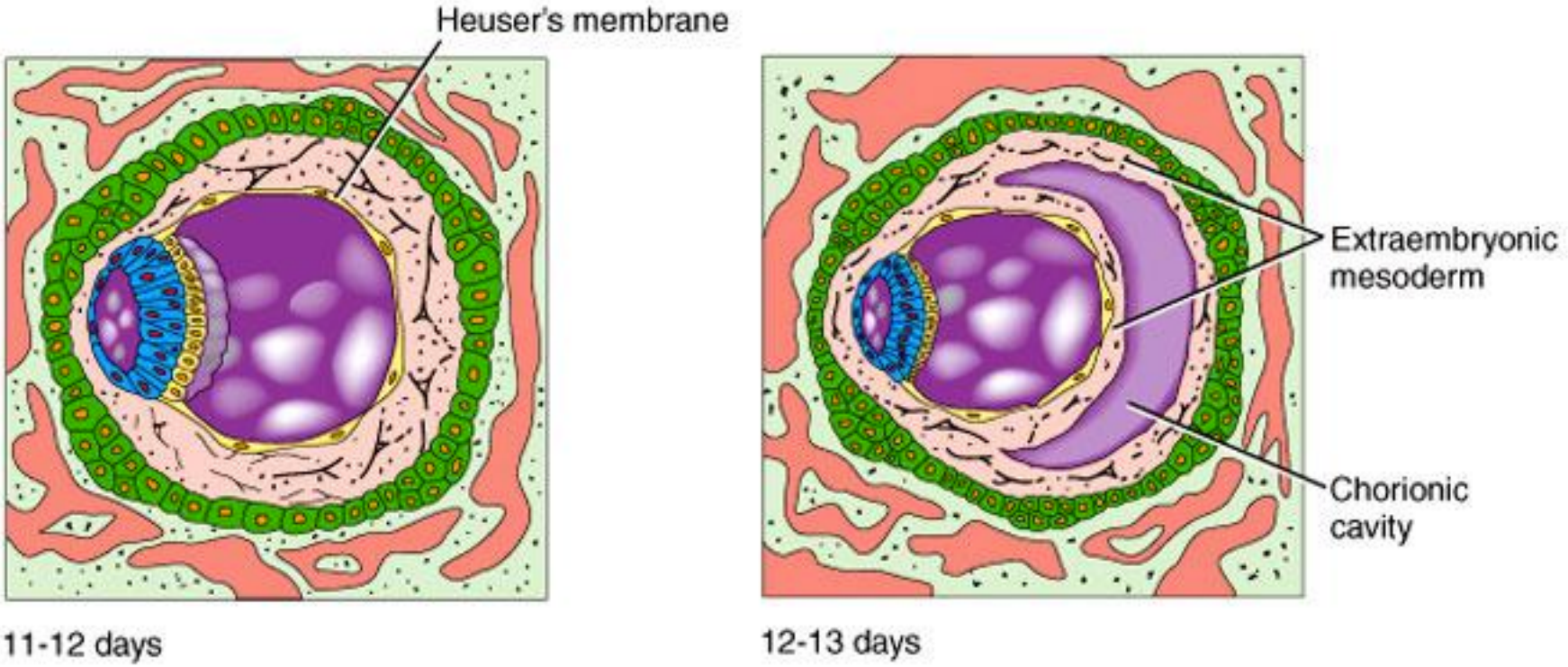


図2-4B, Cに相当する原図

9日ヒト胚子の画像

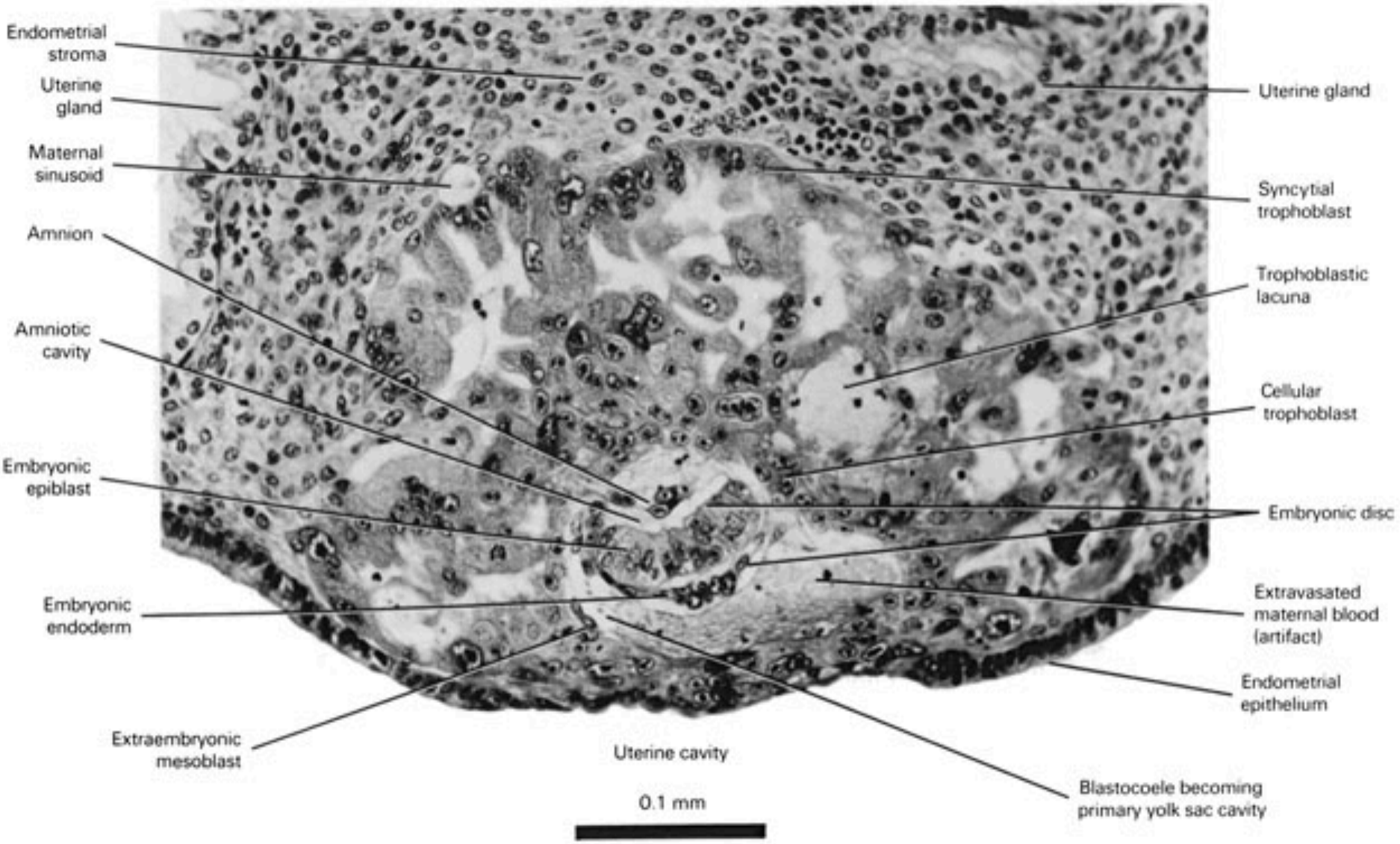


図2-7Bに相当

12日ヒト胚子の画像

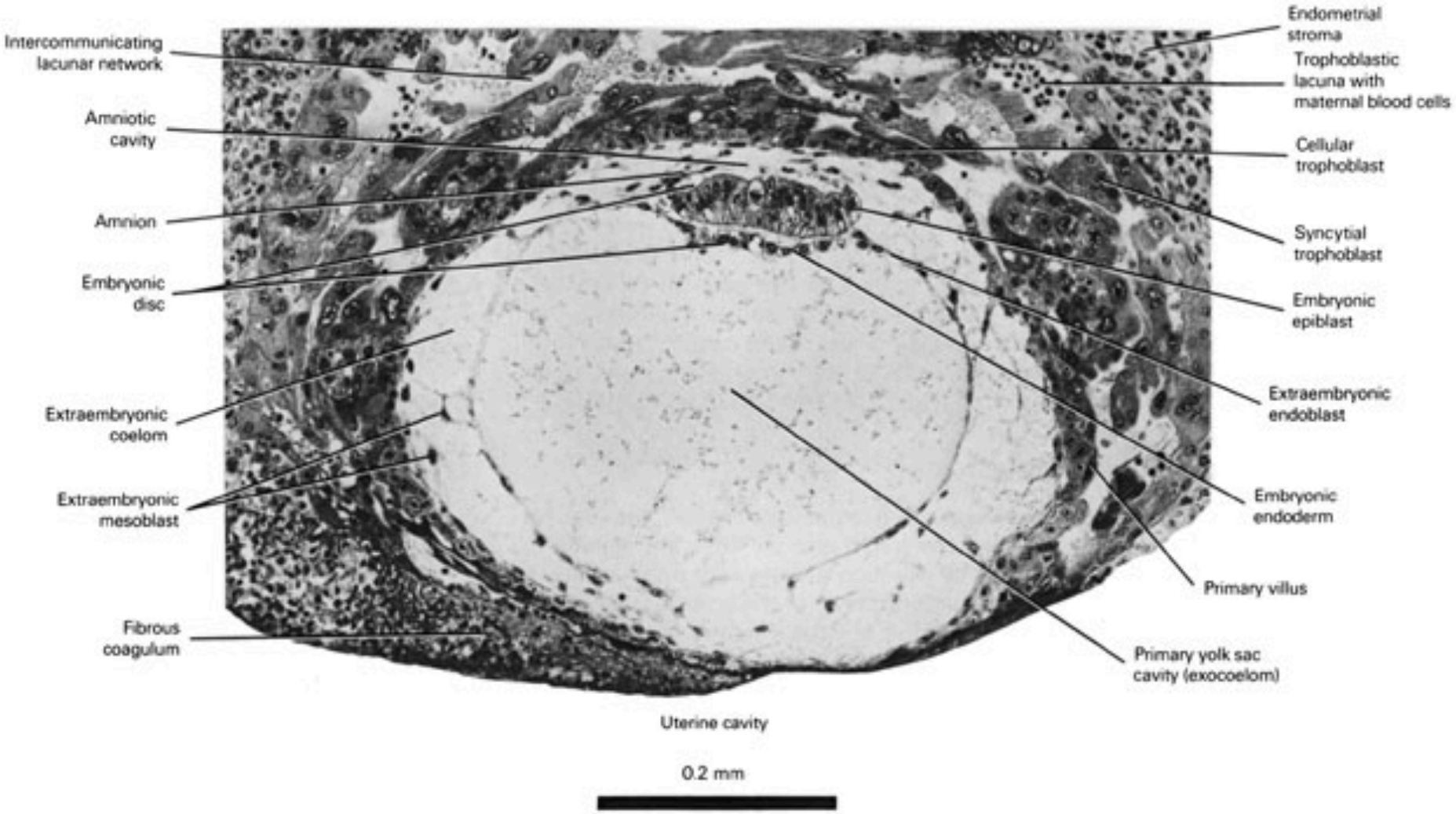


図2-7Bに相当

最新の論文より：長期のヒト胚培養

LETTER

doi:10.1038/nature17948

Self-organization of the *in vitro* attached human embryo

Alessia Deglincerti[1]*, Gist F. Croft[1]*, Lauren N. Pietila[1], Magdalena Zernicka-Goetz[2], Eric D. Siggia[3] & Ali H. Brivanlou[1]

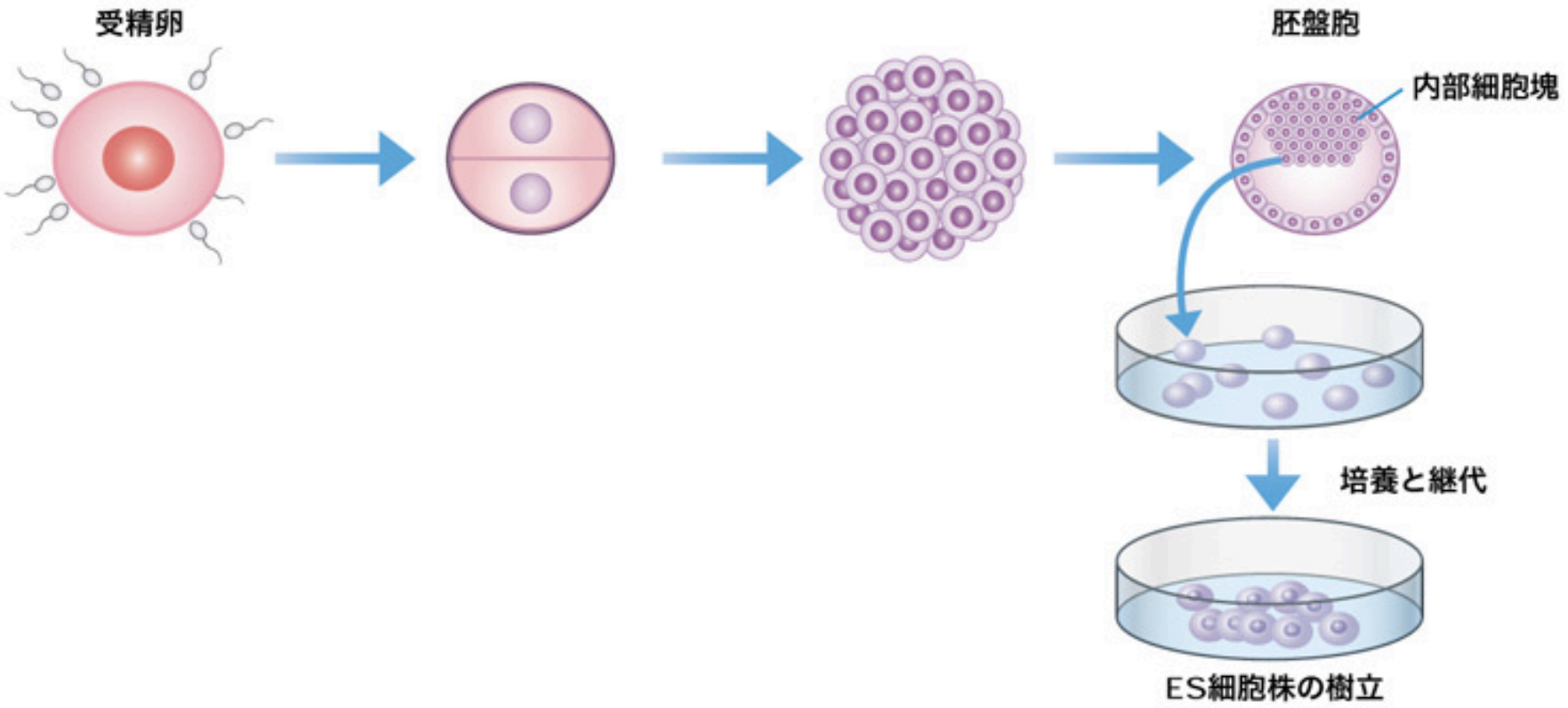
TECHNICAL REPORT

nature cell biology

Self-organization of the human embryo in the absence of maternal tissues

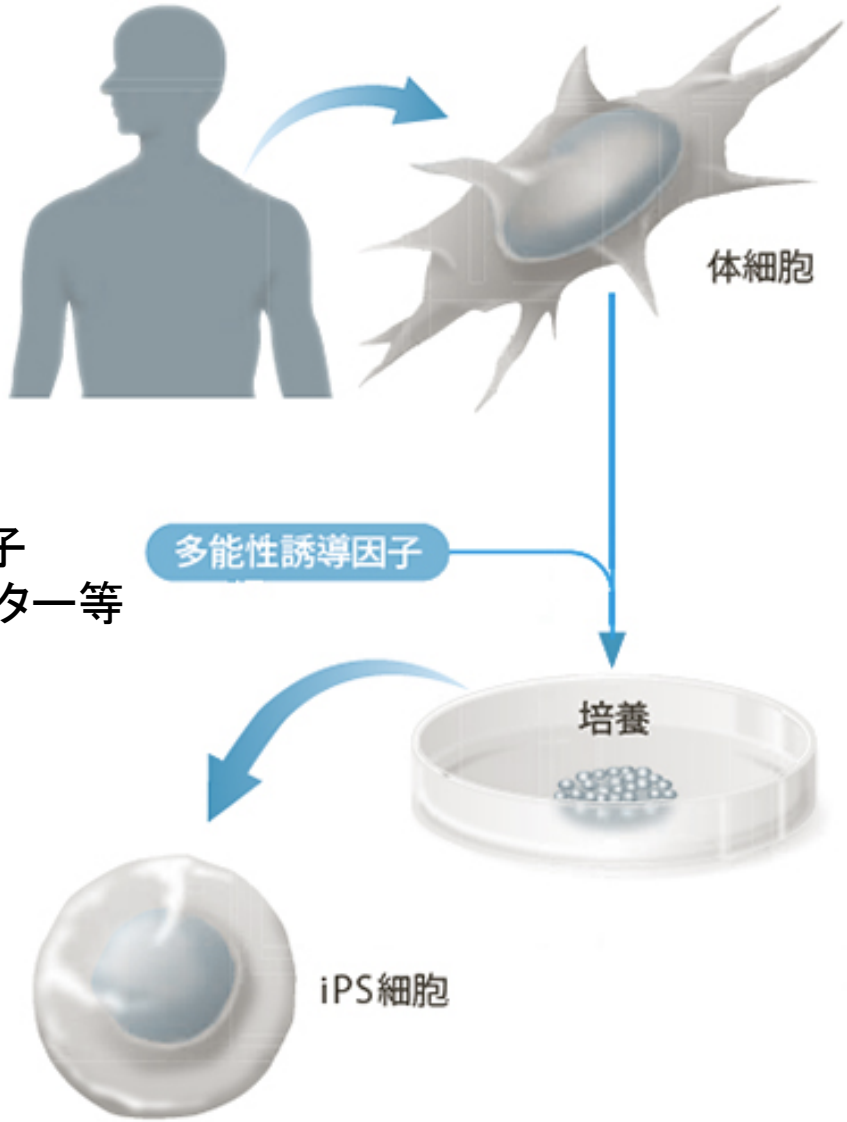
Marta N. Shahbazi[1,5], Agnieszka Jedrusik[1,5], Sanna Vuoristo[1,5], Gaelle Recher[1,6], Anna Hupalowska[1], Virginia Bolton[2], Norah M. E. Fogarty[3], Alison Campbell[4], Liani G. Devito[2], Dusko Ilic[2], Yakoub Khalaf[2], Kathy K. Niakan[3], Simon Fishel[4] and Magdalena Zernicka-Goetz[1,7]

胚性幹細胞（ES細胞）の作り方



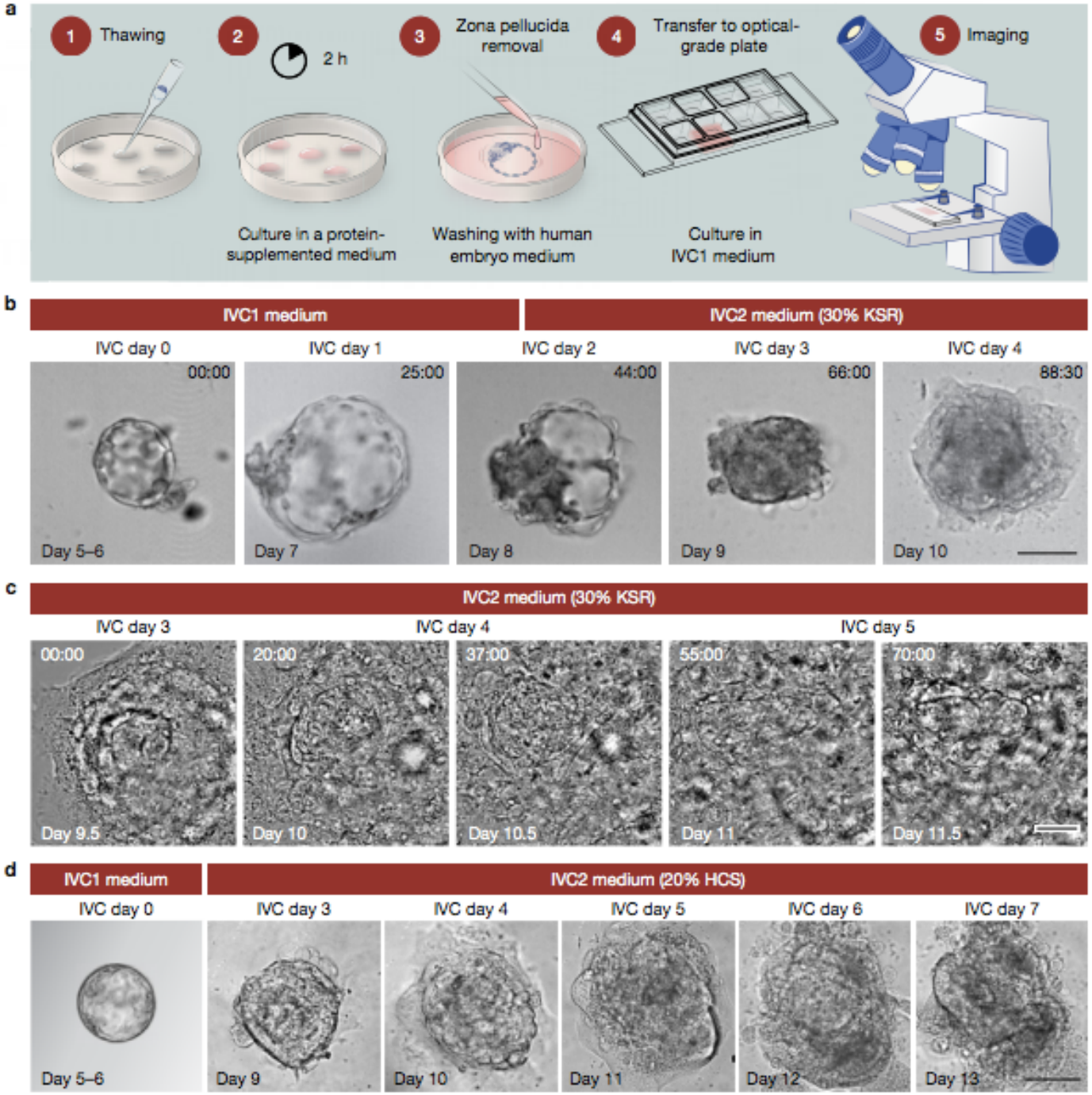
http://www.natureasia.com/ja-jp/jobs/tokushu/detail/7

誘導多能性幹細胞（iPS細胞）の作り方



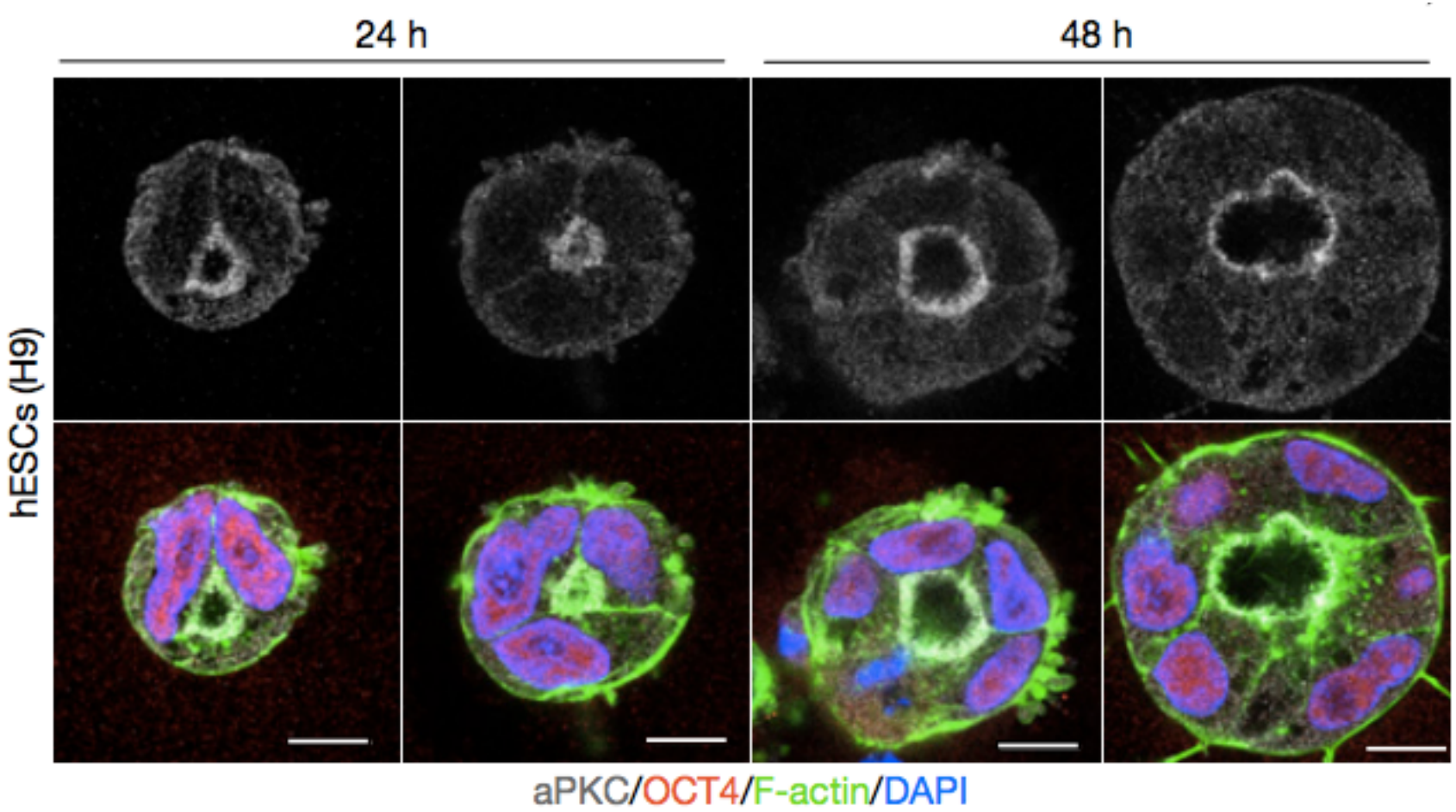
詳しく知りたい方はこちらなど↓

http://www.cira.kyoto-u.ac.jp/j/faq/ips-master.html

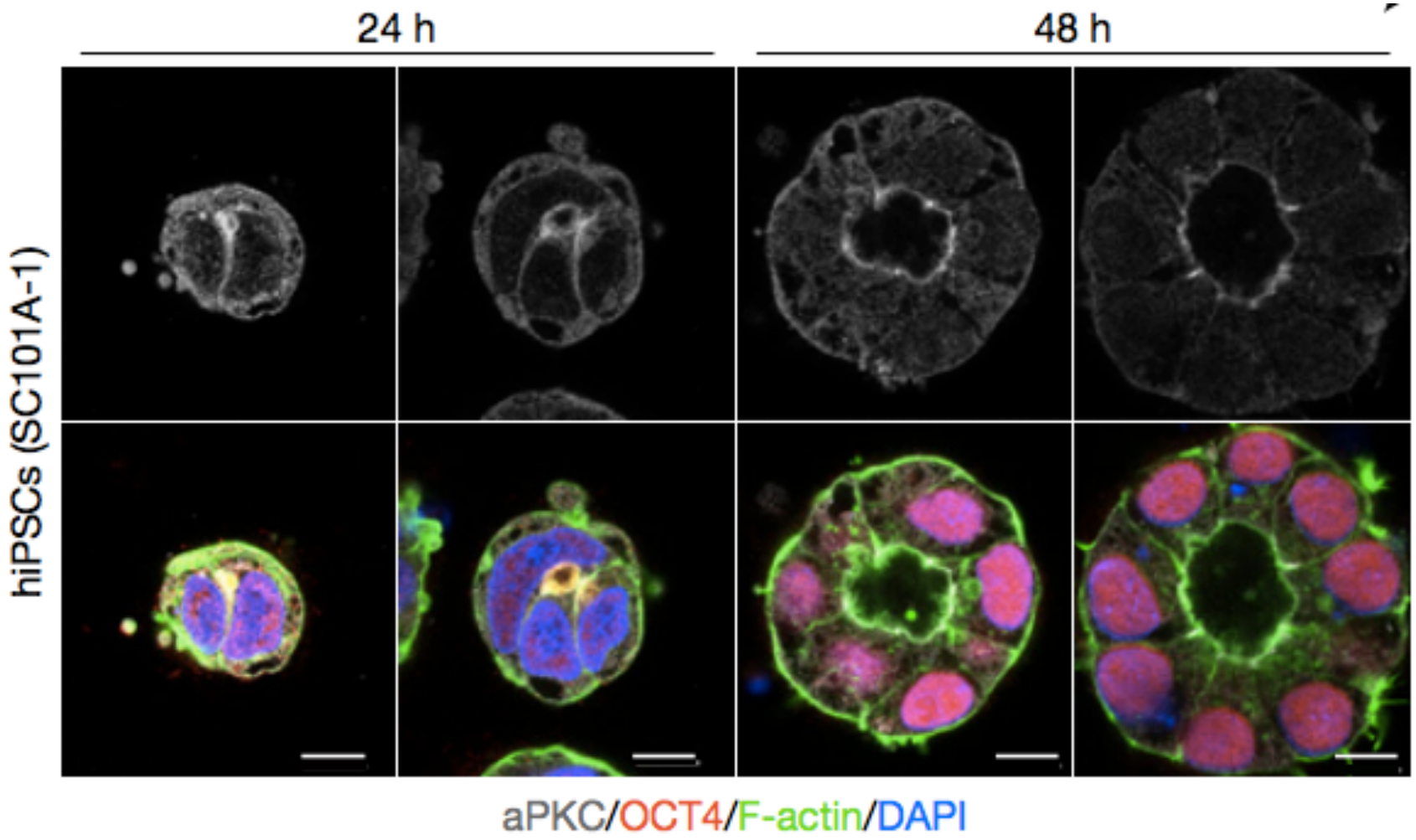

a
1 Thawing
2
2 h
3 Zona pellucida removal
4 Transfer to optical-grade plate
5 Imaging
Culture in a protein-supplemented medium
Washing with human embryo medium
Culture in IVC1 medium
b
IVC1 medium
IVC2 medium (30% KSR)
IVC day 0
IVC day 1
IVC day 2
IVC day 3
IVC day 4
00:00
25:00
44:00
66:00
88:30
Day 5–6
Day 7
Day 8
Day 9
Day 10
c
IVC2 medium (30% KSR)
IVC day 3
IVC day 4
IVC day 5
00:00
20:00
37:00
55:00
70:00
Day 9.5
Day 10
Day 10.5
Day 11
Day 11.5
d
IVC1 medium
IVC2 medium (20% HCS)
IVC day 0
IVC day 3
IVC day 4
IVC day 5
IVC day 6
IVC day 7
Day 5–6
Day 9
Day 10
Day 11
Day 12
Day 13

NCB, 2016

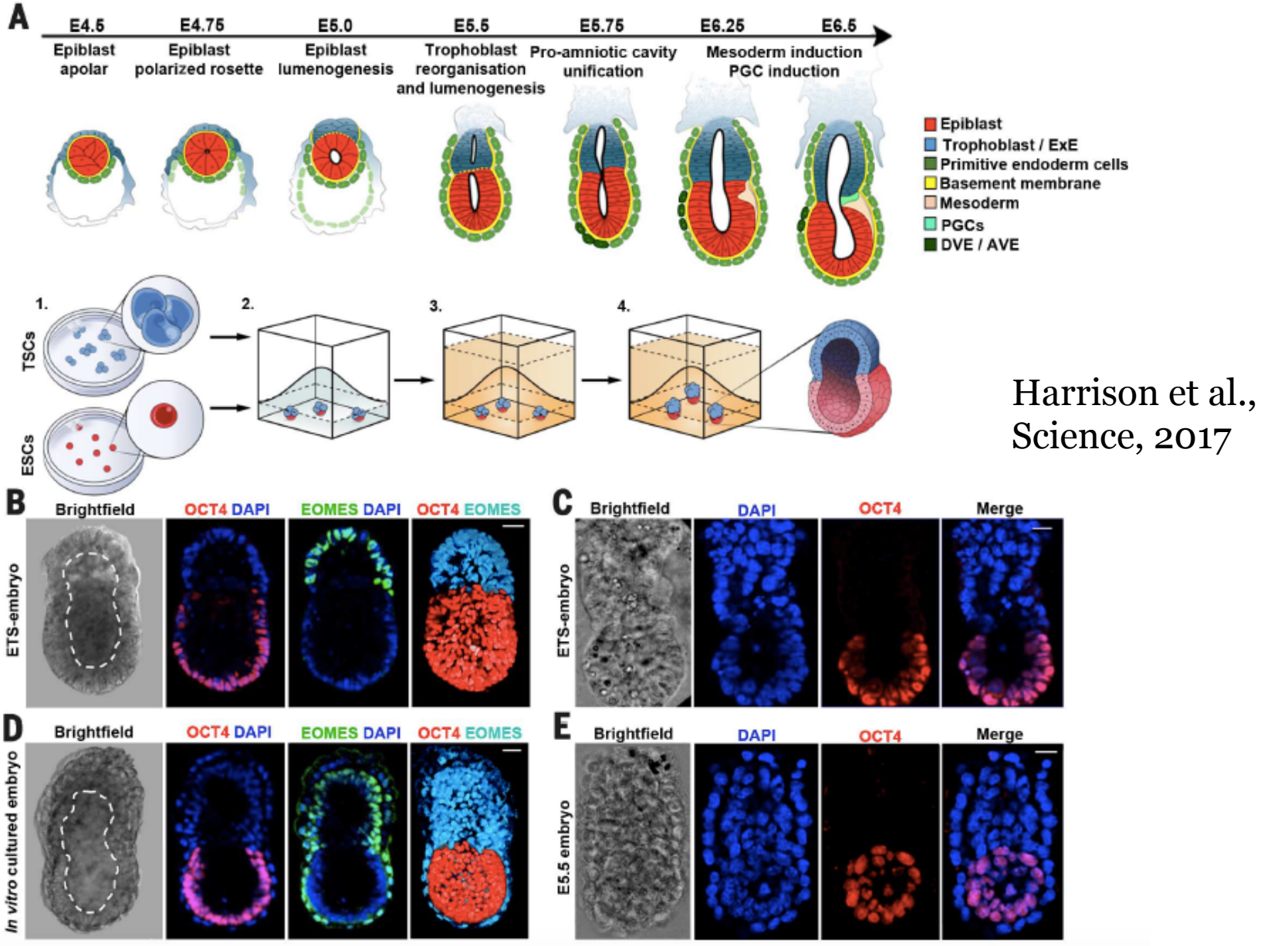
最新の論文より：ヒトES細胞培養



最新の論文より：ヒトiPS細胞培養



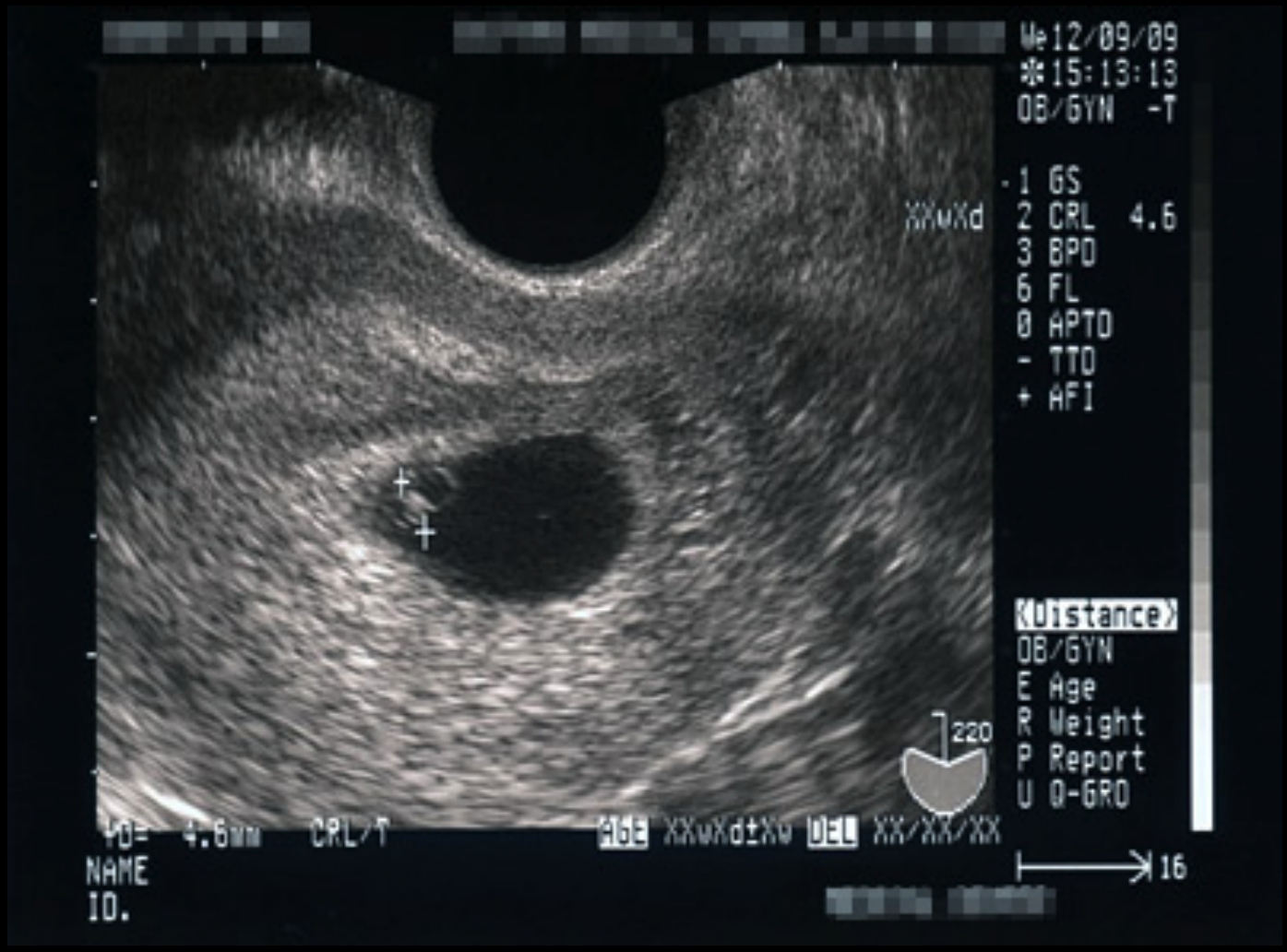
さらに最新の論文より：中胚葉形成まで成功！



Harrison et al., Science, 2017

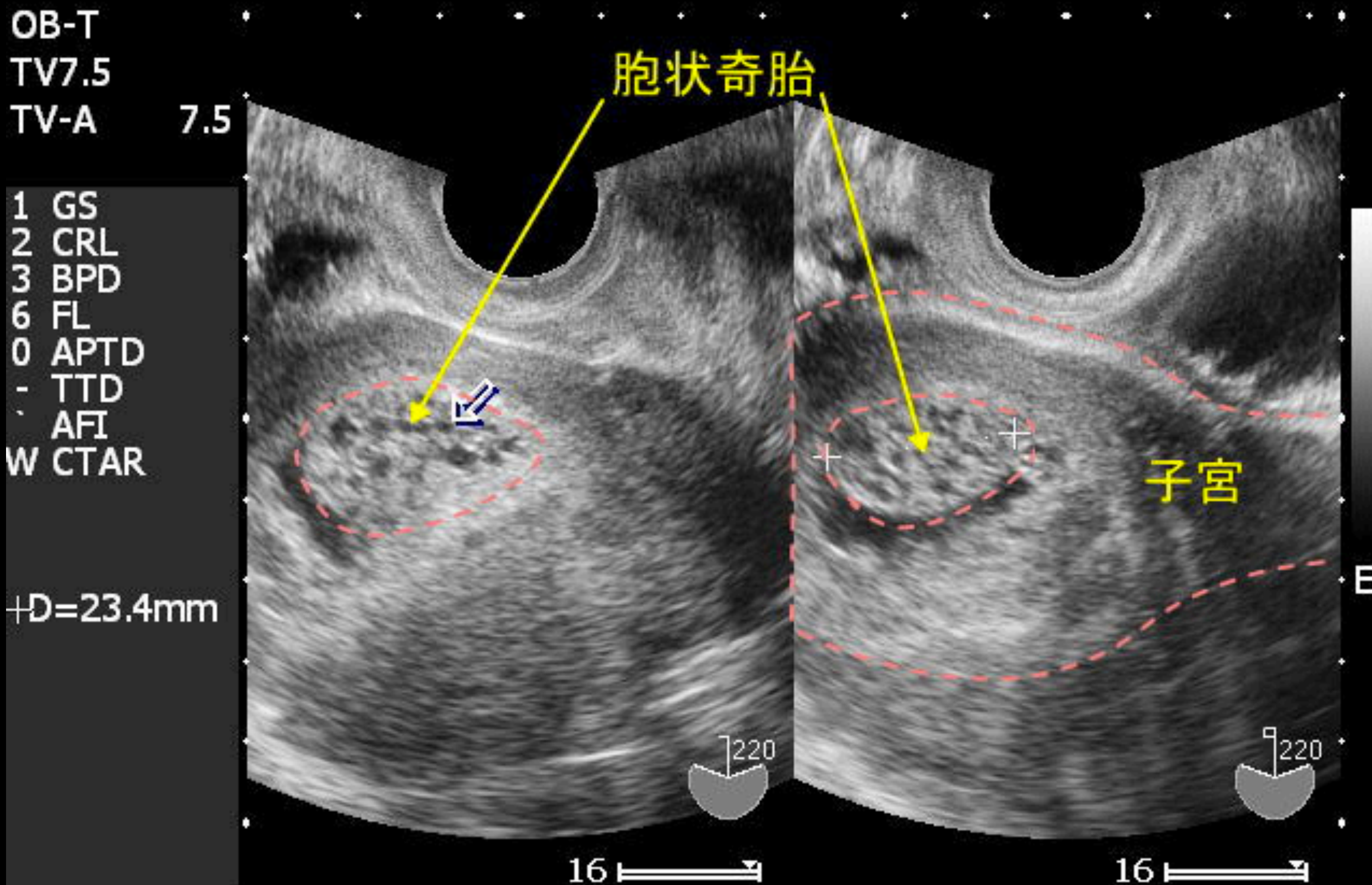
胎児エコー画像

妊娠２ヶ月＝発生2週頃

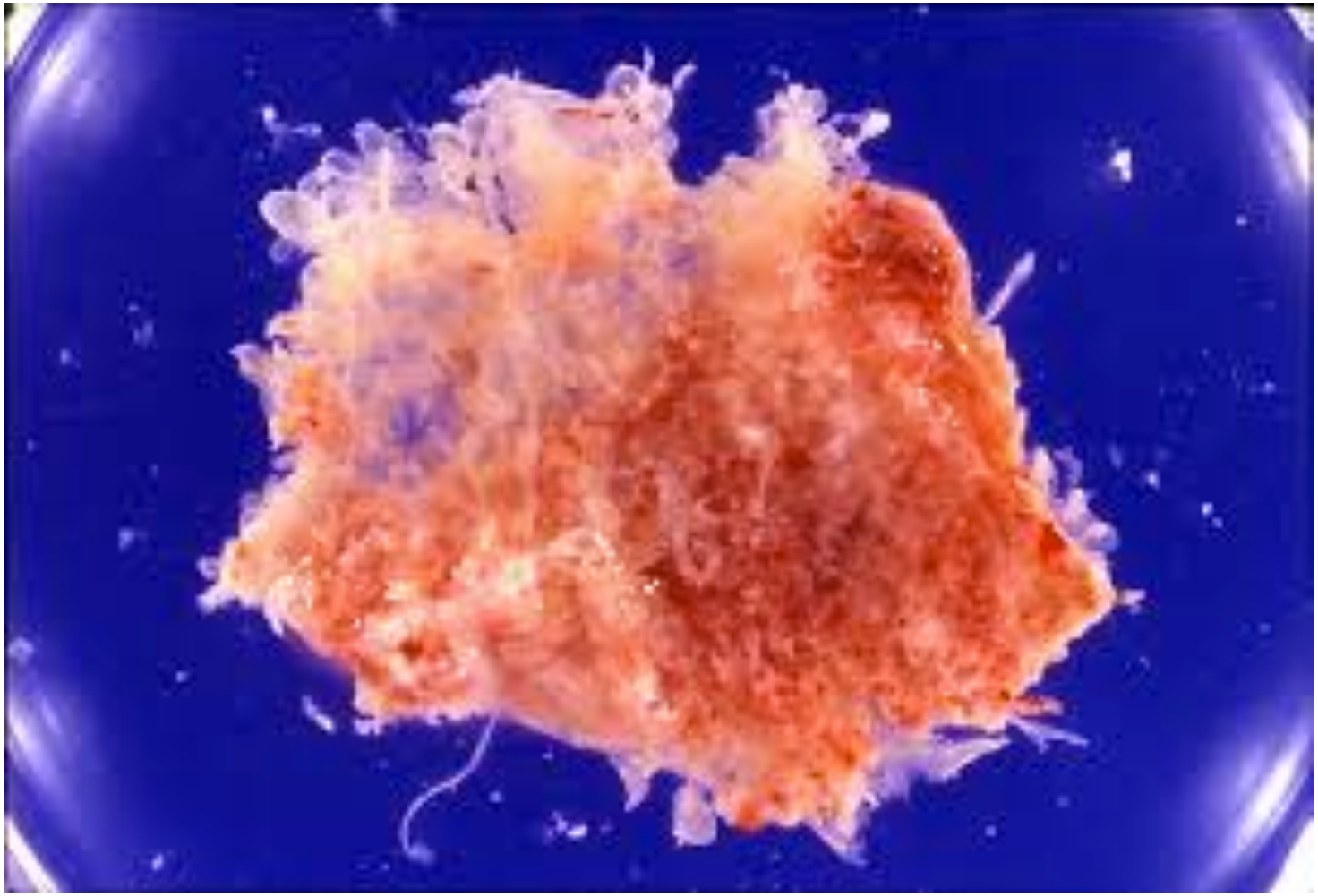


妊娠・出産サイトより

胞状奇胎

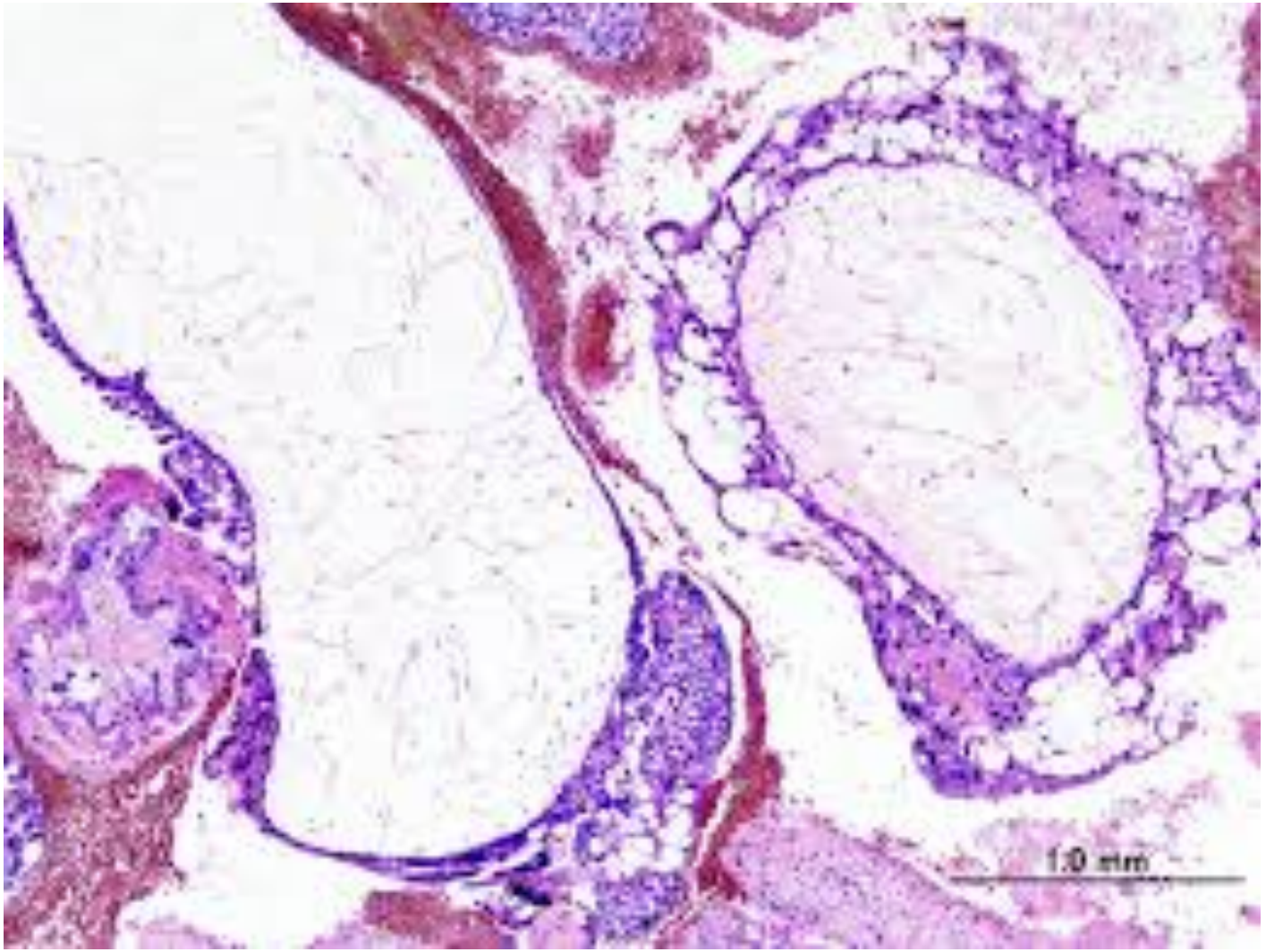


胞状奇胎



東京女子医科大学産婦人科学講座より

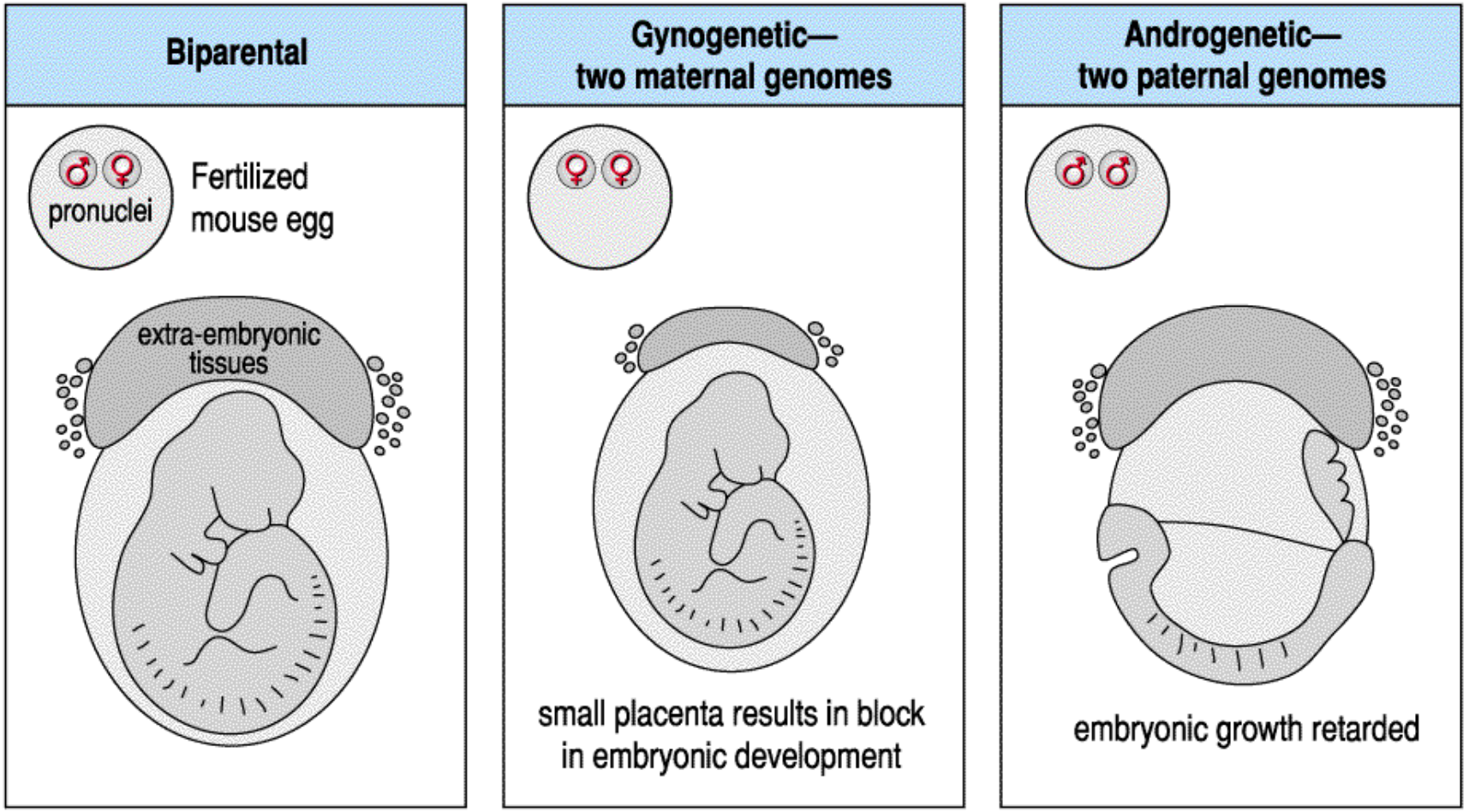
胞状奇胎の組織像



Wikipediaより

父母両方のゲノムが必要！

両親に由来するゲノム　２つの母親由来ゲノム　２つの父親由来ゲノム

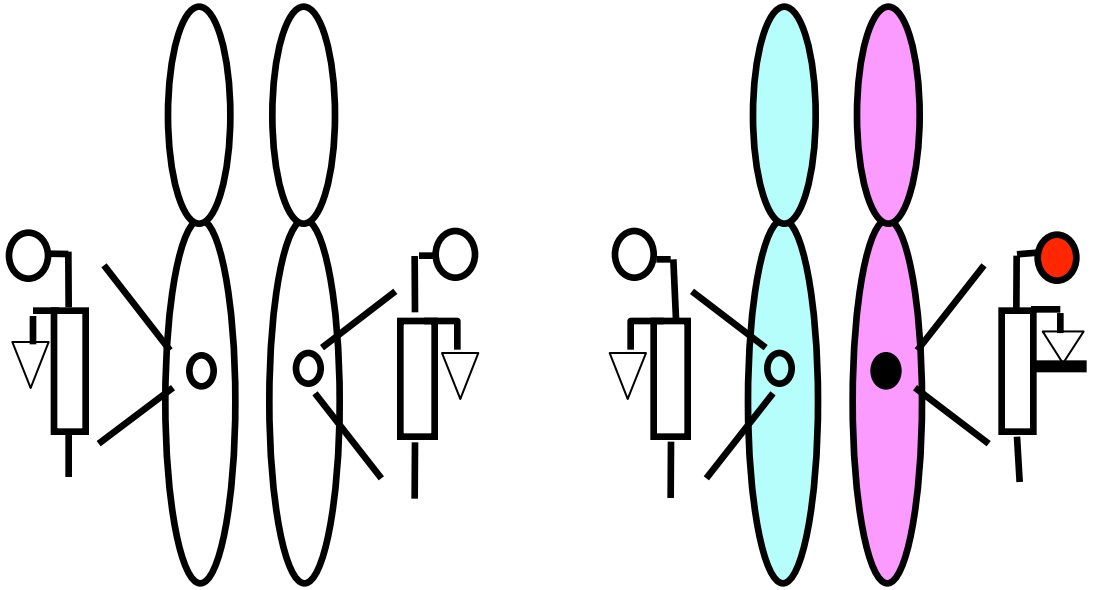


胞状奇胎になる

Wolpert, Developmental Biology, 2nd ed

ゲノム刷り込み現象（imprinting）

父（または母）由来の染色体上でのみ働く遺伝子がある



通常の遺伝子　　刷込み遺伝子

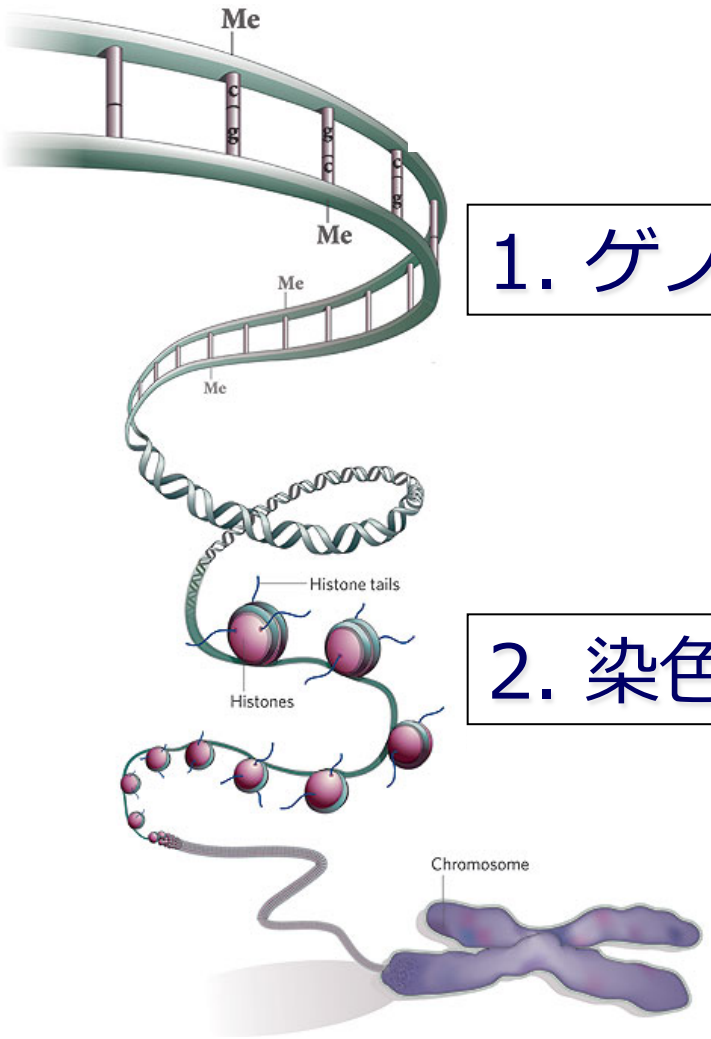
DNAのメチル化による発現の抑制など

山梨大学久保田健夫教授のスライドを改変

“インプリンティング”の由来

コンラッド・ローレンツ
1973年にノーベル生理学医学賞受賞（Psychology Wikiより）

エピジェネティクスの２つの要素



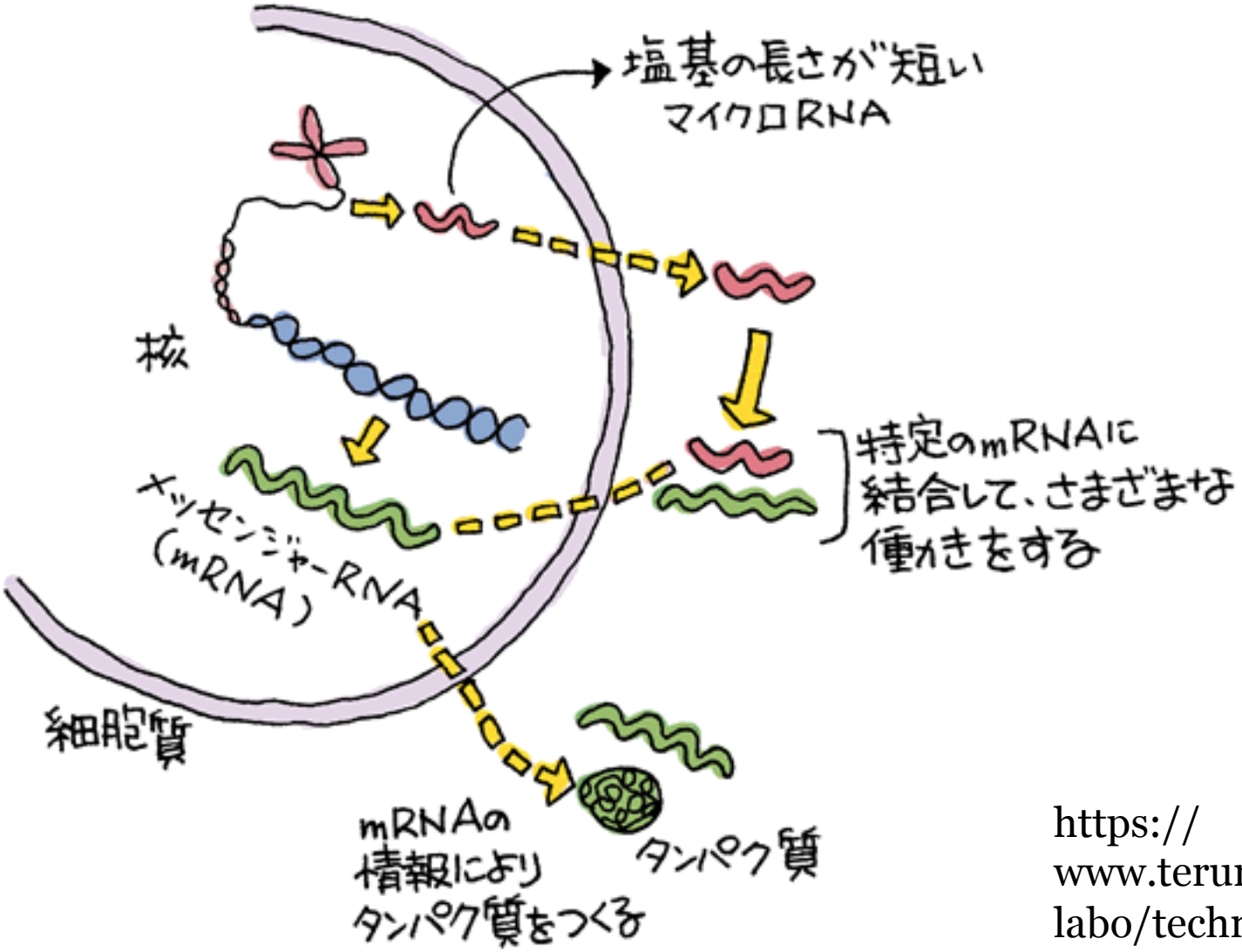
1. ゲノムDNAのメチル化

ゲノム配列がハードドライブなら
エピゲノム修飾はソフトウエア

2. 染色体ヒストン蛋白の化学修飾

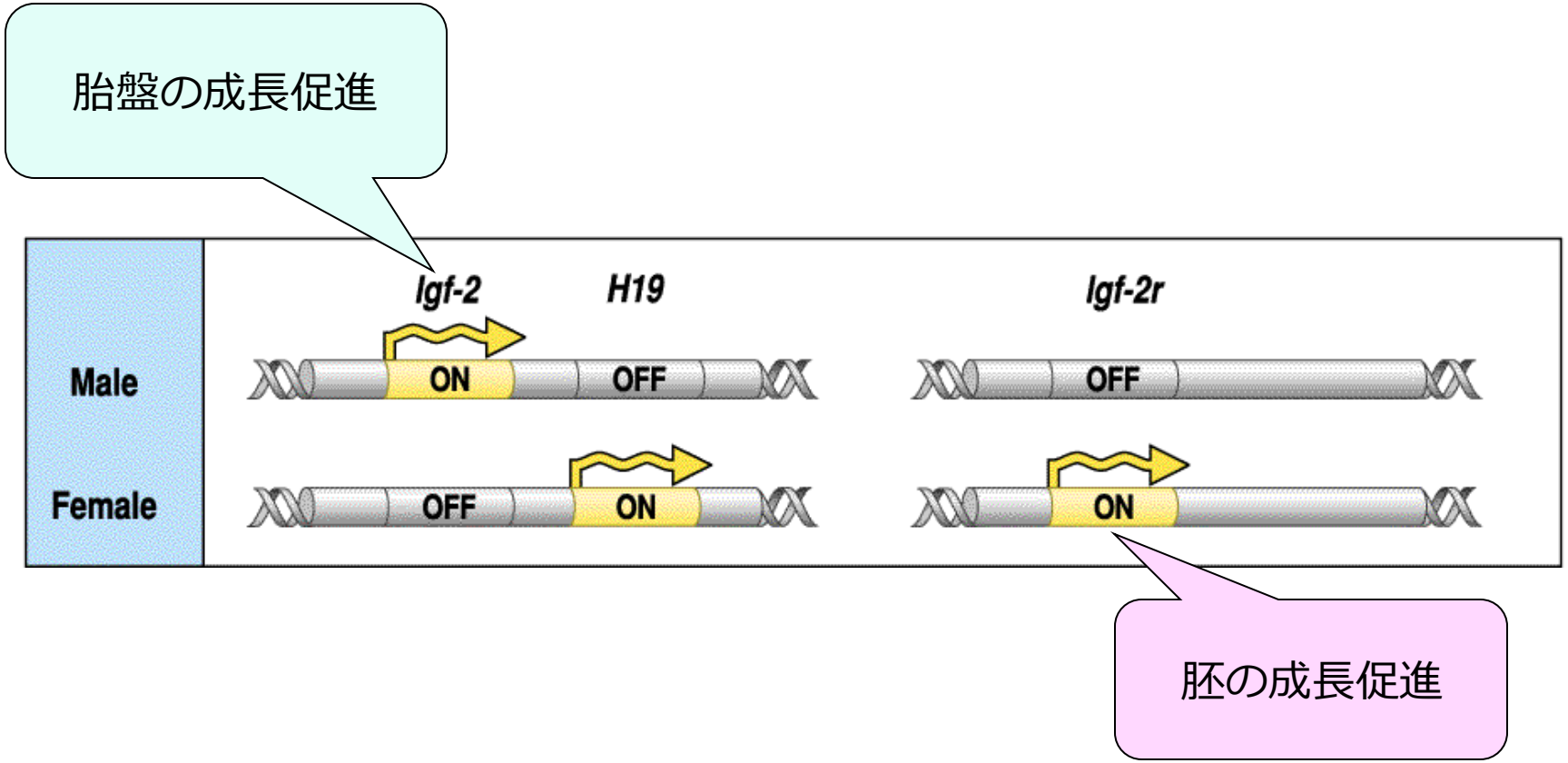
Nature 441： 143-145 , 2006

microRNA



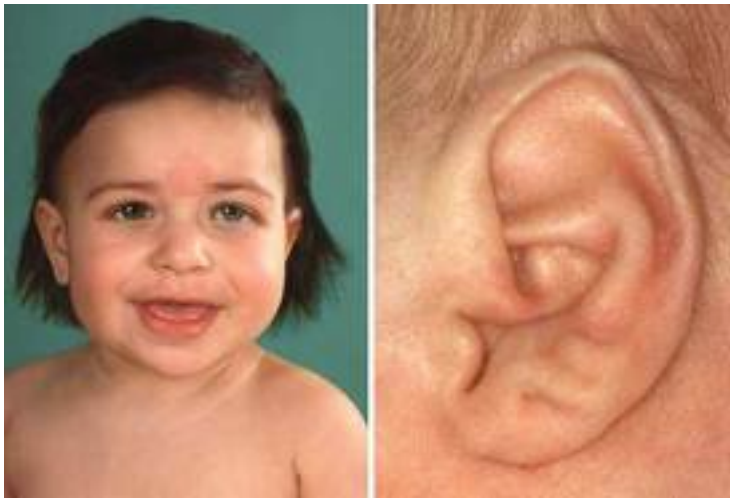
https://www.terumozaidan.or.jp/labo/technology/25/index.html

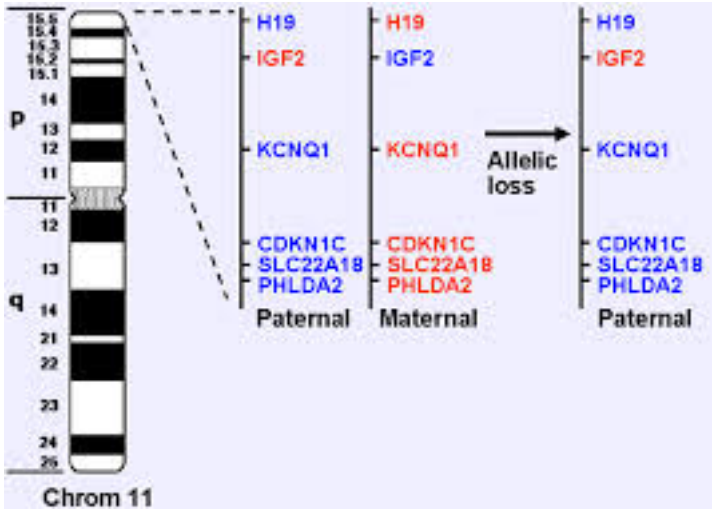
ゲノム刷り込み現象



Wolpert, Developmental Biology, 2nd ed

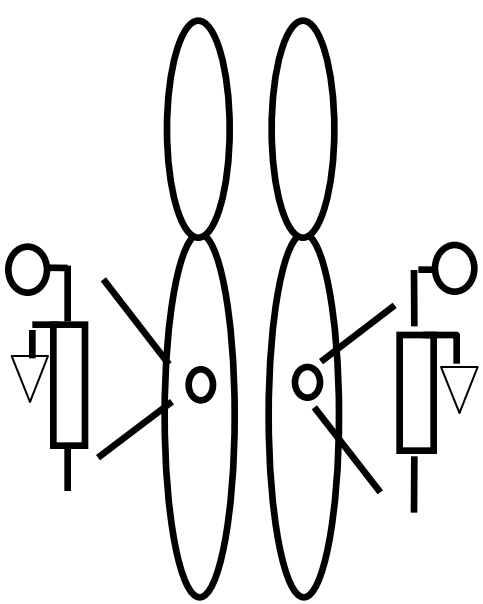
Beckwith-Wiedemann症候群



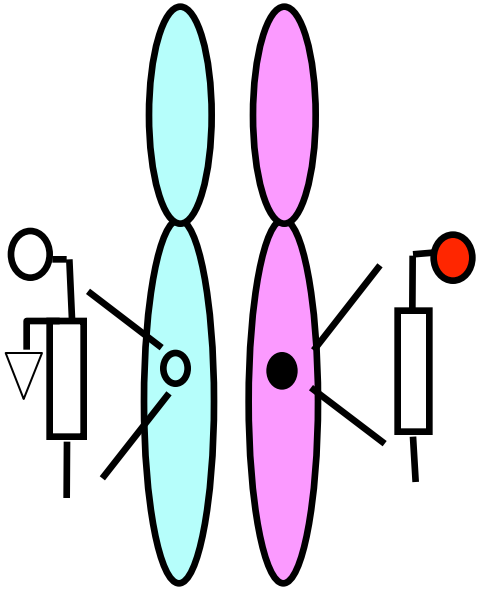


- 常染色体劣性遺伝病
  - 11p15.5
  - 頻度1/13,700
- 巨大児出生
  - 平均3.900g
- 臍帯脱出(E)・巨舌(M)・巨体(G)を主徴
- Wilmus腫瘍の合併例
- インプリンティング異常
  - 11p15遺伝子座がすべて父方由来
  - 父性片親発現を示すIGF2遺伝子の発現過剰による

遺伝子レベル・染色体レベルの
ゲノム刷り込み現象

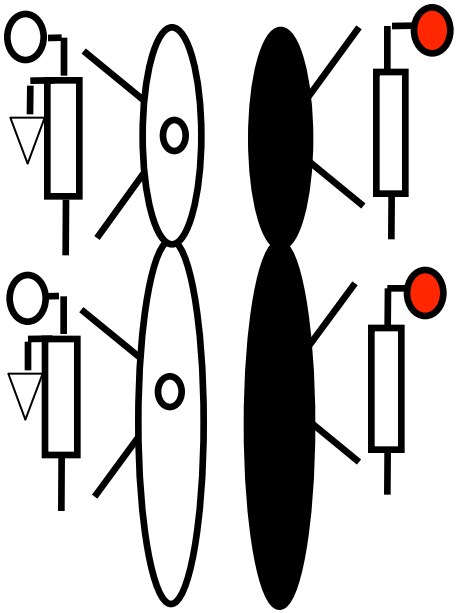


通常の遺伝子



ゲノム刷り込み遺伝子

遺伝子レベルの抑制

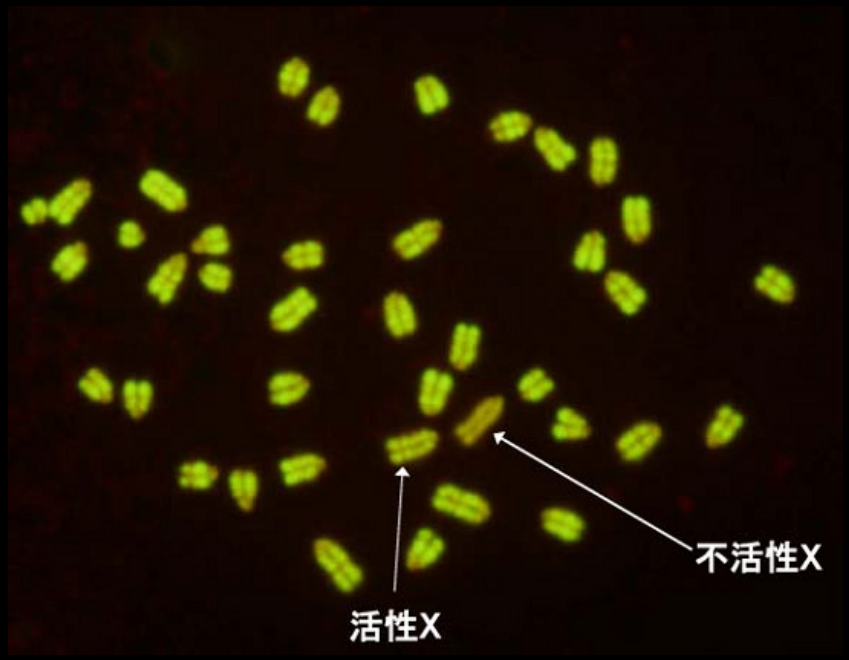


X染色体の不活化

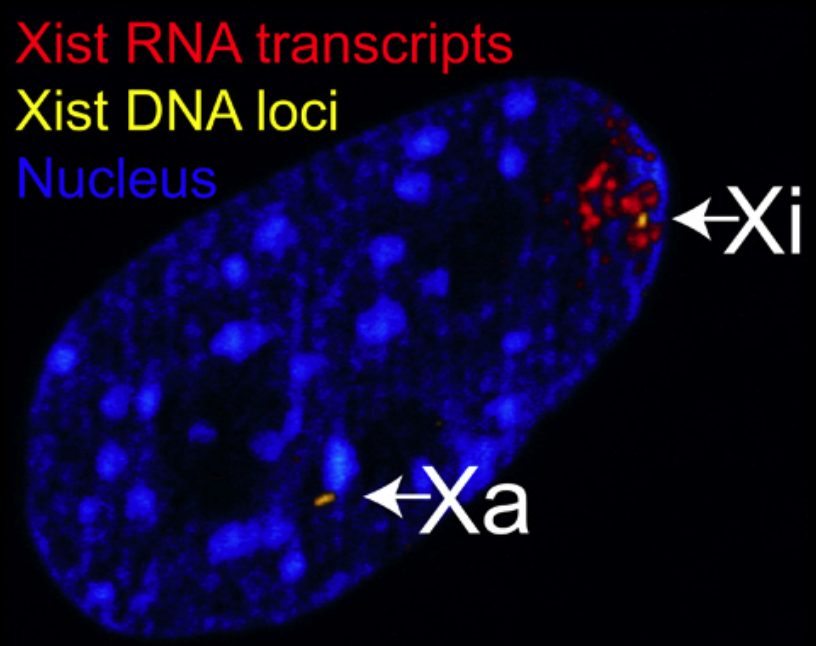
染色体レベルの抑制

山梨大学久保田健夫教授のスライドを改変

X染色体不活性化

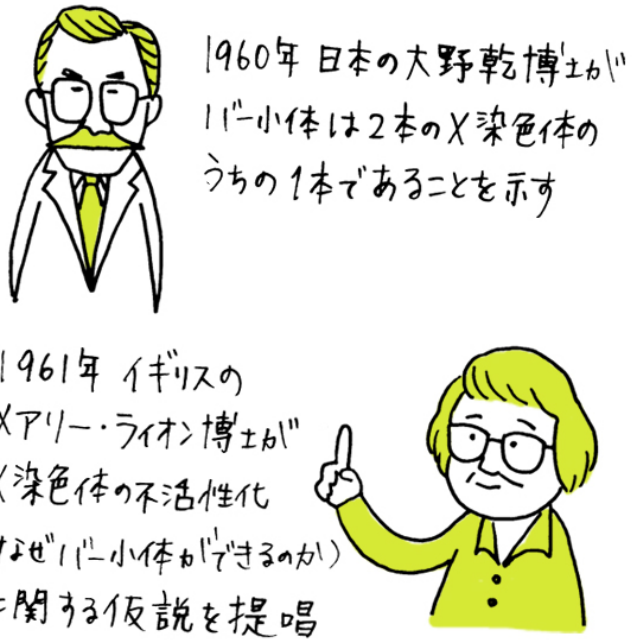


遺伝研：遺伝学電子博物館より



Wikipediaより

「X染色体不活性化」の創始者



Mary Frances Lyon
(1925-2014)

北大分子細胞生物学教室HPより

Mary F. Lyonの論文（Nature, 1961）

of the year gave the same symptoms; (e) on *L. esculentum* × *L. pimpinellifolium* the symptoms were identical both in the inoculation from the vine and from diseased *I. holstani*.

From *I. holstani* the isolate has so far been transmitted to tobacco (varieties White Burley and Samsun) and to *Petunia*, by sap and by *Myzodes persicae*; to *Nicotiana glutinosa*, *Datura stramonium*, *Vigna sinensis* and *I. holstani* by sap. The percentage infection in the transmission from these species to the same species or to the other species that gave positive results in the inoculation from *I. holstani*, is higher than in the transmission from *I. holstani*.

We are trying to transmit the isolates from the herbaceous plants to grape vine. For this work we use symptomless grape vines, selected during three years and belonging to varieties that appeared to be very receptive to the 'infectious degeneration' in previous experiments on transmission by grafting from vine to vine.

Other work in progress is the identification of the isolates.

No rod-shaped virus particles were seen in a series of observations, using the electron microscope, with exudates obtained by Johnson's method and with drops prepared with Brandes's dipping method both with diseased grape vines (leaves, shoots and roots) and with infected herbaceous plants.

E. Baldacci
A. Amici
P. Bonola
E. Betto
G. Fogliani
E. Refatti

Istituto di Patologia vegetale,
Università di Milano.

[1] Amici, A., Baldacci, E., and Refatti, E., *Ann. Facoltà Agraria Milano (N.S.)*, **7**, 41 (1958).

GENETICS

Gene Action in the *X*-chromosome of the Mouse (*Mus musculus* L.)

Ohno and Hauschka[1] showed that in female mice one chromosome of mammary carcinoma cells and of normal diploid cells of the ovary, mammary gland and liver was heteropyknotic. They interpreted this chromosome as an *X*-chromosome and suggested that the so-called sex chromatin was composed of one heteropyknotic *X*-chromosome. They left open the question whether the heteropyknosis was shown by the paternal *X*-chromosome only, or the chromosome from either parent indifferently.

The present communication suggests that the evidence of mouse genetics indicates: (1) that the heteropyknotic *X*-chromosome can be either paternal or maternal in origin, in different cells of the same animal; (2) that it is genetically inactivated.

The evidence has two main parts. First, the normal phenotype of *XO* females in the mouse[2] shows that only one active *X*-chromosome is necessary for normal development, including sexual development. The second piece of evidence concerns the mosaic phenotype of female mice heterozygous for some sex-linked mutants. All sex-linked mutants so far known affecting coat colour cause a 'mottled' or 'dappled' phenotype, with patches of normal and mutant colour, in females heterozygous for them. At least six mutations to genes of this type have been reported, under the names mottled[3,4], brindled[3], tortoiseshell[1], dappled[4], and 26*K*[2]. They have been thought to be allelic with one another, but since no fertile males can be obtained from any except, in rare cases, brindled, direct tests of allelism have usually not been possible. In addition, a similar phenotype, described as 'variegated', is seen in females heterozygous for coat colour mutants translocated on to the *X*-chromosome[7,8].

It is here suggested that this mosaic phenotype is due to the inactivation of one or other *X*-chromosome early in embryonic development. If this is true, pigment cells descended from cells in which the chromosome carrying the mutant gene was inactivated will give rise to a normal-coloured patch and those in which the chromosome carrying the normal gene was inactivated will give rise to a mutant-coloured patch. There may be patches of intermediate colour due to cell-mingling in development. The stripes of the coat of female mice heterozygous for the gene tabby, *Ta*, which affects hair structure, would have a similar type of origin. Falconer[9] reported that the black regions of the coat of heterozygotes had a hair structure resembling that of the *Ta* hemizygotes and homozygotes, while the agouti regions had a normal structure.

Thus this hypothesis predicts that for all sex-linked genes of the mouse in which the phenotype is due to localized gene action the heterozygote will have a mosaic appearance, and that there will be a similar effect when autosomal genes are translocated to the *X*-chromosome. When the phenotype is not due to localized gene action various types of result are possible. Unless the gene action is restricted to the descendants of a very small number of cells at the time of inactivation, these original cells will, except in very rare instances, include both types. Therefore, the phenotype may be intermediate between the normal and hemizygote types, or the presence of any normal cells may be enough to ensure a normal phenotype, or the observed expression may vary as the proportion of normal and mutant cells varies, leading to incomplete penetrance in heterozygotes. The gene bent-tail, *Bn* [10], may fit into this category, having 95 per cent penetrance and variable expression in heterozygotes. Jimpy, *jp*, is recessive, suggesting that the presence of some normal cells is enough to ensure a normal phenotype, but Phillips[11] reported one anomalous female which showed the jimpy phenotype. Since it showed the heterozygous phenotype for *Ta* this animal cannot be interpreted as an *XO* female; it is possible that it represents an example of the rare instance when by chance all the cells responsible for the jimpy phenotype had the normal gene inactivated.

The genetic evidence does not indicate at what stage of embryonic development the inactivation of one *X*-chromosome occurs. In embryos of the cat, monkey and man sex-chromatin is first found in nuclei of the late blastocyst stage[12,13]. Inactivation of one *X* at a similar stage of the mouse embryo would be compatible with the observations. Since an *XO* female is normally fertile it is not necessary to postulate that both *X*-chromosomes remain functional until the formation of the gonads.

The sex-chromatin is thought to be formed from one *X*-chromosome also in the rat, *Rattus norvegicus*[14], and in the opossum, *Didelphis virginiana*[15]. If this should prove to be the case in all mammals, then all female mammals heterozygous for sex-linked mutant genes would be expected to show the same phenomena as those in the mouse. The coat of the tortoiseshell cat, being a mosaic of the black and yellow colours of the two homozygous types, fulfils this expectation.

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Genetic Basis for Graft-against-Host Immunological Reactions between Two Inbred Lines of Chickens

It has been established that the enlargement of the embryonic spleen which follows the injection of adult chicken blood into chick embryos is due, at least in part, to a proliferation of cells derived from the injected blood[1,2]. Cock and Simonsen[3] have shown that virtually no splenic enlargement occurs when the blood-donor and embryonic recipients are members of the same inbred line of chickens. The phenomenon of splenic enlargement seems to be fundamentally immunological in nature, and due to donor cells proliferating in response to those host antigens which differ from any in the donor.

It should be possible, by injecting blood from adult birds from one parental line into F_2-generation and back-cross embryos between two inbred lines, to analyse the antigenic difference of the other parental line. Assuming that the antigens of the parental lines are dominantly determined and that they segregate in crosses between the lines in a Mendelian fashion, then a proportion of F_2-generation embryos, and of embryos of the back-cross to the parent of the blood-donating line, will be expected to lack those genes which determine antigens occurring exclusively in the non-blood-donating line. The proportion of embryos which lack these genes will be $(\frac{1}{4})^n$ in the F_2-generation and $(\frac{1}{2})^n$ in the back-cross, where n is the number of pairs of genes involved. Since splenomegaly will occur only when the recipient embryo possesses antigens foreign to the donor cells, these are also the proportions of embryos in the respective crosses which will show no splenic enlargement. All F_1 hybrids and embryos of the back-cross to the parent of the non-blood-donating line will receive the genes which determine antigens peculiar to the non-blood-donating line and all these embryos will therefore show splenic enlargement. Thus, an estimate of the value of n can be obtained by observing the proportion of F_2 and back-cross embryos which show no splenic enlargement. The genetic basis for this method is essentially similar to that used in analysing histo-compatibility differences between inbred strains of mice using tumour transplantation[3], and skin transplantation[4].

The method outlined above has been used to observe antigenic differences between the Rensheath *C*- and *I*-inbred lines of White Leghorns[4]. Both lines have been brother–sister mated annually for more than twenty generations. Chick embryos were injected intravenously at 15 days of incubation with 0·1 ml. of citrated blood from *I*-line cocks, and killed 4 days later and their spleens weighed. The embryos injected were: *C*-line embryos, *I*-line embryos, the F_2-generation ($CI \times CI$ and $CI \times IC$), and the back-crosses ($C \times CI$ and $C \times IC$, $I \times CI$ and $I \times IC$). In designating the crosses, the male parent is stated first. Two *I*-line cocks were used as blood-donors to the F_2 and back-cross embryos and experiments with each donor were performed twice. Only a small number of *C* embryos were available for injection, but the marked splenic enlargements obtained indicate that *C* tissues are antigenic to *I* cells. A small number of *I*-line embryos injected with *I*-blood showed no splenomegaly. So far, we have had no F_1-hybrids to test, but Cock and Simonsen[3] have obtained splenic enlargement after injecting *I*-blood into newly hatched $C \times I$ chicks. The patterns of spleen weights obtained after injecting *I*-blood into F_2 and back-cross embryos were similar in each of the four experiments, and the results have been pooled in Fig. 1. The proportions of spleens in the different crosses showing no enlargement are shown in Table 1, and these results are compared with the theoretical frequencies expected for 1, 2 and 3 pairs of dominant genes determining antigens peculiar to the *C*-line. The proportions best fit the expectancy for one pair of genes, and the results suggest, therefore, that the *C*-line carries one antigen (capable of stimulating splenic enlargement) which is absent from the *I*-line. The results also fit the expectancy if the *C*-line possesses one dominantly determined antigen and one recessively determined antigen foreign to the *I*-cells. In this case, the proportion of unenlarged spleens in the F_2-generation would be 18·75 per cent (the proportion falls in a series $(\frac{3}{4})^{n_1} \times (\frac{1}{4})^{n}$, where n_1 is the number of pairs of recessive genes and n is the number of pairs of dominant genes). The proportion of unenlarged spleens in the back-crosses would remain unchanged. However, until we have other evidence for the

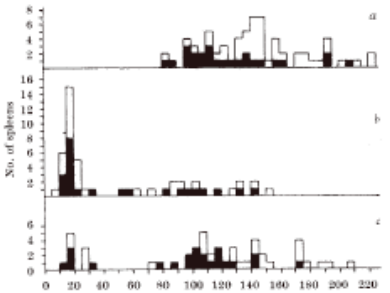
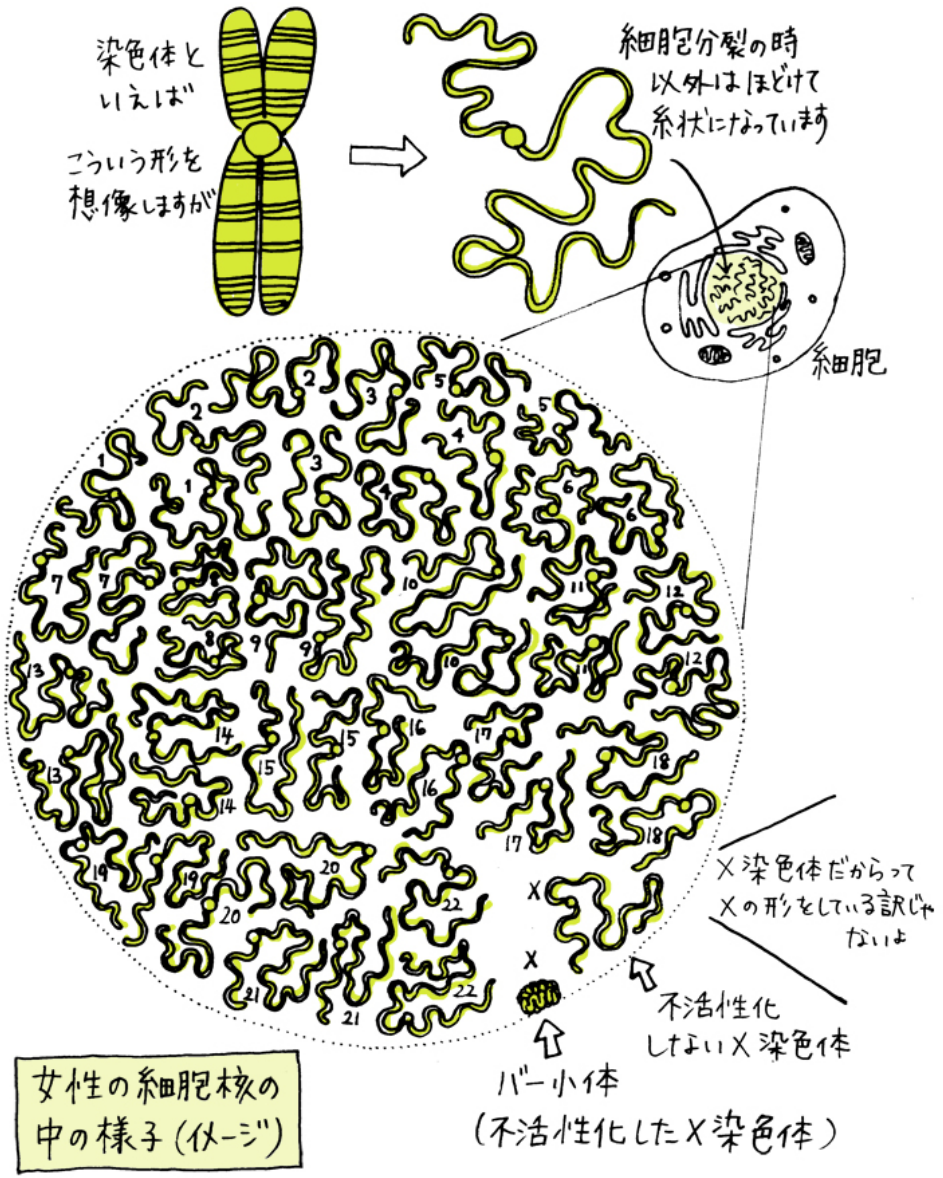


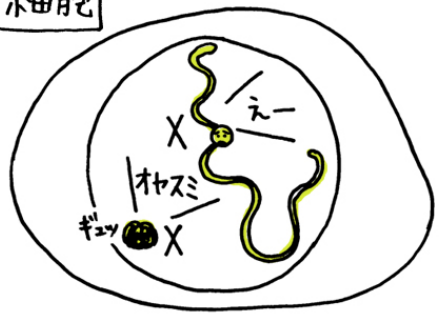
Fig. 1. Distribution of spleen weights obtained after injecting *I*-line cock-blood into F_2-generation and back-cross embryos. Solid squares, male spleens; open squares, female spleens. (*a*) $C \times CI$ and $C \times IC$ embryos; (*b*) $I \times CI$ and $I \times IC$ embryos; (*c*) $CI \times CI$ and $CI \times IC$ embryos

「X染色体不活性化」の意義



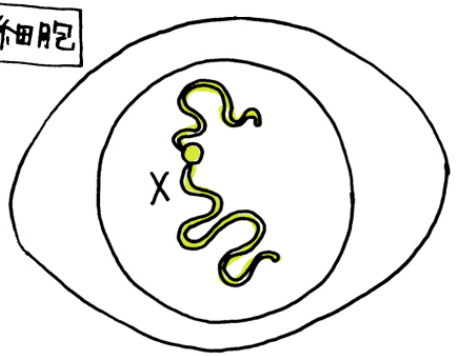
生きるために必要なのは
X染色体1本分の遺伝情報なので

女性の細胞



Xのうち1本が「凝縮」して不活性化
もう1本のXだけ働く

男性の細胞

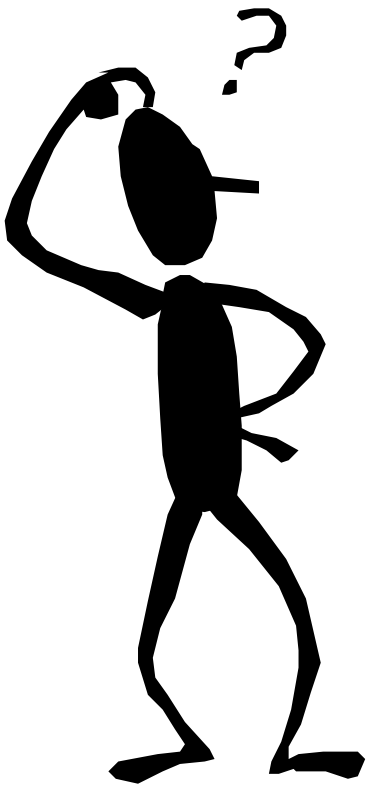


Xが1本働いている

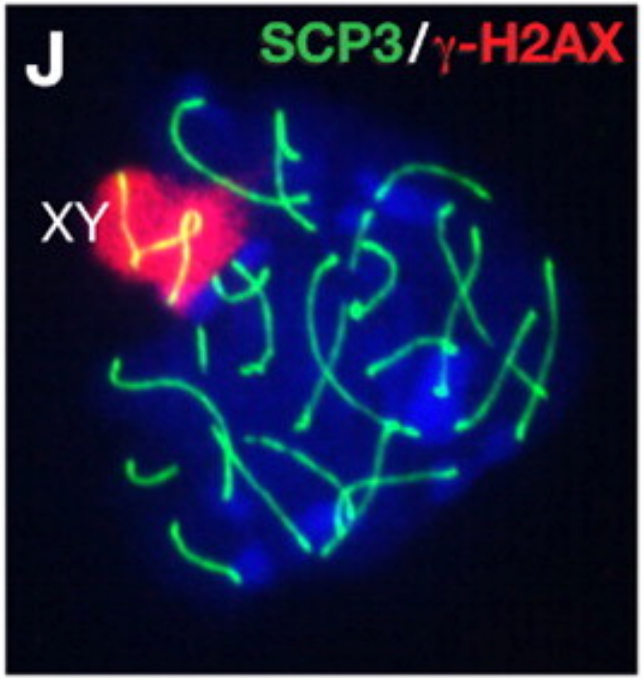
三毛猫は雌のみ！


父ネコ
X染色体を持つ
精子
卵子
母ネコ
黒になる遺伝情報を
もつX染色体
受精
茶色になる遺伝情報を
もつX染色体
XXの受精卵
細胞分裂
細胞がある程度増えたところで
X染色体のどちらか1本が不活性化。
細胞ごとに選ばれる。
メスの
子ネコ
誕生
X染色体の不活性化パターンに
合わせて黒と茶のまだら模様が
できる
※常染色体にある白毛の遺伝
情報が働くと、三毛猫になる

X染色体不活性化(XCI)はいつ生じるか？

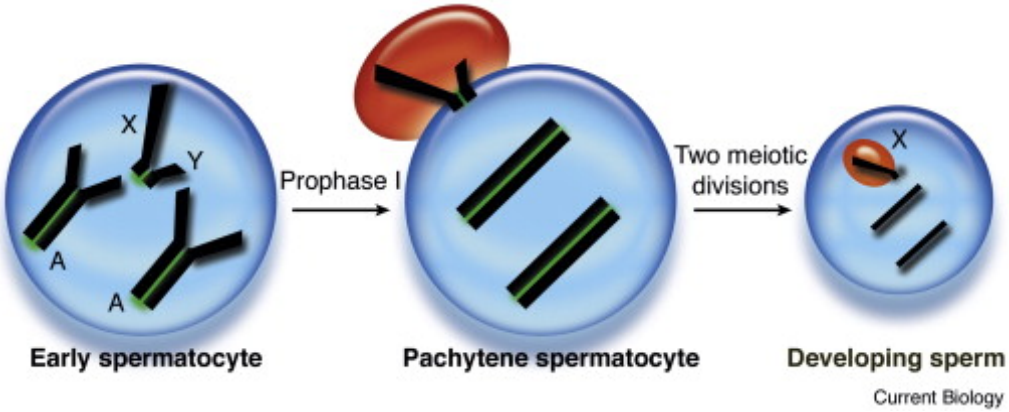


XY bodyで父方X染色体不活性化！



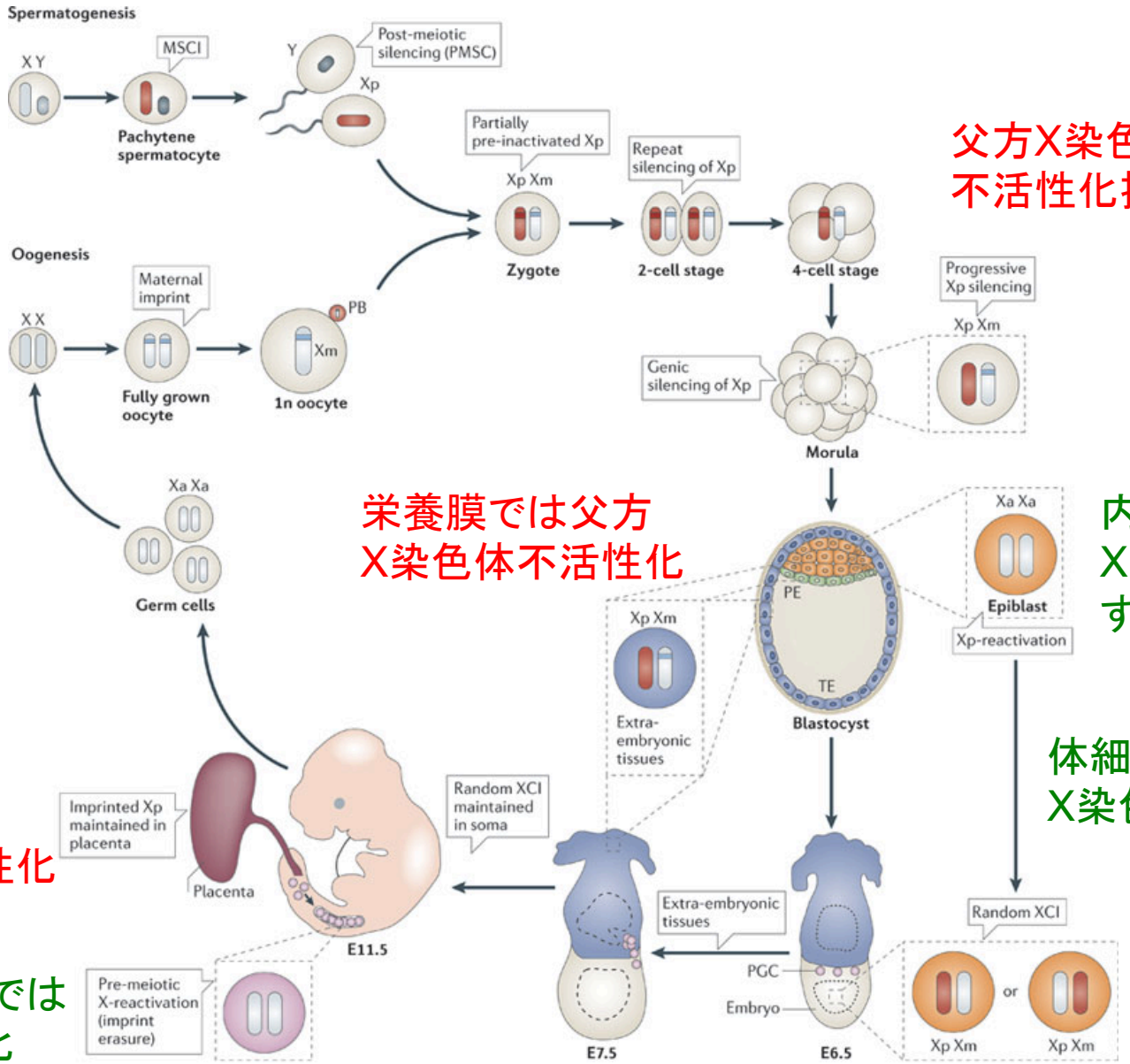
Kuznetsov et al., 2007

XY body



精母細胞では
X染色体不活性化

Nature Reviews Molecular Cell Biology 12, 815-826 (December 2011)



父方X染色体
不活性化持続

栄養膜では父方
X染色体不活性化

内部細胞塊では
X染色体
すべて賦活化

体細胞ではﾗﾝﾀﾞﾑな
X染色体不活性化

胎盤では父方
X染色体不活性化

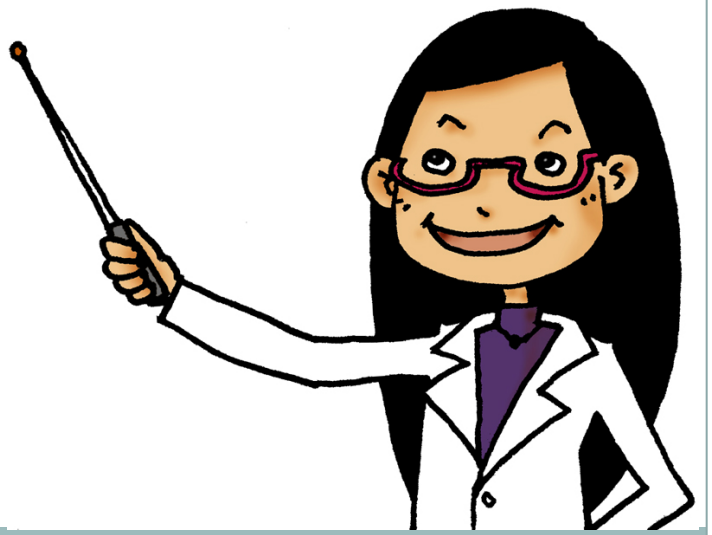
始原生殖細胞では
X染色体賦活化

インプリンティングとX染色体不活性化

- 正常な胚発生には父方・母方両方のゲノムが必要
- インプリンティング（刷り込み）
  - 父方の遺伝子発現→胎盤形成に重要
  - 母方の遺伝子発現→胎児の発生に重要
- インプリンティング異常による疾患
  - 例：ベックウィズーウィーデマン症候群
    - 父方の11p15.5領域が重複
    - DNAメチル化の異常など
- X染色体不活性化
  - 女性のX染色体はランダムに片方が不活性化されている
  - 精子形成の間にX染色体が不活性化
    - 子の胚体外組織に受け継がれる

クイズ！

胎盤は母体由来か、胎児由来か？
その理由、根拠は？
出席カードに書いて下さい。



講義予定

- 5/29(1)：ガイダンス、序章
- 5/29(2)：第1章（配偶子形成・受精・発生第1週）
- 5/29(3)：第2章（発生第２週：二胚葉）
- 6/5(4)：第3章（三胚葉～軸形成）
- 6/5(5)：第4章（神経管形成・神経堤細胞）
- 6/5(6)：第5章（形態形成・動物モデル）（吉川助教）
- 6/12(7)：第6章（胎盤・羊水）
- 6/12(8)：第7章（皮膚・皮膚付属器）
- 6/12(9)：特別講義「先天異常」（安田先生）

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