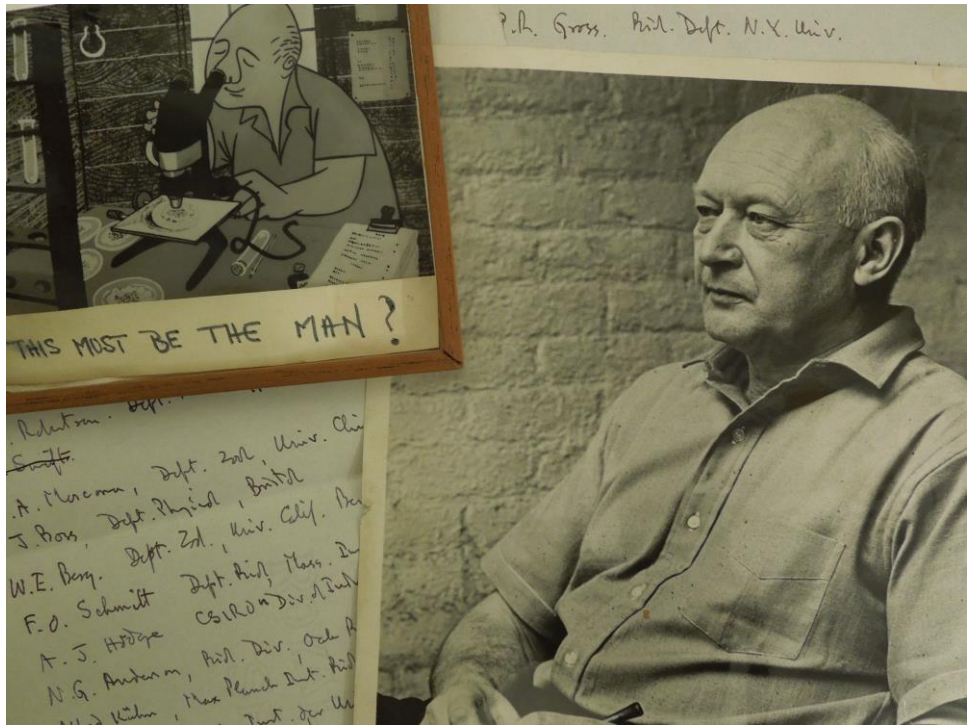
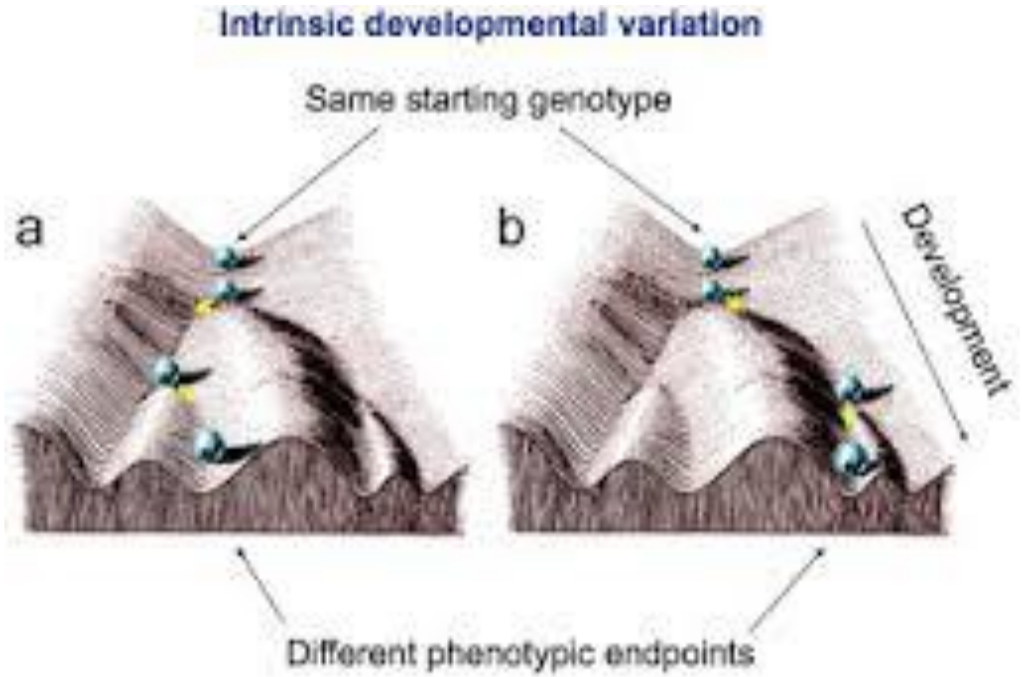
Epigenetics in embryonic development

The term Epigenetics was coined by Waddington to define it study of “causal mechanisms by which the genes of the genotype bring about phenotypic effects.” Waddington (1942)

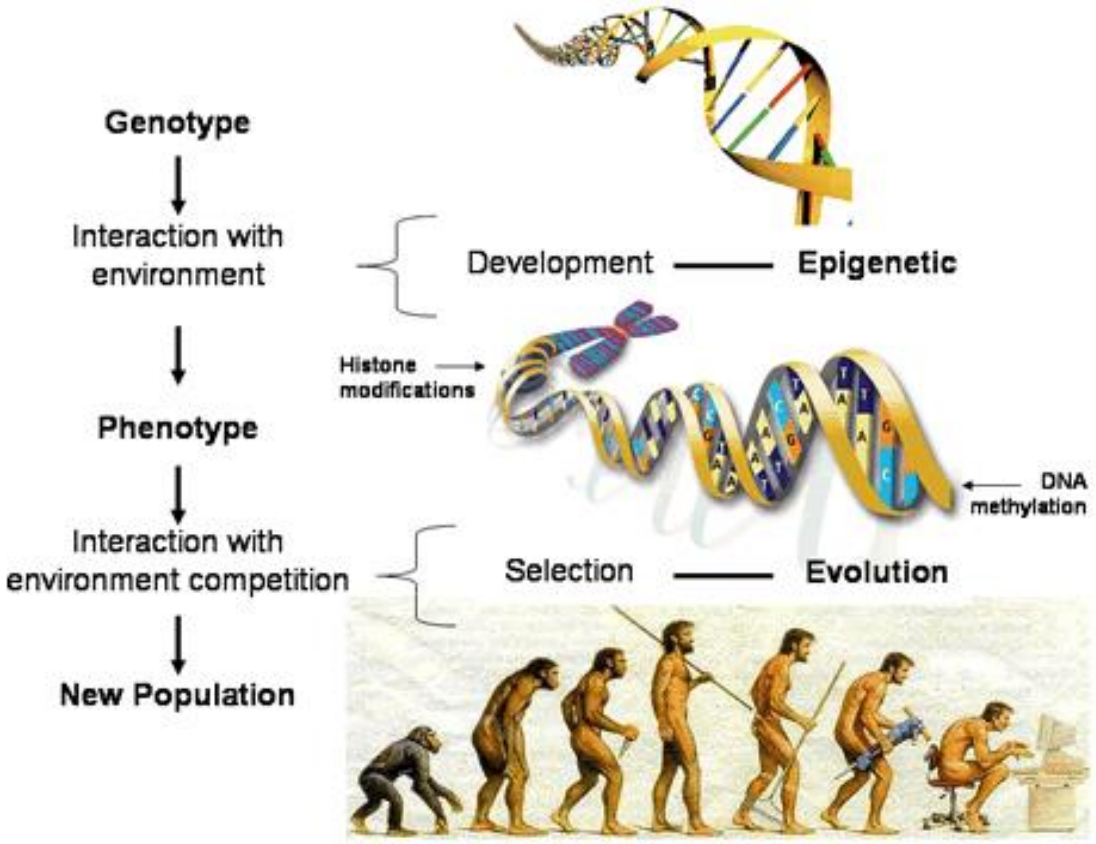




The course of development of an organism is determined through the interaction of genes with other genes and with the external environment

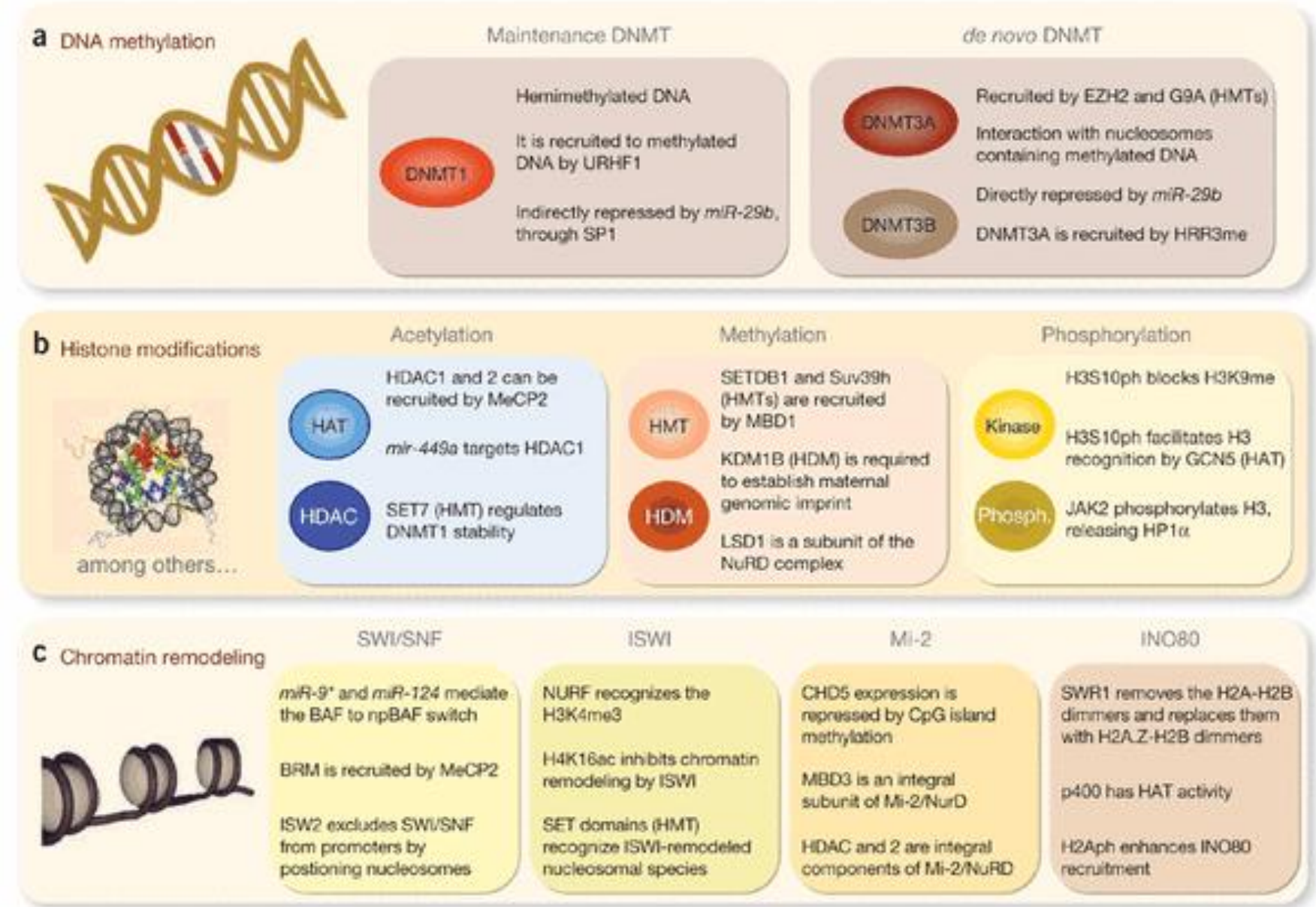
Evolution of the concept

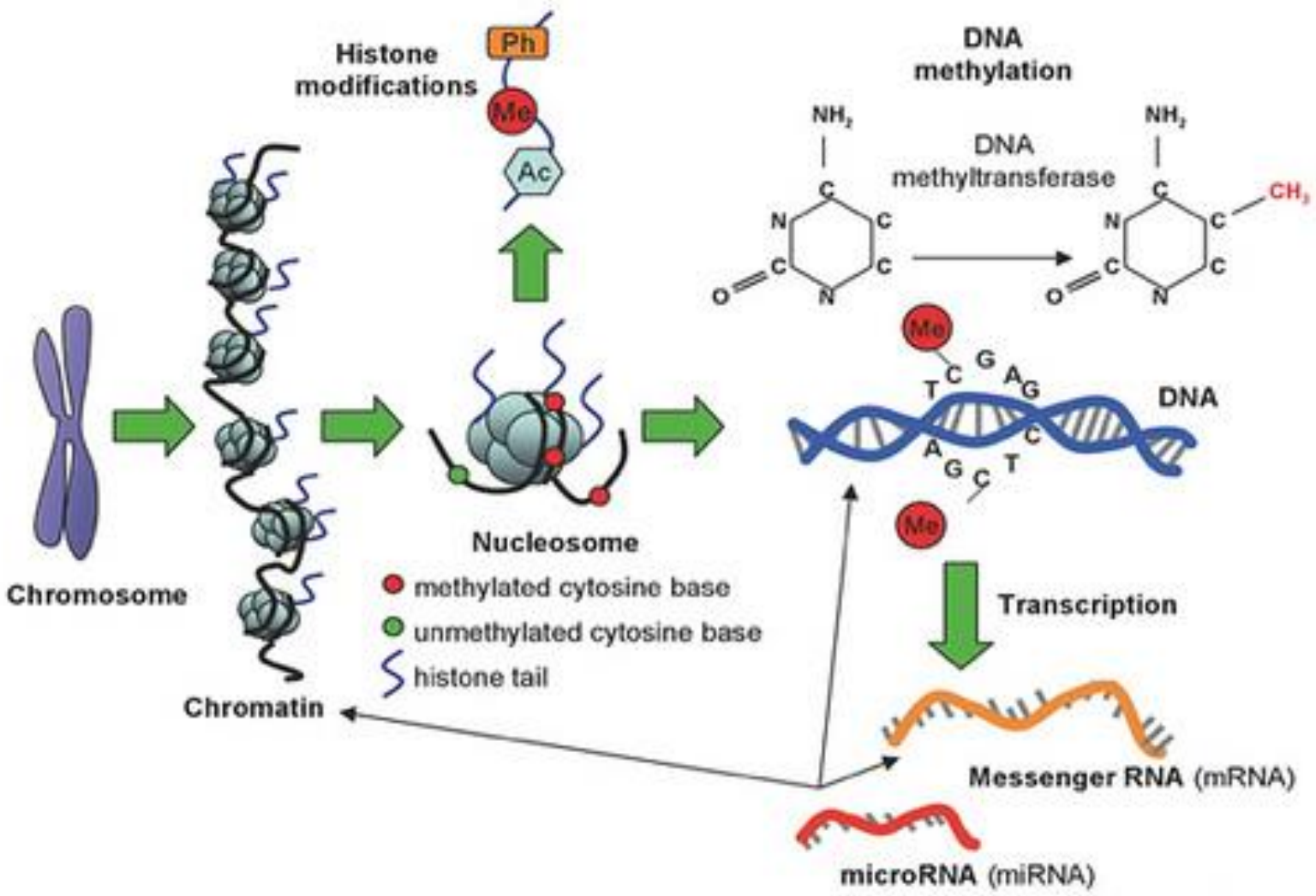
«The study of mototically and/or meiotically heritable changes in gene function that cannot be expalined by change DNA sequence»



Reversible and heritable changes in gene function that occur without changing the DNA sequence

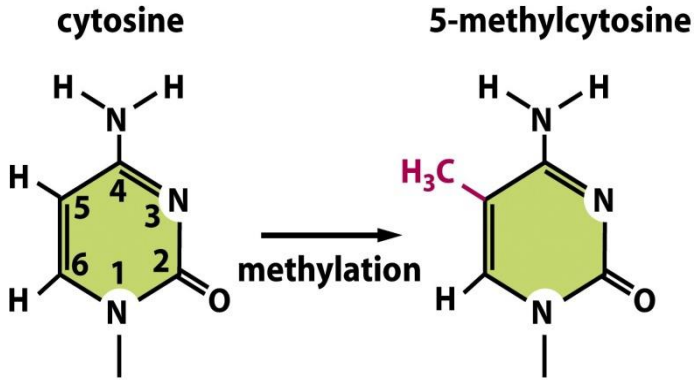
Epigenetic modifications





These modifications cause the degree of accessibility of the DNA to decrease the transcription factors by altering the activity of this gene.

Methylation Marks Regulation

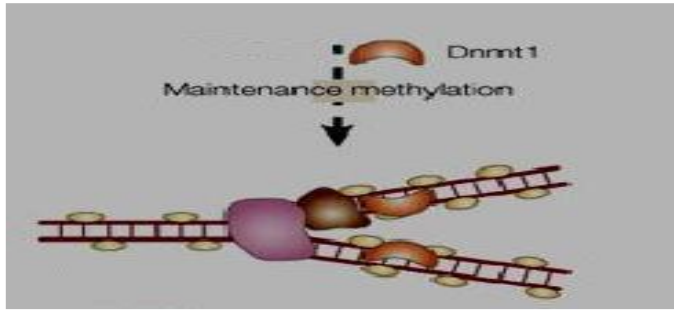


METHYLATION Affects the cytosines in position 5, of the CG dinucleotide sequences located mainly near the transcription start sites, constituting regions called CpG islands

TISSUE-SPECIFIC

SOMATIC CELLS

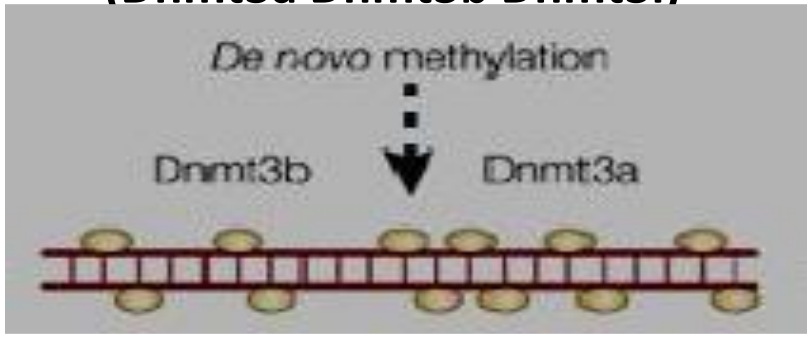
It is transmitted stably by mitosis
Maintenance Methyltransferase
(Dnmt1)



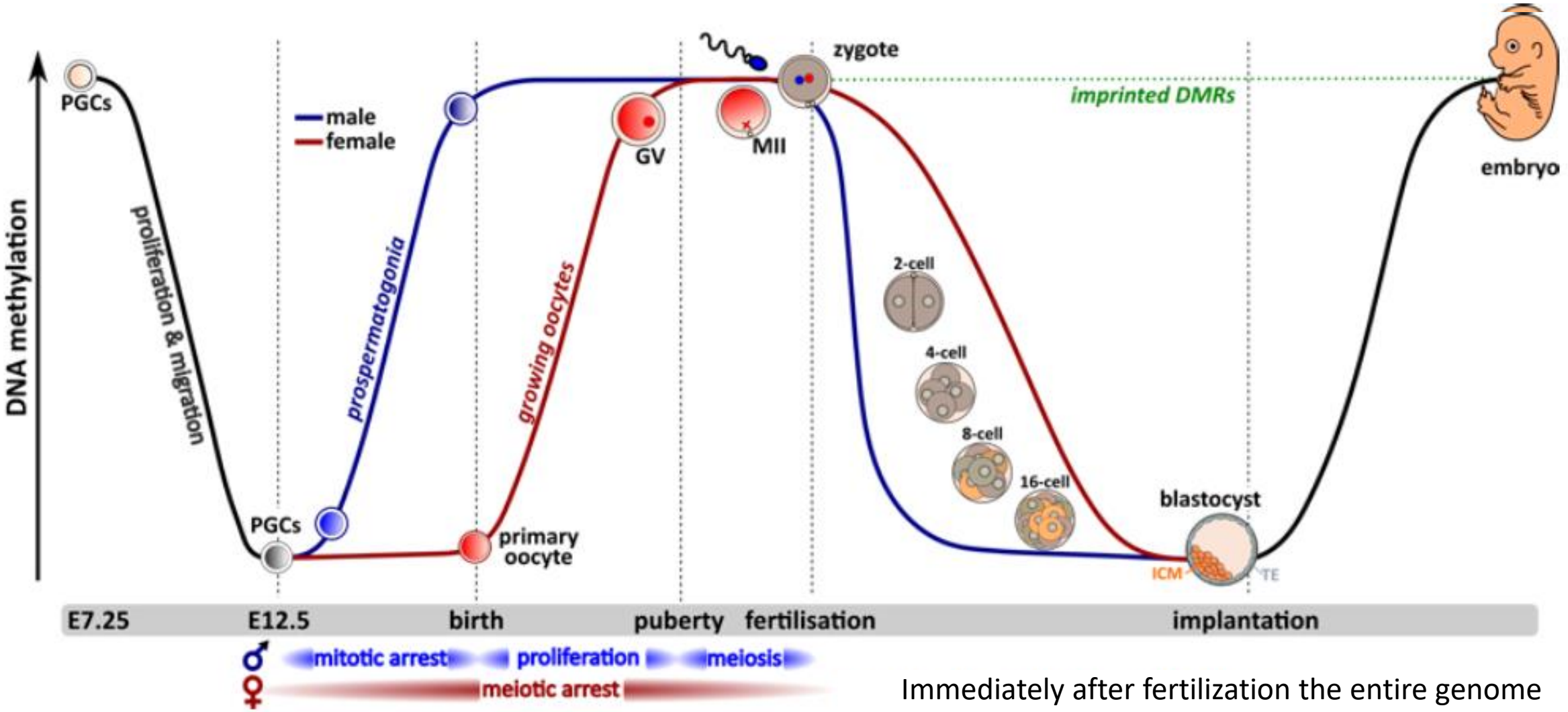
DEVELOPMENT-SPECIFIC

GAMETS

It is transmitted stably by mitosis
Maintenance Methyltransferase
(Dnmt3a Dnmt3b Dnmt3l)

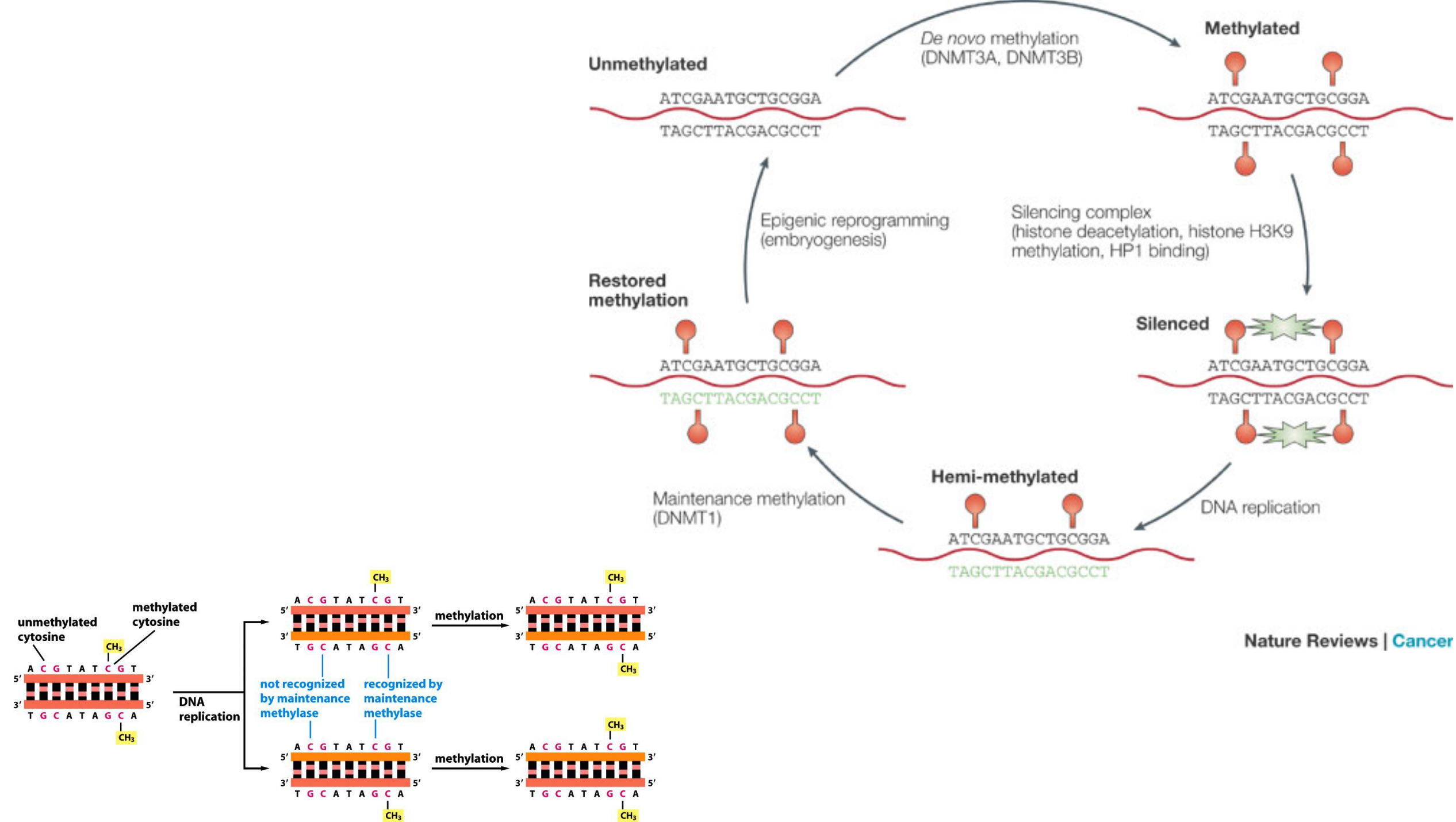


DNA METHYLATION DYNAMICS



During gametogenesis, methylation is canceled and subsequently restored based on the sex of the subject (**DE NOVO METHYLATION)**

Immediately after fertilization the entire genome undergoes a wave of demethylation **(DEMETHYLASE)**


Unmethylated
ATCGAATGCTGCGGA
TAGCTTACGACGCCT
De novo methylation
(DNMT3A, DNMT3B)
Methylated
ATCGAATGCTGCGGA
TAGCTTACGACGCCT
Silencing complex
(histone deacetylation, histone H3K9
methylation, HP1 binding)
Silenced
ATCGAATGCTGCGGA
TAGCTTACGACGCCT
DNA replication
Hemi-methylated
ATCGAATGCTGCGGA
TAGCTTACGACGCCT
Maintenance methylation
(DNMT1)
Restored
methylation
ATCGAATGCTGCGGA
TAGCTTACGACGCCT
Epigenic reprogramming
(embryogenesis)
unmethylated
cytosine
methylated
cytosine
DNA
replication
not recognized
by maintenance
methylase
recognized by
maintenance
methylase
methylation
methylation
Nature Reviews | Cancer

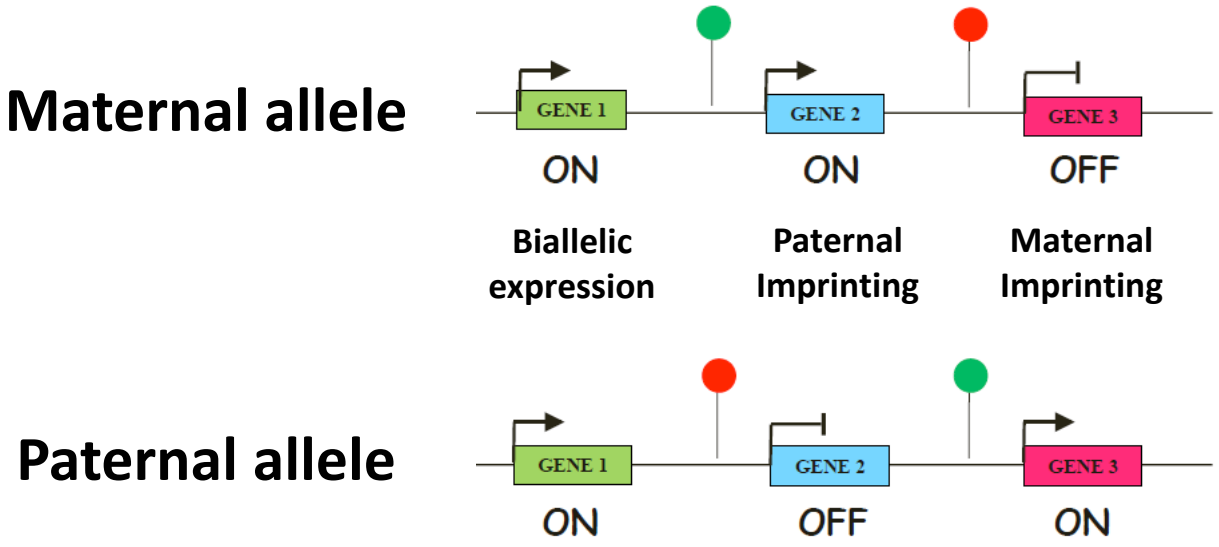
Governing processes of epigenetics

- Gene expression
- Cell Differenziation and embryogenesis
- X chromosome inactivation
- Genomic Imprinting
- Suppression of the mobility of transposable and retroviral elements
- Cancer

GENOMIC IMPRINTING

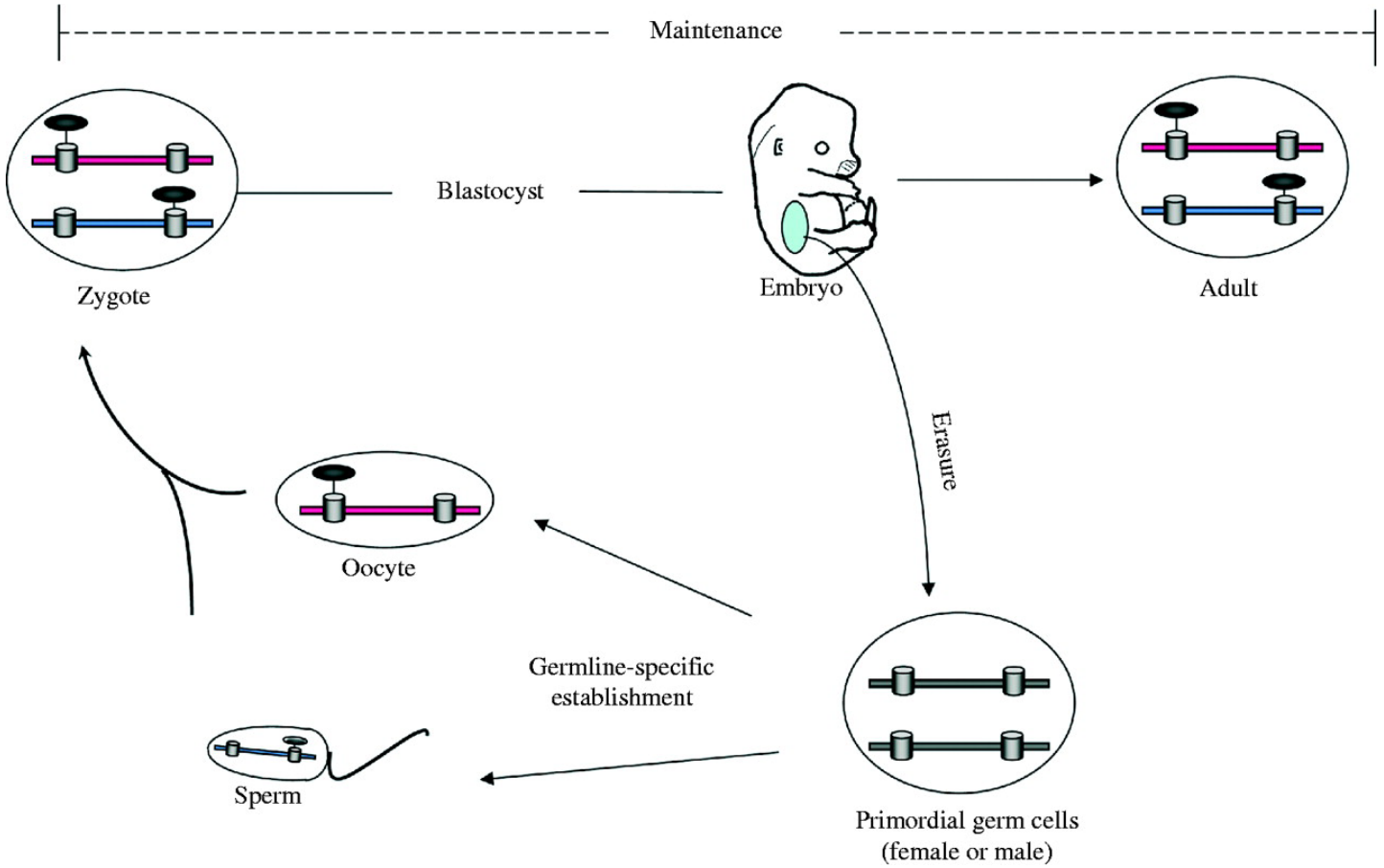
Epigenetic expression process leading to the monoallelic expression of a group of genes according to their parental origin

An **imprinted gene** is silenced on one of the 2 alleles through DNA methylation mainly by the so-called "imprintig controll regions" (ICRs)



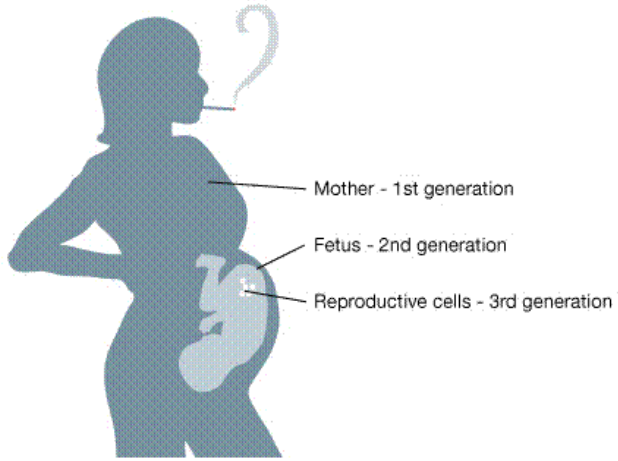
Imprinting in 3 steps

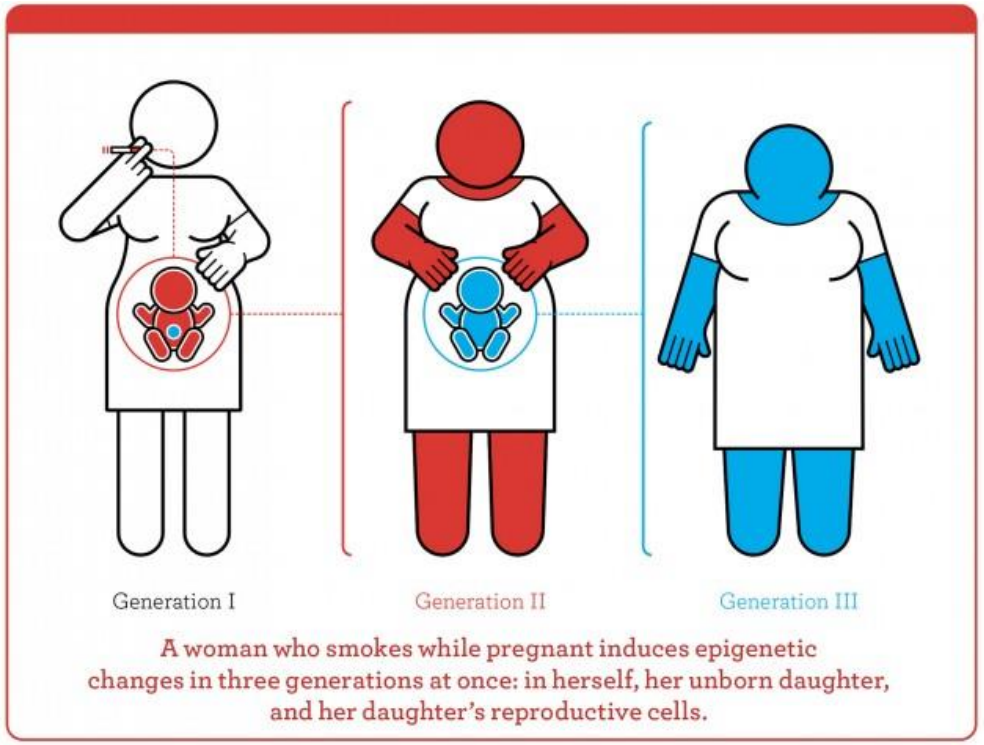
1. The imprinting marks are established in the gametes according to the sex of the individual
2. Brands are maintained during embryogenesis and throughout the individual's life
3. The imprinting marks are erased in the primordial germ cells



Epigenetics during pregnancy:

Intergenerational/parental effect:
3 generations at once are exposed to the same **environmental** conditions
(diet, toxins, hormones, stress).





Evidence of the existence of imprinting in humans

There are two human pathologies comparable to parthenogenetic and androgenic zygotes:

Teratomas: 2n, maternal origin

Hydatidiform mole: 2n, Paternal origin

Triploid (3n):

They are all abortions but ...

Phenotype of 2nP 1nM (a hyperplasia of the extraembryonic structure and absence of the embryo) is different from that of the 2nM 1nP (almost absent extraembryonic structures and normal embryo)

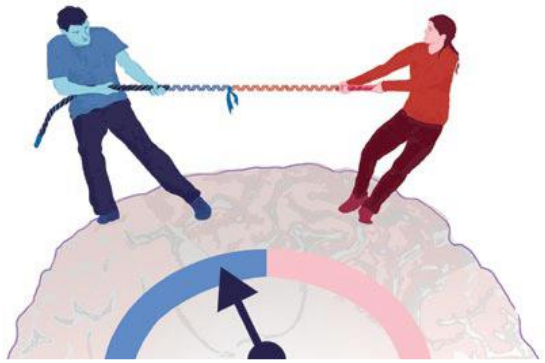
Uniparental chromosome disomies (**UPD**: both chromosomes of a couple provided by the same parent) have different phenotypic effects dictated by the sex of the parent who provided the chromosome pair

Imprinted genes play a fundamental role during embryonic and fetal development

Paternal Gene → **Placenta and extraembryinic annex**

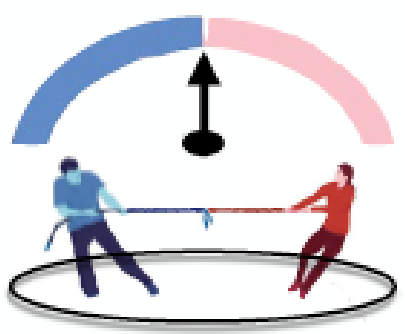
Maternal Gene → **Embryo**

Paternal conflict



→ **The equal contribution of both parent are necessary for the correct embryonic develompent**

PARENTAL CONFLICTING THEORY

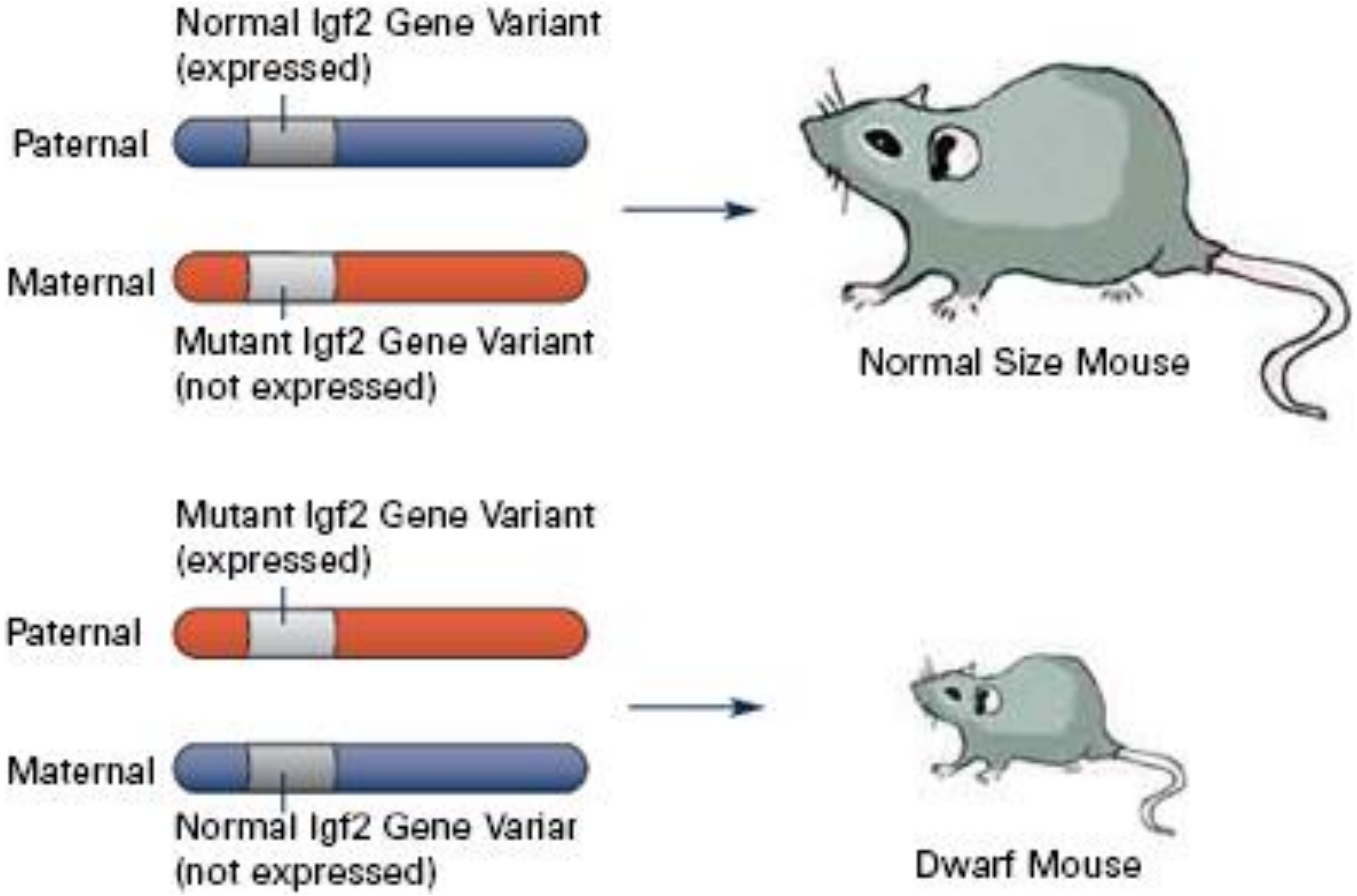


Male strategy:
force female to invest all of her energy into his offspring (immediate pay-off)
Female strategy:
save energy for future offspring (long term storage)

EXAMPLE:

The IGF2 gene is a maternal imprinted gene that plays an important role during fetal development.

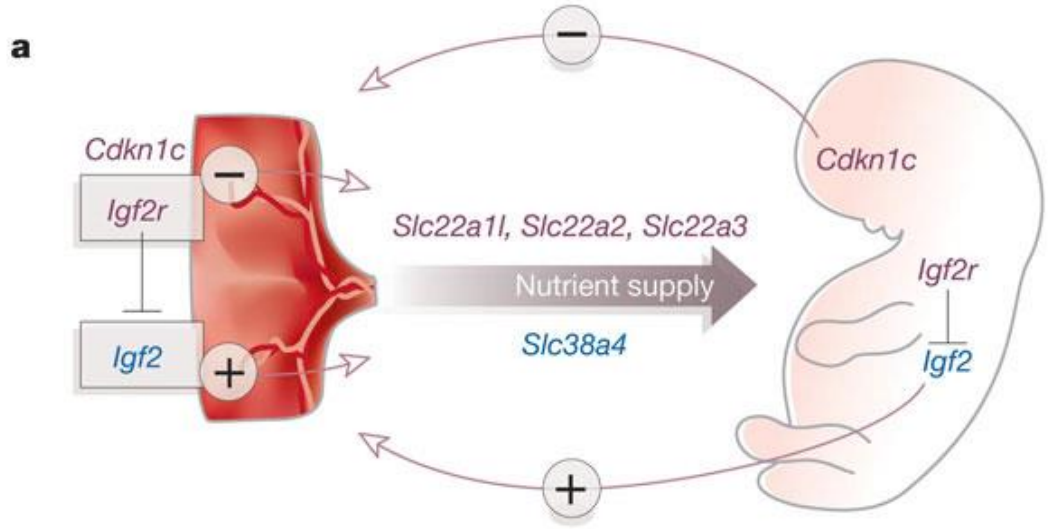
Only the paternal copy of IGF2 is transcribed and has importance for the phenotype



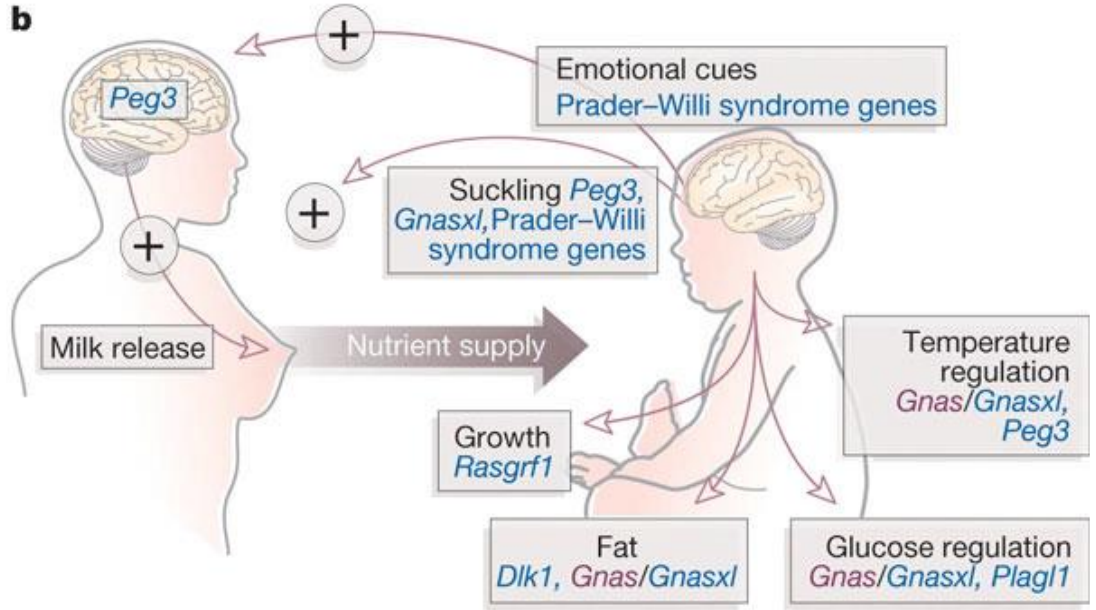
Mutant mice for paternal copying are half the size of a normal mouse at birth

Mutant mice are normal

Imprinting is involved in various stages of an individual's life



It affects the **development** of the fetus and placenta by regulating the exchange of nutrients between mother and fetus



Influences the **postnatal behavior** of the mother and infant (breastfeeding and maternal care)

c

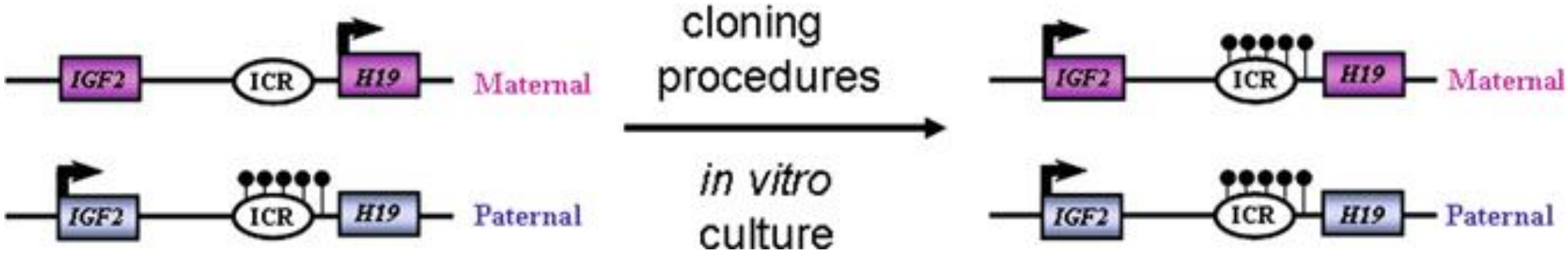
New evidence: «The imprinted brain theory»

It affects the behavior of an adult man

IN VITRO CULTURE AND EMBRYO MANIPULATION

ANOMALIES IN FETAL AND EMBRYONIC DEVELOPMENT

Epigenetic defects

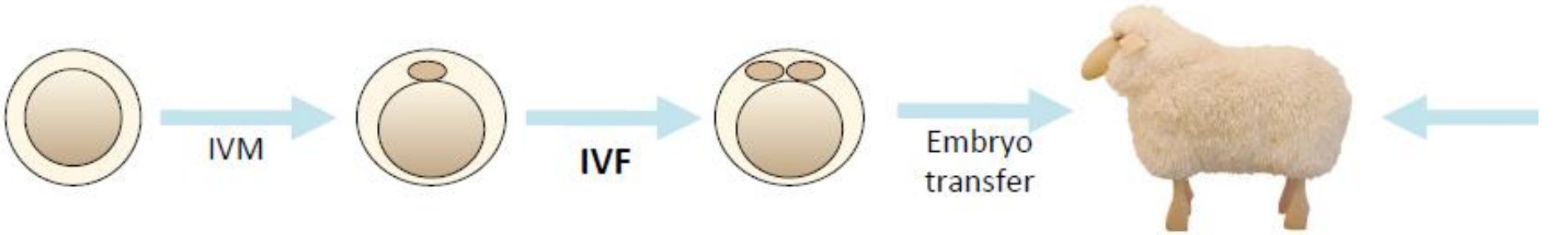


If the methylation status of ICRs is not stably maintained during development, growth-related disorders such as Beckwith-Weidmann Syndrome can be generated

Imprinting loss (LOI): protein level doubled compared to normal

Expression loss: protein absent

EXPERIMENTAL DRAWING



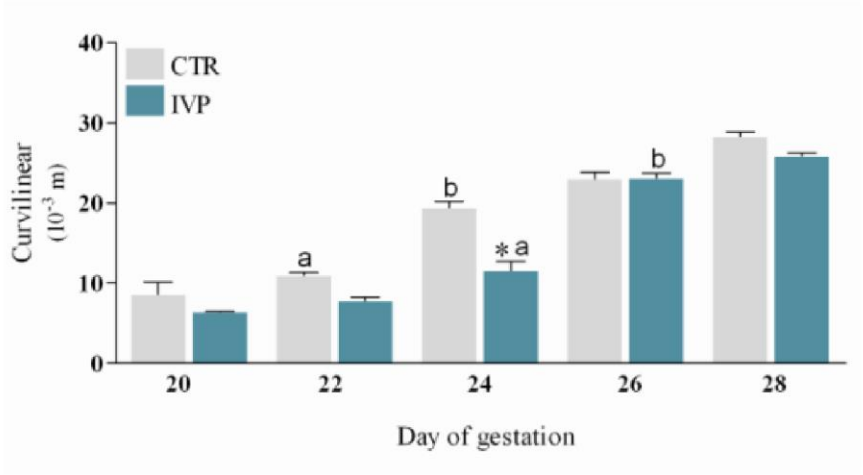
Sample collection 20-28 days
(Fetuses and Placenta)

AIM

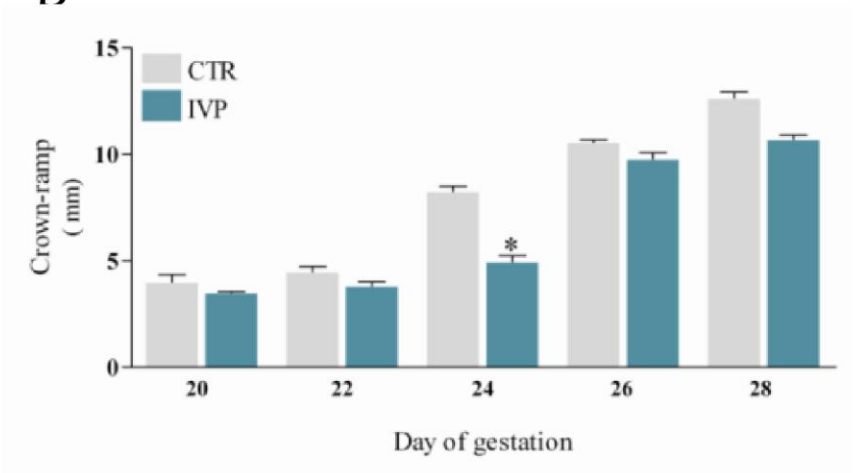
Evaluate the presence of any epigenetic defects in the development of sheep embryos produced in vitro

IVF FETUSES: reduced fetal growth

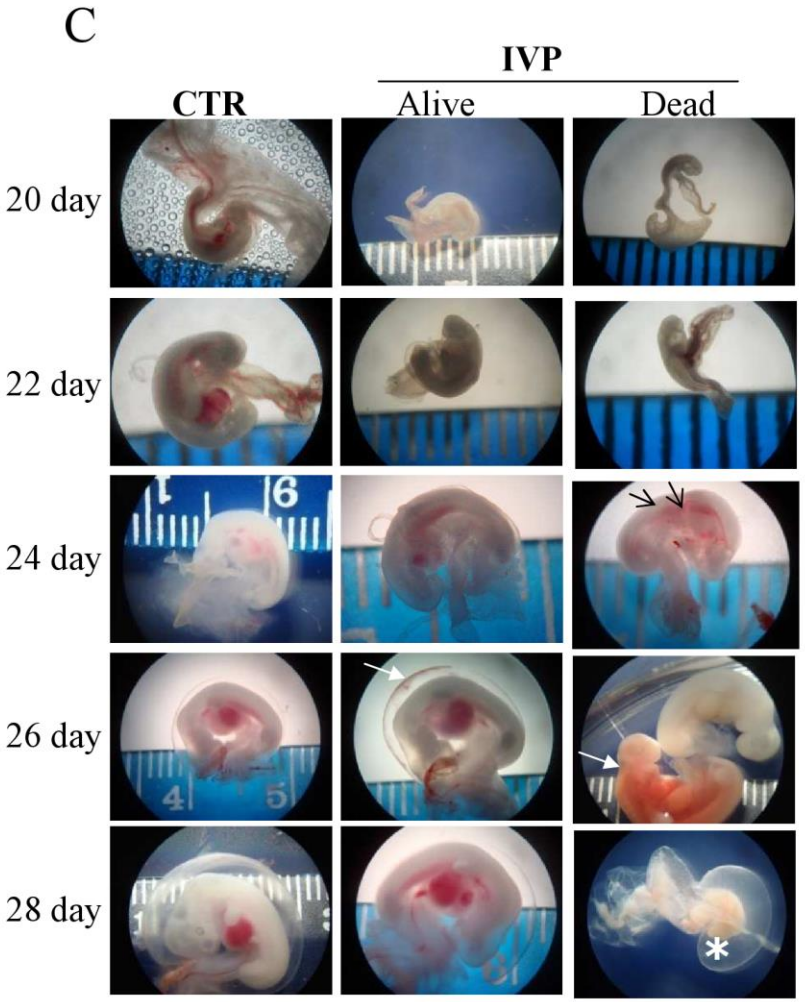
A



B

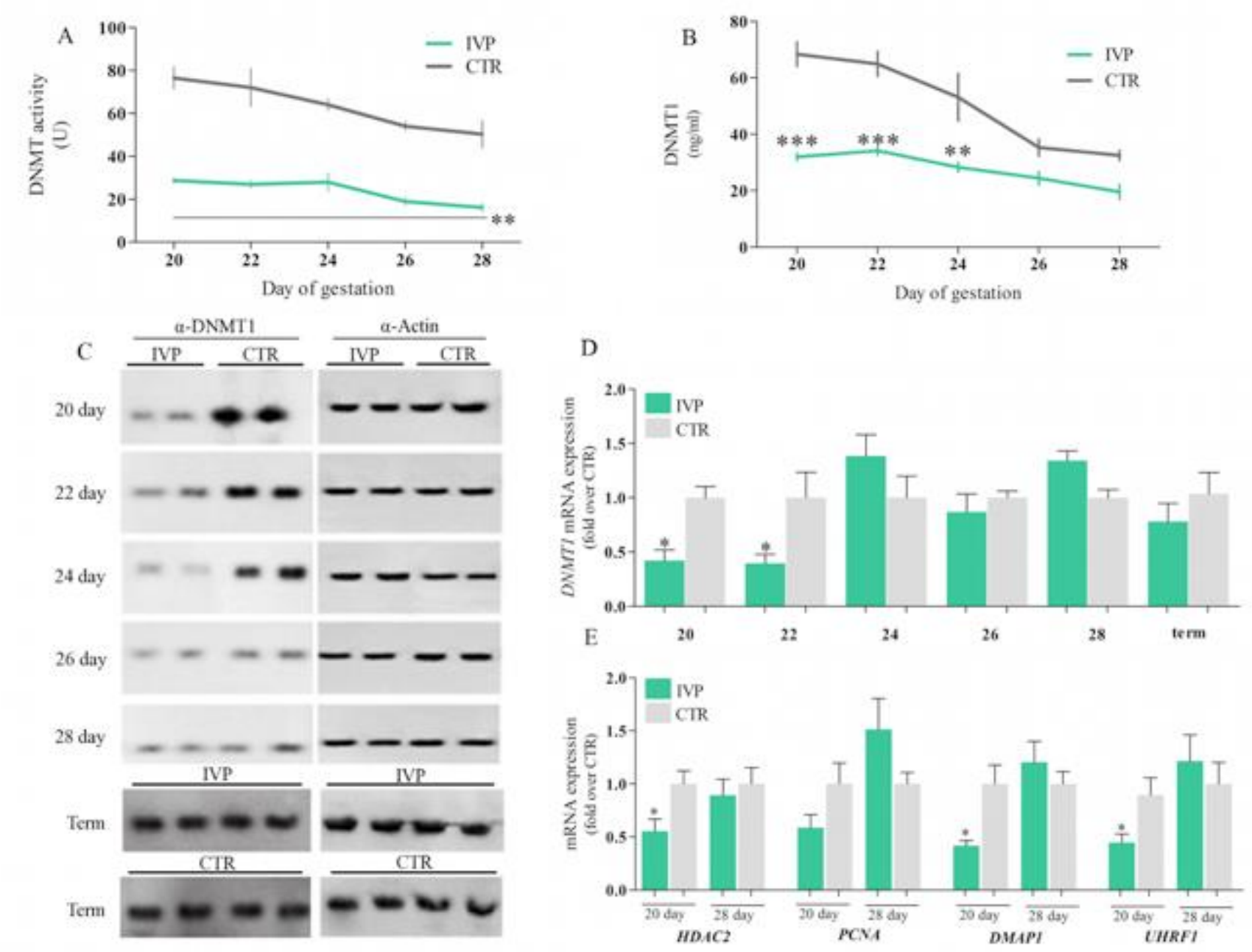


C



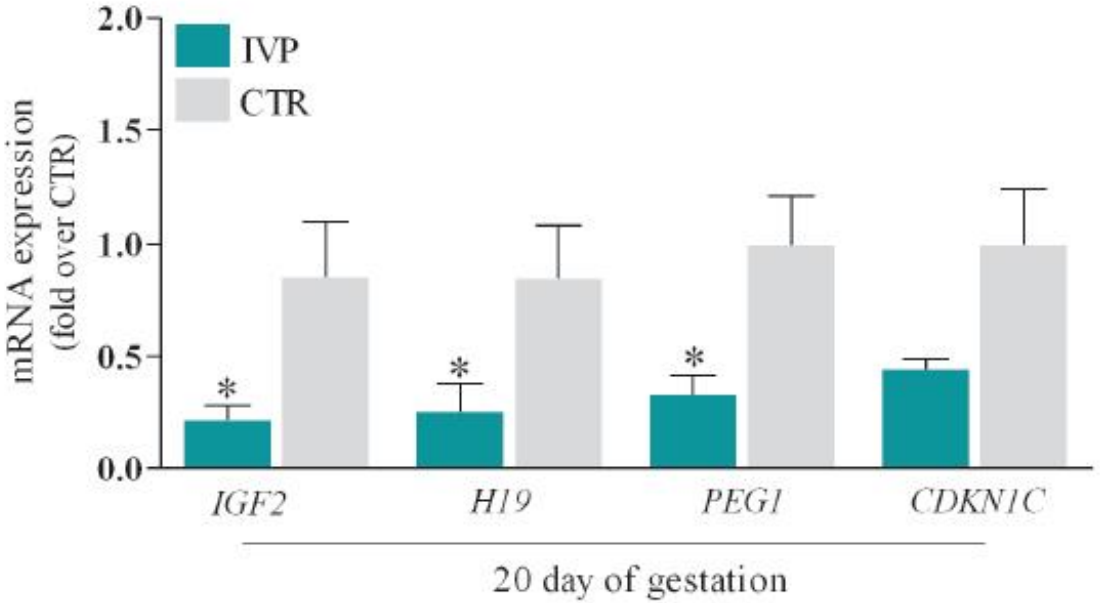
Placentas obtained from IVF fetuses:

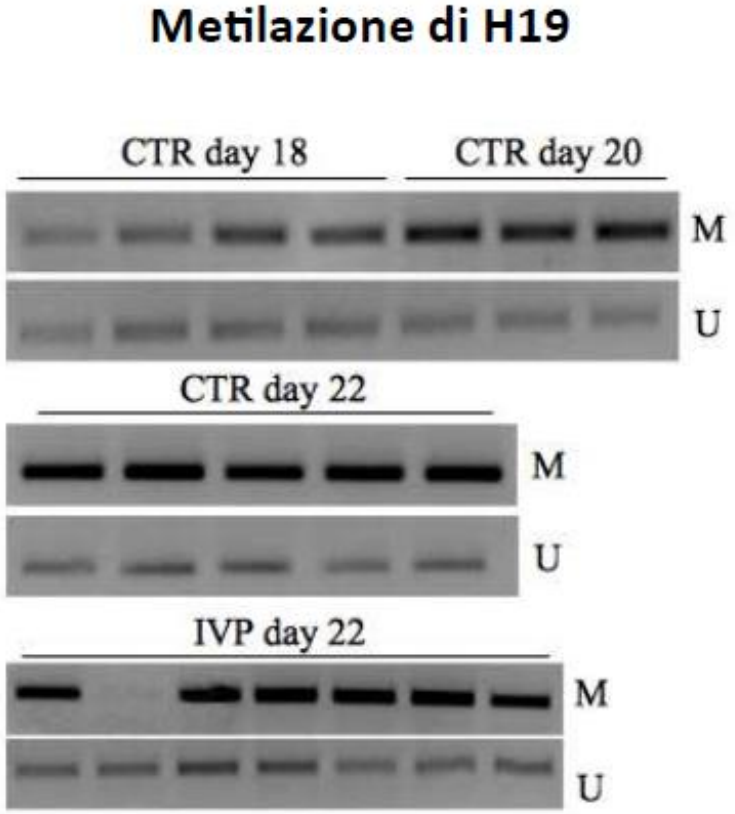
Defects in the regulation of the methylation maintenance mechanism (DNMT1 and its cofactors)



Placentas obtained from IVF fetuses:

Defects in the regulation of imprinted genes
(Defects in **expression** and **methylation**)





human reproduction

(Ptak et al; 2013)

MONOPARENTAL EMBRYO

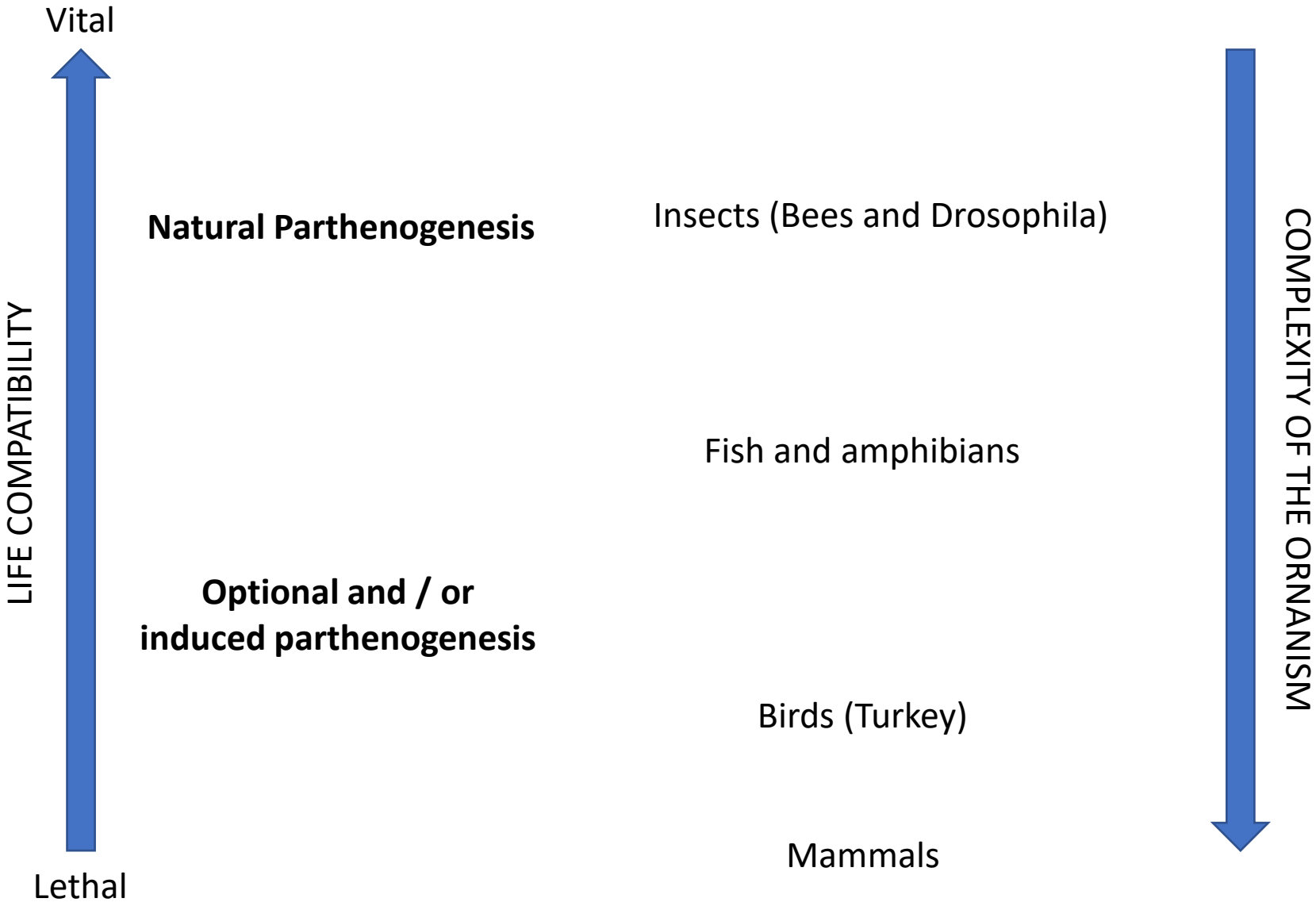
androgenotes

Embryos (2n) composed solely of a **paternal genome**

Parthenogenotes

Embryos (2n) composed solely of a **maternal genome**

Parthenogenesis: the production of an embryo from a female gamete without any contribution from a male gamete, with or with- out the eventual development into an adult…..



Parthenogenetic activation can occur:

- Mechanical stress
- Chemical agents such as Ethanol, Strontium, calcium ionophores

Increase in the intracytoplasmic concentration of Ca similar to that induced by the entry of the spz

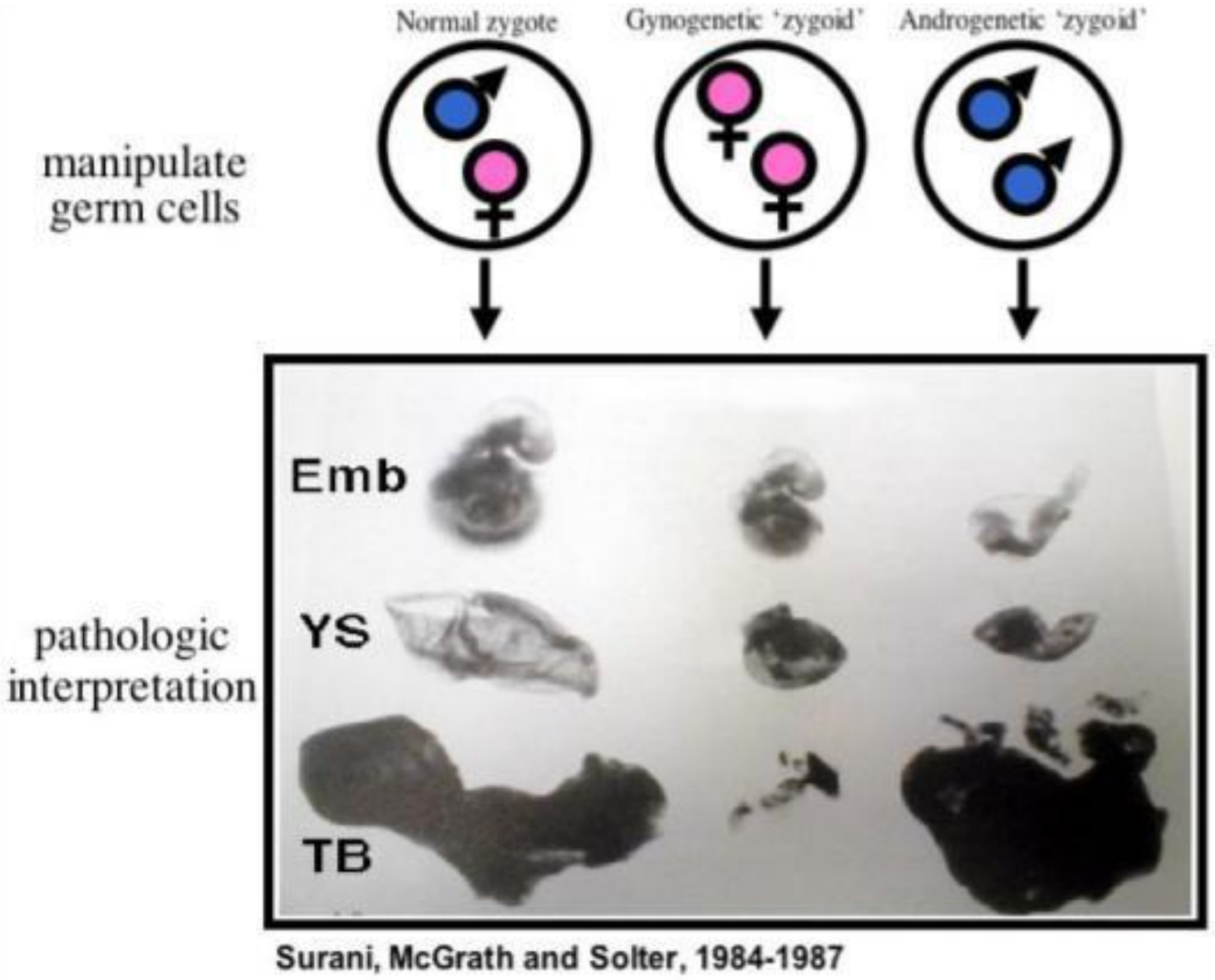
FISHES

Diploidin restoration in 1n zygotes can occur spontaneously or be induced by chemical, thermal or compression treatments

AMPHIBIANS

It occurs by mechanical activation of the oocyte

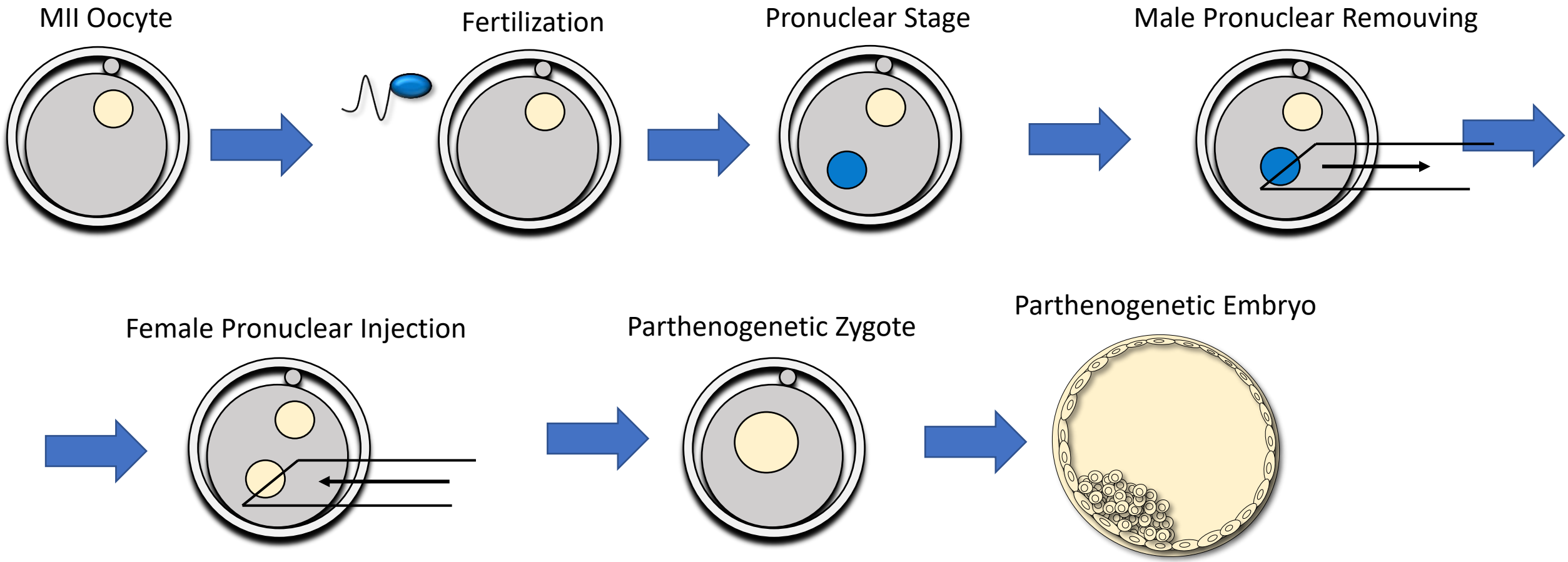
# Unequal contribution of paternal and maternal genome



Surani, McGrath and Solter, 1984-1987

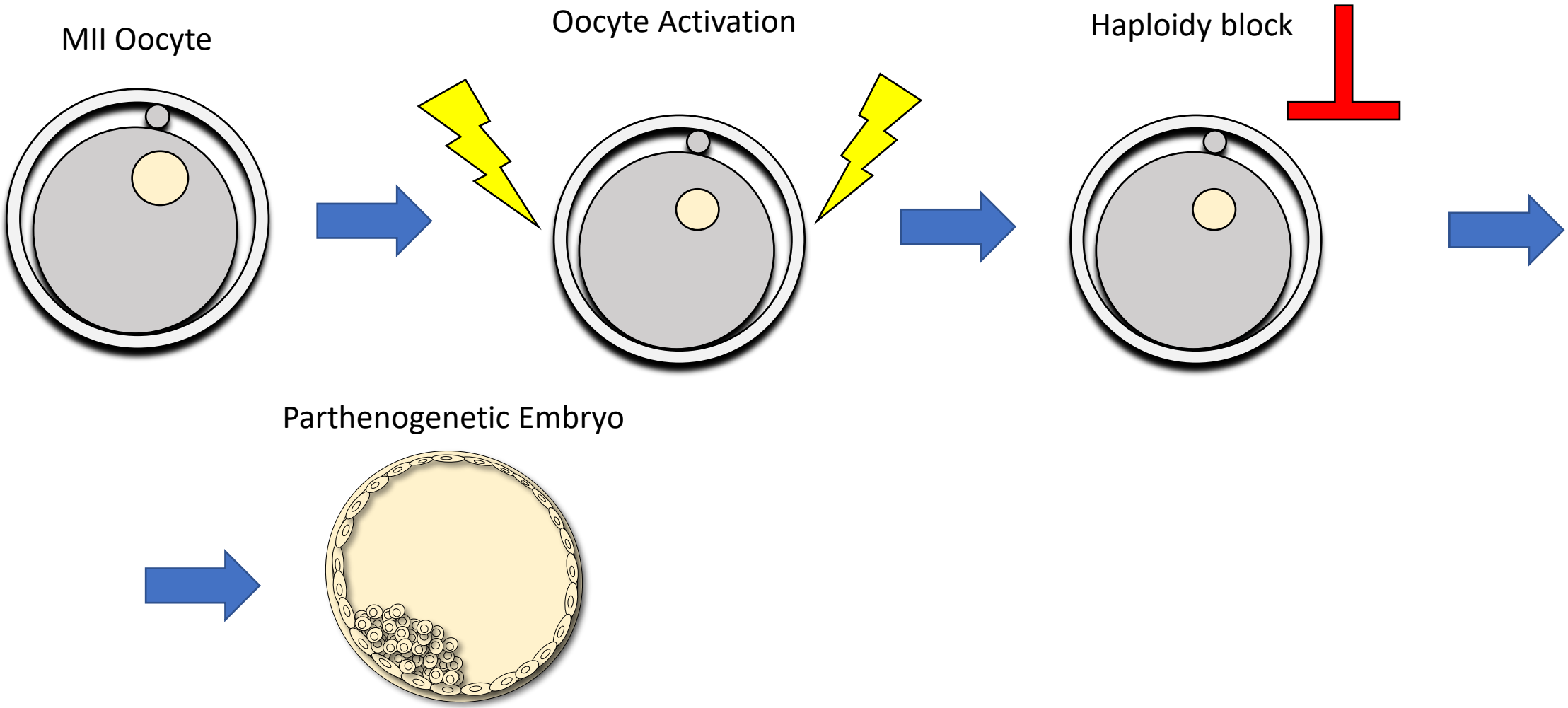
Parthenogenetic Embryo Production

PRONUCLEAR TRANSFER



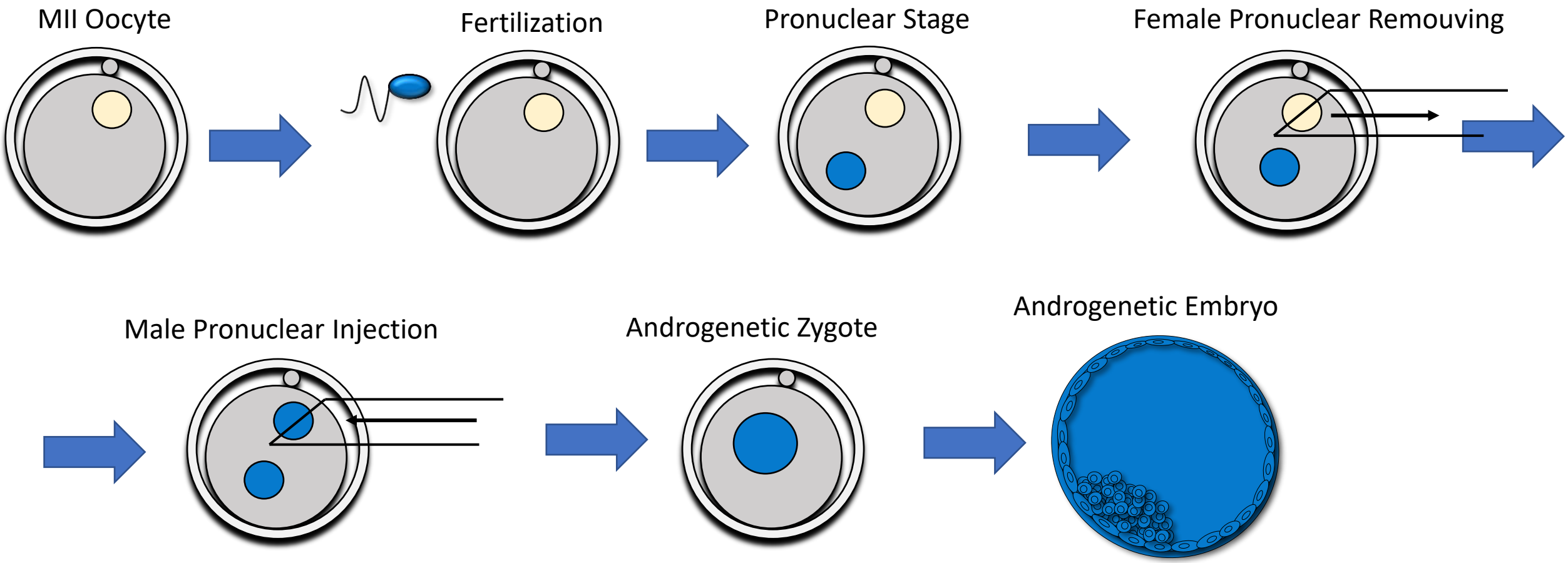
Parthenogenetic Embryo Production

CHEMICAL ACTIVATION

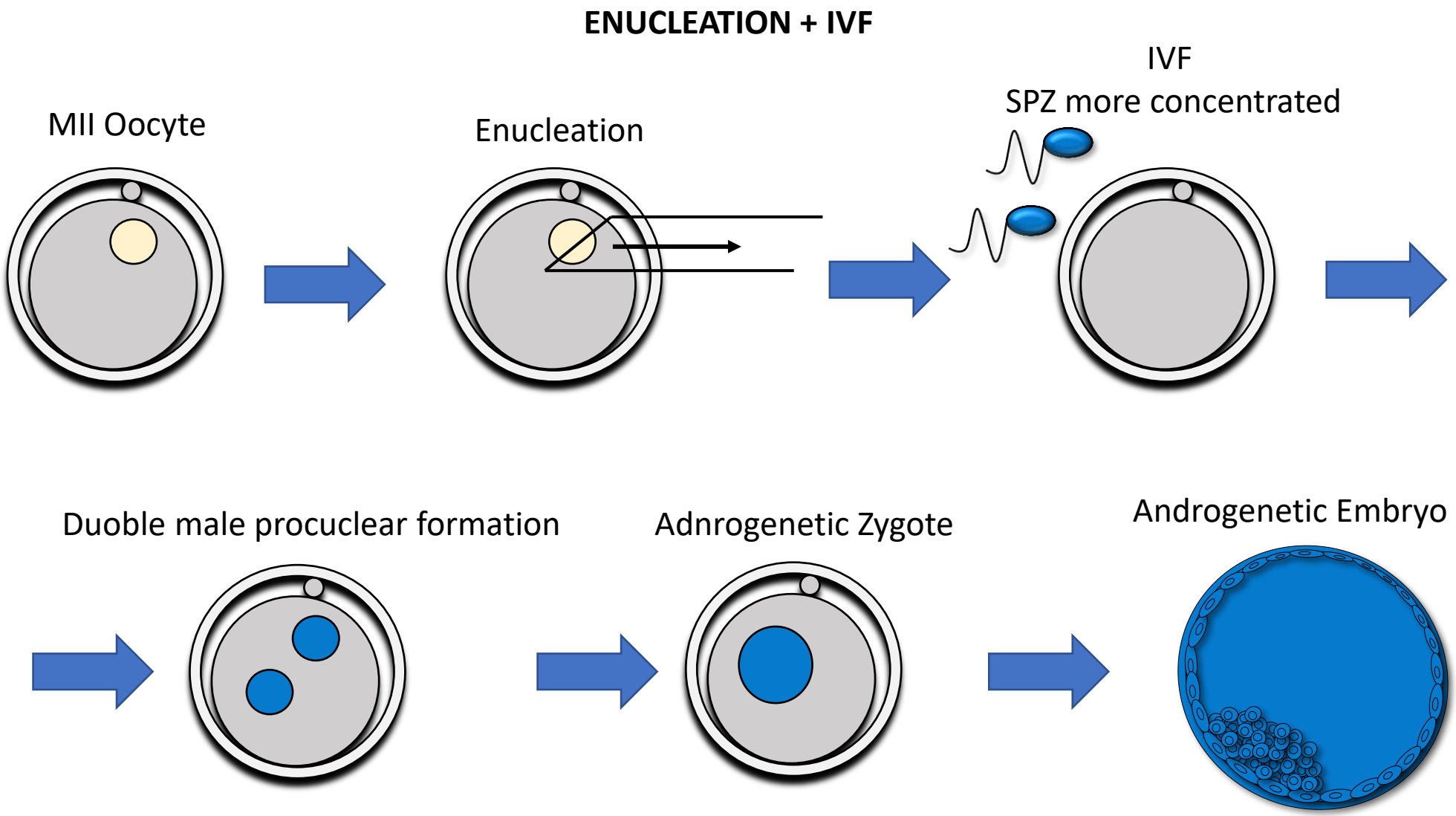


Androgenetic Embryo Production

PRONUCLEAR TRANSFER

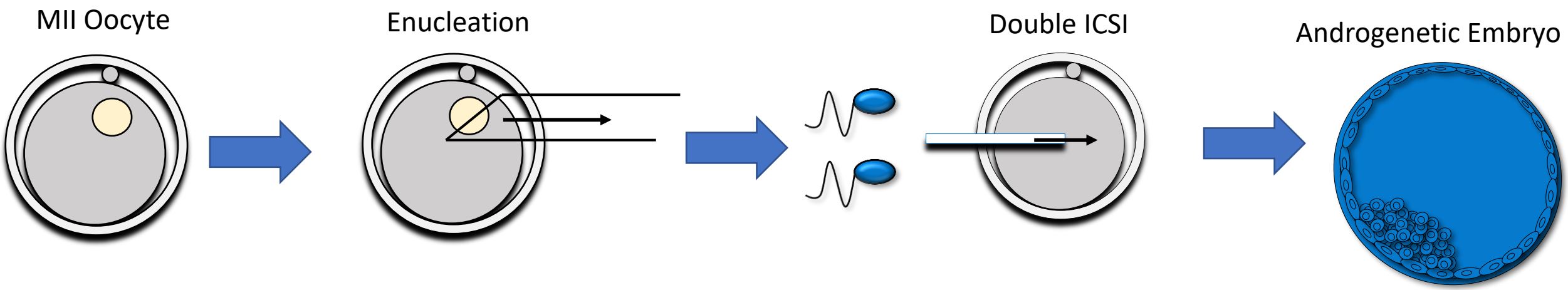


Androgenetic Embryo Production

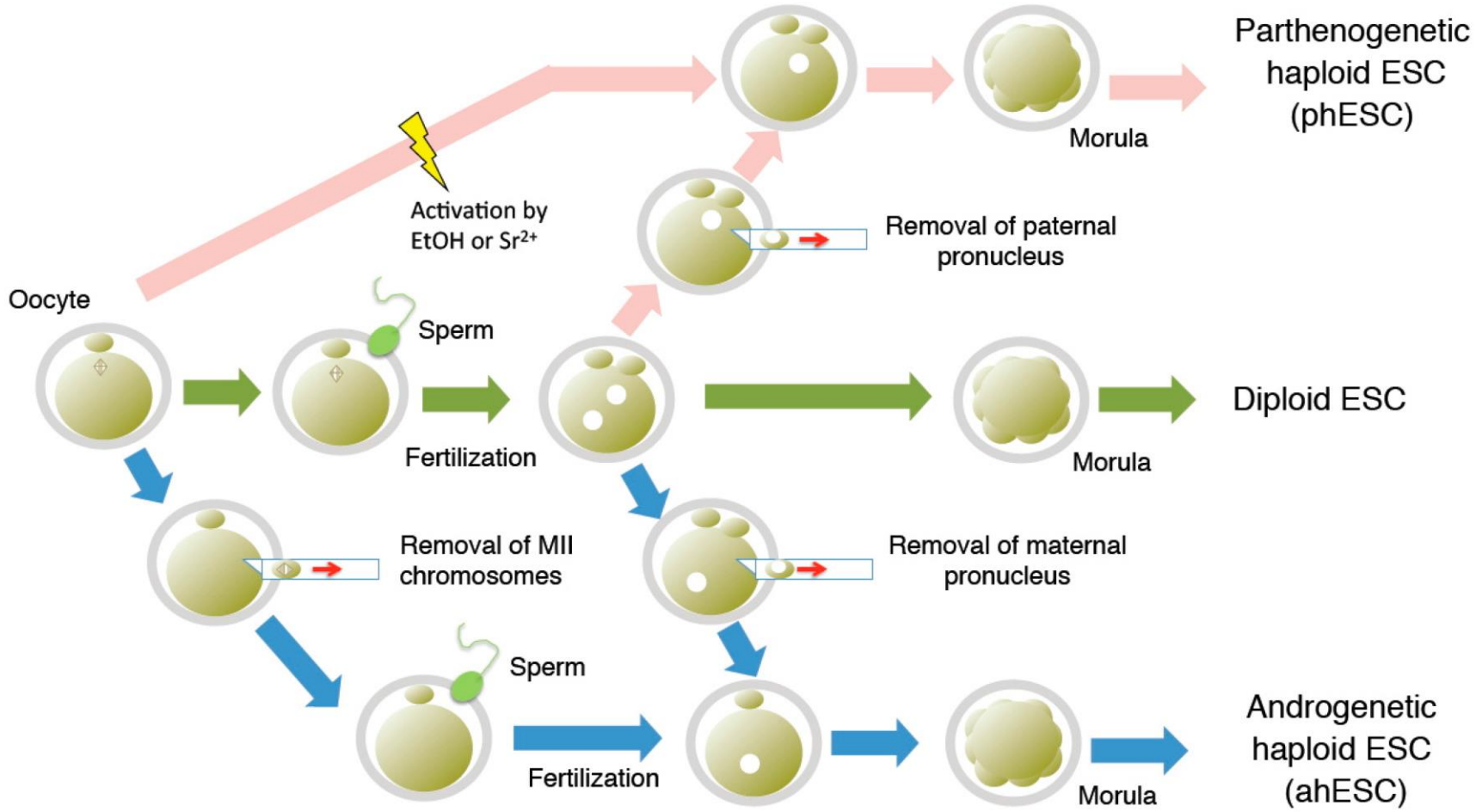


Androgenetic Embryo Production

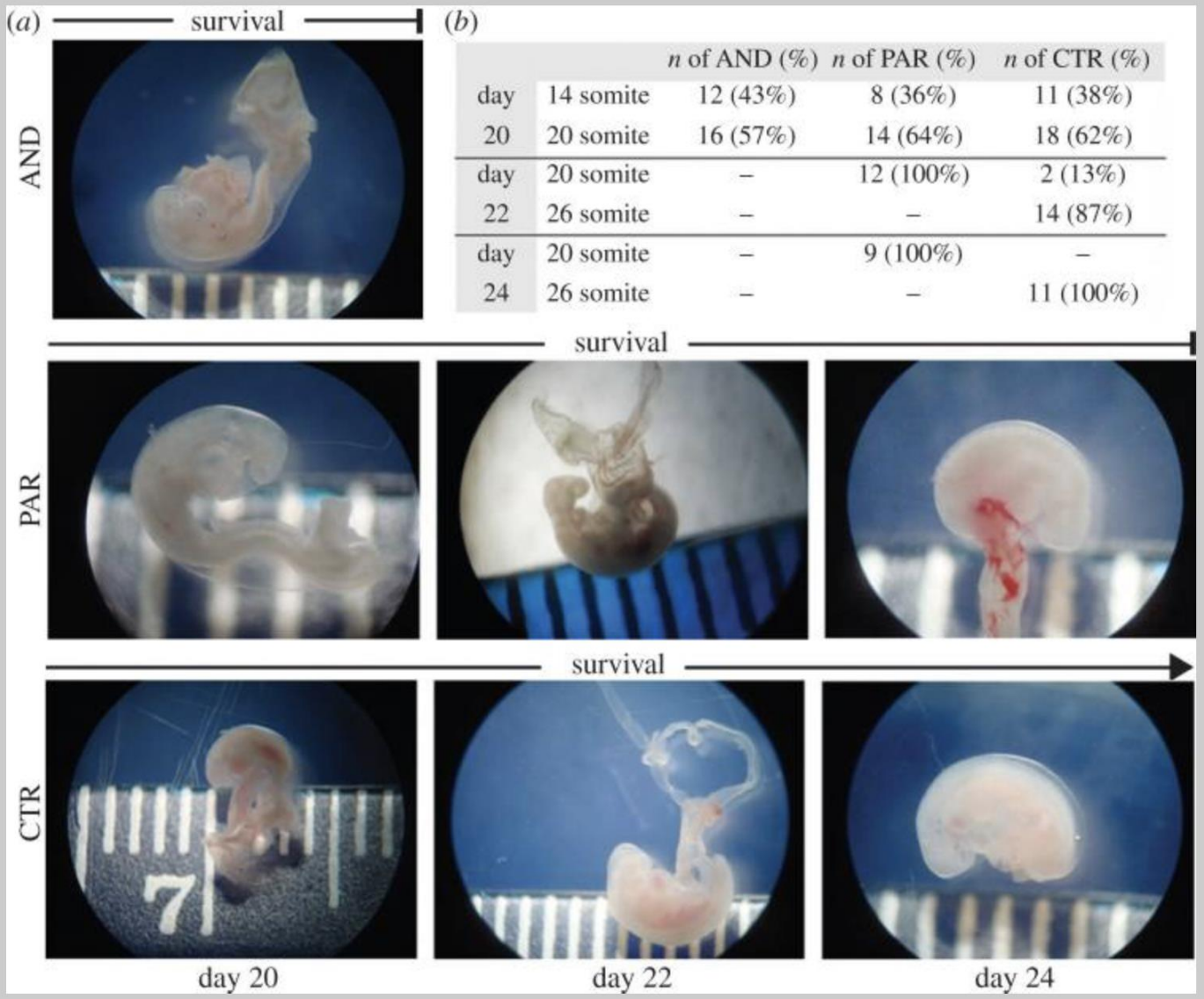
ENUCLEATION + DOUBLE ICSI



Monoparental embryos: source of haploid ESC



Sheep Monoparental

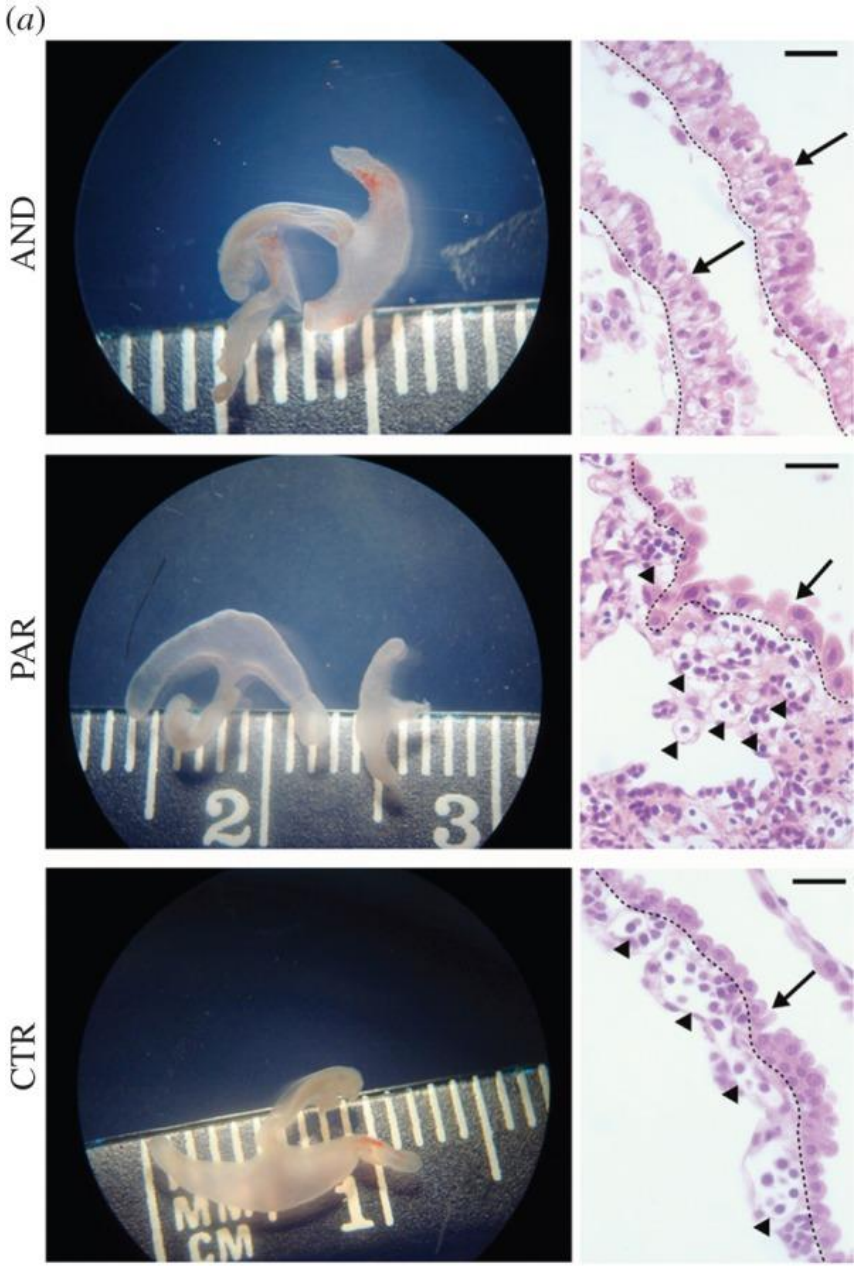


(b)

		n of AND (%)	*n* of PAR (%)	*n* of CTR (%)
day 20	14 somite	12 (43%)	8 (36%)	11 (38%)
	20 somite	16 (57%)	14 (64%)	18 (62%)
day 22	20 somite	–	12 (100%)	2 (13%)
	26 somite	–	–	14 (87%)
day 24	20 somite	–	9 (100%)	–
	26 somite	–	–	11 (100%)

Delay of fetal development in PAR embryos

Developmental skills linked to parental origin:
PAR to 24 days gestation
ANS to 20 days of gestation



Morphological anomaly of the trophoblast

Absence of blood vessels: no maternal-fetal communication