EPIGENETIC IN ART

Are we influencing it?

Asia Renzi

Biotechnologies of Human Reproduction

Gynecology – IC Management of Infertile Couple

2019 - 2020

1978
Louise Brown

IVF ICSI PGS

8 Million
babies

The advent of in vitro fertilization (IVF) in animals and humans implies an **extraordinary change in the environment** where the beginning of a new organism takes place.

WHAT IS EPIGENETICS ?

DNA Methylation

Histone Regulation

HOW CAN ART INFLUENCE EPIGENETICS?

Ovarian Stimulation

In Vitro Maturation

ART Technique

In Vitro Culture

Embryo Manipulation: PGD and Transfer

Cryopreservation

WHAT IS EPIGENETICS ?

Genetics

- Laws of inheritance
- Transmission of characters from parents to offspring

Epigenetics

1939, Conrad Waddington

Relevance of the environment in development.

WHAT IS EPIGENETICS ?

Epigenetics is the study of several processes that can alter gene expression without changing the DNA sequence.

- Regular and Natural Occurrence
- Age
- Environment / Lifestyle
- Disease State

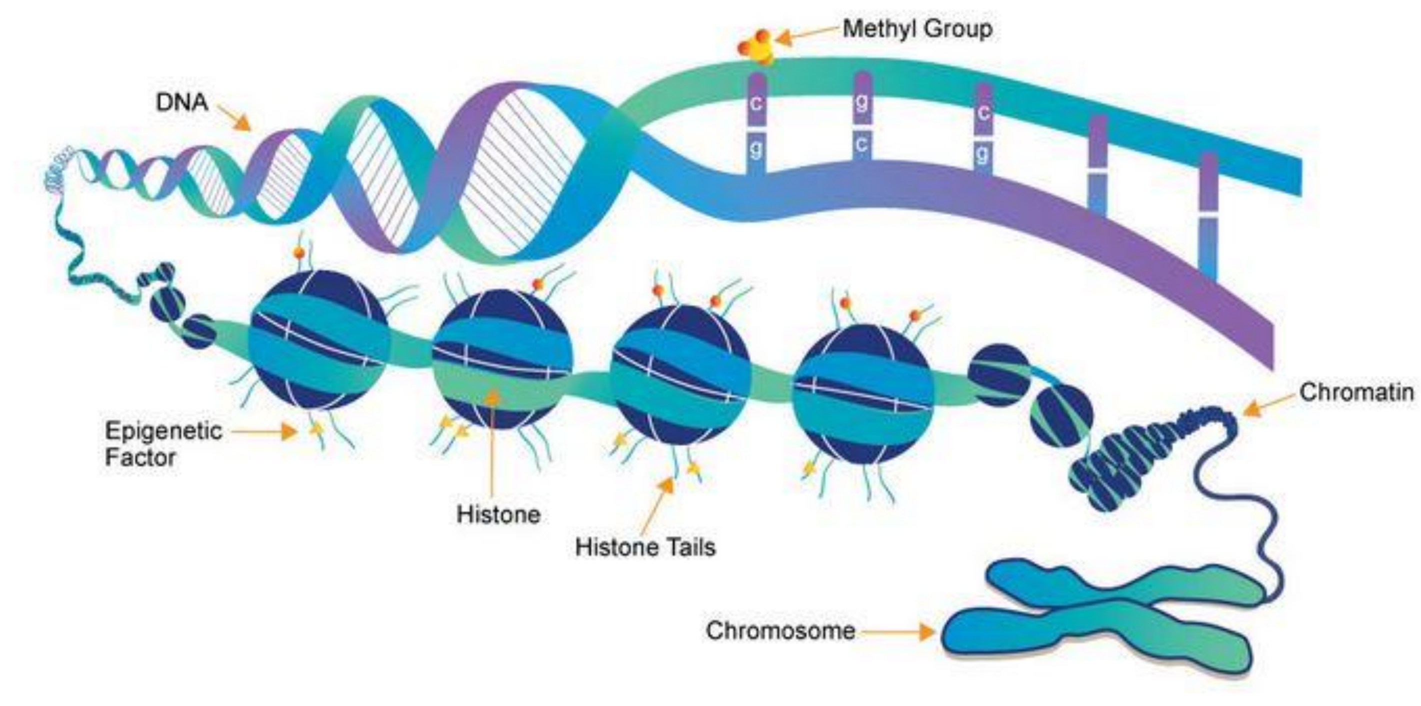
Main epigenetic modifications:

I. DNA Methylation
II. Histone Modification
III. Micro-RNA (miRNA) / Non-coding RNA (ncRNA) – associated gene silencing
IV. Higher order packaging of DNA around nucleosomes

Nucleosome: 147 bp of DNA associated with an octomeric core of histone protein.

2 H3-H4 dimers +
2 H2A-H2B dimers.

N-terminal histone tails protrude from nucleosomes.



Representation of the chromatin structure, including histones and DNA, which become available to epigenetic marks.

H1 histone
=
Linker histone

Nucleosome spacing determines chromatin structure

Heterochromatin
Euchromatin

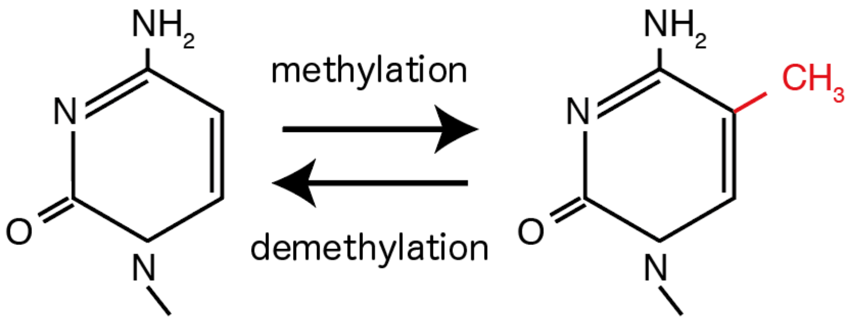
Chromatin structure and gene accessibility to transcriptional machinery are regulated by modifications to both DNA and histone tails.

Covalent attachment of a methyl group to the C5 position of cytosine residues in CpG dinucleotide sequences

CpG methylation can suppress transcription by

- The block of DNA recognition and binding by some transcription factors.
- Other factors that may preferentially bind to methylated DNA, blocking transcription factor access.

DNA methylation



Cytosine
for switching ON the gene expression

methylated Cytosine
for switching OFF the gene expression

Gene regulation in embryonic stem cells

X-chromosome inactivation

Imprinting

Repression of transcription of repeat elements and transposons

Restriction of the expression of some tissue-specific genes during development and differentiation maintenance

DNMT 1

de novo methylation

DNMT 3A
DNMT 3B

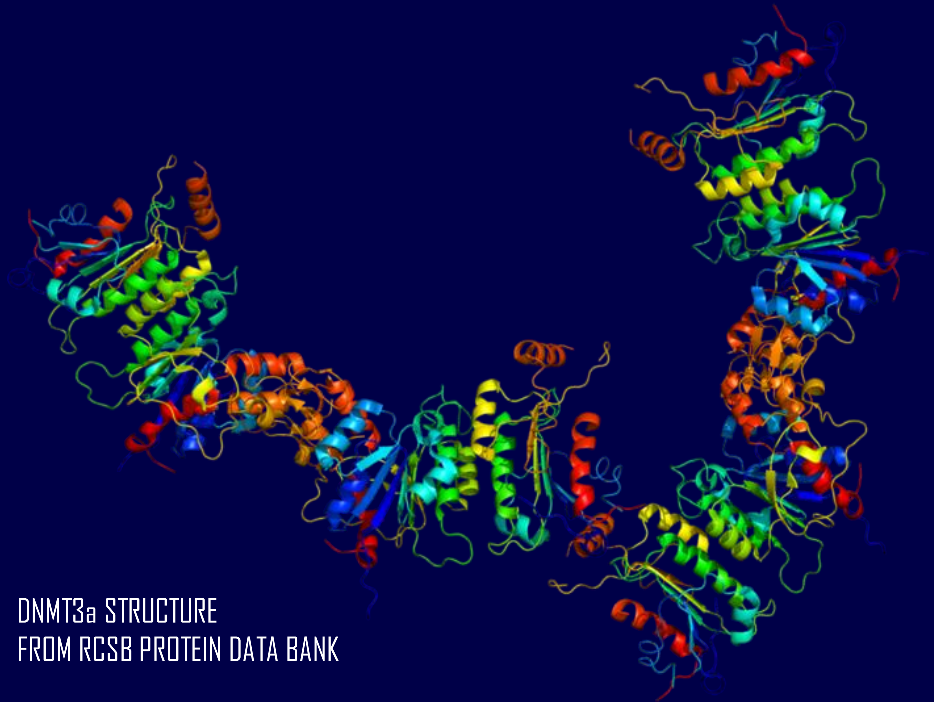
DNMT1 recognizes hemi-methylated DNA and adds methyl groups to the non-methylated daughter strand formed during replication.

Maintenance of methylation during subsequent replication cycles.

A non-genetic trait (DNA methylation) and its effects on gene expression can be passed from cell to cell.

Methylation can be considered a long-term, relatively stable, epigenetic trait.

The effects contribute to maintain the cellular phenotype.



DNMT3a STRUCTURE
FROM RCSB PROTEIN DATA BANK

In imprinting and X-inactivation CpG methylation represses gene expression in some chromosomal regions.

Genomic imprinting

Epigenetic phenomenon that causes genes to be expressed in a parent-of-origin-specific manner.

Prader-Willi syndrome, chromosome 15 (15q11-q13)

Angelman syndrome, chromosome 15 (15q11-q13)

Silver-Russell syndrome, chromosome 11 (11p15.5)

Beckwith–Wiedemann syndrome, chromosome 11 (11p15.5)

In imprinting, clusters of genes in a chromosomal region are coordinately inhibited by methylation of a **differentially methylated regions (DMRs**, imprinting center**).**

During imprinting: expression of **oocyte-specific factors to mark maternal chromosomes.**

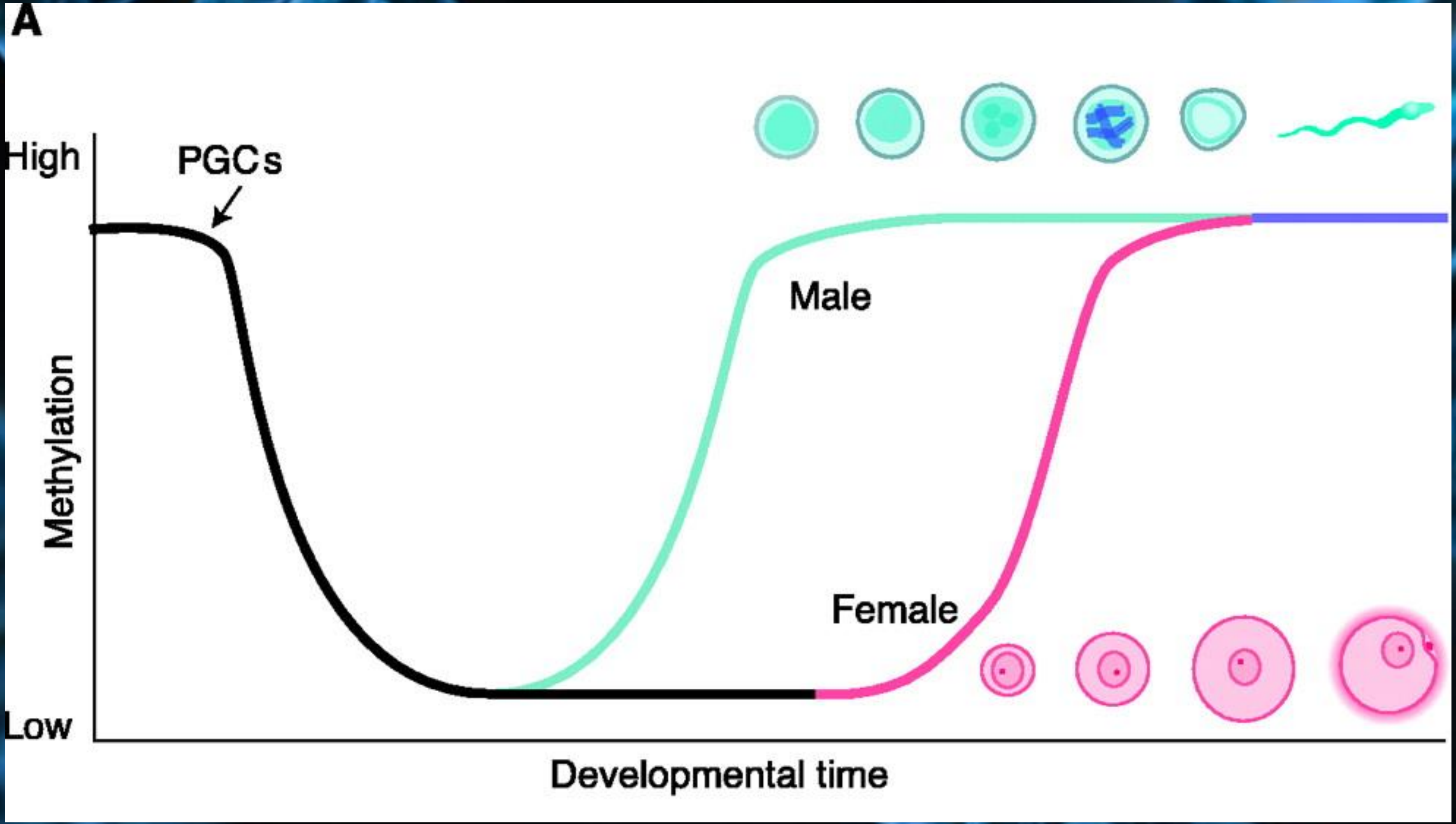
In both imprinting and X-inactivation, the expression of long **non-coding RNAs** may also play a regulatory role.

During development, the pattern of CpG methylation changes in a predictable manner.

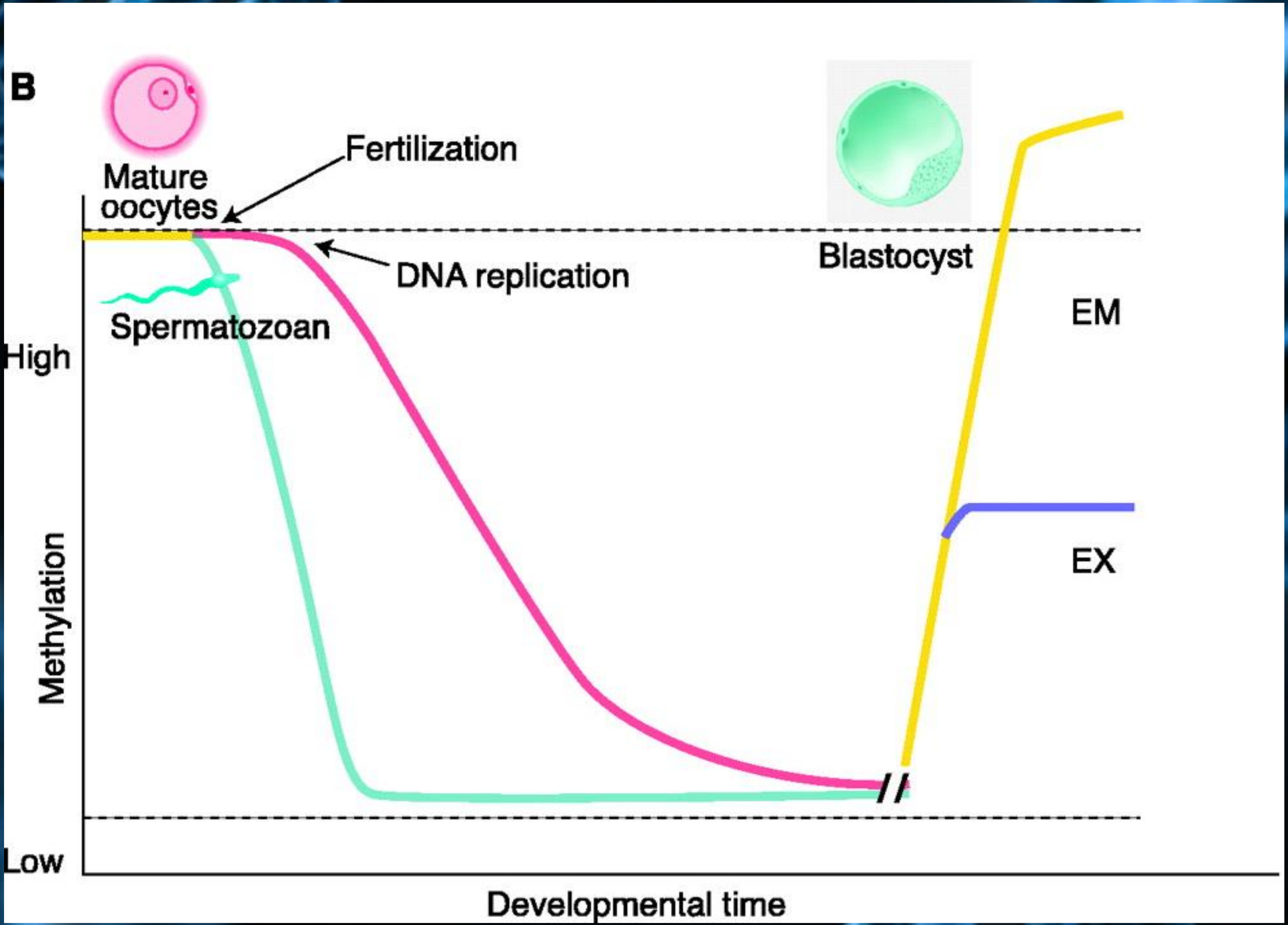
In mammals there are at least two developmental periods in which methylation patterns are reprogrammed genome wide, generating cells with a broad developmental potential:

1. Reprogramming in germ cells
2. Reprogramming in preimplantation embryos

Both epigenetic reprogramming events in germ cells and in early embryos are critical for imprinting and can affect imprinting.



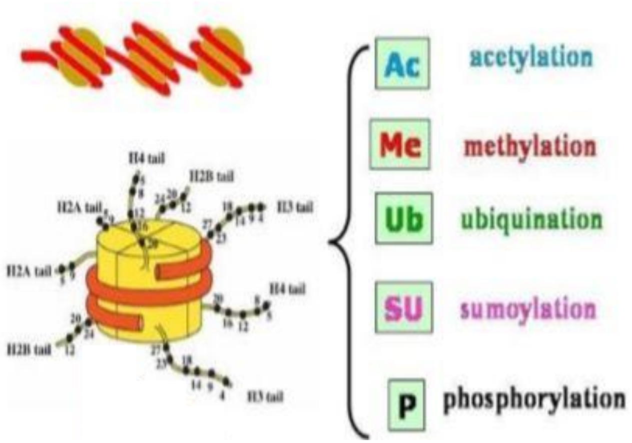
Reprogramming in Germ Cells



Reprogramming in Early Embryos

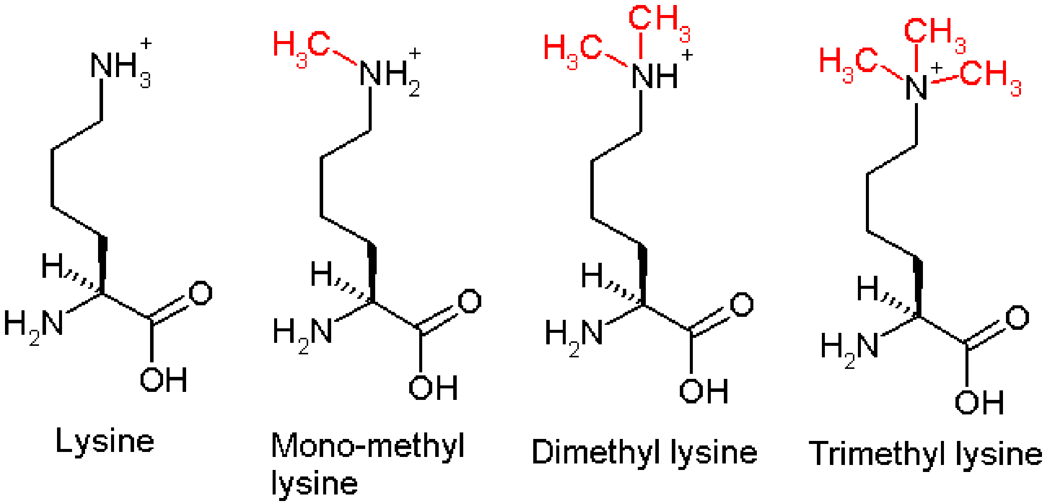
Histones can be post-translationally modified to restructure chromatin in many ways.
'Histone Code Hypothesis' suggests that different combinations of histone modifications may regulate chromatin structure and transcriptional status.

Histone regulation



Histone acetylation of lysine residues in H3 and H4 tails is most consistently associated with promoting transcription.

Low histone acetylation + CpG methylation is associated with heterochromatin .



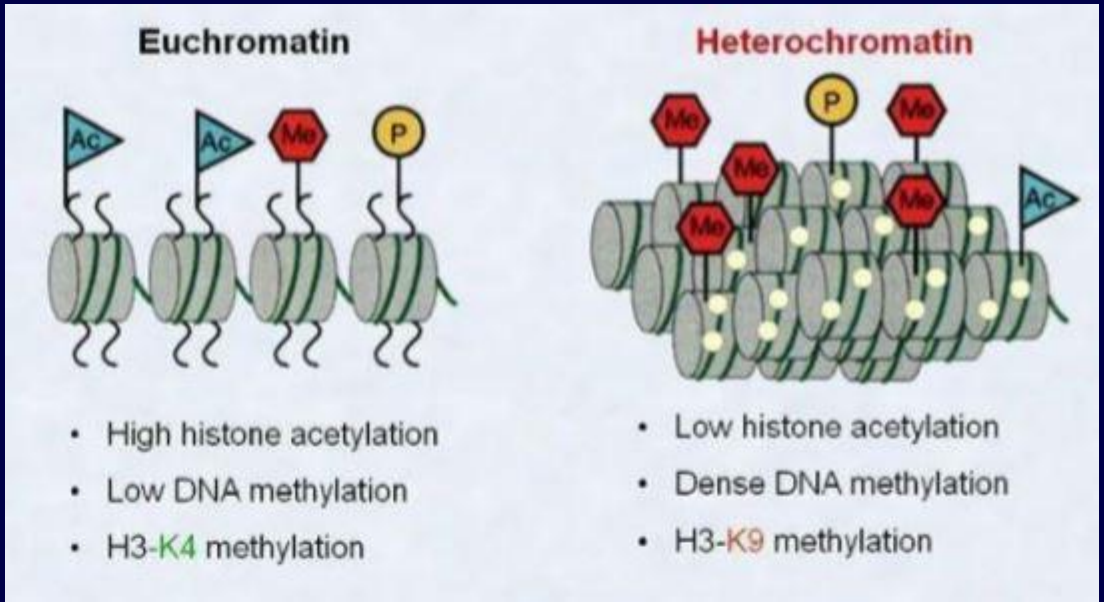
Lysine residues can also be mono-, di-, or tri-. **methylated**

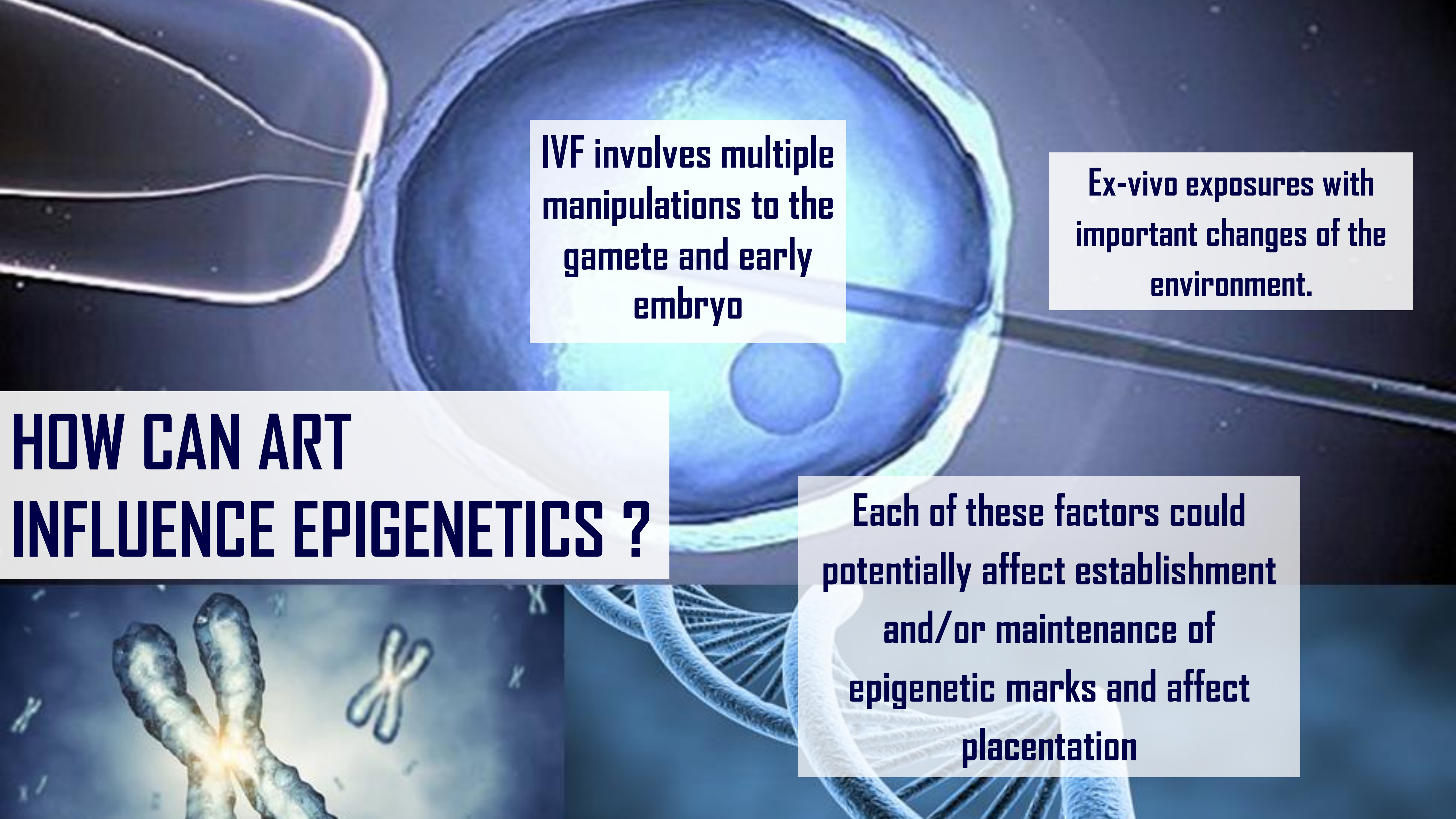
Different effects on transcription.

H3K27me3
H3K9me — Silencing

H3K4me3
H3K36me3 — Transcriptional activity

DNA Methylation and histone modifications help to compartmentalize the genome into domains of different transcriptional potentials.



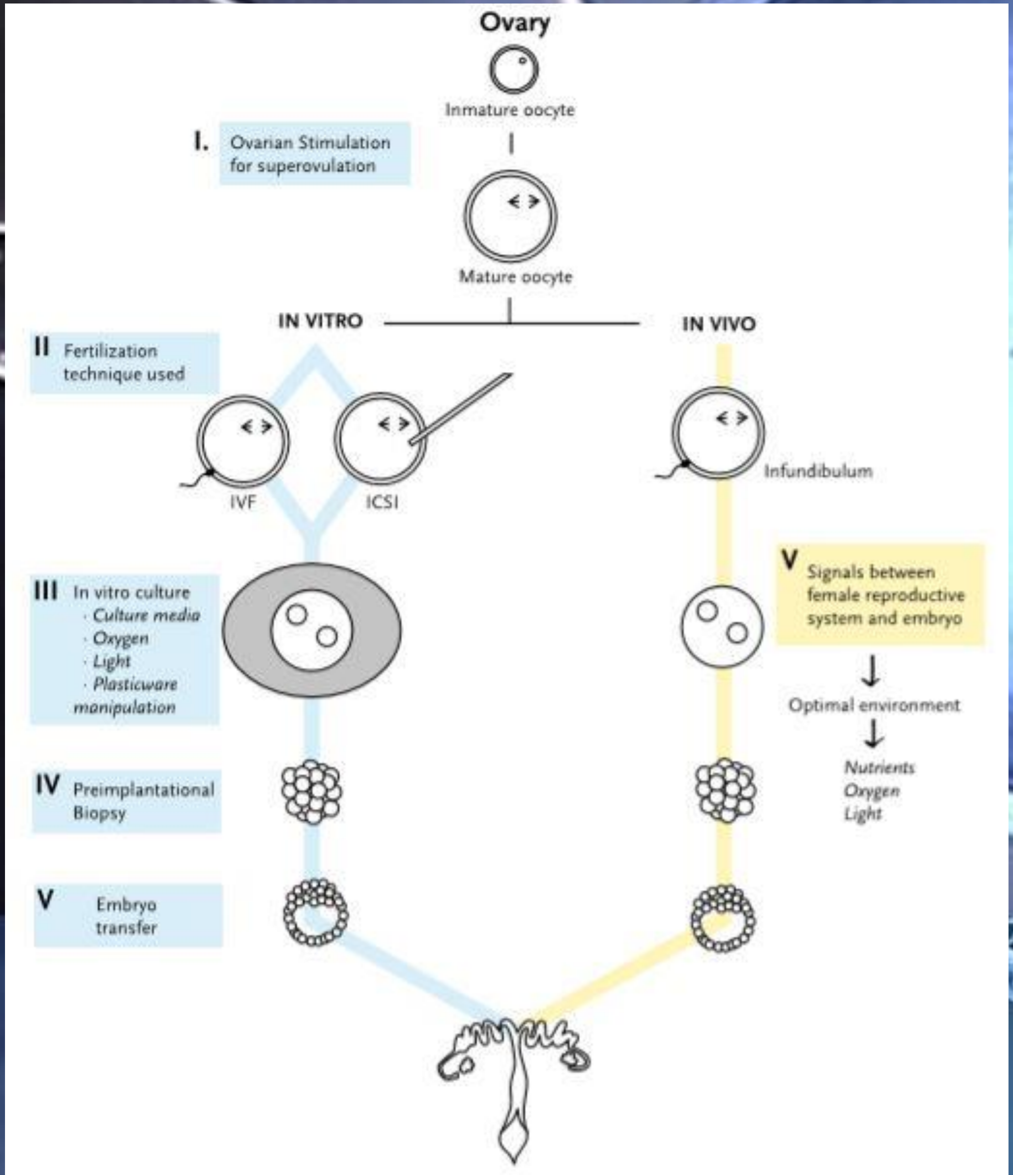


HOW CAN ART INFLUENCE EPIGENETICS ?

IVF involves multiple manipulations to the gamete and early embryo

Ex-vivo exposures with important changes of the environment.

Each of these factors could potentially affect establishment and/or maintenance of epigenetic marks and affect placentation



Many processes and techniques associated with ART can alter the epigenetic reprogramming of gametes, embryos and normal mammal development.

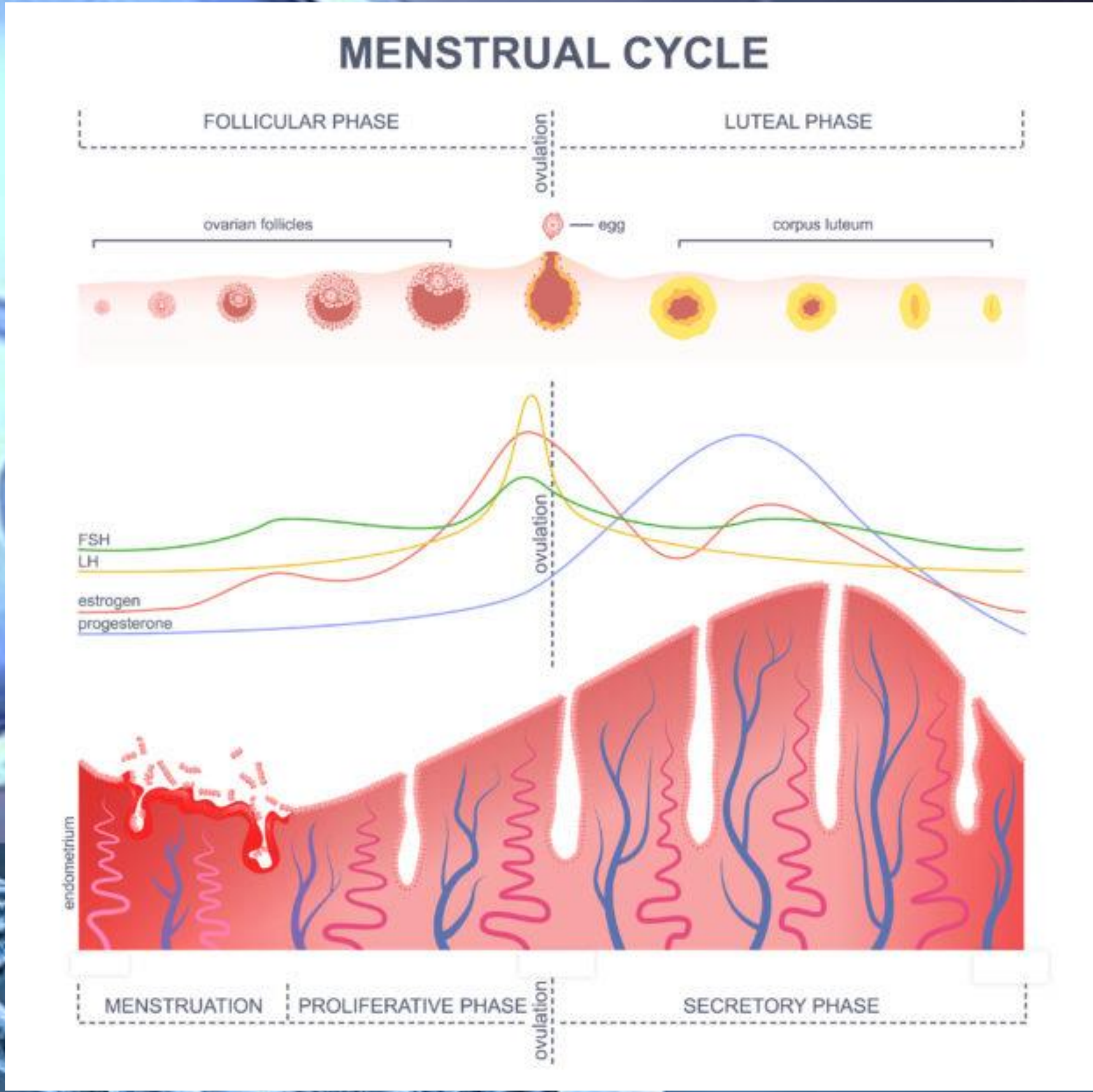
- Ovarian stimulation
- In vitro maturation (IVM) of gametes.
- Fertilization technique.
- In Vitro culture.
- Embryo manipulation: pre-implantation genetic diagnosis (PGD) and embryo transfer.
- Cryopreservation

Ovarian Stimulation

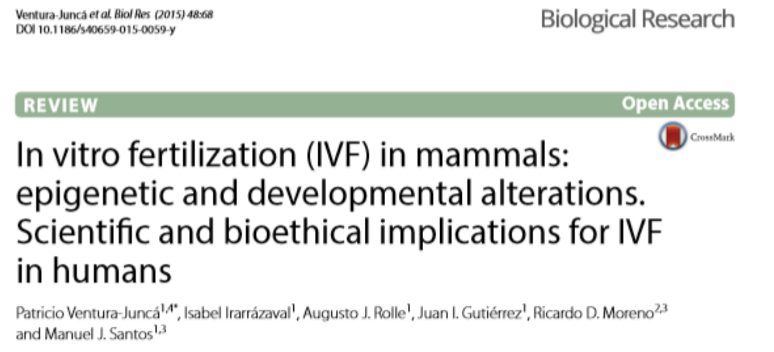
Superovulation makes the ART process more productive.

Administration of exogenous gonadotropins to stimulate oocyte growth.

Epigenetic establishment in oocytes occurs in a growth and maturation dependent manner.



Ovarian Stimulation


Ventura-Juncá *et al. Biol Res* (2015) 48:68
DOI 10.1186/s40659-015-0059-y
Biological Research
REVIEW Open Access

In vitro fertilization (IVF) in mammals: epigenetic and developmental alterations. Scientific and bioethical implications for IVF in humans

Patricio Ventura-Juncá[1,4*], Isabel Irarrázaval[1], Augusto J. Rolle[1], Juan I. Gutiérrez[1], Ricardo D. Moreno[2,3] and Manuel J. Santos[1,3]

The analysis of the genomic imprinting of eggs obtained from super-ovulation shows disorders in some imprinted genes.

The hormone dose used for this procedure seems to be important in the degree of methylation.

In human oocytes, selected imprinted genes including H19, PEG1 and KCNQ1OT1 show abnormalities in methylation following superovulation.

This significant amount of information has enhanced efforts to IMPROVE THE SUPEROVULATION PROTOCOLS in animals to DIMINISH ITS NEGATIVE EFFECTS.

In Vitro Maturation

In vitro maturation (IVM) refers to maturation in culture of immature oocytes.

Positive results have been reported, but in vitro maturation is not yet become a mainstream fertility treatment and still considered experimental.

Analysis of DNA methylation status of imprinted genes H19, Mest/Peg1 and Igf2R during in vitro maturation of mouse oocyte show:

- a loss of methylation at the Igf2R locus and Mest/Peg1 locus
- a gain of methylation at the H19 locus.

Similar results have been found in humans.

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Best Practice & Research Clinical Obstetrics and Gynaecology 44 (2017) 90–104
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8

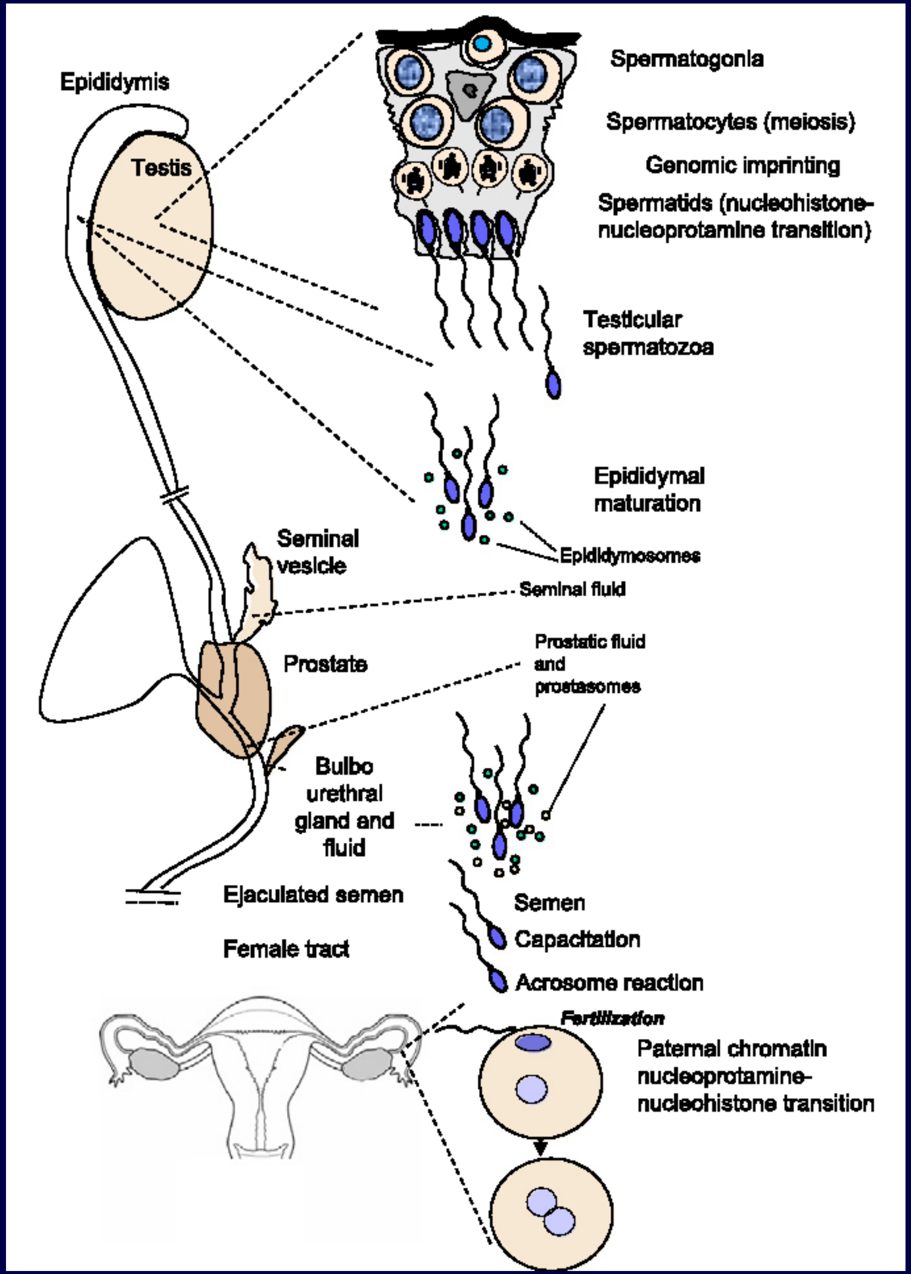
Genetic and epigenetic risks of assisted reproduction

Ziru Jiang [a], Yinyu Wang [a], Jing Lin [a], Jingjing Xu [a], Guolian Ding [a, b], Hefeng Huang [a, b, *]

[a] The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[b] Institute of Embryo-Fetal Original Adult Disease, Shanghai Key Laboratory for Reproductive Medicine, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

The genetic risks of IVM are not very clear yet.
It has been shown that the level of alteration depends on the time and the composition of culture media.

What about sperm?



- Epididymal sperm have the epigenetic reprogramming already completed, in contrast to what happens in egg maturation.
- Sperm culture has not been associated with epigenetic alterations.
- The eventual epigenetic alterations that may be found in sperm have been associated with male infertility.

A new challenge is presented when in animal and human ICSI is performed with immature sperm from the testes.

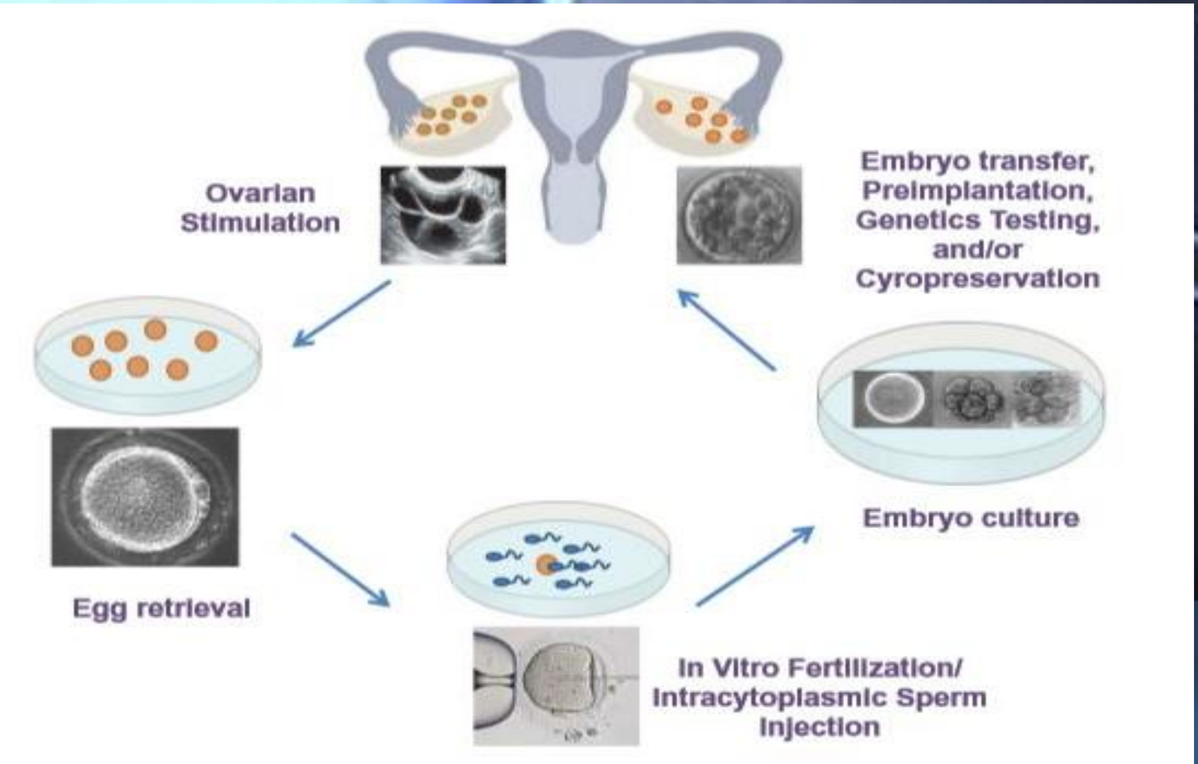
Technique

The kind of ART technique used can alter the epigenetic reprogramming and eventually development.

ICSI

1992, Palermo et al.
Great success but not many previous experimental testing.

Involves fertilization by injection of a single sperm directly into an oocyte.



ABOUT ICSI

ICSI **evades natural selection** at the oocyte membrane.

Bypasses physiological events like sperm capacitation, acrosomic reaction and membrane fusion.

Allows **genetically and structurally abnormal sperm** to fertilize eggs and pass abnormal genetic materials to the children.

Involves physical disruption of the oocyte membrane and the introduction of extraneous material.

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ICSI correlates to **asynchronous remodeling of chromatin decondensation** of the male pronucleus in primates, mice and cattle.

Mice produced by ICSI compared to those produced by regular IVF have long-lasting **transcriptome disturbances** that are maintained until the neonatal stage

Mouse ICSI blastocysts, compared to in vivo conceived groups, have a **reduction in the inner mass** cells and significant **differences in gene expression** related to cell function, development and metabolism.

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Up to date these alterations have not correlated with changes in the phenotypic profile or with transgenerational effects.

Some studies did not find any differences in preimplantation development in IVF or ICSI-produced mice compared to naturally conceived mice.

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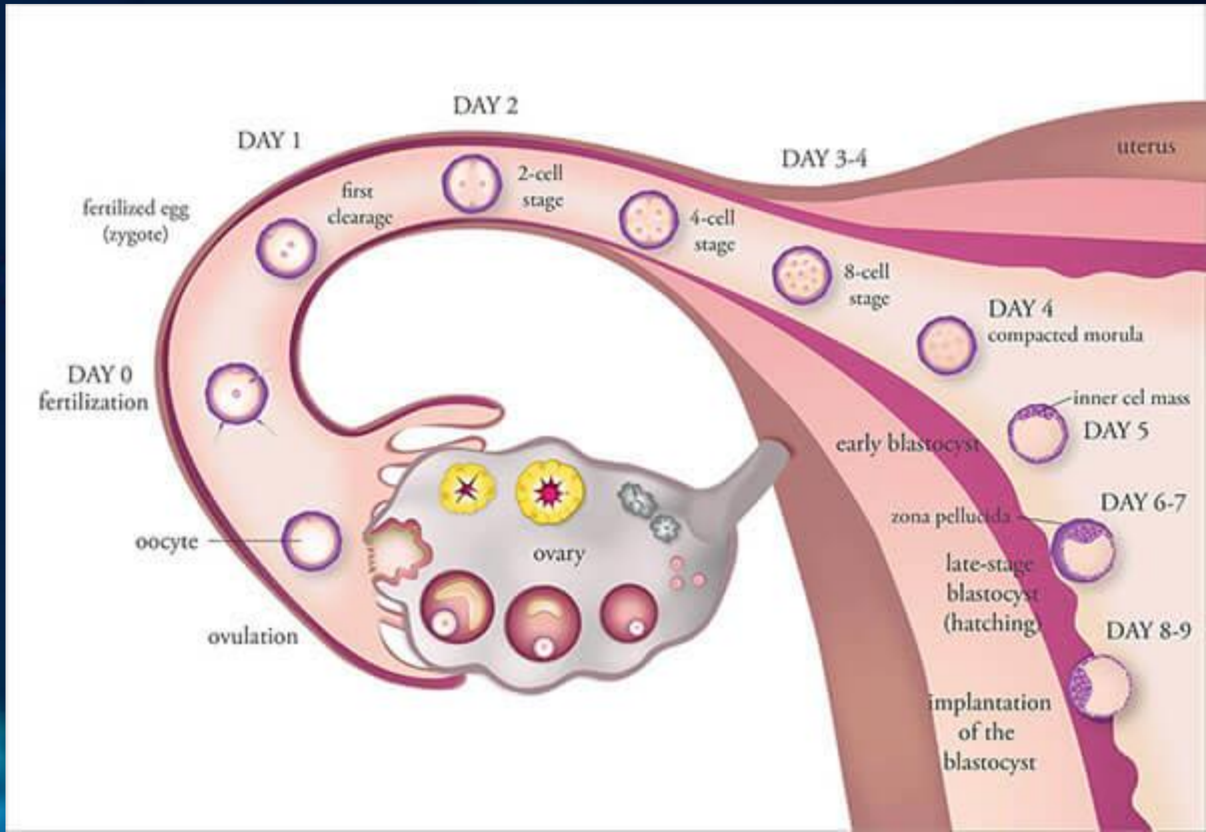
Different studies reported, with heterogeneous results, a correlation between ICSI and syndromes like **AS**, **BWS**, and **Silver-Russel** syndrome.

Different protocols used for ICSI in different species and studies can explain the different results.

In Vitro Culture

Suboptimal culture media affect the percentage of implantation and the survival of embryos that could achieve implantation.

Most relevant factor in the alterations of epigenetic reprogramming and development of animal embryos produced by ART.



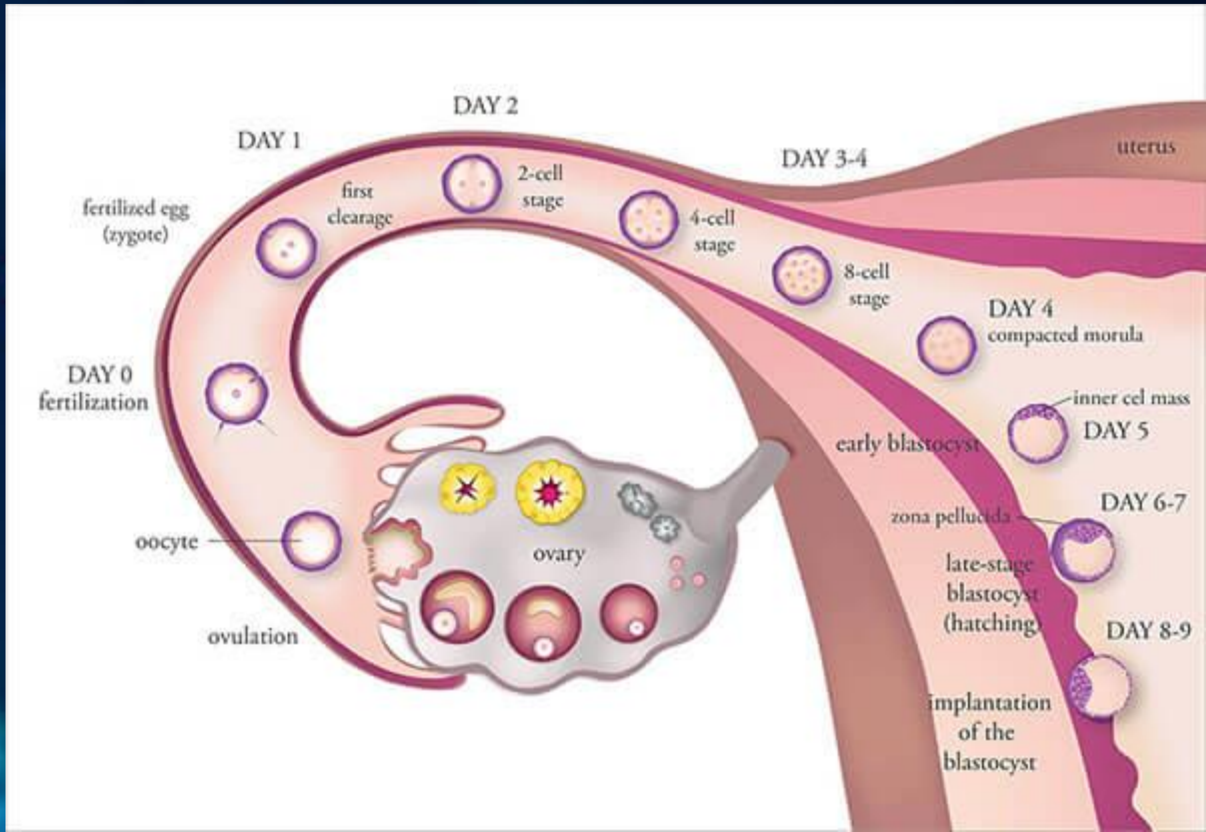
↓ Development speed

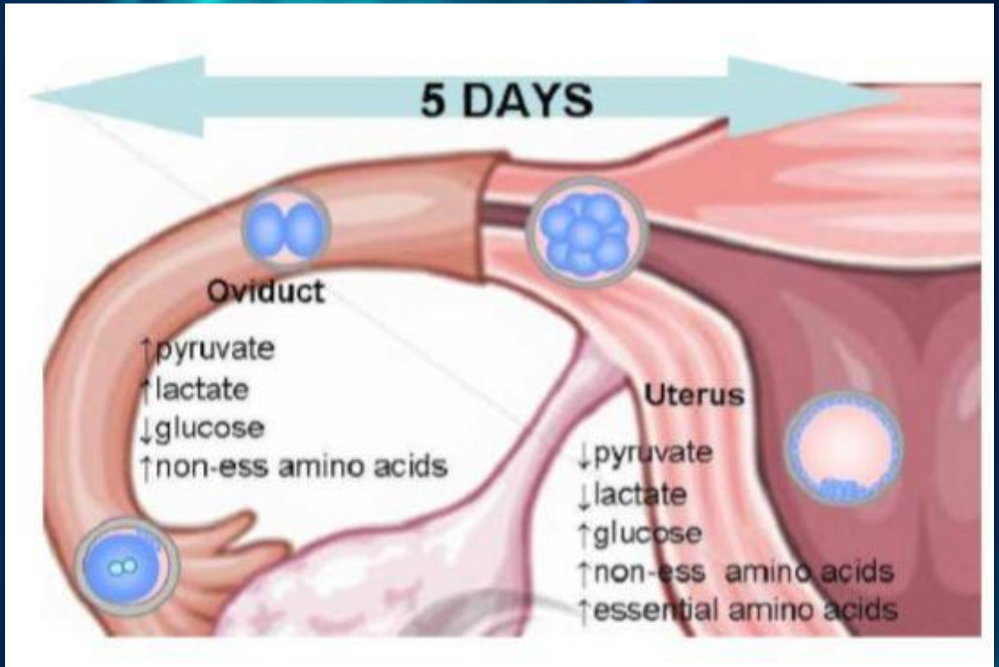
↓ Embryo quality

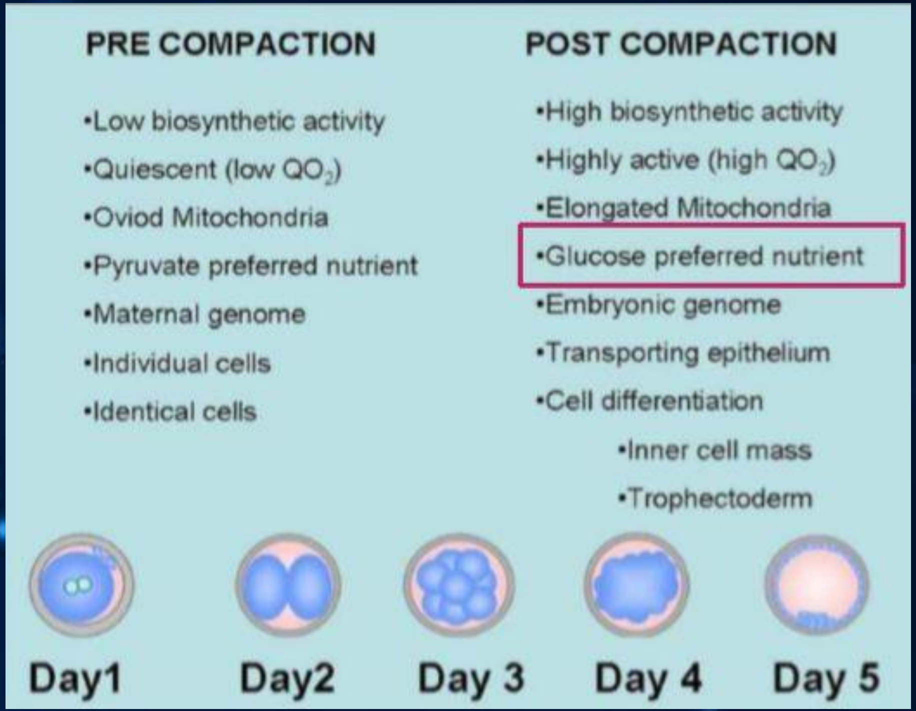
↓ Trophoblast development

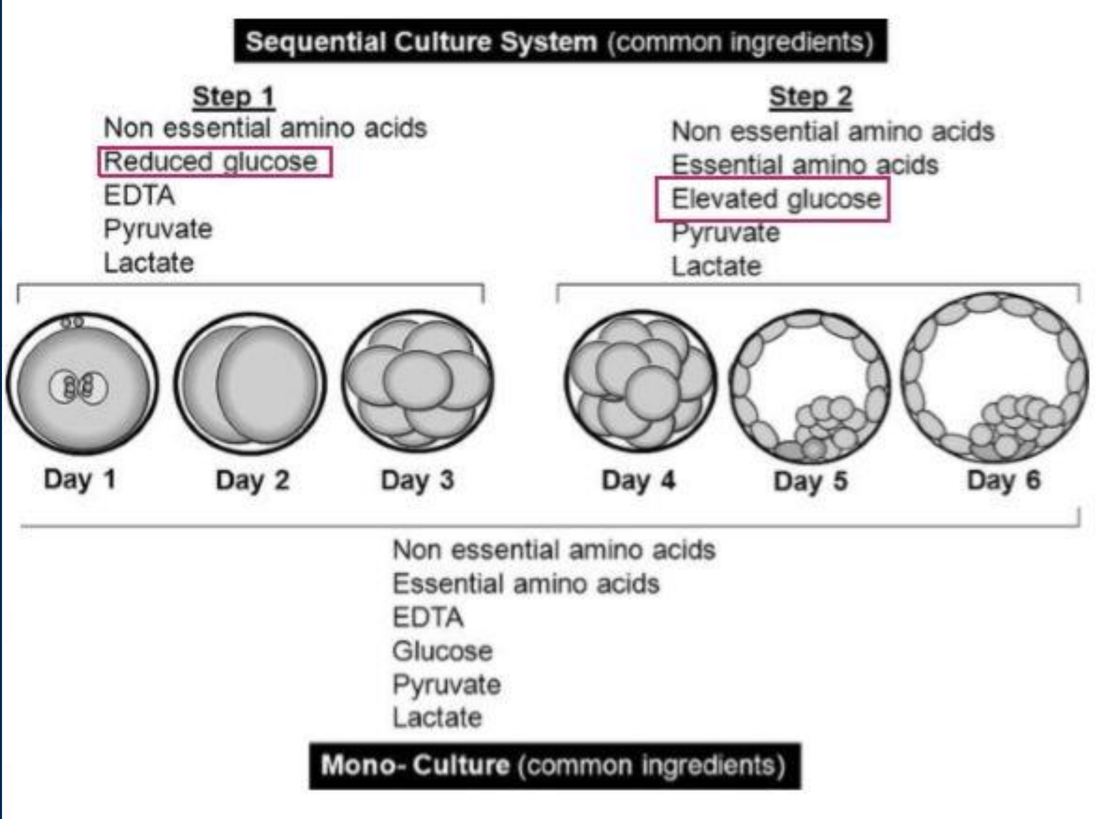
Abnormal preimplantation epigenetic reprogramming.

In Vitro Culture




5 DAYS
Oviduct
↑pyruvate
↑lactate
↓glucose
↑non-ess amino acids
Uterus
↓pyruvate
↓lactate
↑glucose
↑non-ess amino acids
↑essential amino acids


PRE COMPACTION
•Low biosynthetic activity
•Quiescent (low QO2)
•Oviod Mitochondria
•Pyruvate preferred nutrient
•Maternal genome
•Individual cells
•Identical cells
POST COMPACTION
•High biosynthetic activity
•Highly active (high QO2)
•Elongated Mitochondria
•Glucose preferred nutrient
•Embryonic genome
•Transporting epithelium
•Cell differentiation
•Inner cell mass
•Trophectoderm
Day1
Day2
Day 3
Day 4
Day 5



Standardization of embryo culture conditions and culture media is important concern in ART.

One important introduction are time-lapse incubators

Undisturbed culture system

↓ Embryo manipulation

↓ Changes in temperature and gas concentrations

No clear definition of superiority (if any) of one with respect to the other.

Differences in the mRNA pattern and development speed between embryos produced in vitro and in vivo.

Different expressions of imprinted genes as H19 depending on the culture medium.

Effects in the postnatal and adult period.

The first and most relevant alteration in phenotype in animals produced by IVF is the Large Offspring Syndrome (LOS).
Many features of LOS are similar to those found in the Beckwith–Wiedemann (BWS) syndrome in humans.
It is of special interest that the epigenetic alterations in the LOS are very similar to those found in the BWS.

The impact of culture medium on the outcome of ART is today undoubtedly a major constraint for these techniques in mammalian species.

Is important to find the best conditions of culture medium that can minimize its deleterious effects on epigenetic reprogramming and development.

EMBRYO MANIPULATION:

PGD

The goal of PGD is to help couples to avoid termination of pregnancy for a genetically abnormal fetus and conceive healthy babies.

PGD allows selection of embryos in couples at risk of transmitting monogenic diseases and chromosome number or structural abnormalities.

PGD methodology may confer some adverse effects on mothers and offspring

AND EMBRYO TRANSFER

Epigenetic risks of PGD

Research on mice models has revealed that biopsy for PGD causes abnormal development of the resulting offspring.

Blastomere removal procedures affected placental functions.

More studies are needed to arrive at a **well-informed** conclusion on the risks of PGD.

with higher risk of preterm birth
ow birth weigh.

The parental genetic condition rather than the PGD procedure posed a risk of adverse obstetric and neonatal outcomes in humans.

This suggests that exposure of the embryo to minimal environmental changes in temperature; humidity or pH can in itself lead to aberrant epigenetic regulation.

The process of embryo transfer itself, which is minimally invasive, has been shown to affect **DNA methylation at imprinted genes.**

EMBRYO TRANSFER

EMBRYO TRANSFER

Isolation of the effects of embryo transfer on mice independently of other factors.

Study of the methylation profile of ten imprinted genes

A control group of embryos was conceived in vivo, not cultured or transferred

The embryo transfer group conceived in vivo that was transferred without going through culture had an **aberrant expression of imprinted genes compared to the control group.**

In the embryo **cultures + transfer**, the effects of transfer was increased by culture as shown by the **bigger number of genes with aberrant allelic expression in embryonic and extraembryonic tissues**

CRYOPRESERVATION

Frozen-thawed embryo transfer is an indispensable element in ART and has maximized the effectiveness of fertility preservation.

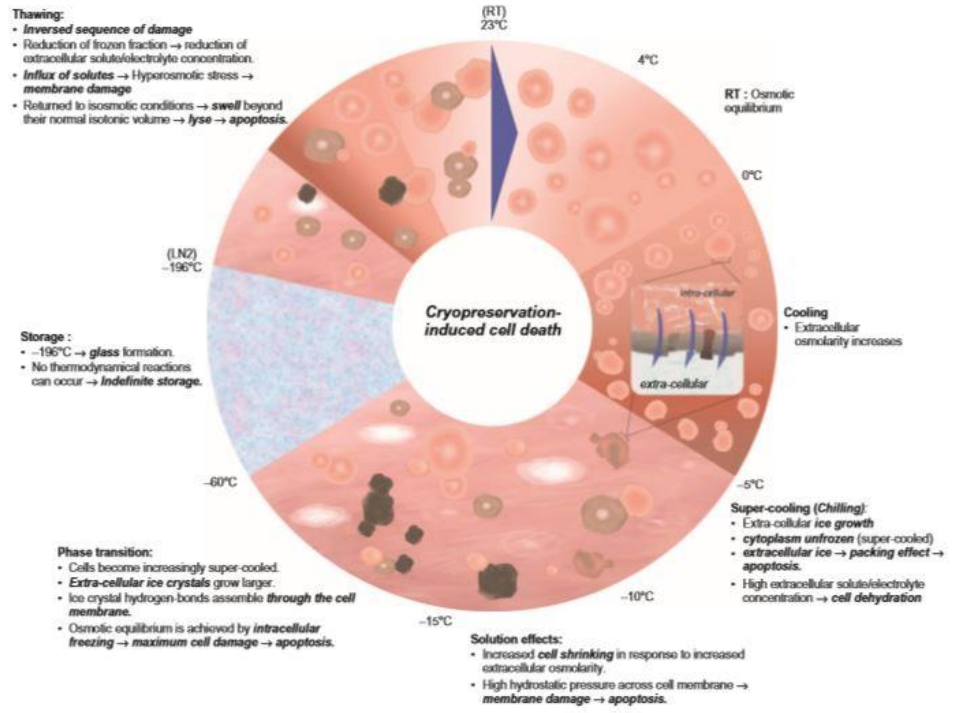


Figure 2 Schematic of physical events underlying the freezing, storing and thawing.

Two possibilities:

- Slow-freezing
- Vitrification

Higher ongoing pregnancy rate than fresh-embryo transfer.

The genetic and epigenetic risks of frozen-thawed embryo transfer are still uncertain.

CRYOPRESERVATION

Genetic Risks

Vitrification induces a **transient increase in DNA breaks** and a possible sporadic change in CpG methylation in mouse oocytes.
DNA fragmentation is regarded as an indicator of DNA damage to assess the efficiency of cryopreservation.

Epigenetic risks:

Comparison between embryos cryopreserved on day 3 using a slow-freezing DMSO protocol and control fresh embryos to determine the expression levels of de novo DNMT (DNMT3a and 3b) and the global DNA methylation levels.

The results suggested that the DNMT expression patterns could be disturbed after cryopreservation but it seems to be **reinstated in a timely**

Further studies are needed to confirm the effect of cryopreservation on genomic imprinting in human oocytes and embryos.

↓ **H3K4me3**

Frozen thawed bovine blastocysts

↑ **H3K27me3**

This phenomenon may be caused by cellular stress, especially oxidative stress.

So, what can we say about the existing data regarding the effect of assisted reproductive technologies on the epigenome?

The majority of studies examining epigenetic changes are conducted in samples resulting from live births at the time of delivery.

Examination of chorionic villus samples from first trimester pregnancies.

The authors did not observe any significant methylation differences between the IVF and controls in the CVS samples.

Several other studies have examined epigenetic changes in specimens following spontaneous abortions, though these data must be interpreted with caution as the samples may be from ‘abnormal’ pregnancies. Results from these studies vary significantly.

A subfertility diagnosis may be responsible for epigenetic differences seen in patients undergoing ART.


EPIGENETICS
https://doi.org/10.1080/15592294.2019.1646572
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REVIEW
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Epigenetic changes and assisted reproductive technologies

Sneha Mani [*a], Jayashri Ghosh [*b], Christos Coutifaris[a], Carmen Sapienza[b], and Monica Mainigi [a]

[a]Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA; [b]Fels Institute for Cancer Research and Molecular Biology, Temple University, Philadelphia, PA, USA

The wide variation in the ARTs protocols used in different clinics and the lack of proper survey tools may be the causes of diverging points of views and of this inconclusive situation.

The fertility status and life history of couples are also important factors as significant differences are observed between donors.

However, the influence of IVF in epigenetic changes is confirmed by several animal data.

ART may contribute to epigenetic changes in the offspring.

It is critical to identify modifiable elements in our protocols to minimize the risks to our patients and their offspring.

Ethical prioritization of ART

Other investigations to optimize the ART procedures and reduce the risks.

CONCLUSIONS

THANK YOU !