Are we influencing it?
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.
Epigenetics is the study of several processes that can alter gene expression without changing the DNA sequence.

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

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


N-terminal histone tails protrude from nucleosomes.
Covalent attachment of a methyl group to the C5 position of cytosine residues in CpG dinucleotide sequences.

DNA 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.

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
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.
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.
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 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.

<table>
<thead>
<tr>
<th>Lysine</th>
<th>Mono-methyl lysine</th>
<th>Dimethyl lysine</th>
<th>Trimethyl lysine</th>
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<tbody>
<tr>
<td>H3K27me3</td>
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<td>H3K9me</td>
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<td>Silencing</td>
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<td>Transcriptional activity</td>
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DNA Methylation and histone modifications help to compartmentalize the genome into domains of different transcriptional potentials.
Ex-vivo exposures with important changes of the environment.

IVF involves multiple manipulations to the gamete and early embryo.

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

HOW CAN ART INFLUENCE EPIGENETICS?

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
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.
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.

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.
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.
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.
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.
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.
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.
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.
Abnormal preimplantation epigenetic reprogramming.

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

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

One important introduction are time-lapse incubators.

Undisturbed culture system

Embryo manipulation

Changes in temperature and gas concentrations
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.
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.
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.

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

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

PGD is not associated with higher risk of preterm birth and low birth weight.

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.
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.

Higher ongoing pregnancy rate than fresh-embryo transfer.

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

Two possibilities:
- Slow-freezing
- Vitrification
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.
The results suggested that the DNMT expression patterns could be disturbed after cryopreservation but it seems to be reinstated in a timely manner.

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

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

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.
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.
THANK YOU!