Human parthenogenetic stem cells

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Parthenogenesis is a biological phenomenon by which embryonic development occurs without paternal contribution (Vrana et al, 2003).

From the greek παρθένος *parthenos*, "virgin", + γένεσις *genesis*, "birth".

While common is some plants, insects and reptiles, parthenogenesis is not a naturally occurring form of reproduction in mammals.

Mammalian oocytes can, however, undergo a limited parthenogenetic development.

Mammalian oocytes can undergo a limited parthenogenetic development after activation by a physical, chemical or electrical stimulus.

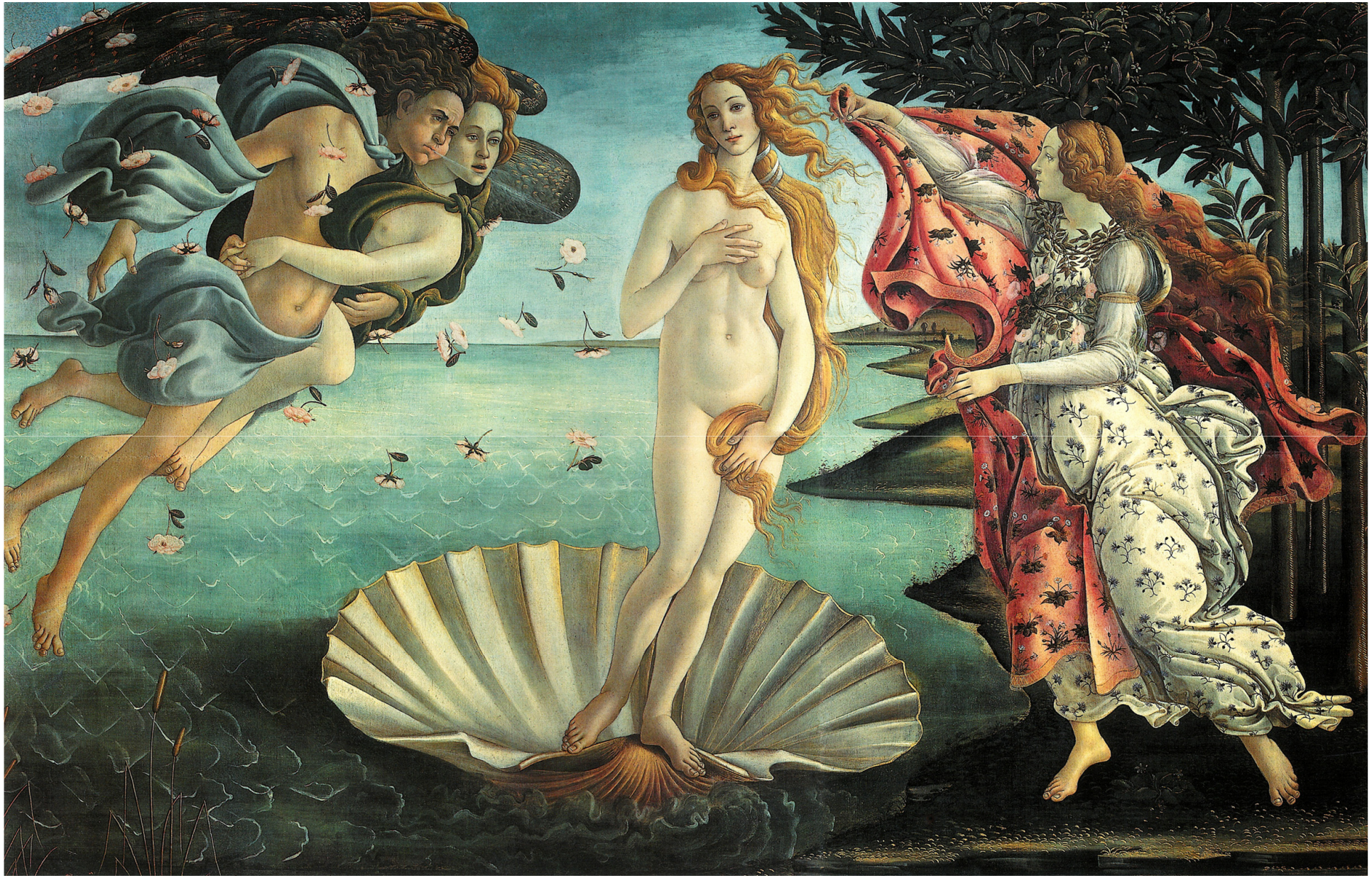
Development does not proceed to term and but it stops around implantation.

There is a consistent hypo-development of placenta in parthenogenetic pregnancies.

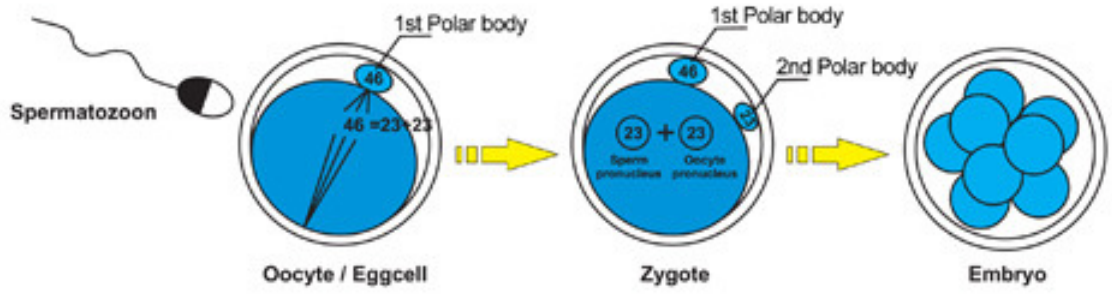
Main reason thought to be altered expression of paternally expressed genes, in particular imprinted genes.

Species	**Maximum development (days)**	**Pregnancy length (days)**	**Reference**
Mouse	10	21	Surani *et al*. 1986
Rabbit	10–11	31	Ozil 1990
Pig	29	114	Kure-bayashi *et al*. 2000
Sheep	25	150	Loi *et al*. 1998
Bovine	48	280	Fukui *et al*. 1992
Marmoset monkey	10–12	144	Marshall *et al*. 1998

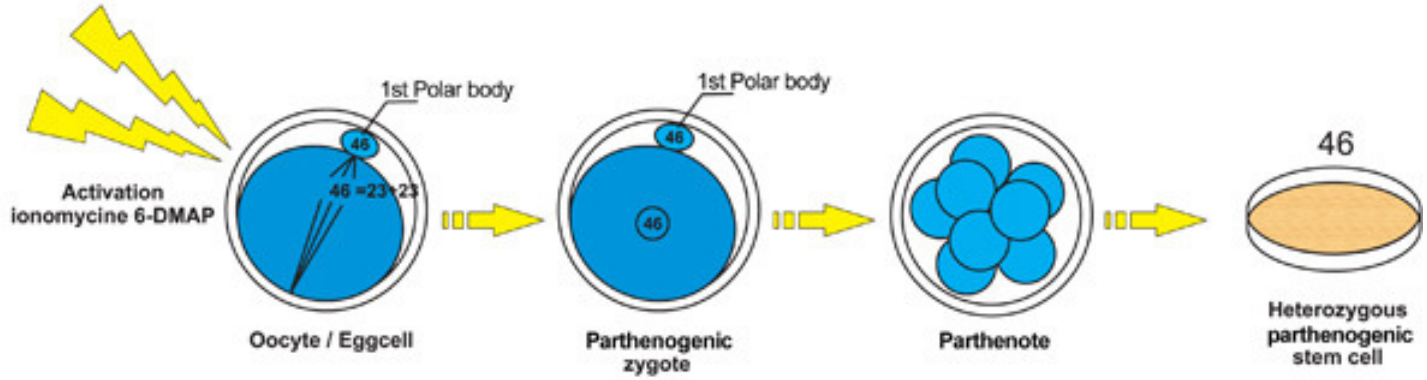
Brevini and Gandolfi, 2008



Fertilization



Parthenogenesis



Activation needs to accomplish two tasks:

1) Reactivation of meiosis → mimic Ca++ oscillation that occur during fertilization

CA++ ionophores such as Ionomicin or A23187, ethanol, strontium chloride, or electrical stimulus.

2) Block the extrusion of the 2nd PB and initiate a mitotic cell cycle

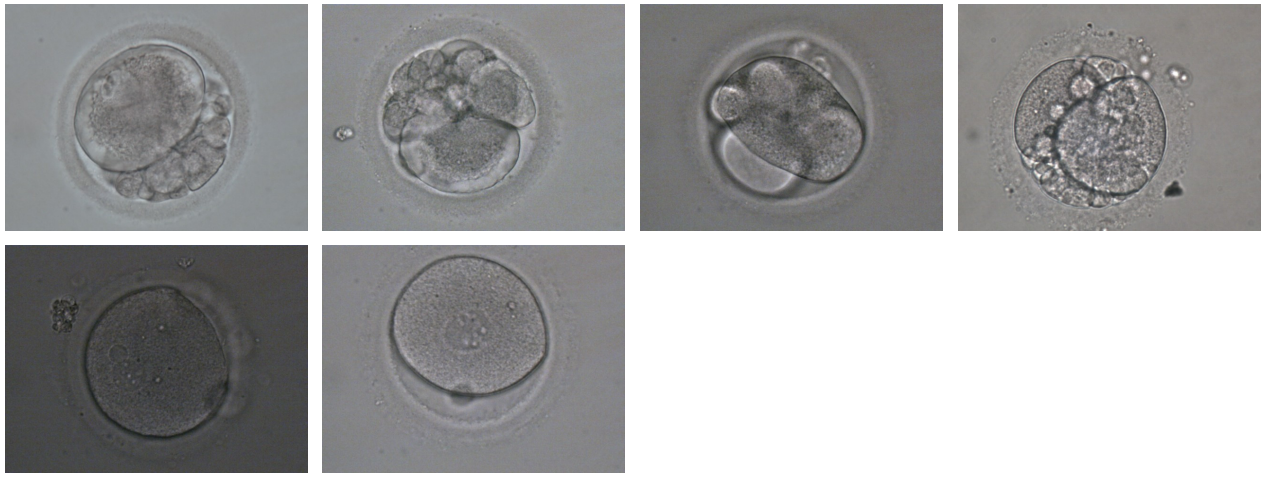
Heat shock, Cytochalasin B (inhibits cytoskeleton movements).

Inhibition of protein kinase, of protein phosphorylation (6-DMAP), or synthesis.

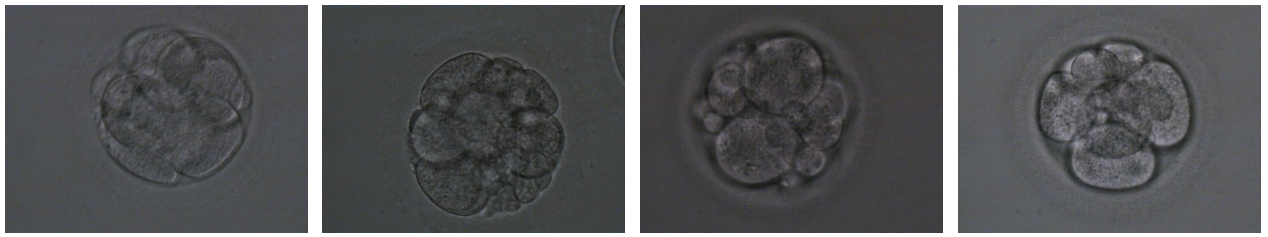
pES cell production in mammalian species

- Human spontaneous activation thought to be the cause of benign ovarian tumors (Coullin, 2005).
- Mouse haploid and diploid pESC derived soon after mouse ESC (Kaufman et al, 1983; Kaufam et al, 1984).
- *Macaca fasciculata* pESC (Cibelli et al, 2002) and *Macaca mulatta* pESC (Dighe et al, 2008)
- Human pESC (Revazova et al, 2007; Lin et al, 2007; Mai et al, 2007; Revazova et al, 2008)
- Mouse pESC form teratomas BUT fail to produce live pups after tetraploid complementation.
- SCNT with mouse pESC fail to produce viable embryos, BUT sequential NT increase gestational length (up to d14.5 pc)

Development of human oocytes after parthenogenetic activation

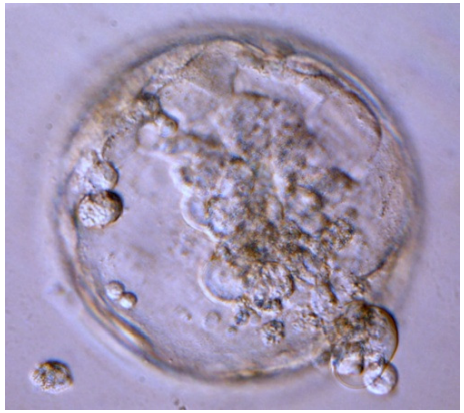


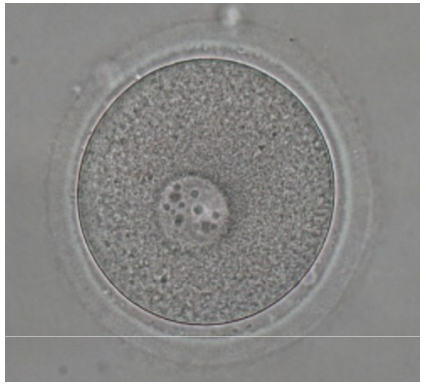
D+1

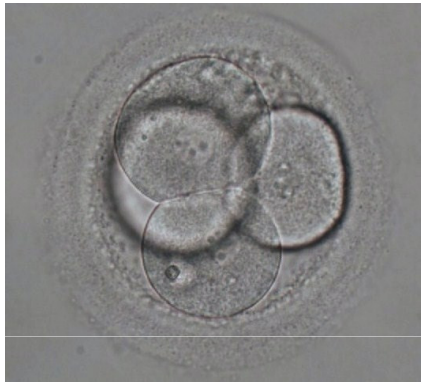


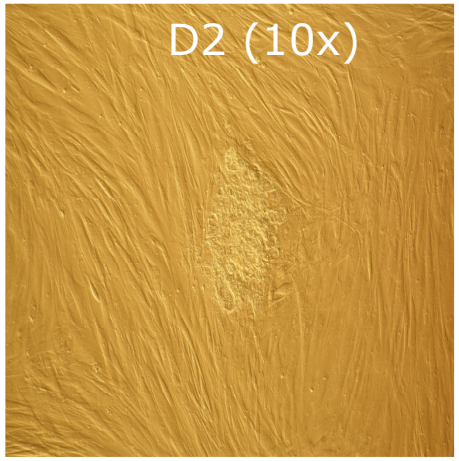
D+3

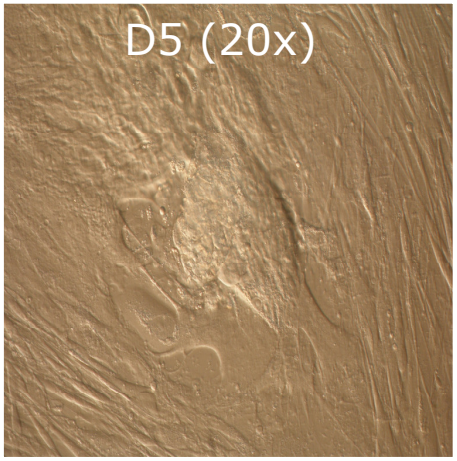
Oocytes	1PN1PB @ 18h	Cleaved @ D2	Blastocysts @ D5	Cell lines
17	12	16 (94%)	6 (37.5%)	1 (6.25%)

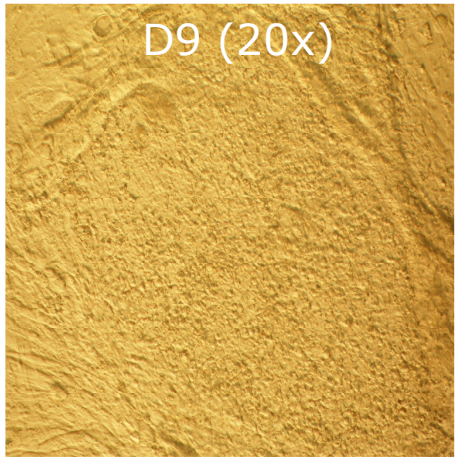


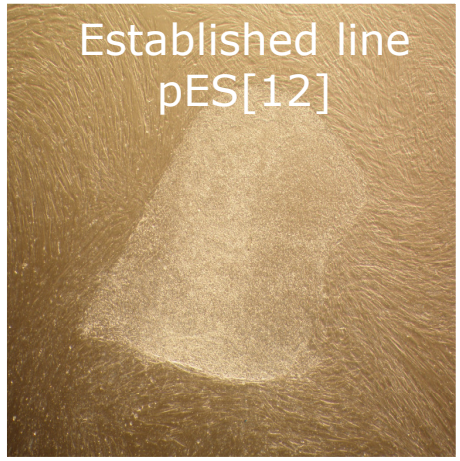


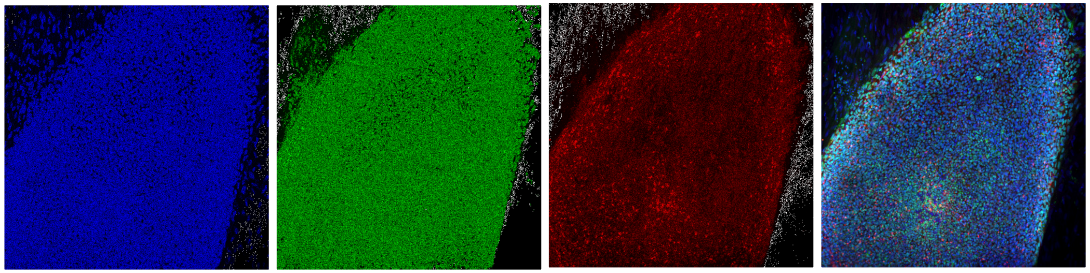




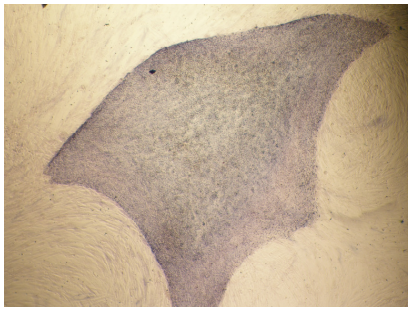


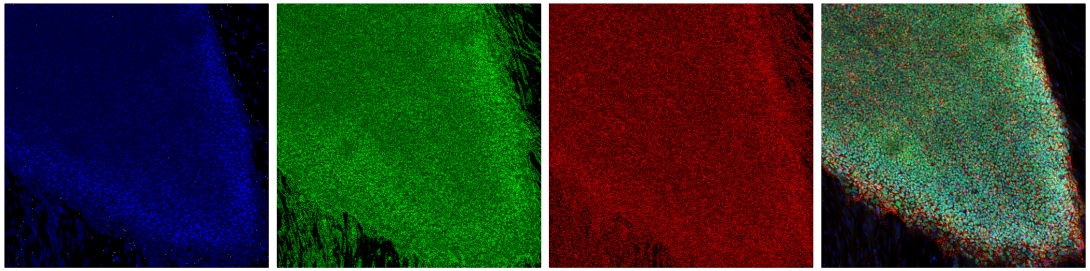




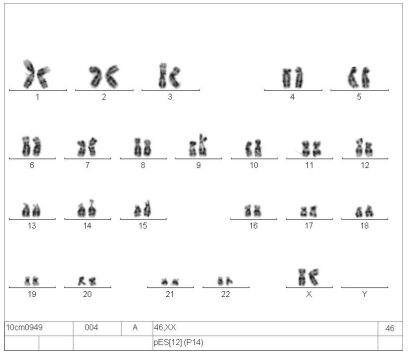


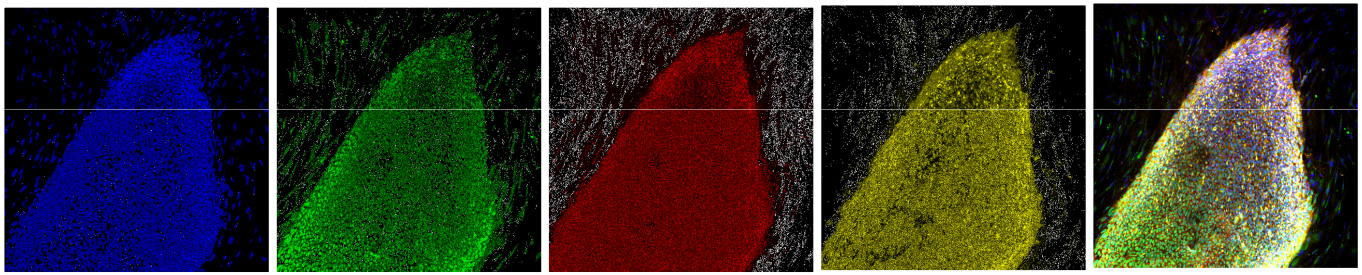
DNA
Nanog
TRA 1-81



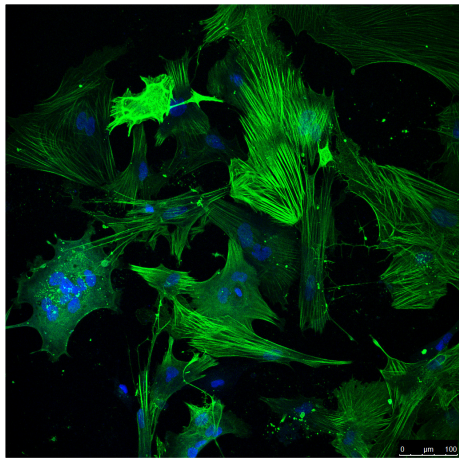


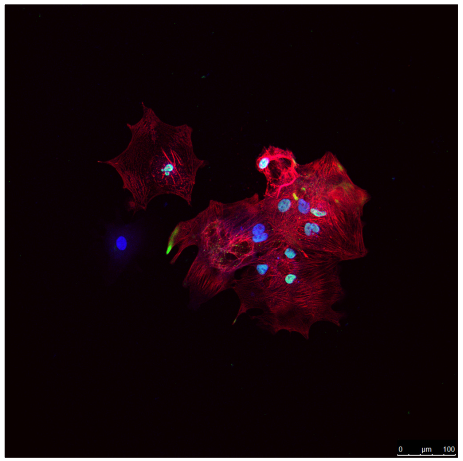
DNA
Oct4
SSEA3

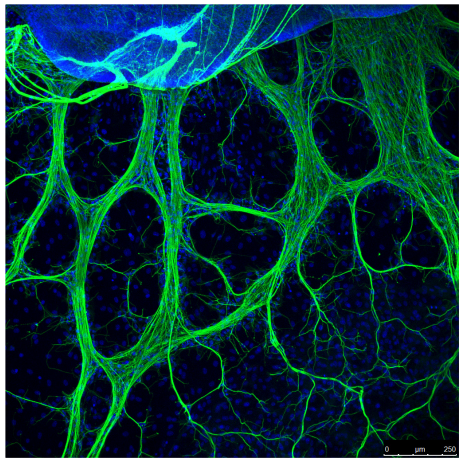


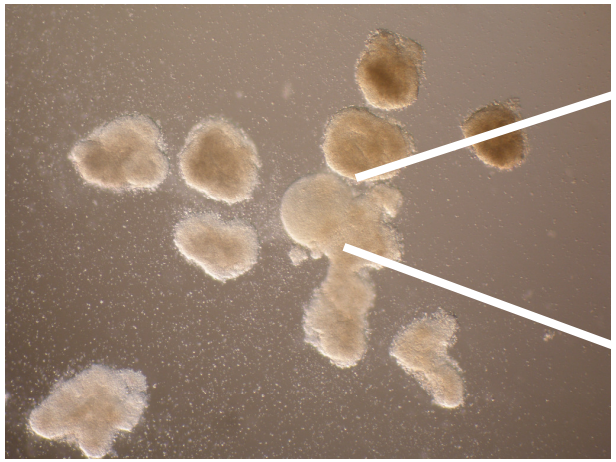


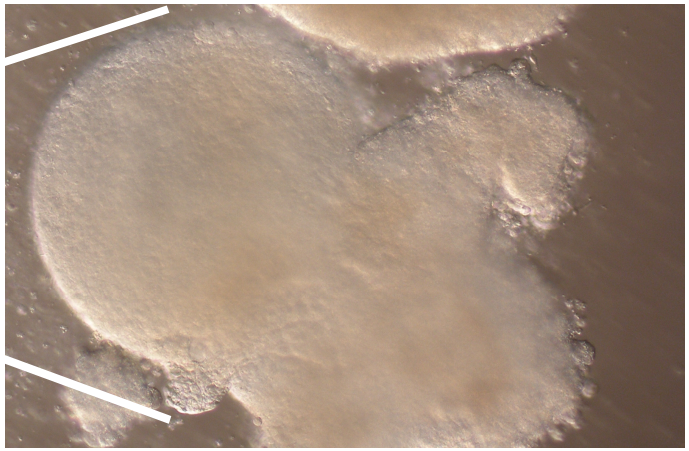
DNA
Sox2
SSEA4
Tra 1-60

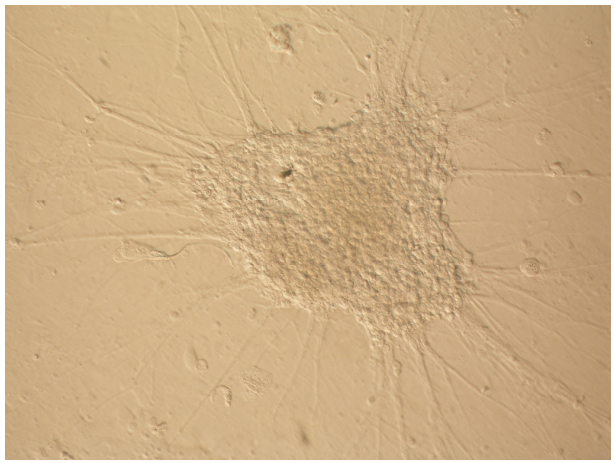


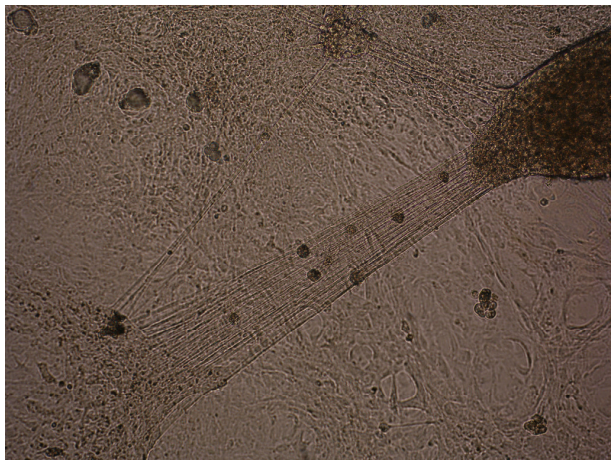












Could we use pES for regenerative medicine?

- Pros:
  - No paternal genome → no fertilization → no “embryo”?
  - No viable embryos to term
  - Differentiating ability to many cell types in vitro
  - Could provide immunologically compatible cells (for the oocyte donor)

- Cons:
  - Genomic imprinting
  - High homozygosis

Genomic imprinting

Every autosomal gene is inherited in 2 copies, one from our mother and one from our father.

In a small subset of genes, one copy is turned off in a parent-of-origin dependent manner.

Thus, the allelic expression of an imprinted gene depends upon whether it resided in a male or female the previous generation.

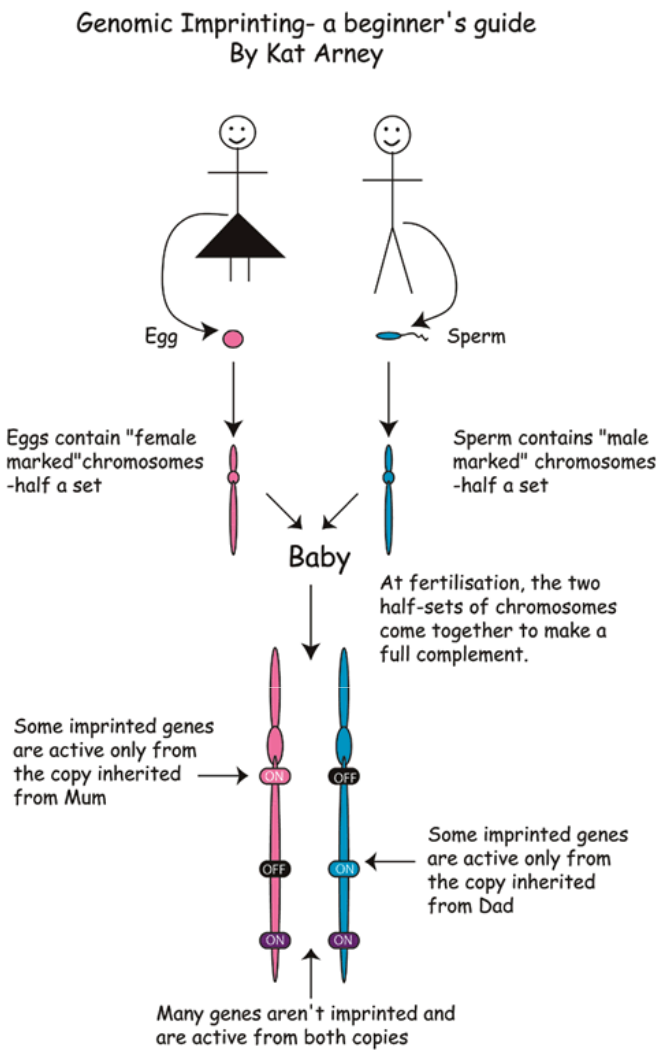
Genomic imprinting evolved in mammals with the advent of live birth.

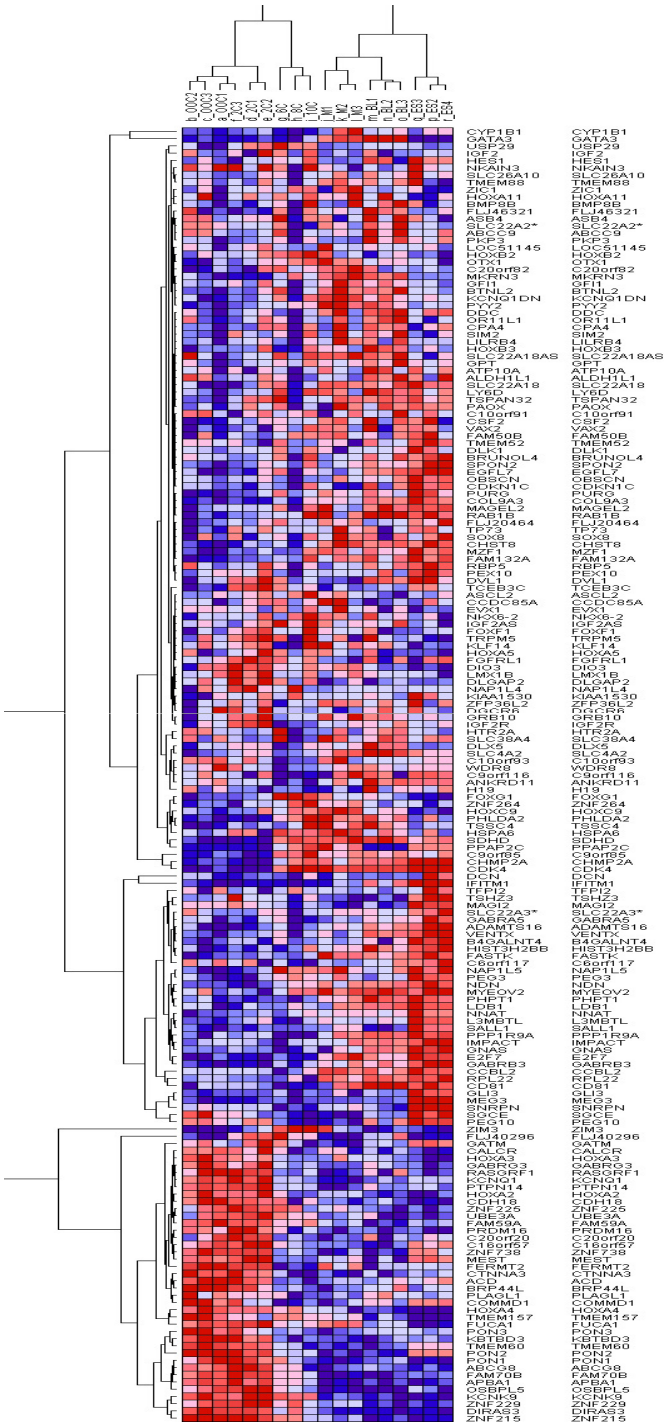
Its evolution apparently occurred because of a parental battle between the sexes to control the maternal expenditure of resources to the offspring (***Haig,1996***).

Paternally expressed imprinted genes tend to promote growth while it is suppressed by those genes that are maternally expressed.

Paternally expressed genes enhance the extraction of nutrients from the mother during pregnancy to gain reproductive advantage for its genes, whereas, the maternal genome seeks to limit it for the same reason.

www.geneimprint.com





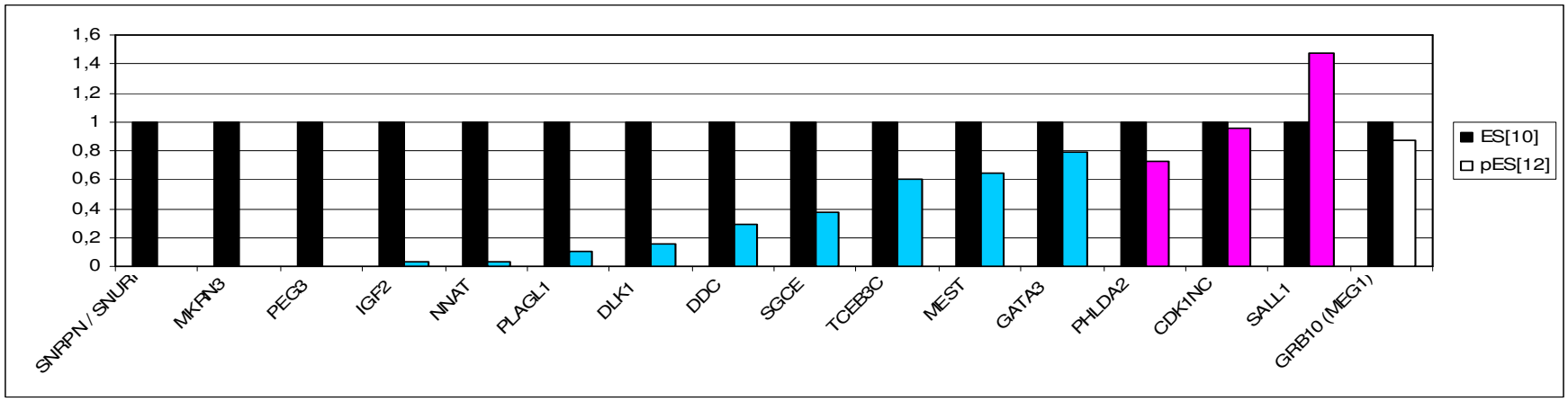
Imprinted genes are monoallelically expressed in a parent-of-origin dependent manner because the same parental allele is always epigenetically silenced *(**Jirtle and Weidman 2007**)*.

- In *Macaca mulatta*, the paternally expressed PEG10, IGF2, DIRAS3, and SGCE were also detected in pESC.

- The transcripts for SNRPN, NDN, MAGEL2, PLAGL1, and MKRN3 were never detected.

- The transcripts for PEG3, MEST and ZIM2 were more variable, detected in some lines and not in other.

- Maternally expressed transcripts were usually expressed at the same level or slightly higher in pESC than ESC.



EBs l.a.p. x 1 week

↓

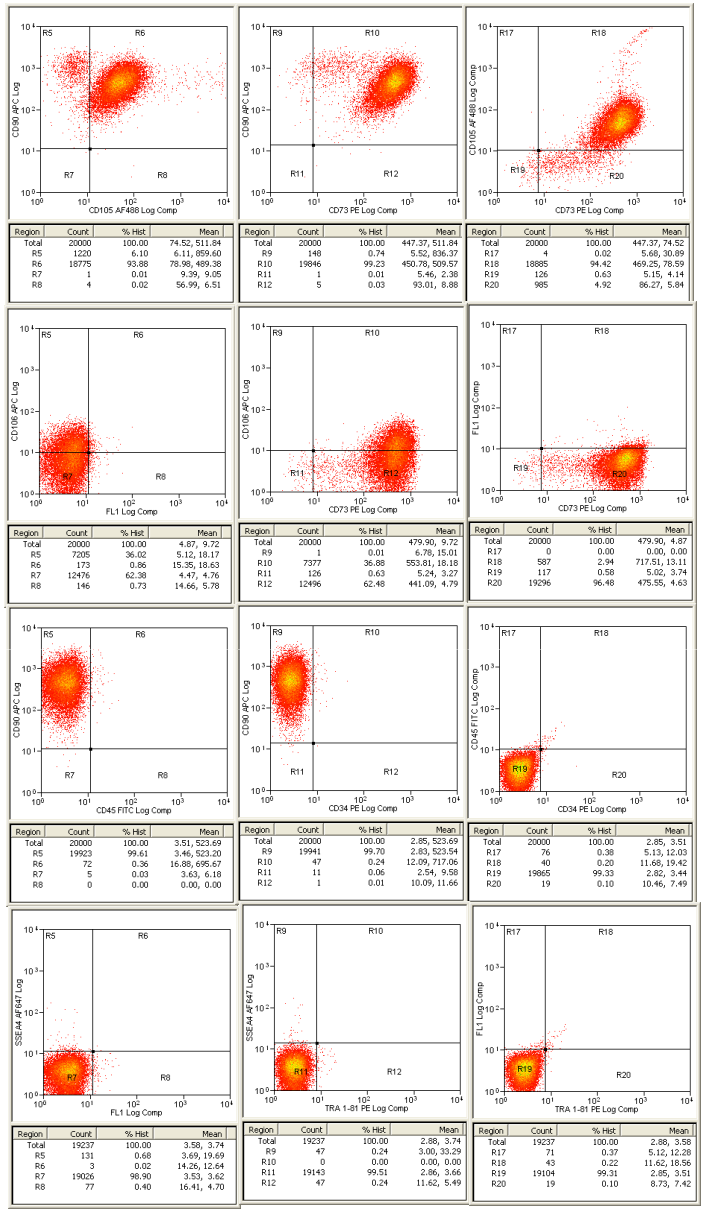
Attach on gelatine

↓

3 weeks selection

↓

Switch to MSC medium



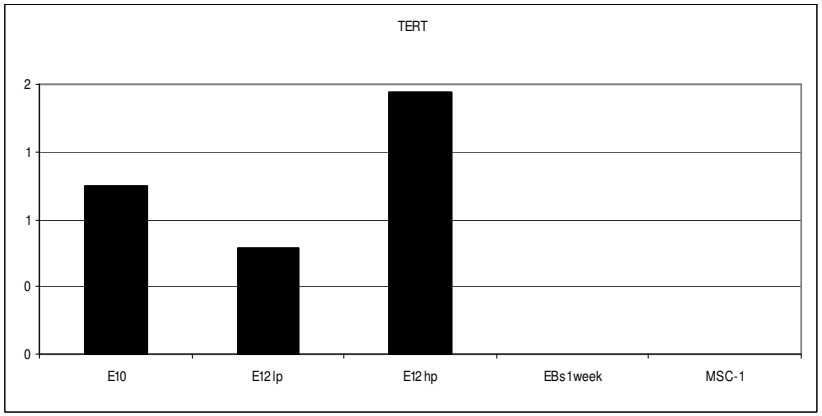
CD105 AF488
CD73 PE
CD90 APC

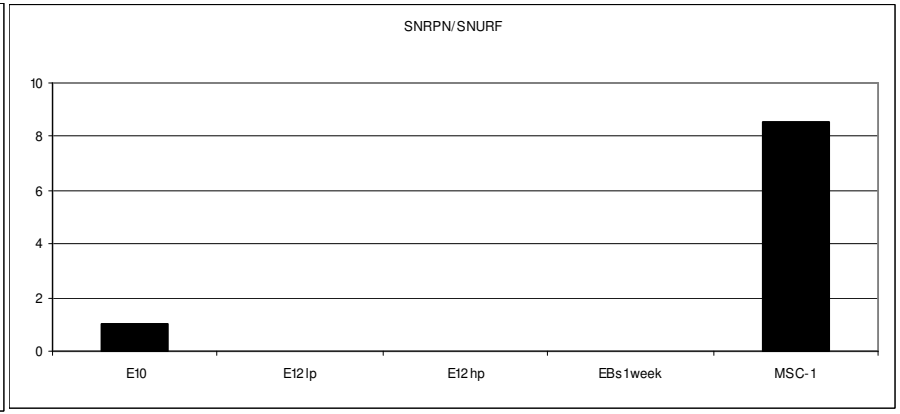
CD73 PE
CD106 APC

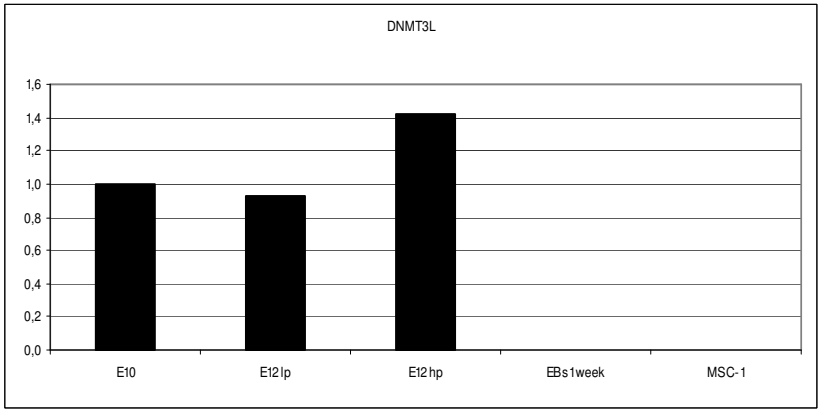
CD45 FITC
CD34 PE
CD90 APC

TRA 1-81 PE
SSEA4 AF647

pES[12] → Diff-MSC







Zygosity of pESC

- In conventional activation, diploidy is achieved by retention of the 2PB.
- The resulting cell line is therefore highly homozygous BUT for crossing over that happened during MI, which can produce heterozygous loci.
- In monkey pESC, homozygosity varies between 40 and 95%.
- In human pESC, homozygosity can reach >95%.
- Most lines are homozygous in HLA loci.

conclusions

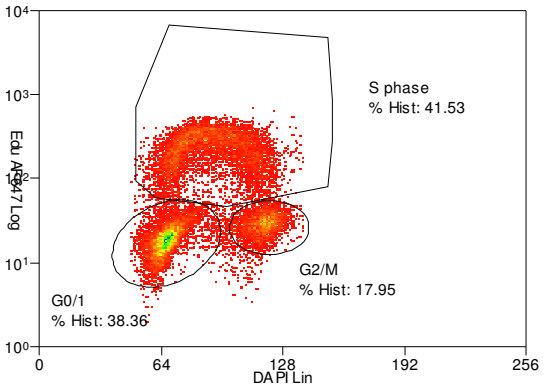
- Parthenogenetic activation can be efficiently used to derive stem cell lines
- The efficiency of line derivation makes it a viable alternative for women of reproductive age (I.e. 1 cycle of ovarian stimulation should suffice for creating a donor matched line)

- This method is considered by most more acceptable than the use of biparental embryos donated for research by couples. No viable fetuses ever reported.

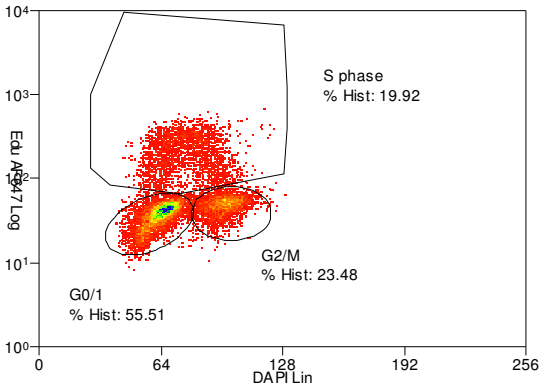
- Monoparental genomic imprinting is conserved after in vitro culture of undifferentiated pESC.
- Altered expression of imprinted genes has been linked with numerous kind of cancer, as well as to complex syndromes with strong emphasis on impaired nervous development/function.

- It is still unclear whether the immunological advantages of parthenogenetic stem cells outweight the limitation of functional homozygosis, as well as homozygosis in most genes.

ES10 biparental XX



pES12 monoparental XX



Cell line	G0/1	S	G2/M
ES10	38	41	18
pES12	55	20	23