2025.10.19 대한생식면역학회 추계학술대회

Epigenetic insights into the impact of assisted reproductive technology on obstetric outcomes

강남차병원 홍유미

Introduction

- **Epigenetic modification of ART**

- **Epigenetic regulation and placenta related pregnancy complication**
- **Imprinting disorder**
- **Long-term childhood outcome**

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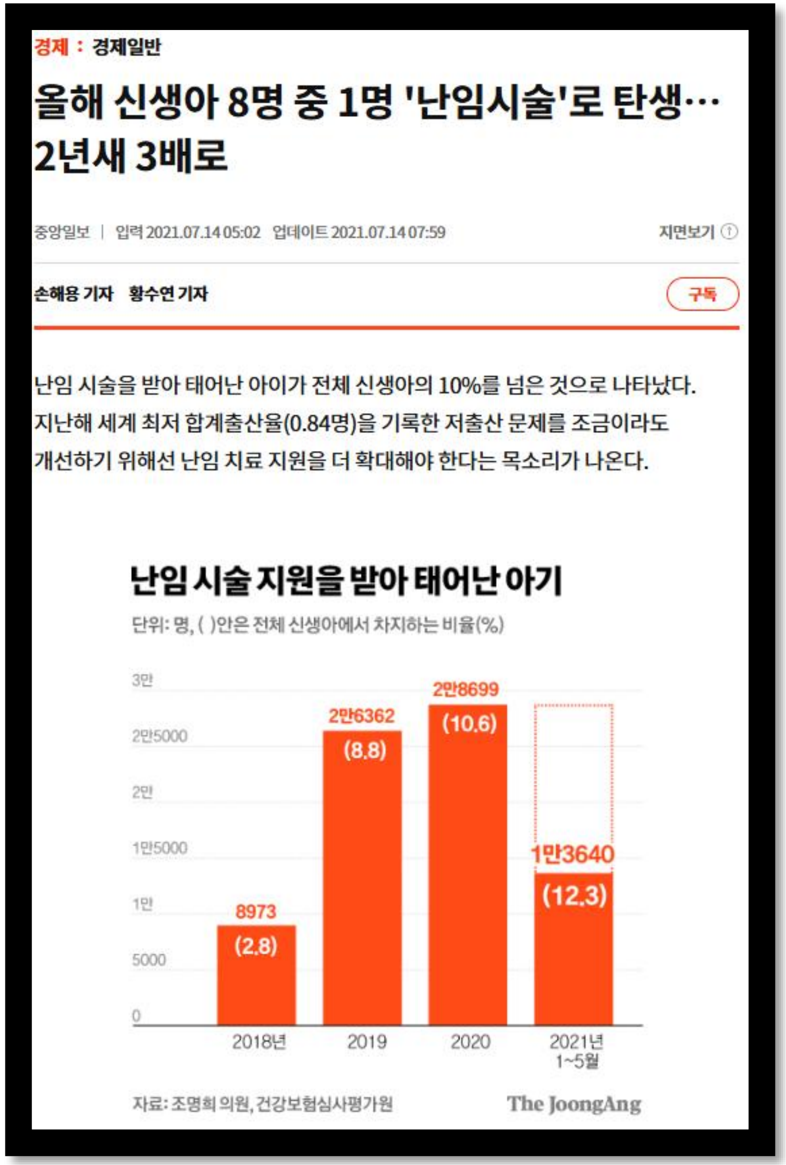
Epigenetics modification of ART



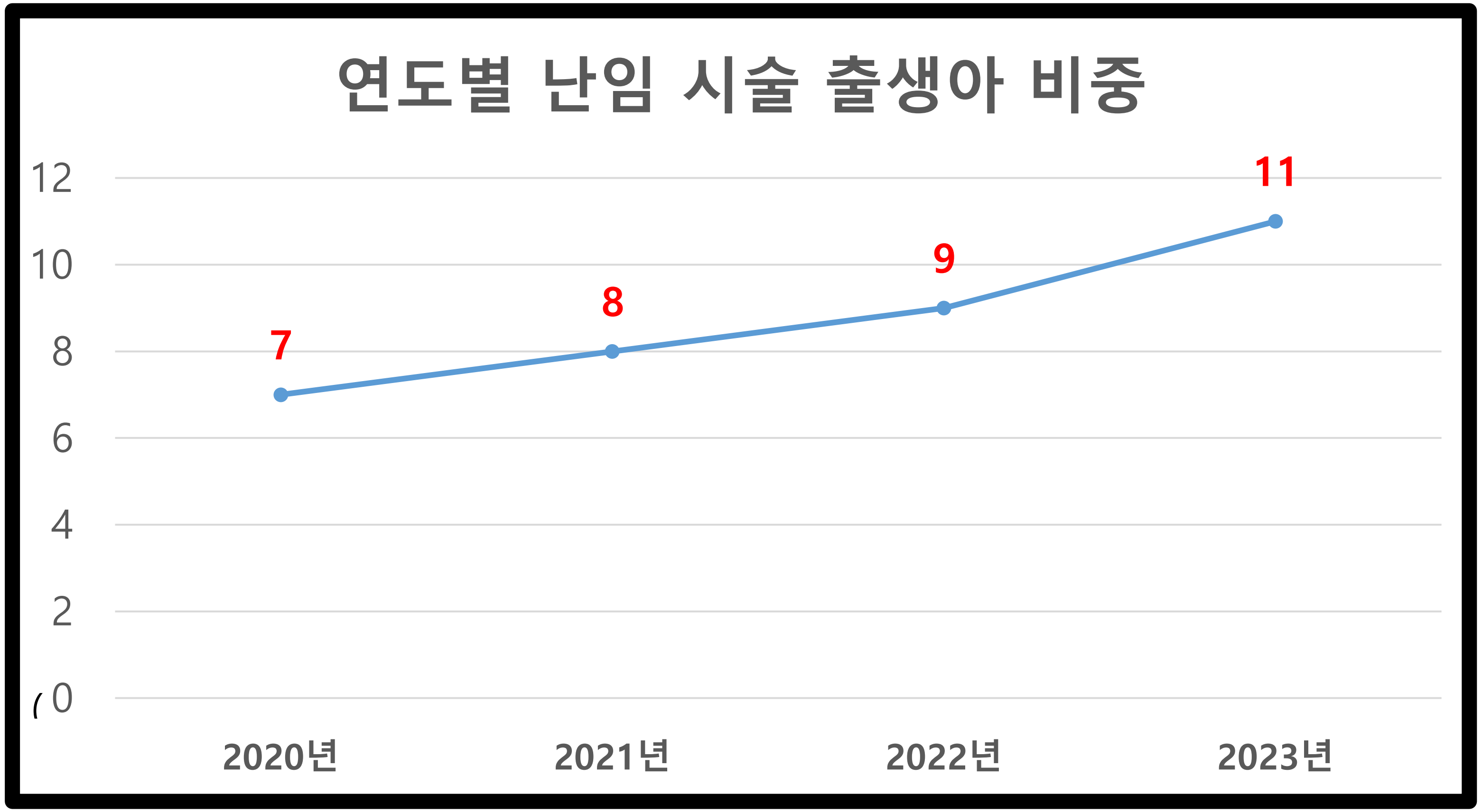
경제 : 경제일반

올해 신생아 8명 중 1명 '난임시술'로 탄생… 2년새 3배로

중앙일보 | 입력 2021.07.14 05:02 업데이트 2021.07.14 07:59 지면보기

손해용 기자 황수연 기자 구독

난임 시술을 받아 태어난 아이가 전체 신생아의 10%를 넘은 것으로 나타났다. 지난해 세계 최저 합계출산율(0.84명)을 기록한 저출산 문제를 조금이라도 개선하기 위해선 난임 치료 지원을 더 확대해야 한다는 목소리가 나온다.



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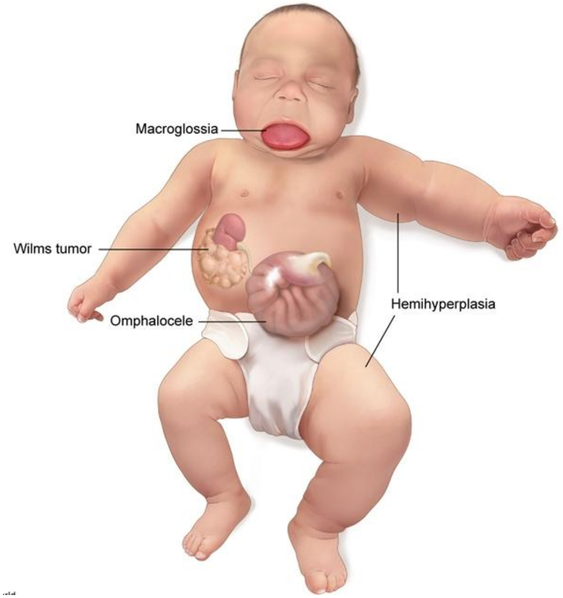
Epigenetics modification of ART



Epigenetics modification of ART

Epigenetics modification of ART

- Epigenome

: The complete set of **chemical modifications** to the DNA and histone proteins of an organism that regulate gene expression **without altering the underlying DNA sequence**.

Pinborg et al, AOGS, 2016: 95

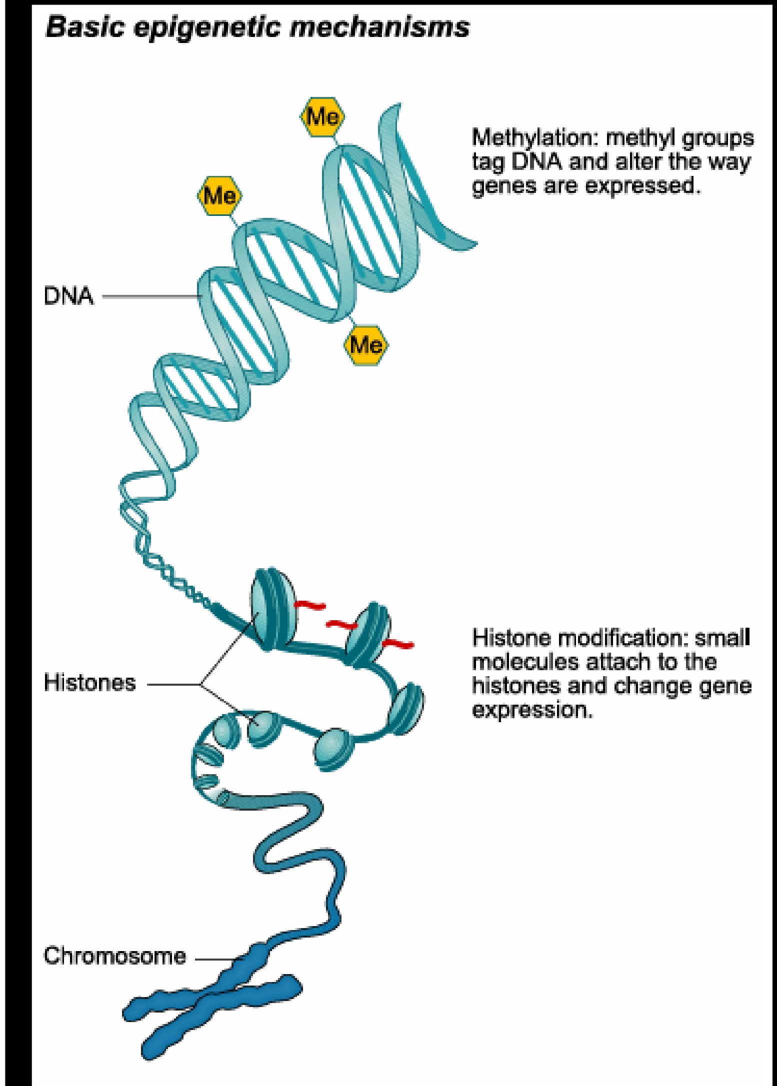
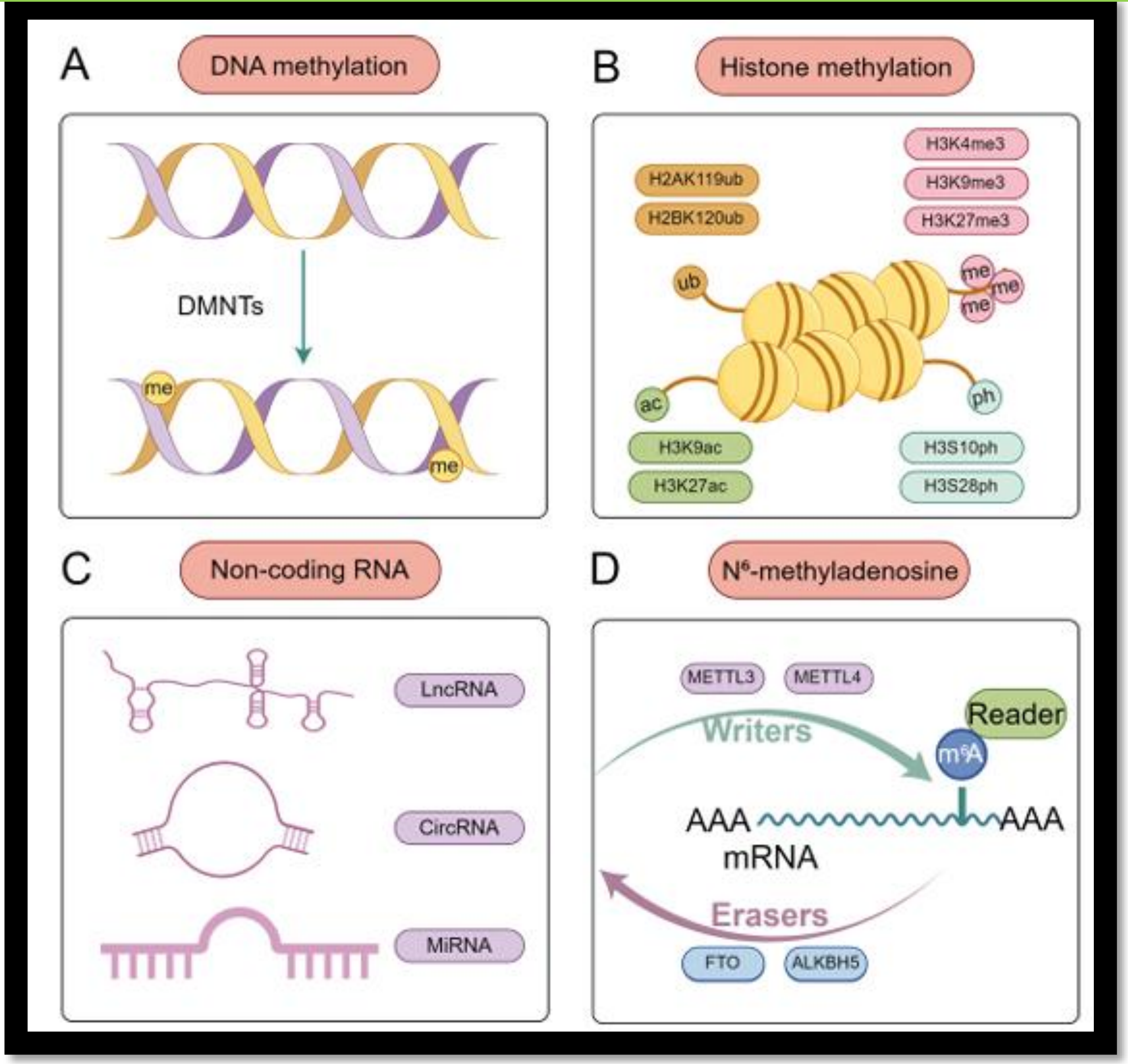


Figure 1. Basic concepts of epigenetics.

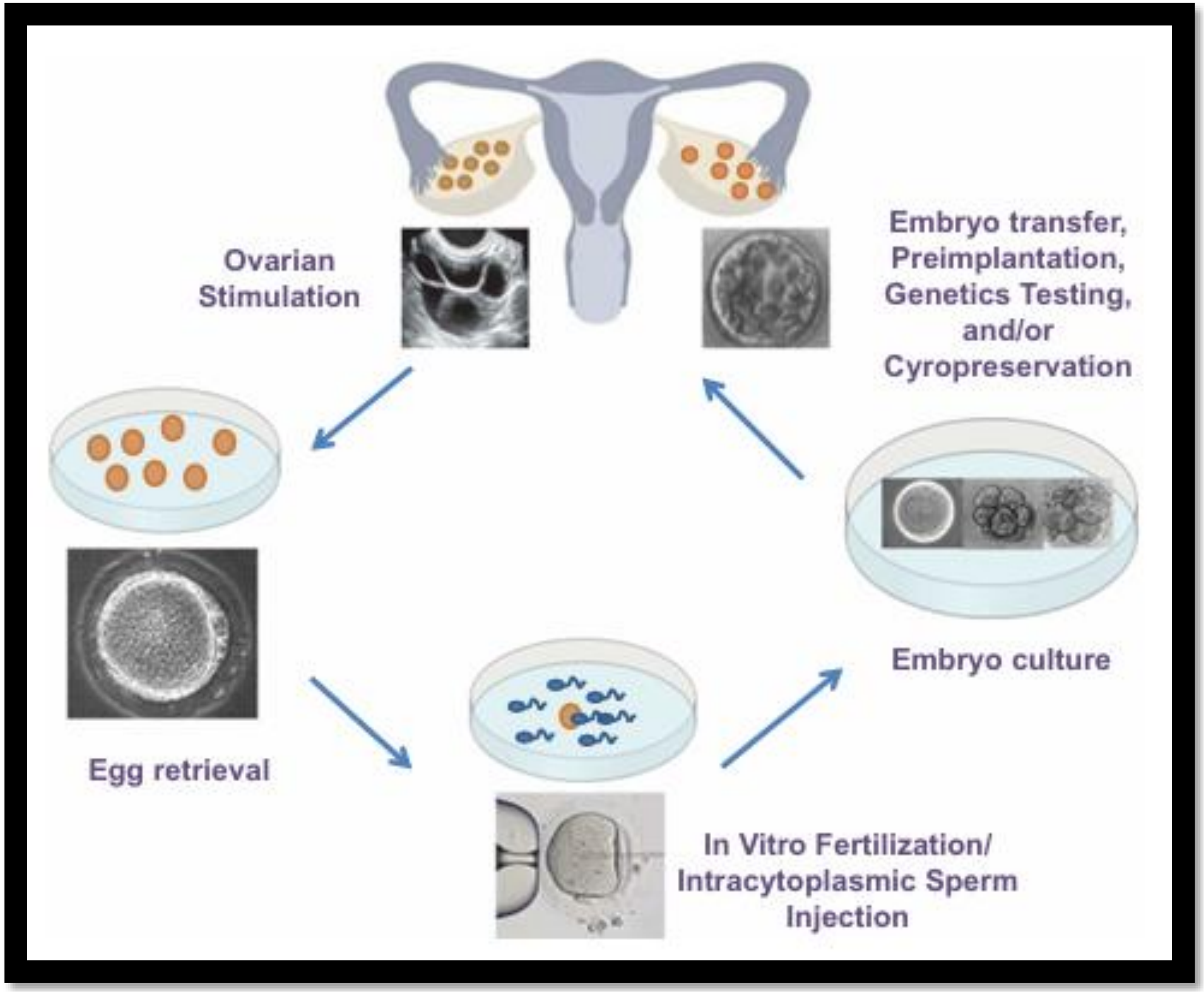
Epigenetics modification of ART

- **DNA Methylation**
- **Histone modification**
- **Non-coding RNA**
- **Nucleosome remodeling**



Xueqin Ma et al, Frontiers in Immunology, 2025;10

Epigenetics modification of ART



Sneha Mani et al, Epigenetics, 2020:15

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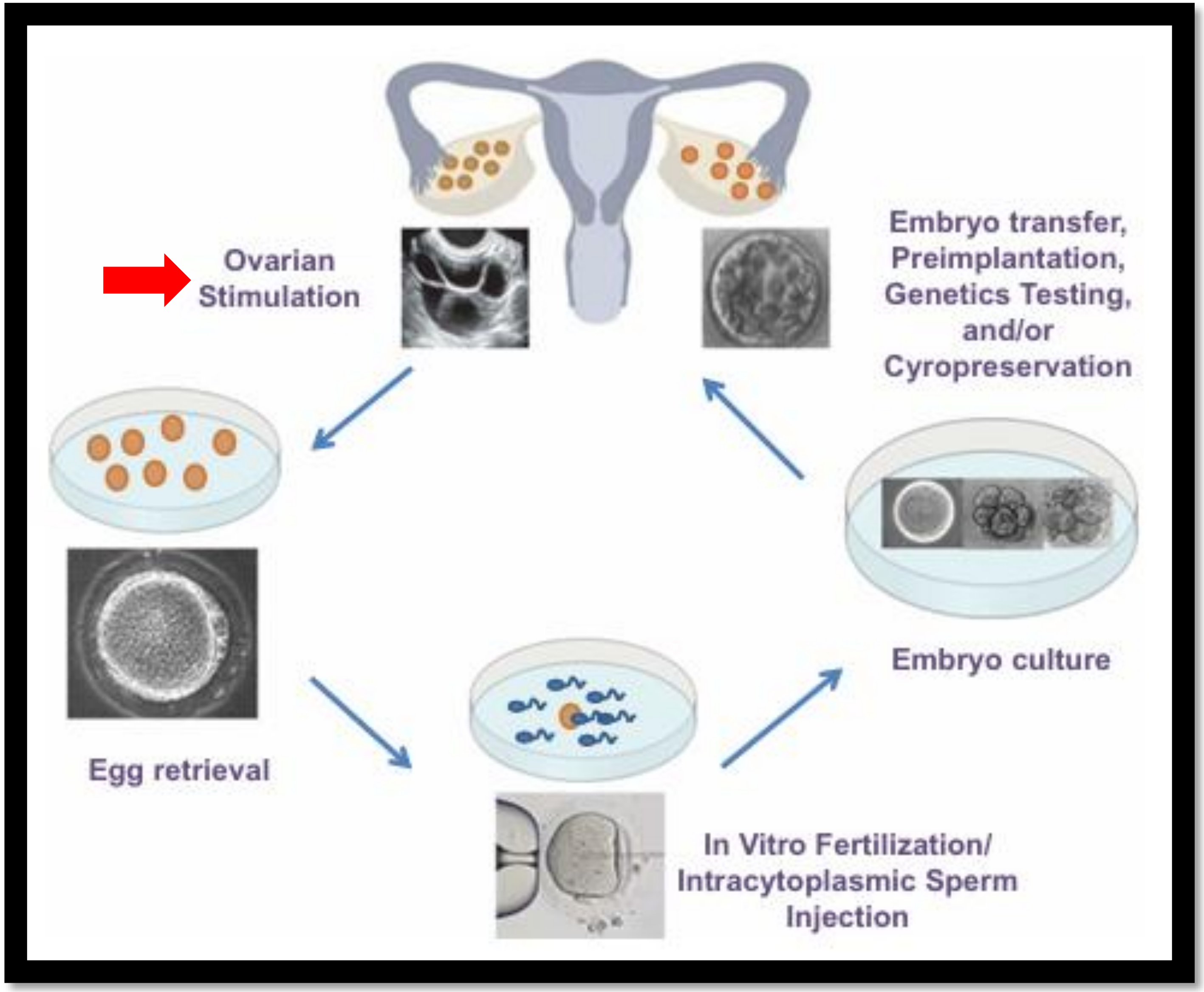

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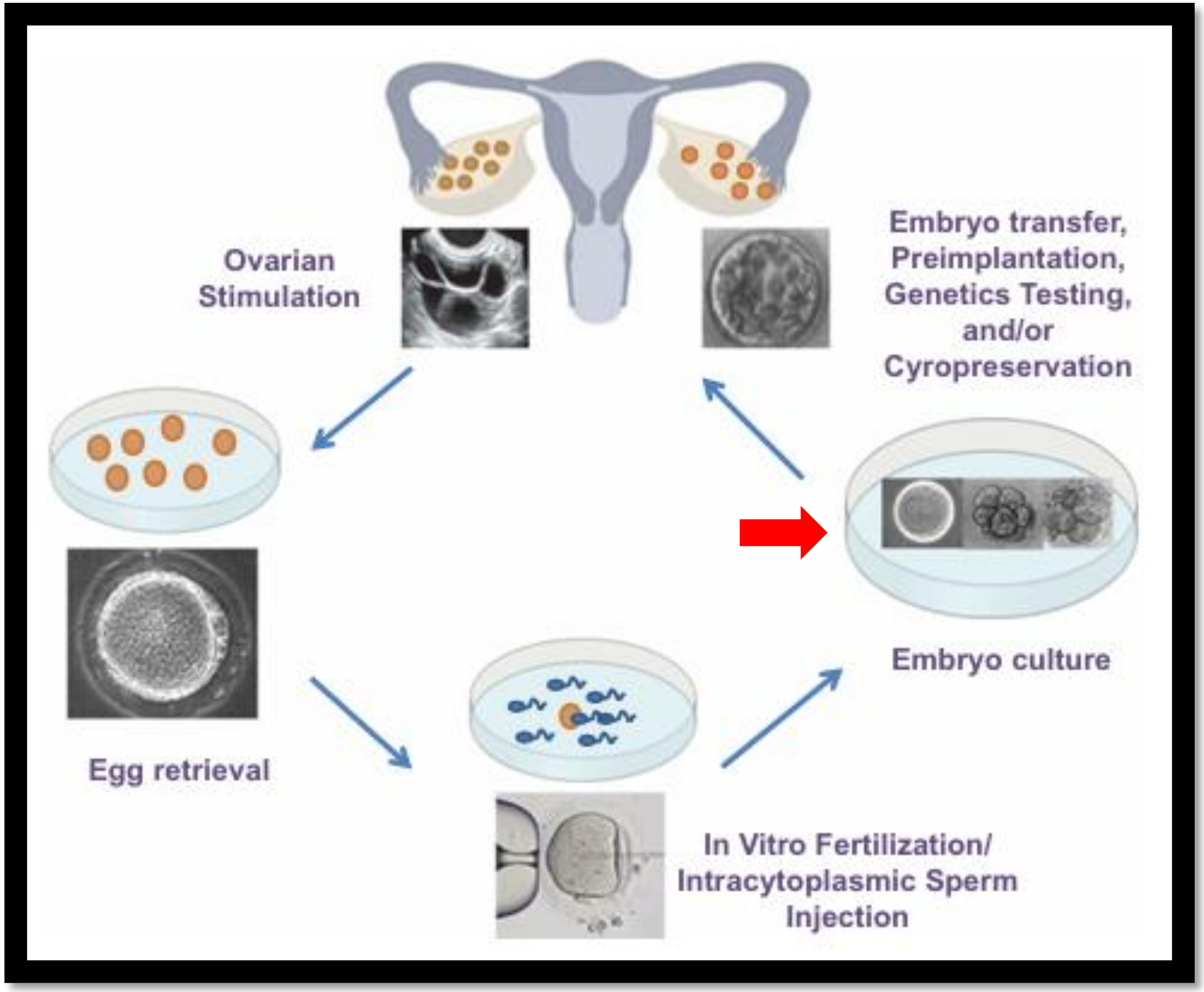

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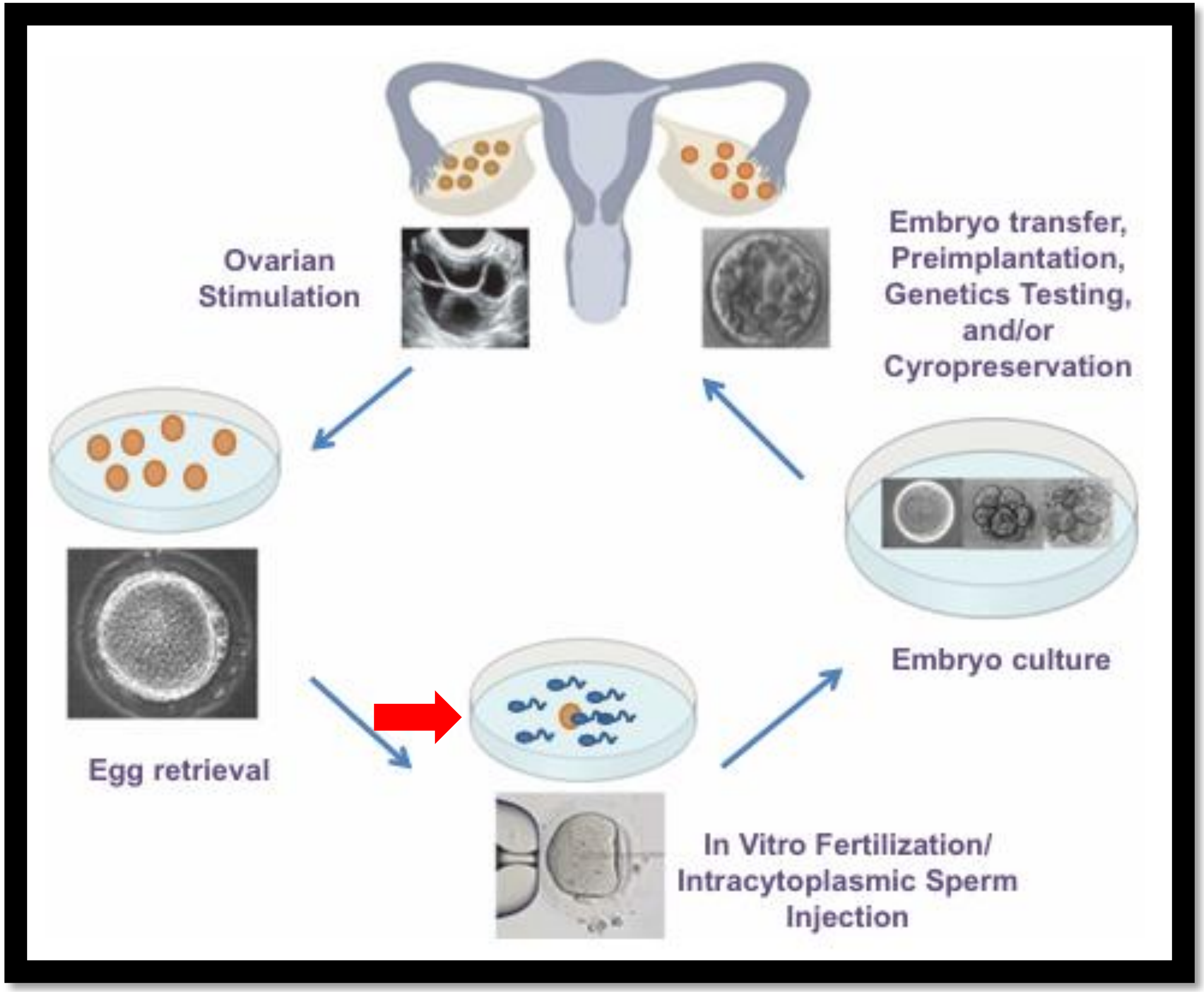

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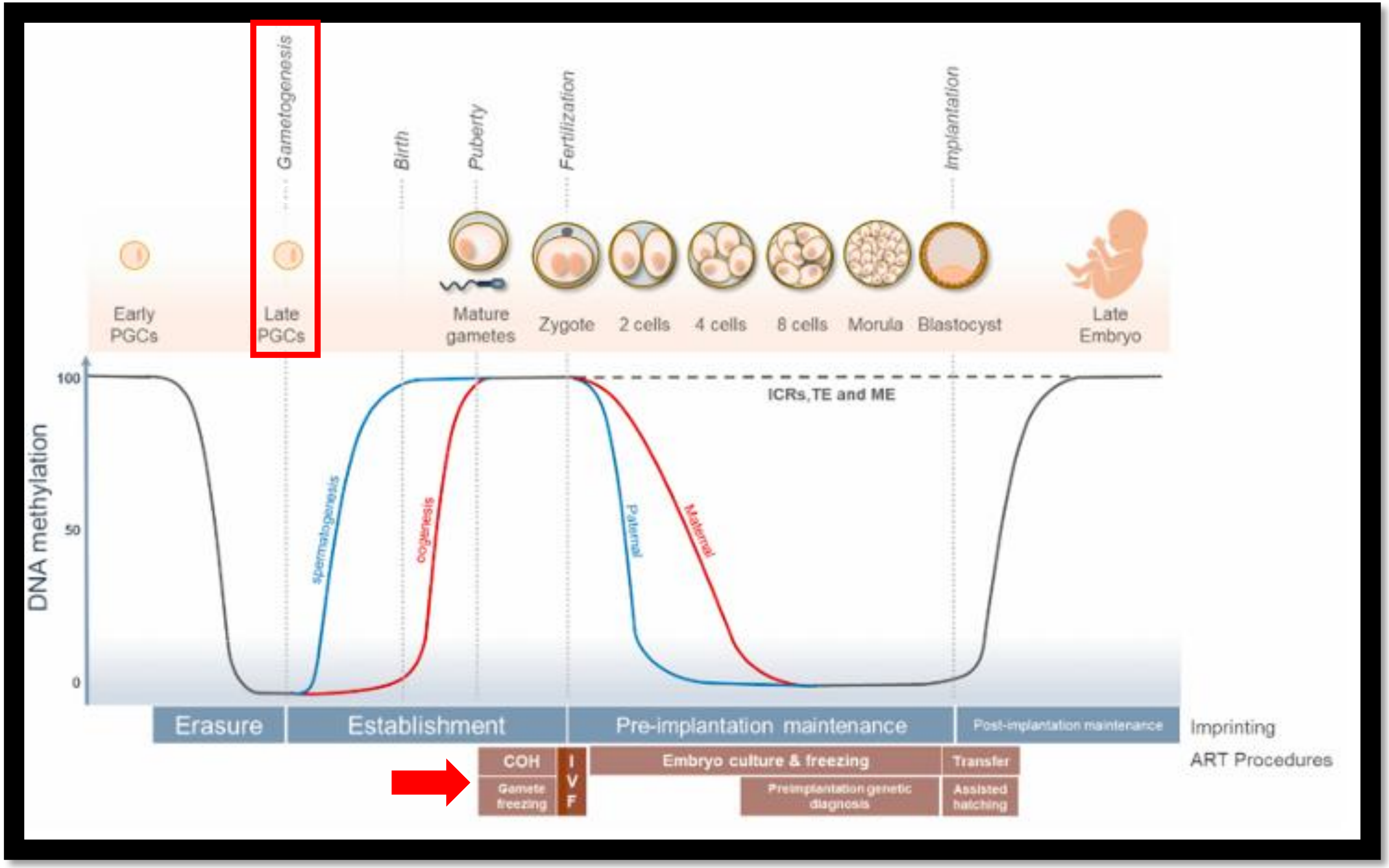

Eguzkine Ochoa, Life 2021; 11, 728

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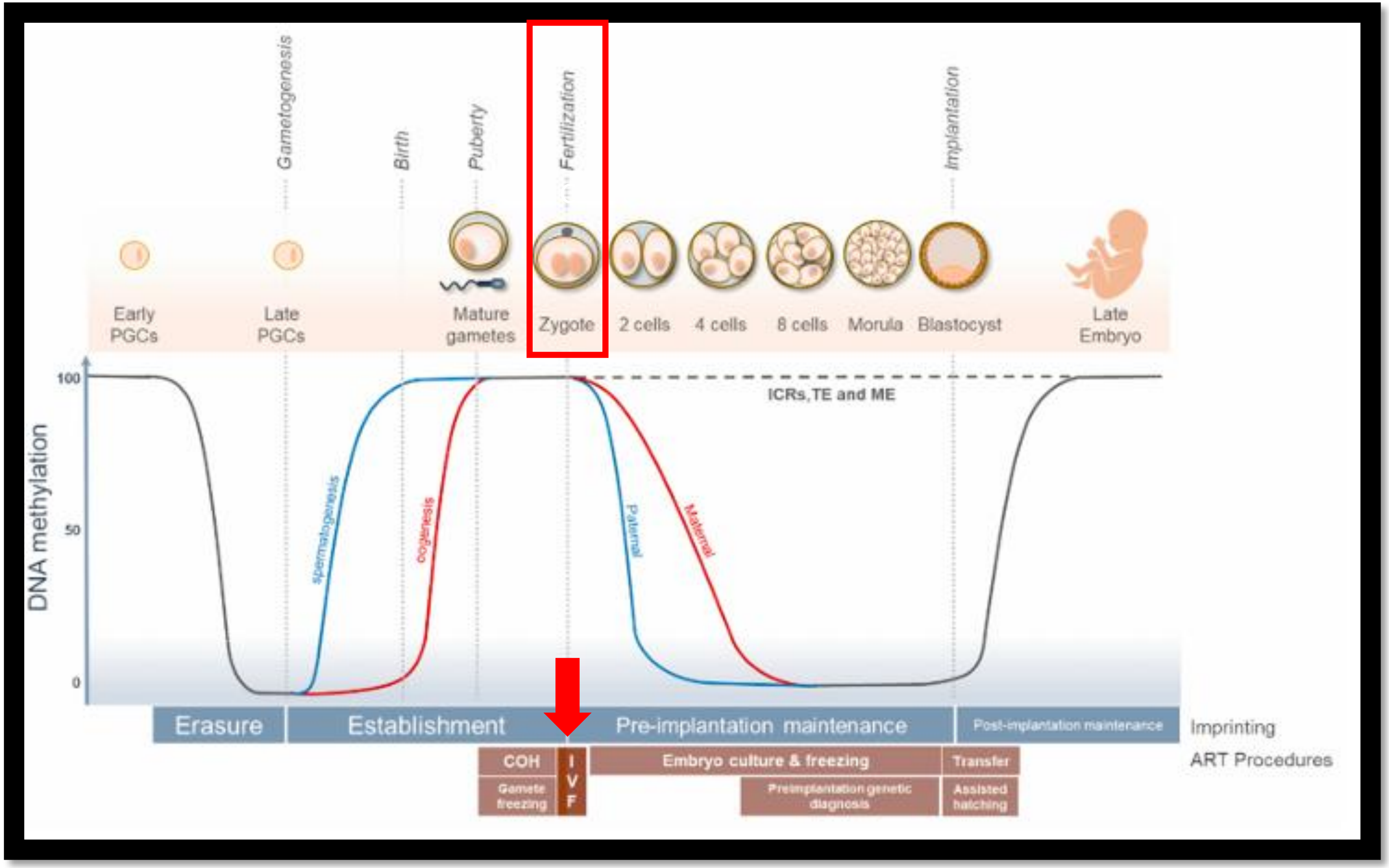

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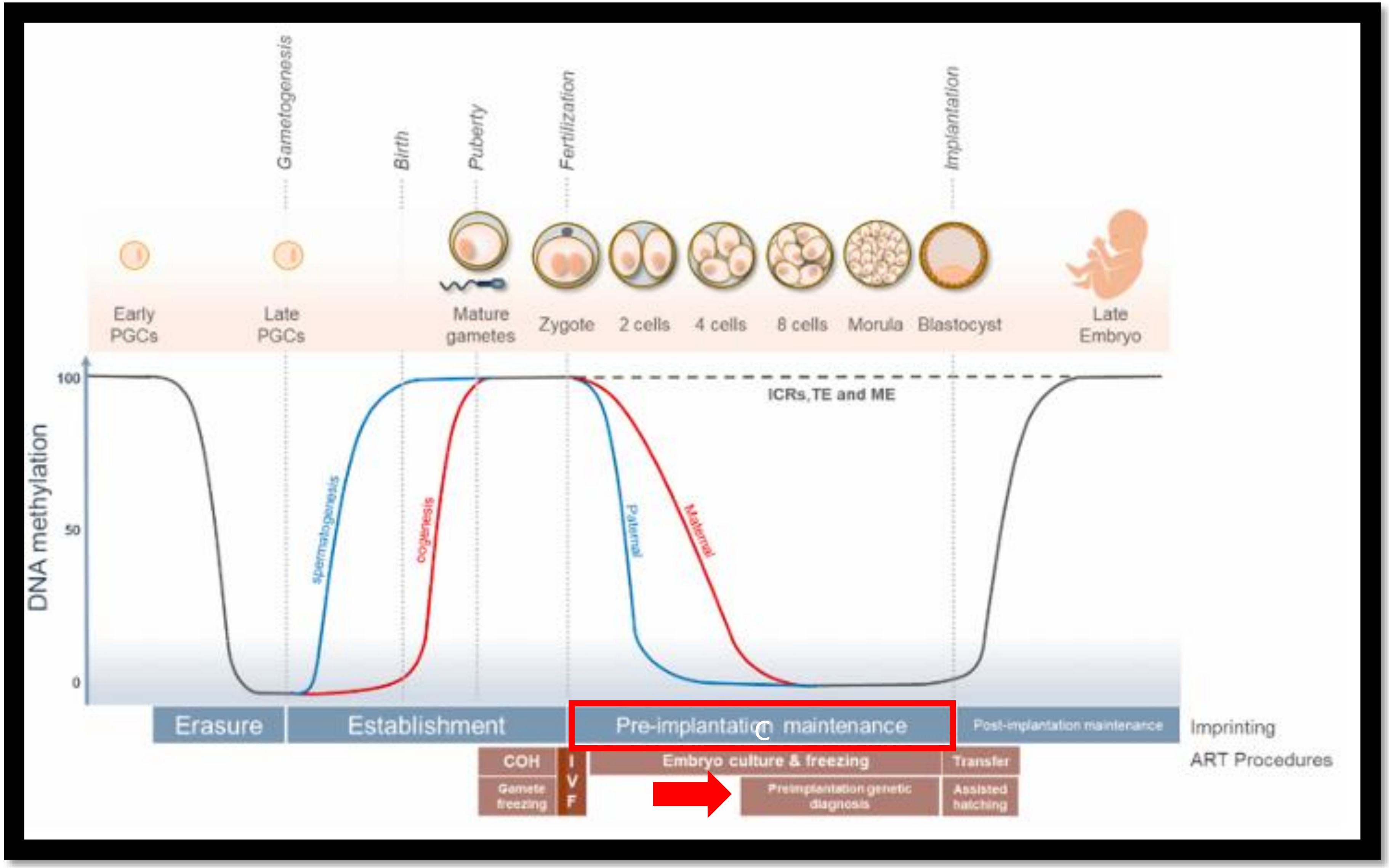

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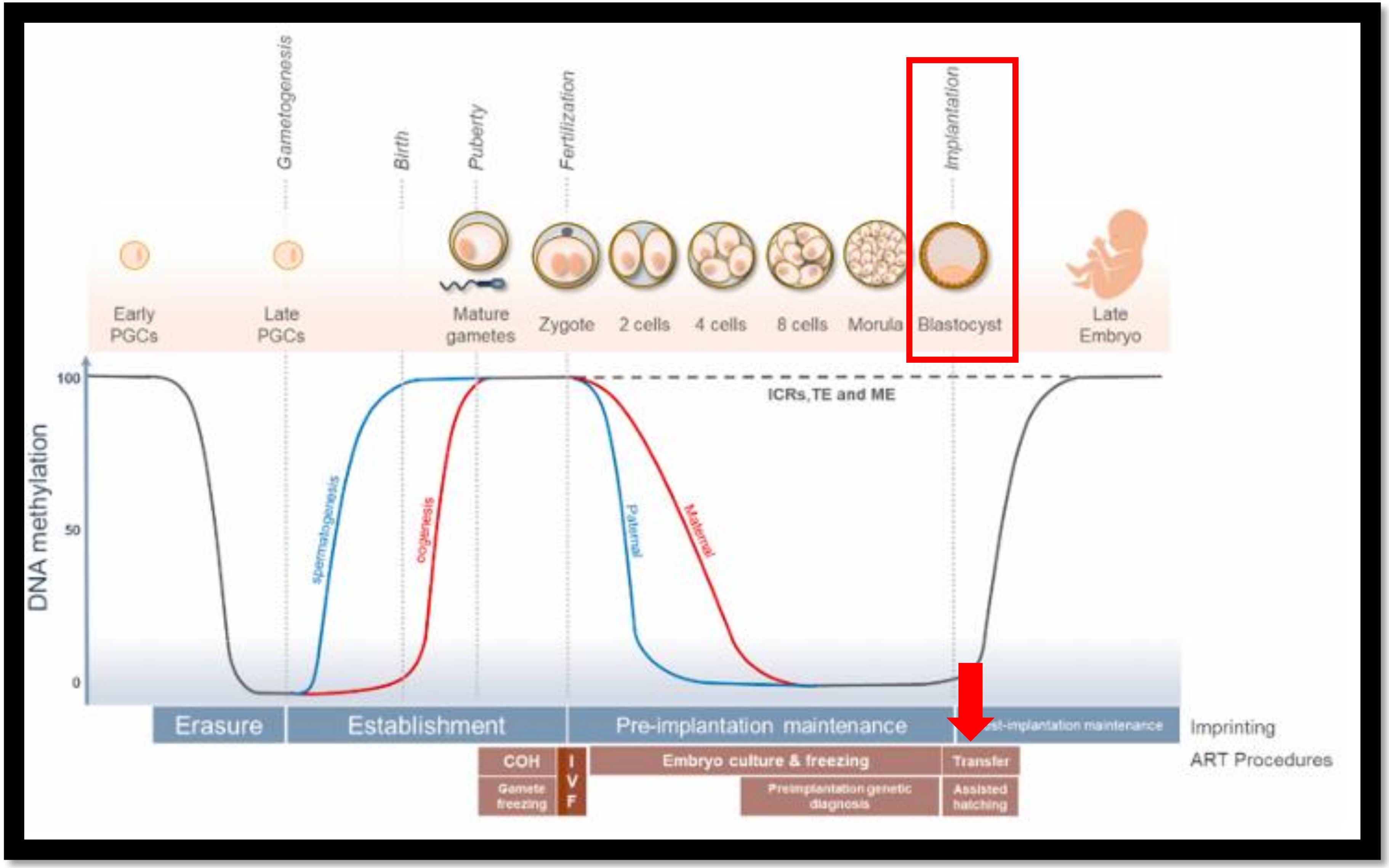

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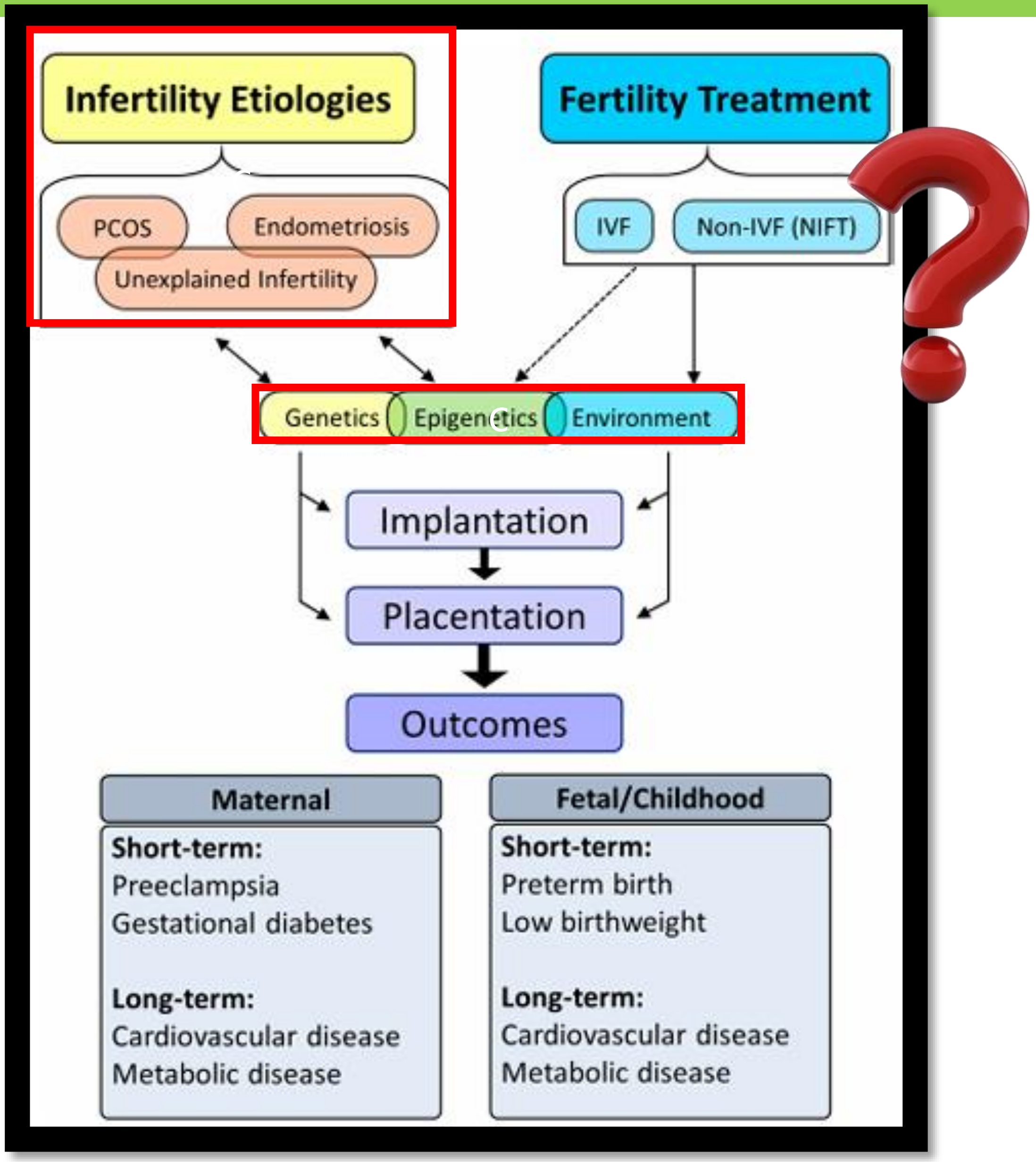
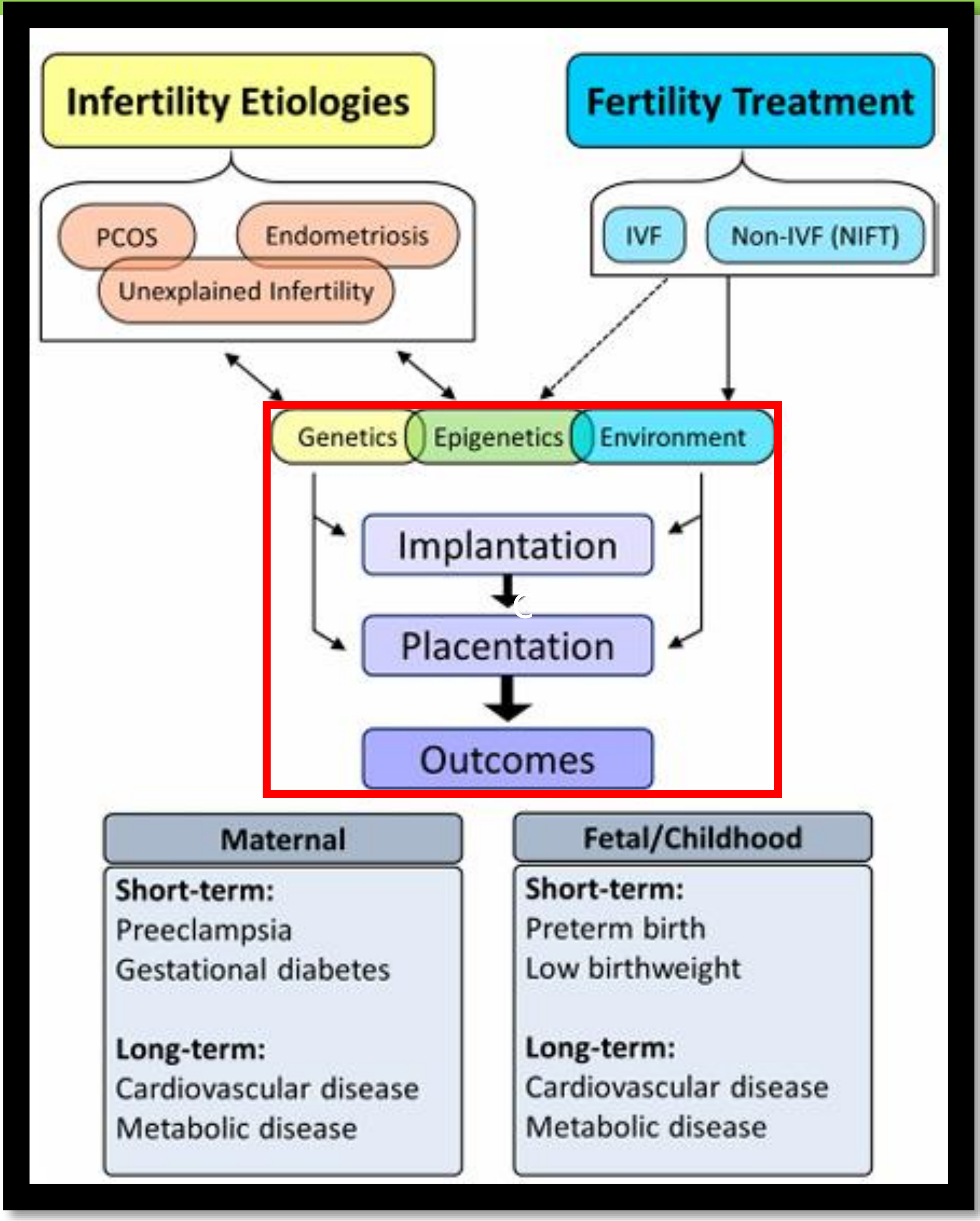


Margareta D et al, J Clin Endocrinol Metab, 2019 104(6)

Epigenetics modification of ART



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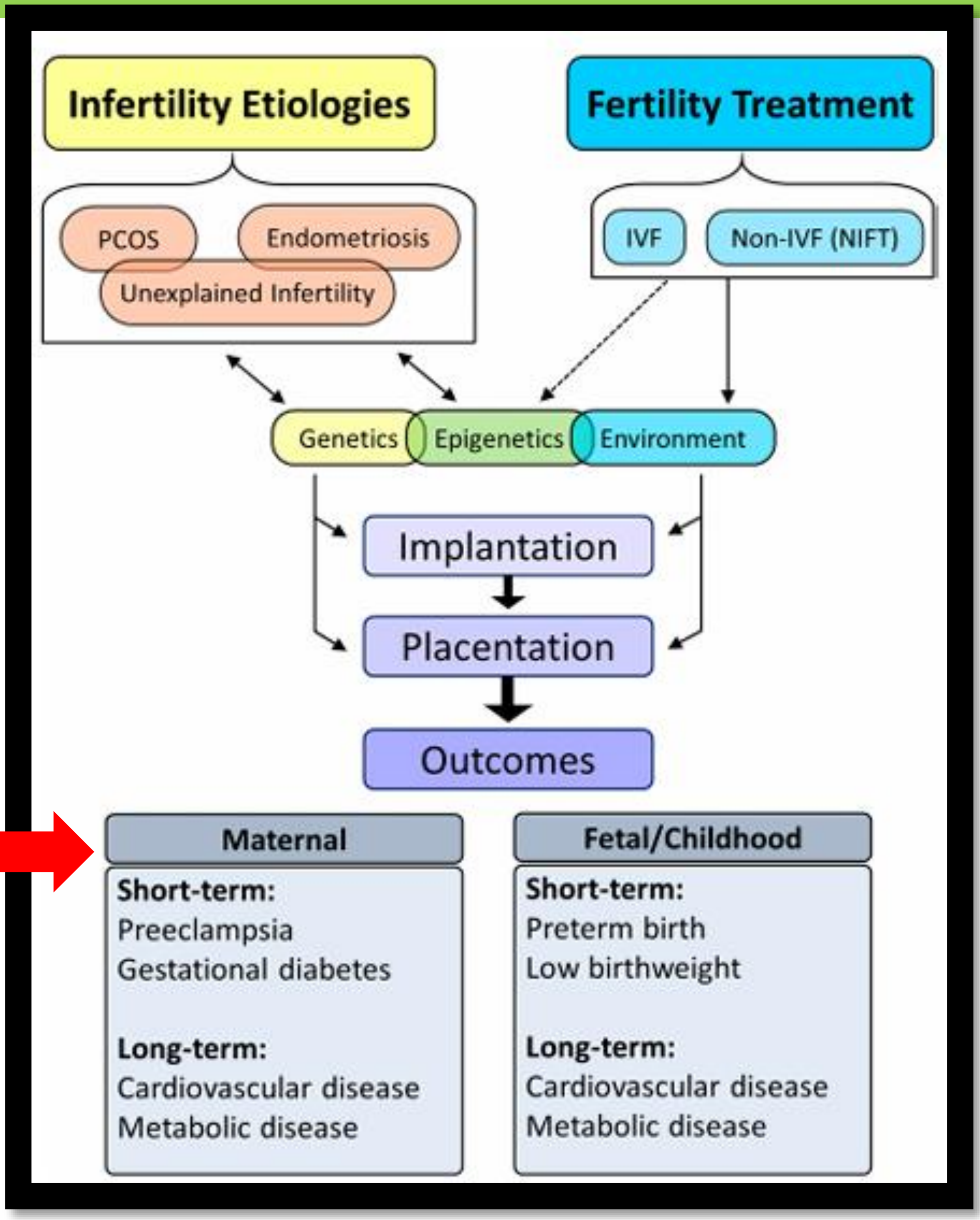

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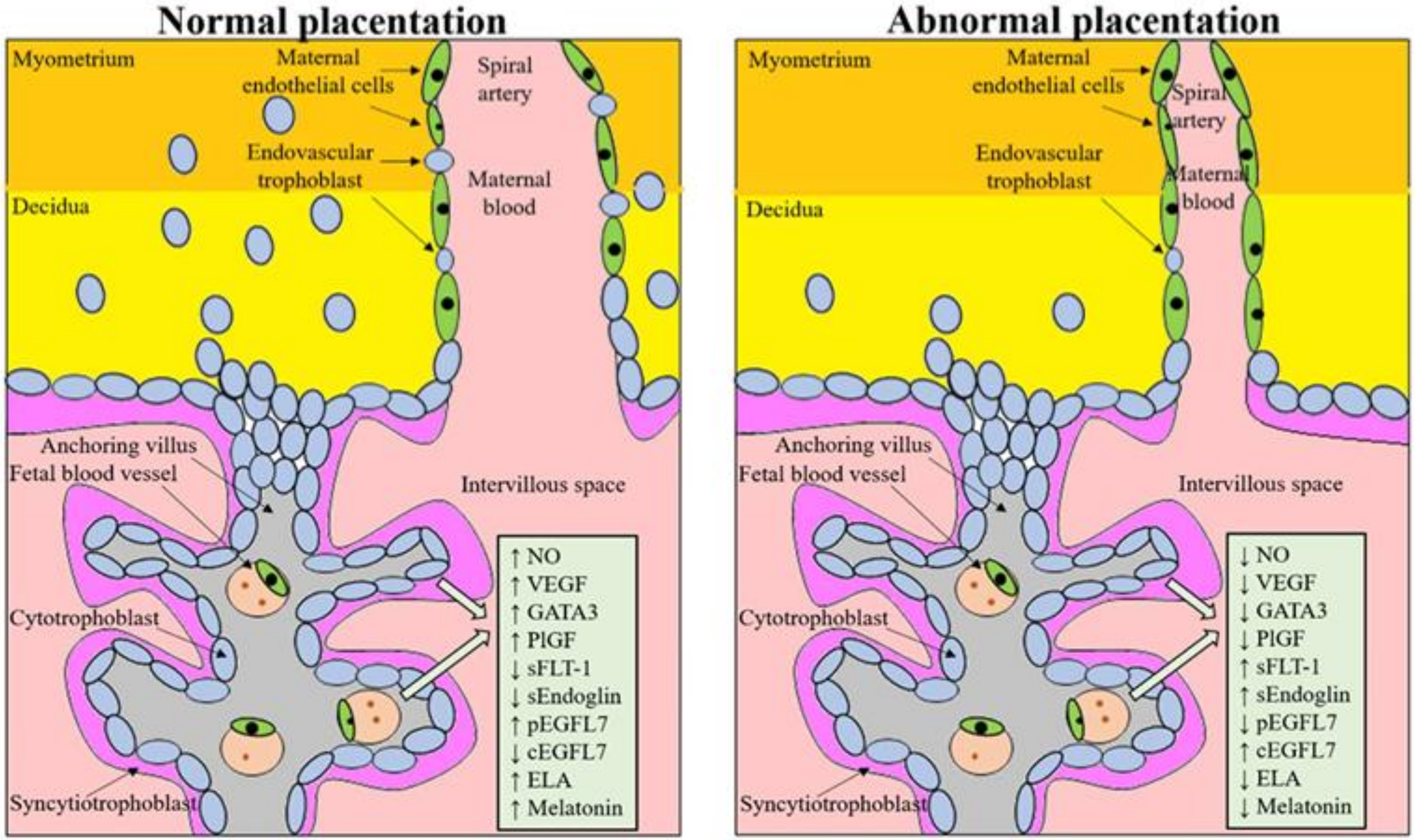
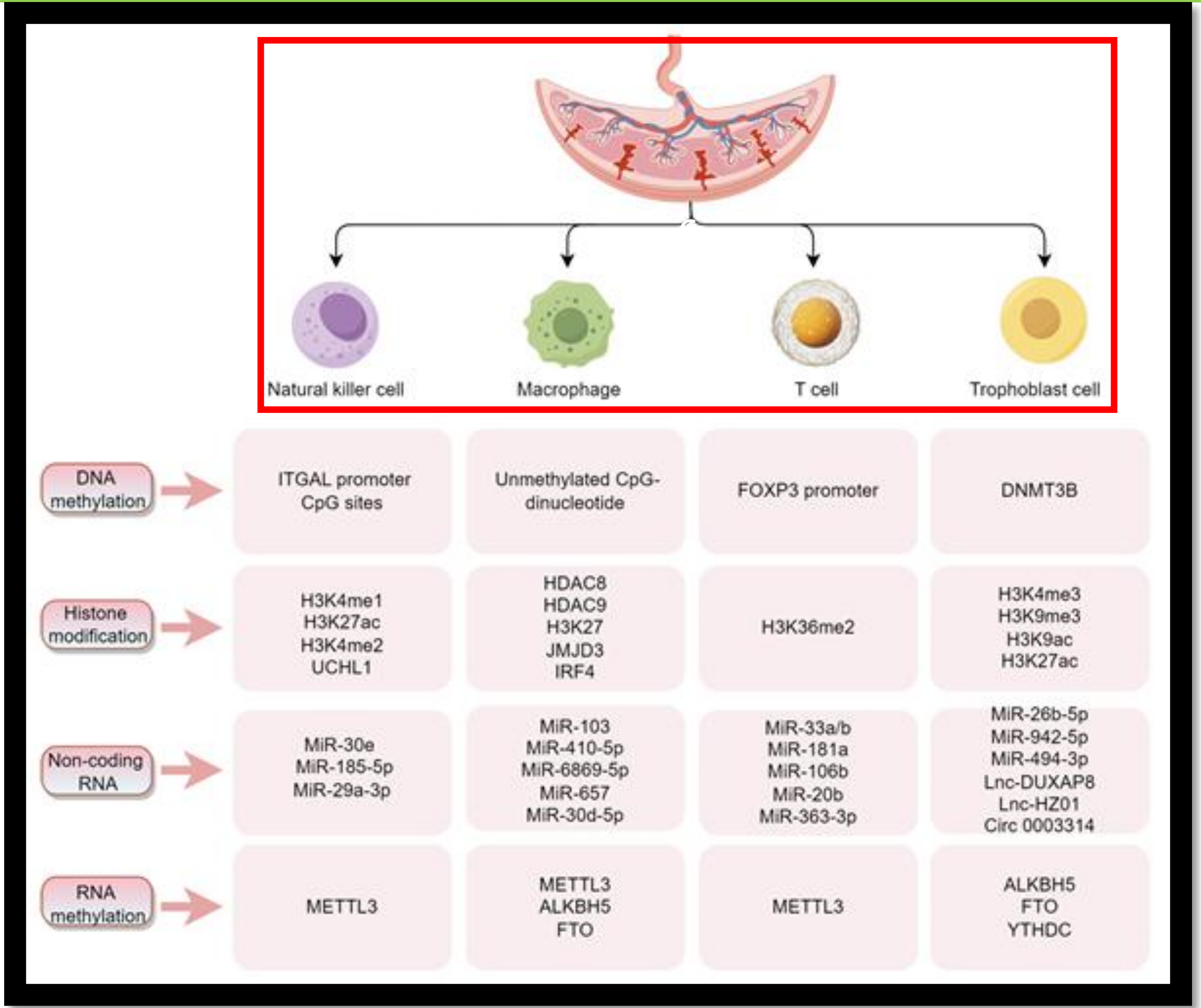
Epigenetic regulation and placenta related pregnancy complication



Claudio Manna et al, Int. J. Mol. Sci, 2022;23

Epigenetic regulation and placenta related pregnancy complication



Xueqin Ma et al, Frontiers in Immunology, 2025;10

Epigenetic modifications	Molecules	Mechanisms	Diseases
DNA Methylation	DNMT3A Downgrade	Mediates the non-dependent induction of DNA methylation by TGFBR1	Early onset severe pre-eclampsia
	Placental growth factor and Fms-associated tyrosine kinase-1 hypomethylation	——	Pre-eclampsia
	DNA methylation at the CPG site	——	Low birth weight
	Placental DNA methylation sites associated with birth weight	——	Perinatal cardiometabolic status of the mother, chronic disease in later life of the offspring
	CD3 methylation in pregnancy	——	Psychiatric symptoms such as depression and anxiety
Histone modification	Deletion of Men1, a member of the histone H3K4 methyltransferase complex	Disruption of terminal differentiation of stromal cells	Embryo resorption and pregnancy failure
	H3K18la downgrade	Influence on endometrial tolerance	Abortion
	Knockout of KAT8	Adjustment of H4K16ac/CDX2 axis	Vulnerable to embryo implantation failure induced miscarriage
	KDM5CK upward	Regulate the expression of TGFβ2 and RAGE	Recurrent spontaneous abortion
	H3K4me3 and H3K9ac downgrades	Regulated by Gal-2 and PPARγ	Pre-eclampsia
	Down-regulation of HDAC 2 expression and activity in monocytes/macrophages	——	Gestational diabetes
	Up-regulation of miR-153-3p	Mediated inhibition of trophoblast function by the IDO/STAT3 pathway	Unexplained recurrent miscarriage

Xueqin Ma et al, Frontiers in Immunology, 2025;10

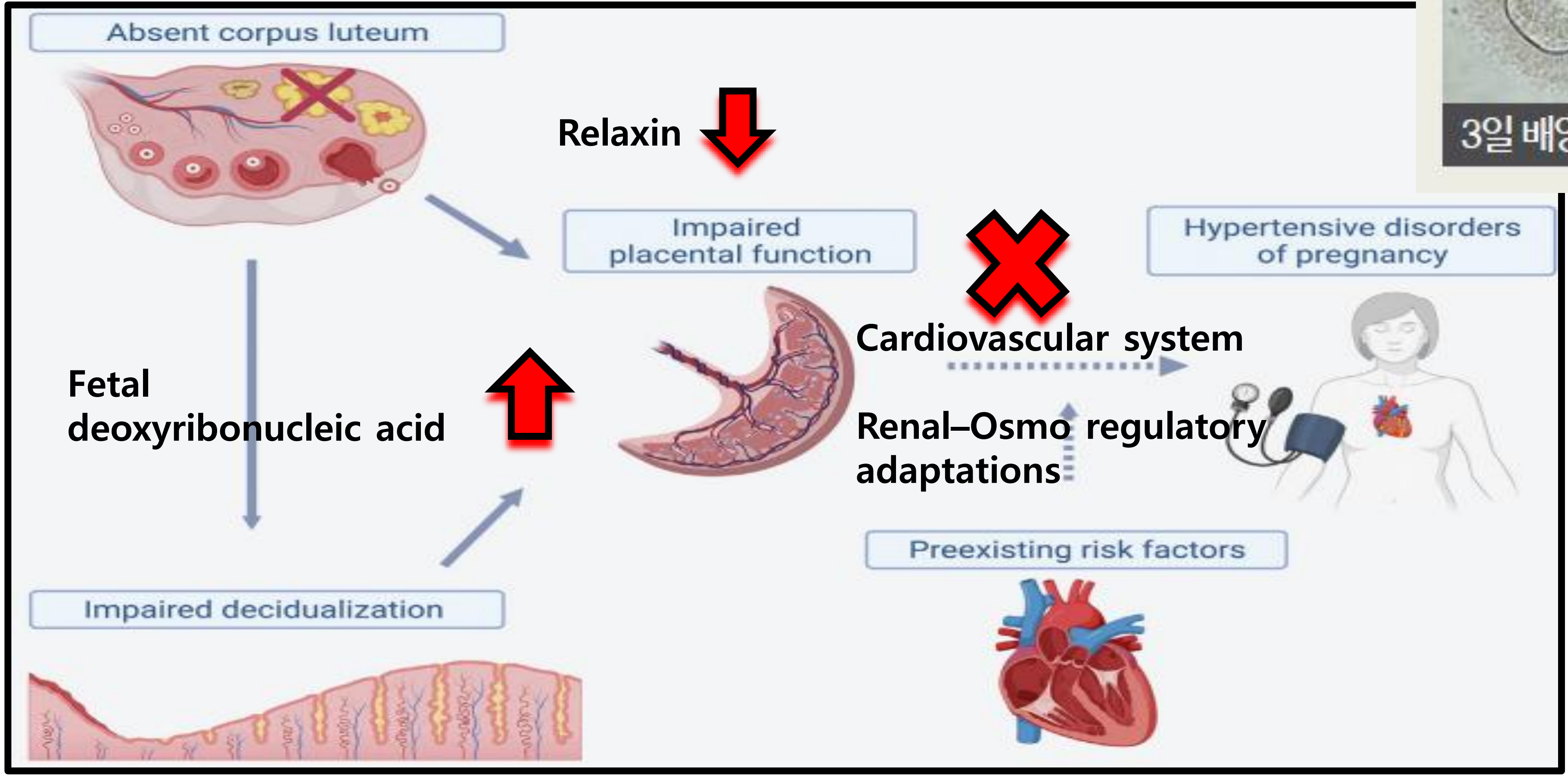
Epigenetic regulation and placenta related pregnancy complication

Non-coding RNA	Up-regulation of miR-185-5p	Reduced VEGF Expression and Angiogenesis in dNK Cells	Recurrent spontaneous abortion
	Up-regulation of miR-515-5P	Reducing HDAC2 levels harms trophoblast cell biological behavior	Recurrent spontaneous abortion
	Up-regulation of miR-23a	Inhibition of HDAC2 and activation of NF-κB impede trophoblast migration and invasion and promote apoptosis	Pre-eclampsia
	Down-regulation of miR-199a-5p	Reduction of VEGFA expression to inhibit trophoblast invasion	Pre-eclampsia
RNA methylation	Upregulation of METTL3	Increase the level of m6A RNA methylation and hnRNPC1/C2 expression in trophoblasts	Pre-eclampsia
	Upregulation of METTL14	Increase the level of m6A RNA methylation and FOXO3a expression and inhibite trophoblast proliferation and invasion	Pre-eclampsia
	Upregulation of RBM15	RBM15 suppresses hepatic insulin sensitivity of offspring of gestational diabetes mellitus mice via m6A-mediated regulation of CLDN4	Gestational diabetes Mellitus
	Down-regulation of m6A methylation level	ALKBH5 regulates CYR61 mRNA stability through m6A dependent mechanism and affects trophoblast function	Recurrent spontaneous abortion
	Down-regulation of METTL3	METTL3 mediated ZBTB4 m6A RNA methylation modification inhibits trophoblast invasion ability	Recurrent spontaneous abortion

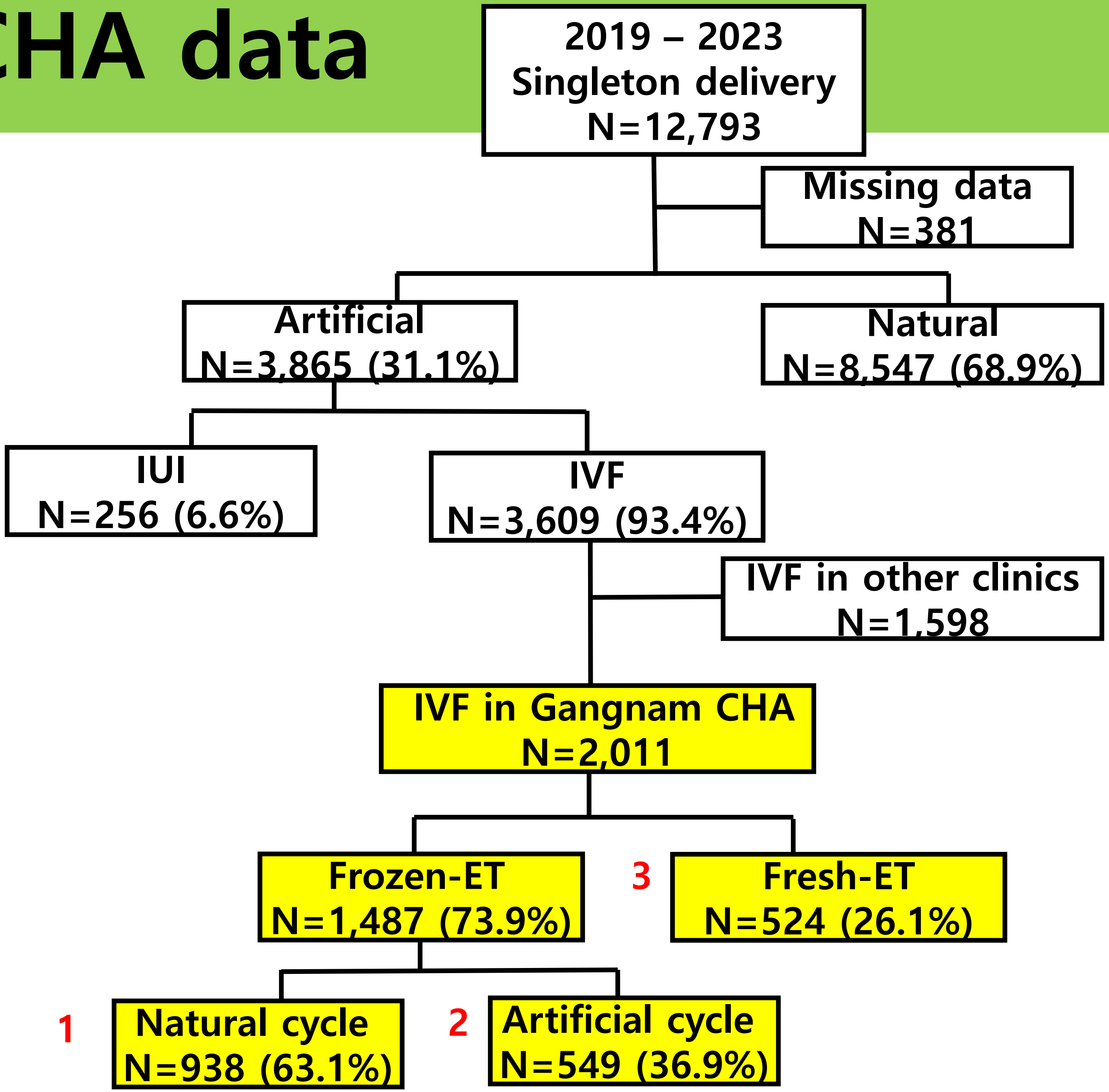
Xueqin Ma et al, Frontiers in Immunology, 2025;10

Epigenetic regulation and placenta related pregnancy complication

- **Proposed mechanism for preeclampsia risk following artificial frozen embryo transfer cycles**



Gangnam CHA data



Gangnam CHA data

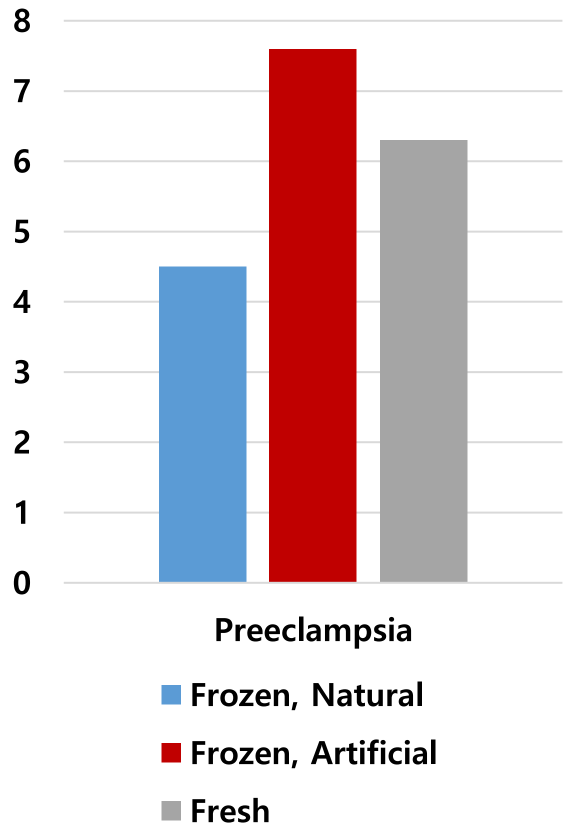
- **Obstetric outcomes**

	Frozen-ET, Natural N=938	Frozen-ET, Artificial N=549	Fresh-ET N=524	*P*
GA at delivery (weeks)	37.7±3.6	37.8±2.8	37.5±3.7	0.883
Birth weight (g)	3141.6 ±544.7	3186.8 ±543.6	3084.4 ±541.5	0.109
Preterm birth, n (%)	81 (8.6)	57 (10.4)	60 (11.4)	0.196
Small for gestational age, n (%)	56 (6.0)	33 (6.0)	36 (6.9)	0.770
Gestational hypertension, n (%)	39 (4.1)	27 (4.9)	24 (4.6)	0.784
Preeclampsia, n (%)	42 (4.5)	42 (7.6)	33 (6.3)	0.036
Gestational diabetes mellitus, n (%)	173 (18.4)	95 (17.3)	104 (19.8)	0.562
Placenta previa, n (%)	70 (7.5)	53 (9.6)	54 (10.3)	0.131

Gangnam CHA data

- **Obstetric outcomes in frozen embryo transfer, artificial cycle**

	Relative risk for Frozen-ET, Artificial vs. other two protocols (95% CI)	*P*
Preterm birth	1.172 (0.984-1.396)	0.075
Small for gestational age	1.073 (0.864-1.333)	0.522
Gestational hypertension	1.061 (0.824-1.367)	0.646
Preeclampsia	1.216 (0.975-1.517)	0.083
Gestational diabetes mellitus	1.037 (0.906-1.188)	0.599
Placenta previa	1.199 (0.998-1.440)	0.052



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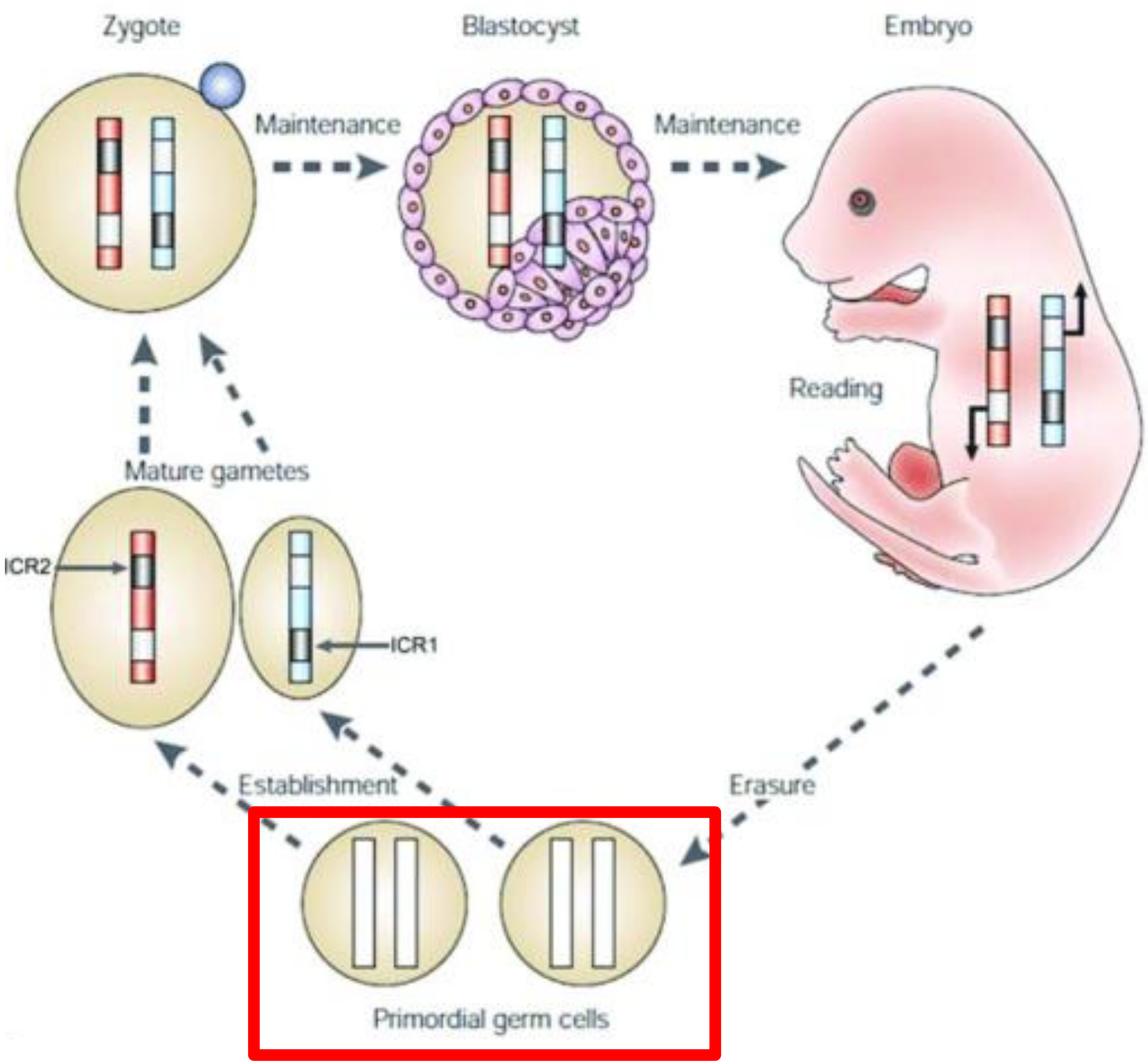
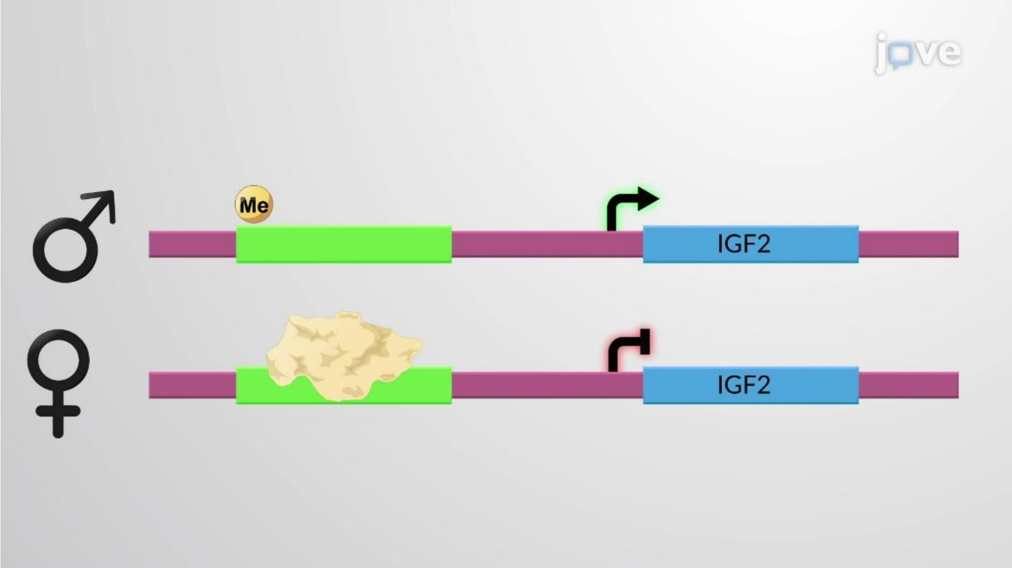
Imprinting disorder





Pinborg et al, AOGS, 2016: 95

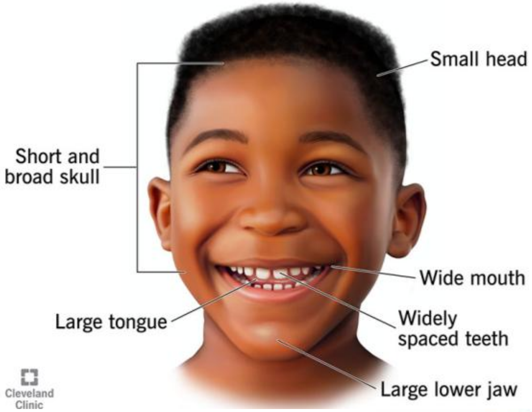
Imprinting disorder

Table 1. Characterization of four human imprinting disorders.

Disorder	Genetic errors	Prevalence	Clinical features
Beckwith–Wiedemann	Chromosome 11p15.5 • Paternal UPD (20–25%) • Methylation defects (50–60%) • Maternal mutations (5%) • Translocation/inversion, duplication and micro-deletions (each <1%) • Unknown (10–20%)	1:15 000	Fetal macrosomia, macroglossia, renal anomalies, midline abdominal wall defects, earlobe creases or ear pits, facial nevus flammeus, neonatal hypoglycemia, and increased risk of childhood cancer
Silver–Russell	Genetically heterogeneous condition, represents a phenotype rather than a specific disorder Chromosome 7-related and 11p15.5 • Maternal UPD (chromosome 7; 5–10%) • Maternal UPD (chromosome 11; <1%) • Methylation defect (chromosome 11; 30–50%) • Duplication (chromosome 7 or 11; rare/unknown)	1: 392 000–1:3000	Intrauterine or postnatal growth retardation, learning disabilities, relative macrocephaly and small triangular face, limb length asymmetry and a variety of minor malformations
Angelman	Chromosome 15q11-13 • Paternal UPD (2–7%) • Maternal deletion (70–75%) • Methylation defect (2–5%) • Point mutations (10%) • Translocation, inversion (<1%) • 10% unknown	1: 20 000–1:12 000	Severe mental retardation, speech impairment, abnormal EEG and seizures, often microcephaly, subtle dysmorphic facial features, jerky movements and hand flapping and happy disposition
Prader–Willi	Chromosome 15q11-13 • Maternal UPD (25–35%) • Paternal de novo deletion (65–75%) • Methylation defect (1–5%) • Micro-deletions and translocations (rare/unknown)	1:30 000–1:10 000	Neurodevelopmental disorder with cognitive disabilities, characteristic facial appearance (narrow temples, elongated face, thin upper lip, prominent nose), low muscle tone, short stature and hyperphagia

Pinborg et al, AOGS, 2016: 95

UPD, uniparental disomy (e.g. two copies of a chromosome, or of part of a chromosome, from one parent and no copy from the other parent).



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Long-term childhood outcome

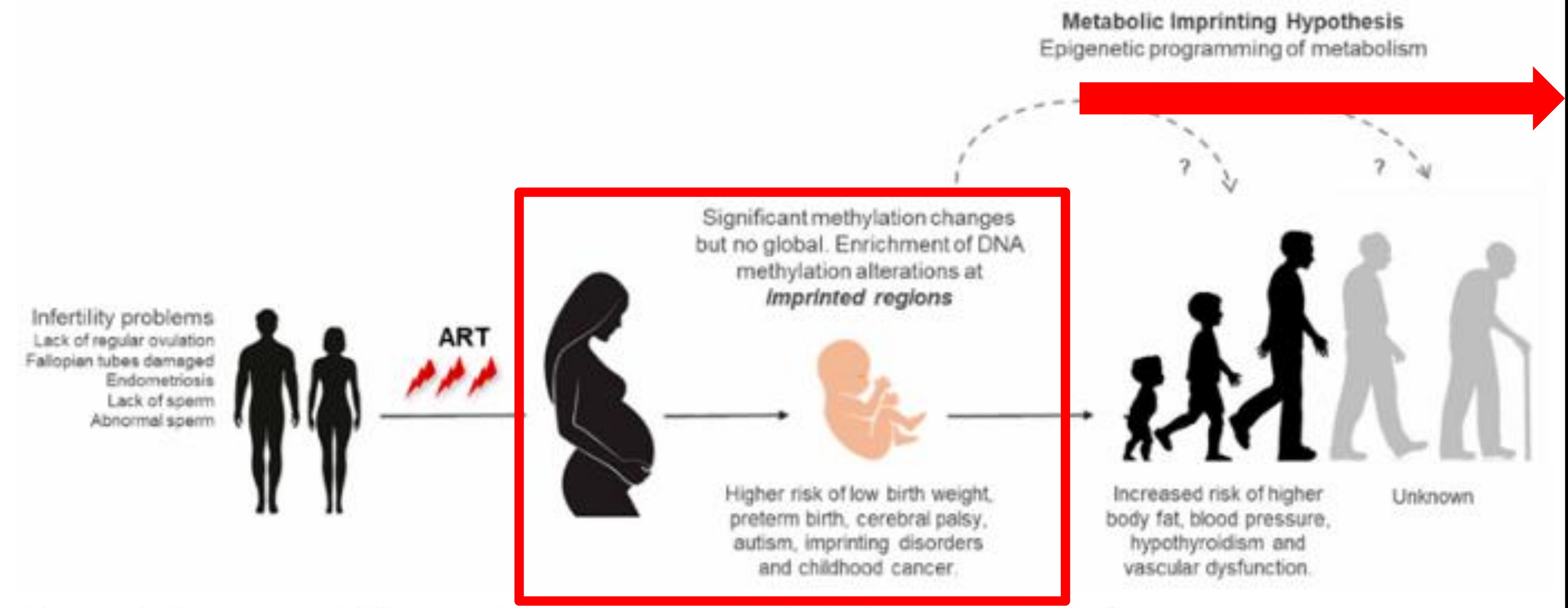


Figure 3. Diagram of the possible impact of ART in long-term health.

Eguzkine Ochoa, Life 2021; 11, 728

Long-term Childhood outcome

Table 1 Studies reporting long-term health outcomes of ART offspring

Disorders	References	Research design and study population	Key findings
Malignancies	Spaan M et. al. [14]	Retrospective cohort study, including 24,268 ART offspring; 13,761 SC offspring; and 9660 naturally conceived offspring of subfertile parents, followed for a mean of 21 years	No significant increase in overall cancer risk in ART offspring, neither compared with naturally conceived children from subfertile women (HR: 1.00, 95% CI: 0.72–1.38) nor compared with the general population (SI, 1.11; 95% CI, 0.90–1.36)
	Spector LG et. al. [15]	Retrospective cohort study, including 146,875 IVF singletons and 2,194,854 SC singletons was followed with a mean (SD) follow-up of 4.5 (2.5) years and 4.7 (2.5) years	An increased risk of hepatic tumors in the IVF group (HR, 2.46; 95% CI, 1.29–4.70); other cancers did not differ
	Hargreave M et. al. [16]	Retrospective cohort study, including 1,085,172 children born in Denmark between 1996 and 2012 and followed up to 2015, with a mean follow-up of 11.3 years	FET was associated with an elevated risk of childhood cancer compared with children born to fertile women (HR, 2.43; 95% CI, [illegible])
Asthma	Wijs LA et. al. [17]	Meta-analysis, including 14 high-quality studies (Newcastle–Ottawa scale ≥ 7)	An increased risk of asthma in ART offspring (RR, 1.28; 95% CI, 1.08–1.51)
	Kallen B et. al. [18]	Registry study of 2,628,728 children born from 1982 to 2007 in Sweden, including 31,918 ART offspring	An increased risk for asthma in IVF offspring (aOR, 1.28; 95% CI, 1.23–1.34)
Obesity	Norrman E et. al. [19]	Retrospective cohort study, including 122,429 ART offspring and 7,574,685 SC offspring, with a mean (SD) follow-up of 8.6 (6.2) years for ART offspring and 14.0 (8.6) years for SC offspring	An increased risk of obesity in ART offspring (HR, 1.14; 95% CI, 1.06–1.23; $p = 0.001$)
Diabetes			
Type 2 diabetes	Norrman E et. al. [19]	Retrospective cohort study, including 122,429 ART offspring and 7,574,685 SC offspring, with a mean (SD) follow-up of 8.6 (6.2) years for ART offspring and 14.0 (8.6) years for SC offspring	No significant increase in the risk of type 2 diabetes in ART offspring (HR, 1.31; 95% CI, 0.82–2.09; $p = 0.25$)
Type 1 diabetes	Norrman E et. al. [20]	Retrospective cohort study, including all 3,138,540 children born in Sweden between 1985 and 2015 with a mean (SD) follow-up of 9.7 (6.4) years for ART children and 16.3 (9.2) years for SC children	An association between FET and type 1 diabetes (aHR, 1.52; 95% CI, 1.08–2.14 frozen versus fresh); no association between ART and type 1 diabetes (aHR, 1.07; 95% CI, 0.93–1.23)
Cardiovascular diseases	Norrman E et. al. [19]	Retrospective cohort study, including 122,429 ART offspring and 7,574,685 SC offspring, with a mean (SD) follow-up of 8.6 (6.2) years for ART offspring and 14.0 (8.6) years for SC offspring	No significant increase of cardiovascular disease in ART offspring (aHR, 1.02; 95% CI, 0.86–1.22; $p = 0.80$)
Thyroid disorders	Sakka SD et. al. [21]	Cohort study, including 106 IVF offspring and 68 SC controls, aged 4–14 years	A significant elevation of serum TSH ($p = 0.046$) in IVF offspring
Neurodevelopmental disorders			
ASD	Davidovitch M et. al [22]	National registry study, including 110,093 males in Israel (born, 1999–2008; ASD, 975, 0.9%)	An association between progesterone hormone treatment and increased risk of ASD (RR, 1.51; 95% CI, 1.22–1.86); no increased risk of ASD for IVF overall
ADHD	Wang C et. al. [23]	National registry study, including 2.4 million children born in Sweden 1986–2012	No significant increase in the risk of ADHD (aOR, 1.03; 95% CI, 0.96–1.10)
ID	Sandin S et. al. [24]	Population-based, prospective cohort study, of 2.5 million births between 1982 and 2007, including thirty 959 (1.2%) IVF offspring, with a follow-up mean of 10 (SD, 6) years	No significant increase in risk of ID among ART offspring (RR, 1.01; 95% CI, 0.83–1.24); ICSI is associated with a higher risk of ID compared with IVF (RR, 1.51; 95% CI, 1.10–2.09)

Siwei Zhang et al, JARG, 2024;41

Further…

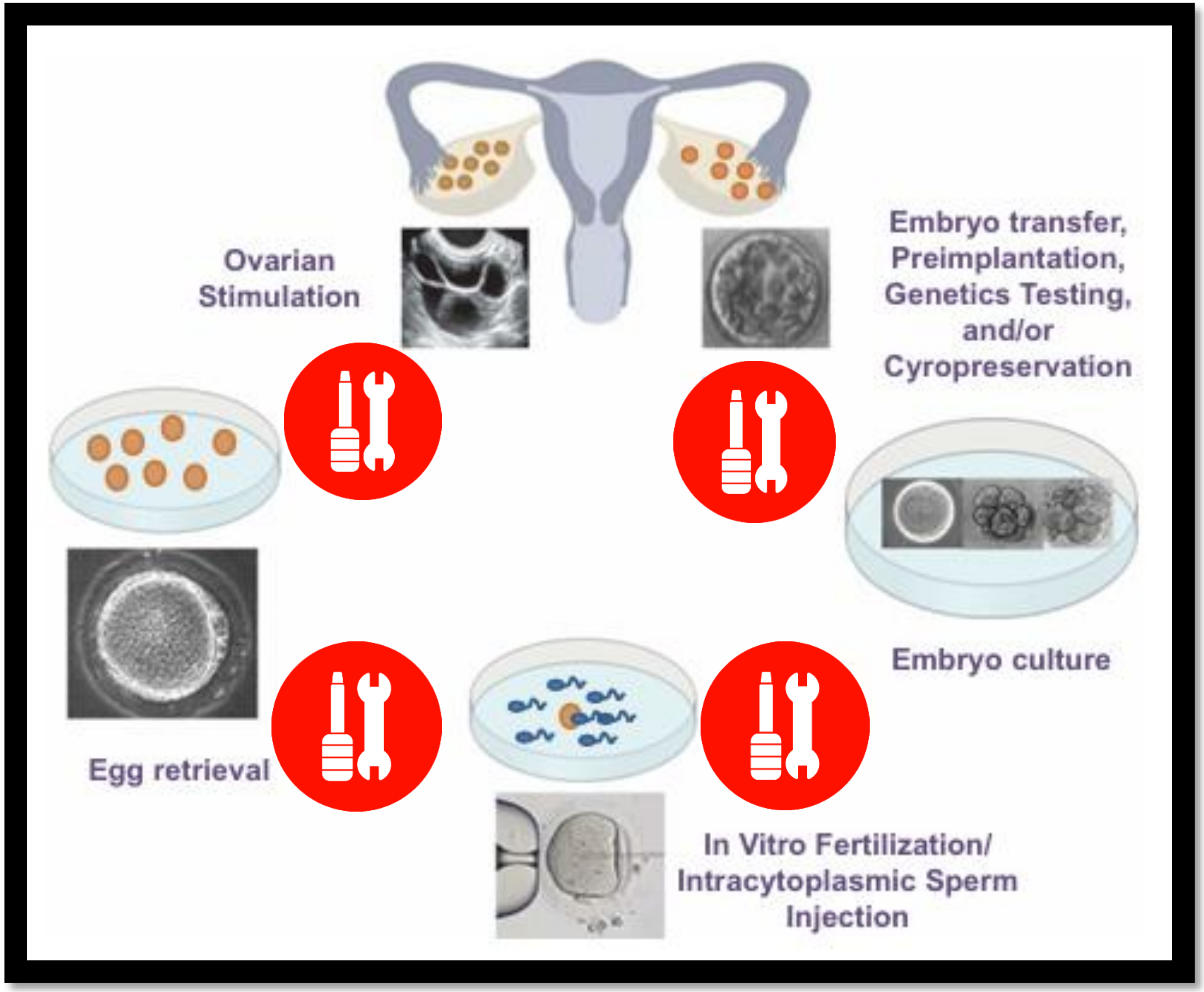
Table 1. Studies using genome-wide approaches to investigate epigenetic differences due to ART

Study	Number of samples	Technical Approach	Genes/regions	Tissue	Outcome	Reference
Katari *et al.*, 2009	10 IVF and 13 naturally conceived	Illumina GoldenGate array	1536 CpG sites in 736 genes	Cord blood, Placenta	Lower mean methylation of IVF at CpG sites in placenta Higher mean methylation of IVF at CpG sites in cord blood	[10]
Camprubi *et al.*, 2013	73 ART and 121 naturally conceived	Illumina GoldenGate methylation array, Bisulfite PCR, Pyrosequencing	25 imprinted DMRs	Cord blood, Placenta	No significant differences in methylation between the two groups	[73]
Castillo-Fernandez *et al.*, 2017	47 IVF and 60 naturally conceived twins	MeDIP-seq	9,592,803 bins (cord blood), 9,285,089 bins (CBMC)	Cord blood, CBMC	Significant methylation differences in *TNP1*, *C9orf3* and *H19/IGF2* regions	[91]
Melamed *et al.*, 2015	10 ART and 8 naturally conceived	Illumina Infinium Human Methylation27K array	27,578 CpGs	Cord blood	733 (2.7%) CpGs significantly different between the two groups. Hypomethylation of 63.2% of the significantly differentially methylated sites in ART	[86]
Hajj *et al.*, 2017	34 IVF, 89 ICSI and 53 naturally conceived	Illumina Infinium Human Methylation450K BeadChip assay, Pyrosequencing	428,227 CpGs, *ATG4C*, *BAZ2B*, *SNORD114-9*	Cord blood	Significant methylation differences in 4730 (0.11%) CpGs between the groups. Hypermethylation of *ATG4C* in ICSI and IVF. Significant methylation difference in *SNORD114-9* CpG between ICSI and controls.	[88]
Estill *et al.*, 2016	94 ART (IUI/ICSI) and 43 naturally conceived	Illumina Infinium Human Methylation450K BeadChip assay	394,454 probes	Fetal blood spots	Significant difference in methylation profiles of the groups.	[87]
Litzky *et al.*, 2017	18 IVF, 79 subfertile and 158 naturally conceived	Illumina Infinium Human Methylation450K BeadChip assay	42 genes (1730 CpGs)	Placenta	No significant relationship between CpG methylation and fertility status.	[89]
Choufani *et al.*, 2018	44 ART and 44 naturally conceived	Illumina Infinium Human Methylation450K BeadChip assay, Pyrosequencing	414,320 probes, *LINE-1*	Placenta	No statistically significant methylation differences between the two groups.	[90]
Xu *et al.*, 2017	5 IVF, 5 NIFT and 5 naturally conceived	Illumina Infinium Human Methylation450K BeadChip assay	485,148 probes	CVS (first trimester)	No significant differences in overall methylation between the groups.	[95]

MS-PCR: Methylation specific PCR; MSED-qPCR: Methylation sensitive enzymatic digestion associated with real-time PCR; SeQMA: Sequence – based quantitative methylation analysis; COBRA: combined bisulfite PCR restriction analysis; SNuPe: single nucleotide primer extension; IP RP HPLC: ion pair reverse phase high performance liquid chromatography; MS-SNuPe: Methylation sensitive single nucleotide primer extension; COH: Controlled ovarian hyperstimulation; MSQ-PCR: Methylation sensitive quantitative polymerase chain reaction; MeDIP: Methylated DNA immunoprecipitation; CBMC: Cord blood mononuclear cells; LUMA: Luminometric methylation assay; Bio-COBRA: Combined bisulfite restriction analysis coupled with Agilent 2200 TapeStation platform; NIFT: non-IVF fertility treatments

Sneha Mani et al, Epigenetics, 2020:15

Further...



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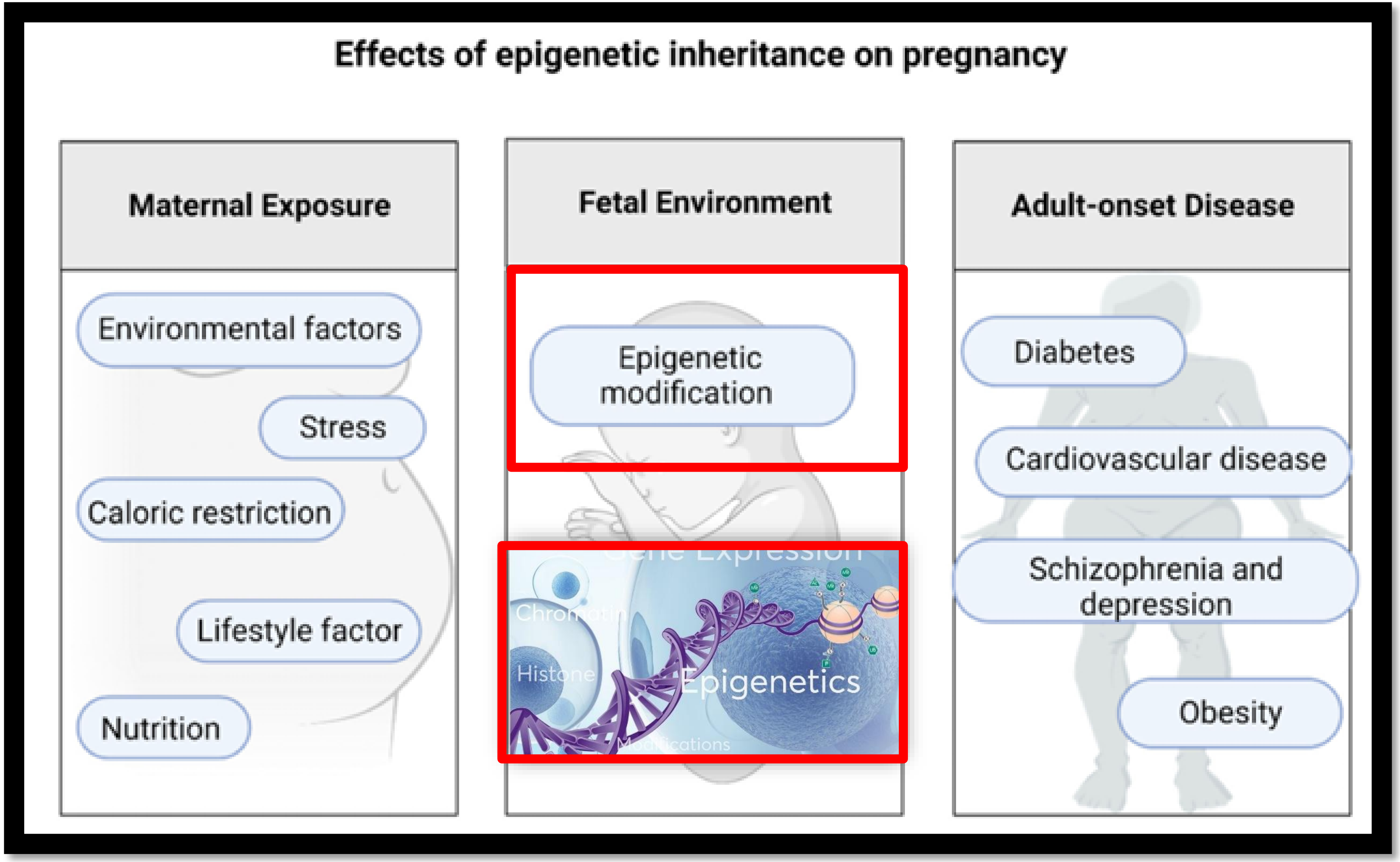
- **Long-term childhood outcome**

Summary


Effects of epigenetic inheritance on pregnancy
Maternal Exposure
Environmental factors
Stress
Caloric restriction
Lifestyle factor
Nutrition
Fetal Environment
Epigenetic modification
Chromatin
Histone
Epigenetics
Adult-onset Disease
Diabetes
Cardiovascular disease
Schizophrenia and depression
Obesity