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REVIEW

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Epigenetic mechanisms in maternal-fetal crosstalk: inter- and trans-generational inheritance

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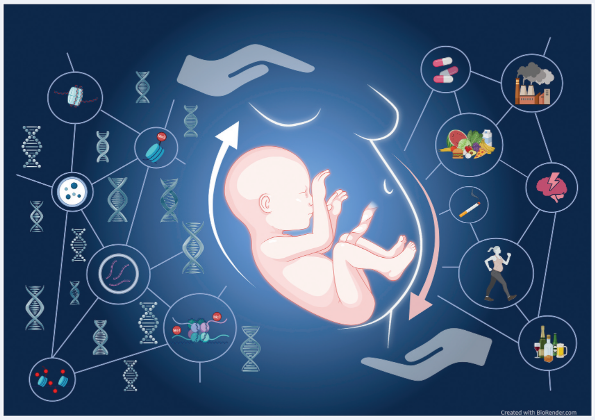
ABSTRACT

Epigenetics as a composite language capable of translating signals from the environment into heritable DNA modifications is revolutionizing the approach to biology and medicine. In the field of human reproductive physiology, many epigenetic marks have been decoded in the different stages of development, from gametogenesis to embryo development, pregnancy and its maintenance. These epigenetics marks are mainly ascribable to DNA methylation/demethylation processes, histone modifications, and non-coding RNAs mediated signals. Epigenetic modifiers such as DNA methyltransferases (DNMTs) and Ten-eleven translocation (TET) enzymes are involved in gene expression regulation by modifying the DNA methylation landscape, the first and best characterized epigenetic mark. In the context of human reproduction, epigenetics signals depict the mother-fetus crosstalk in which the maternal milieu dialogs with the growing embryo to establish an appropriate placental-fetal interface for a successful pregnancy. We here outline key features of the epigenetic crosstalk during the early embryo development and pregnancy establishment. The potential of inter- and trans-generational inheritance of specific epigenetic traits in response to environmental hints is also discussed. Finally, we move forward into future translational developments of epigenetics by discussing the potential use of epigenetic-based treatments (epidrugs) as upcoming therapeutic approaches in advanced personalized reproductive medicine.

PLAIN LANGUAGE SUMMARY

The genetic information we need to develop, grow-up, and live is carried by the DNA, a molecule present in all cells of living beings that contains, as a sort of instruction book, the letters that codify for the different proteins we need. Each gene can be imagined as a specific paragraph of the immense DNA book providing the specific information for a specific protein. Changes in letters or in the sequence of letters determine modifications of the instructions carried by the DNA. In response to environmental factors (such as physical or chemical agents) and personal habits (such as diet, physical activity), DNA may undergo a different type of modifications called "epigenetic modifications." Using the same metaphor of the book, we can think of epigenetic modifications to the DNA sequence as spaces, punctuation, and accents on words: they do not change the sequence of letters that make up the word, but rather the format of the text. Epigenetic characteristics change throughout life and can be passed from a dividing cell to its daughter cells, and from parents to offspring influencing development, health, and disease risk. The study of epigenetic modifications will allow the identification of appropriate behaviors and the development of pharmacological treatments.



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Article highlights

- Epigenetic mechanisms link environmental variations and gene regulation
- Epigenetic dynamics are crucial during development and after birth in determining health and disease
- The maternal environment and the developing embryo communicate using an epigenetic-based language
- The transmission of epigenetic marks may result into inter- and trans-generational inheritance
- Epigenetic-based treatments (Epidrugs) may represent a breakthrough in human reproduction

1. Introduction

Epigenetics plays a crucial role during embryo development in driving cell lineage commitment, in establishing cellular identity and tissue specification, with after birth effects in determining individual health. The term Epigenetics was proposed for the first time by Conrad Hal Waddington in the 1940s as complex landscape of interactions between the environment and the genome that lead to the production of phenotype [1,2]. Only more recently, in 2008, a new definition has been proposed: "*a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence.*" Since then, the relevance of epigenetics has massively increased, resulting into a field that is now changing our comprehension of gene regulation, heredity, and modern biomedicine [3,4]. As a paradigm of the "revolution" that epigenetics is driving, epigenetics relies on genome modifications not resulting in altered DNA sequence, able to give rise to alternative gene activities and functions that may be transmissible and durable, outlining a complex biological context that is developing over time [5].

Normally, the epigenome undergoes continuous remodeling in which epigenetic marks are dynamically established and erased in response to exogenous or endogenous stimuli overall shaping genome architecture [6]. The epigenetic marks are the result of several molecular paths, among these an important and well-studied mechanism is the cytosine methylation, which plays important roles such as transcriptional regulation, through silencing and tuning gene promoters, genomic imprinting, and individual stress response and coping [7,8]. Other epigenetic modifications that allow the regulation of gene expression and reprogramming, also during gametogenesis and embryo development, include histone post-translational modifications, which makes DNA accessible or not to transcriptional factors [9–11], and the regulation of gene activity by non-coding RNAs (ncRNAs) [6,12].

DNA methylation represents a key epigenetic control mechanism in mammalian development, with a dynamic that shapes two fundamental phases: primordial germ cell (PGCs) specification and zygote progression upon fertilization. PGCs are the embryonic precursors of mature gametes that originate in the epiblast in response to bone morphogenic protein signals [13–15]. The molecular and epigenetic changes occurring in the developing embryo are driven by extra-embryonic tissues and microenvironmental triggers, able to induce a distinctive genetic and epigenetic regulatory landscape for PGCs specification [15]. In humans, PGCs proliferate and maturate during their migration to the developing gonads approximately at 4 weeks of development, resulting into precursors able to differentiate into functional mature gametes at puberty. Notably, to erase parental epigenetic memory, a specific PGCs epigenetic reprogramming starts immediately following specification and continues up to PGCs arrival in the developing gonads [16].

We are referring to a complex dynamic set of events that includes the extensive erasure of global DNA methylation marks (leading to less than 5% residual DNA methylation), in concomitance with independent DNA methylations and histone modifications to preserve crucial transcriptional control. This extensive DNA demethylation leads to the comprehensive removal of most parental somatic signatures and those epigenetic marks in PGCs necessary for totipotency reprogramming and subsequent embryonic development [17,18]. After erasure, in later stages of development (before birth in male and after birth in female), de novo methylation will ensure during the individual gametogenesis a sex-specific reestablishment of epigenetic signatures, including the methylation marks at imprinted loci [19] (Figure 1). Moreover, histone post-translational modifications represent additional epigenetic-based key mechanisms of gene regulation. Histones molecular changes, including acetylation, methylation, phosphorylation, and ubiquitination, overall result in chromatin structural organization allowing the switch between hetero- and euchromatin architecture [20], also influenced by external environmental triggers. Among the identified histone modifications we can mention histone H3 trimethylation at lysine 4 and 27, and histone H3 acetylation at lysine 9 (see review [21]), contributing to functional states such as open or closed chromatin, active or repressed genes, poised or operative enhancers [22]. Of note, the majority of gene body CpG islands results methylated (≥55% methylation), and this state is specifically associated with enrichment of trimethylated histone H3 lysine 36 (H3K36me3), and absence of trimethylated histone H3 lysine 4 (H3K4me3) and trimethylated histone H3 lysine 27 (H3K27me3), suggesting a close link between gene body methylation and histone marks [23,24].

The ncRNAs system is a main epigenetic regulatory complex of different ncRNA clusters classified into long ncRNAs (lncRNAs, longer than 200 nucleotides) and short ncRNAs (sncRNAs, less than 200 nucleotides) including miRNAs, siRNAs, piRNAs, tsRNAs (tRNA derived sncRNAs), and rsRNAs (rRNA derived sncRNAs). A growing body of evidence highlights the role of ncRNAs in gene expression control [12], in the epigenetics of embryo development and in maternal-fetal crosstalk, including the establishment and maintenance of imprinted genes expression [25]. The role of ncRNAs in the regulation of PGCs epigenetic landscape has been mainly investigated in animal models that allowed the characterization of selected miRNAs (e.g., let-7 miRNA family, miR-17/92, miR-181, miR-290 cluster), piRNAs, and endo-siRNAs, while in humans (excluding some piRNAs) this is a field still in its infancy [26].

During pregnancy, fetal membranes and decidua undergo epigenetic modifications in which chromatin changes are involved in tissue gene regulation [22]. Therefore, embryo development and pregnancy progression underlie the regulatory effect of specific pathways that are tightly regulated by environmental-epigenetic signals. In this line, the mutual communication between the developing inner cell mass of Embryonic Stem Cells (ESC), the trophectoderm and the endometrial epithelium is mediated by a network of epigenetic molecular factors able to trigger the different phases of the reproductive process. In terms of delivery systems involved in maternal-fetus communication, Extracellular Vesicles (EVs), secreted by both embryonic and maternal cells, have recently attracted attention as epigenomic modifiers [27], with a role in pregnancy and a potential therapeutic target for infertility treatments.

The influence of maternal environment on offspring development and phenotype is overall referred as the "maternal effect," in which the intrauterine milieu may result into a non-genetic transfer of marks and signatures to the developing embryo and across generations.

The present review is focused on the role of epigenetic mother-fetus crosstalk during early embryonic development and pregnancy establishment, highlighting the key mediators and modifications in controlling the specific developmental phases. Finally, we discuss on how environmental hints may result into epigenetic inter- or trans-generational inheritance, and how epigenetic treatments (epidrugs) may be considered upcoming therapeutic approaches in the field of human reproductive medicine.

1.1. Zygote-genome-activation (ZGA) and Blastocyst Dormancy

Upon fertilization, the primary event of early embryonic development entails the transition from maternally derived gene products (i.e., maternal transcripts) to zygote-genome-activation (ZGA). These are two extremely coordinated cytoplasmic and nuclear reprogramming processes overall referred to as the maternal-to-zygotic transition (MZT) [28]. In humans, oocyte-derived maternal mRNA decay occurs during MZT reaching the major effect at the 8-cells stage, and it has been demonstrated that subgroups of maternal transcripts are sequentially removed by maternal (M)- and zygotic (Z)-decay pathways before and after ZGA [29]. Timing of clearance may depend on the specific mRNA and there might be a time-

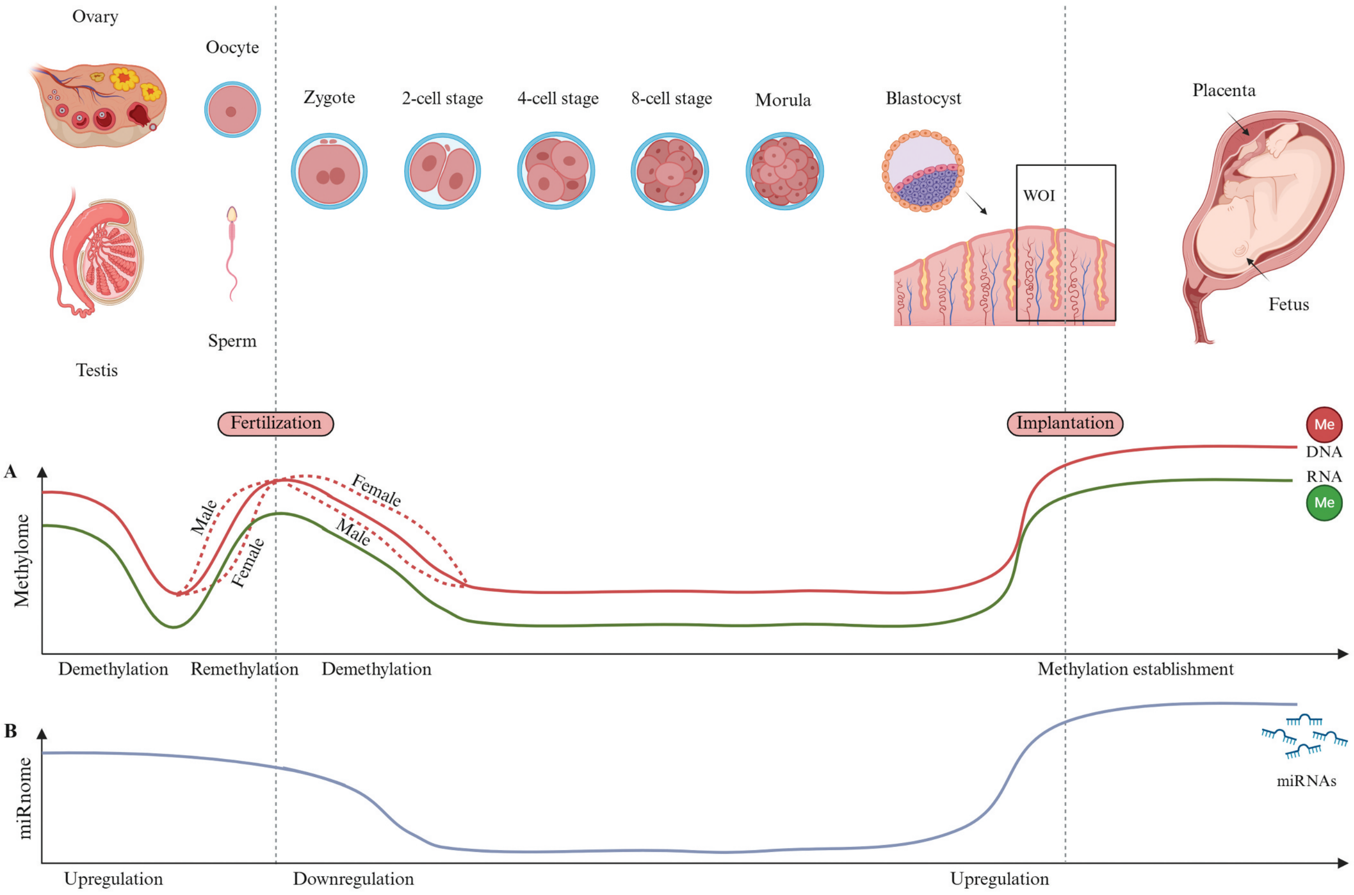

Figure 1. Methylome and miRnome dynamics during human development. (A) PGCs epigenetic reprogramming process lasts up to PGCs arrival in the developing gonads. The process comprises a global erasure of DNA and RNA methylation (red and green line, respectively). This extensive demethylation process leads to the removal of epigenetic marks in PGCs necessary for reprogramming. A remethylation occurs at the time of sperm and oocyte maturation resulting fully differentiated. After fertilization, a new wave of demethylation takes place. Specifically, DNA methylation is sex-differentiated (red dotted lines). DNA re- and de-methylation of the paternal hemigenome is rapid compared to the maternal hemigenome. Concomitant with blastocyst implantation and cell-lineage determination, an epigenetic re-patterning occurs with *de novo* DNA and RNA methylation to establish cell lineage-specific gene expression. (B) miRNome pattern changes immediately after the first embryo division, where 60% of miRNAs are downregulated in the 2-cell stage. At the time of implantation, the miRnome pattern is upregulated again to ensure pregnancy establishment and maintenance. Created in BioRender. Tisato, V. (2025) https://biorender.com/iip4nn0.

window in which maternal and zygotic transcripts synergize in driving embryo development.

At the time of fertilization, sperm and oocyte are fully differentiated cells characterized by specific and distinctive epigenetic marks and signatures. DNA methylation is consistently distributed in sperm genome reaching approximately 90% levels with the exception of definite CpG islands maintained hypomethylated (e.g., those localized to developmental loci such as HOX genes [30,31]), while mature oocytes show a lower global DNA methylation of about 50% [32,33]. In this context, to allow the development of the zygote into embryo, the terminally differentiated parental genomes undergo complete reprogramming to achieve a totipotent state. One of the main epigenetic modifications occurring is an extensive demethylation process to clean up parental epigenetic marks, except for imprinting control regions (ICR) and specific repetitive sequences that keep their methylation status. Differently from what observed in mouse developmental studies, the major genome-wide demethylation wave in humans is completed at the 2-cells stage [34,35]. Actually, the paternal genome is rapidly and actively demethylated by TET enzymes, while the maternal genome undergoes slower passive demethylation resulting from a dilution-effect during cell division in the absence of DNA methylation maintenance by DNMTs that may be unavailable or nonfunctional at this stage [36]. After fertilization the zygote genome is transcriptionally quiescent with a gradual and controlled transcriptional activation characterized by minor and major wave of transcription [37]. In humans, ZGA occurs by the eight-cells stage, though it has been reported that it already starts at the one-cell stage [38], first from paternal genome [39].

In the subsequent developmental stages, an epigenetic repatterning occurs with *de novo* DNA, RNA methylation and chromatin remodeling to establish cell lineage-specific gene expression [40,41]. The targeted reestablishment of DNA methylation patterns is primarily mediated by DNMT3A and DNMT3B, which selectively methylate CpG sites to activate gene regulatory networks governing the specification and maintenance of distinct embryonic lineages, including epiblast, primitive endoderm, and trophectoderm [42,43].

Maternally inherited epigenetic factors sustain the cleavage divisions of zygote over the first 72 hours post-fertilization before a major wave of embryonic genome activation occurs at the 4-cells stage [37]. After genome activation, human embryos undergo compaction to form first the morula and then the early and late blastocyst with a defined inner cell mass, ready for implantation [44]. Similar to chromatin remodeling, histone modifications are also highly dynamic during all stages of embryo development. From 2–4-cells stages, a gain in H3K4me3 levels and loss in H3K27me3 and H3K9me3 is observed [11,45,46]. In this context, sncRNAs are deeply involved in the transition from maternally provided factors into embryo genome activation. This process also accounts for a switch from mainly maternally derived oocytes-piRNAs to embryonic miRNAs expression in cleavage stage embryos coupled with an extensive process of miRNA modification (e.g., 3´ mono- and oligoadenylation) in the zygote [26,47]. Moreover, although less explored than in animal models, sncRNAs seem to contribute to cellular heterogeneity and to the establishment of embryonic lineages by regulating the pluripotency-network axis, as emerged from cell reprogramming models [48,49].

At the preimplantation stage, there may be an interruption of the ongoing developmental processes and undertakes the embryonic diapause, a period of suspension in which blastocyst implantation and development are delayed [50,51]. We are referring to a reversible phase of embryo development concerning over 130 species of mammals (from bears to mice and marsupials), in which the unplanted blastocyst can survive in the uterus in a dormant state [50,51]. Signals and molecular mechanisms involved in diapause establishing and maintenance have been deeply investigated in animal models, also with the aim to identify the embryo-maternal interactions aimed at having an healthy pregnancy [52,53]. Several factors and life-conditions may induce obligate or facultative blastocyst dormancy such as food availability (also depending on seasonal variations), changes in temperature, and day-light length that act in a species-specific manner. The dormant embryos show common peculiar features including low cell proliferation, low metabolic activity, and transcriptional repression, but they still retain the capability of resuming development when exposed to the appropriate signals/conditions [53]. Studies have revealed that maternal signals exert primary regulatory effects on diapause onset and exit and on their timing/duration [51]. In this line, it has been recently demonstrated that soluble factors of maternal origin may intervene in this process as oxytocin, that is able to coordinate the dynamic embryonic development with uterine progression through pregnancy [54].

The control of the embryo fate and diapause entry/exit have been correlated with several factors and mechanisms, including epigenetic processes and key molecular pathways. Multiple epigenetic modifications characterize embryonic development with key signatures that identify the different developmental stages. Regarding diapause, chromatin remodeling in dormant blastocysts results in a comprehensive reduction of activating epigenetic marks including histone-related modifications as H3K36me3, H3K4me3, and H4K5/8/16/12 acetylation, and in an overall increase in repressive histone modifications such as H3K27me3 [55,56]. These epigenetic marks change when dormant blastocysts exit dormancy and become activated with high correlation to gene expression [56,57]. In a recent work, it has been reported that N6-methyladenosine (m6A) RNA methylation by *Mettl3* is involved in this process by promoting global mRNA destabilization and transcription suppression by acting on the oncogene *N-Myc*, resulting in a key anti-diapause factor [58]. miRNAs are also differentially expressed in dormant or activated blastocysts. One of the most studied is the Lethal-7 (let-7) miRNA family, resulted upregulated in mouse dormant embryo compared to the reactivated ones [59]. Of note, Let-7 belongs to the cargo of the maternal extracellular vesicles (endometrial epithelial cells) and induces diapause by suppressing c-myc/mTORC1, and mTORC2 signaling pathways [56]. Moreover, it inhibits the expression of the ornithine decarboxylase1 enzyme involved into polyamines biosynthesis and reactivation of dormant embryos, resulting into one of the main factors of embryonic diapause [60]. Other miRNAs play a role in embryo dormancy [56], among these miRNA-199 and miRNA-181, that target the degradation of *EGR1* gene (highly expressed in diapause) and the glutamine transporters *Slc38a1/Slc38a2*, that are downregulated in diapause [61,62].

Embryo development during the preimplantation phase is very sensitive and crucially depends on the exposure to specific signals, and the role of different signaling pathways for successful embryonic progression has been recently reviewed [63]. In the context of diapause, mTOR plays a crucial role, and its inhibition induces reversible pause of blastocyst development preserving the pluripotent state [64].

In conclusion, animal studies revealed that a productive diapause induction, maintenance, and exit with reactivation and establishment of normal pregnancy needs a viable embryo, a receptive uterus and an efficient molecular interaction between the two. Whether these mechanisms may be conserved and maintained in humans is still a matter of discussion. Hypotheses have been advanced [65], and possible isolated cases of delayed implantation after in-vitro fertilization (IVF) have been reported [66], then, the mechanisms underlying the embryo-maternal crosstalk during diapause have been suggested as useful tools for a better understanding of human ART/IVF approaches. In these techniques, mother and embryo are separated until transfer and this requires a perfect timing and biological coordination that may take advantage from diapause insights [67]. In this line, starting from the evidence that mTOR pathway is a key regulator of diapause in mice, in a recent work Iyer and colleagues have reported new understanding on potential diapause-like dormant state in human [68]. In particular, by using a human stem cell-based model of the blastocyst (blastoids) and blastocyst-stage human pluripotent stem cells, they showed that the inhibition of mTOR leads to the maintenance of cellular identity and drives the onset of a reversible state of restricted proliferation and developmental progression [68]. This suggests that mTOR maintains its role in growth and development regulation of human blastocyst-stage-like cells. These findings are relevant for the comprehensive understanding of human embryogenesis and as potential translational tool in ART procedures. In particular, the modulation of mTOR pathway may be exploited to control and extend the time window at the pre-implantation stage and optimize the embryo-maternal molecular synchronization [68].

1.2. Embryo-endometrial crosstalk during the implantation process: insights on epigenetic signals

Endometrial receptivity plays a pivotal role in successful embryo implantation. Nevertheless, for a successful pregnancy there is the need for a competent blastocyst and appropriate microenvironment conditions, since a poor fetal-maternal interface may result in pregnancy complications or reproductive failure. At the time of blastocyst implantation during the apposition phase the reproductive microenvironment is shaped by both blastocyst-derived and maternal-derived factors. ESCs of the blastocyst secrete extracellular vesicles and nano-sized membrane enclosed vesicles (exosomes) carrying proteins and nucleic acids. Among these, cell surface receptors, signaling proteins, extracellular matrix proteins, mRNAs, miRNAs, and DNA are able to communicate with the trophoblast and activate signaling events including the trophoblast ability to migrate, promoting in turn blastocyst implantation [69]. These molecular mediators coordinate both endometrial receptivity and embryonic epigenetics, and prime epithelial endometrial cell receptivity increasing the adhesive capability of the endometrium [69,70]. Epithelial and stromal cells of endometrium produce and secrete several cytokines and growth factors, such as VEGF, G-CSF, LIF, and Preimplantation Factor (PIF) which regulate embryo growth and development during implantation [71–73]. Moreover, a pivotal role in the regulation of embryo development and trophoblast growth is played by immune cells lodged in the endometrial stroma and in the decidua (Table 1).

Of note, the CD56^{bright} cells derived by NK circulating cell population and Treg cells also play a fundamental role in promoting the immune acceptance of semi-allogenic embryo by maternal immune system, as well as the HLA-G proteins located on trophoblast cells [82,93,94]. In particular, G-CSF and GM-CSF seem to play a direct role in Treg recruitment in the decidua/trophoblast interface, as well as regulating embryo development [81,82]. In this context, the endometrial epithelial cells secrete nano-sized EVs containing proteins and miRNAs [95]. miRNAs are a key element of the communication systems established between the mother and developing embryo and they play a crucial role in regulating hESC self-renewal/pluripotency and embryo development, and some hESC-specific miRNA genes are organized in clusters that represent key regulatory networks conserved in mammals [96]. The evidence that zygote and mature oocyte have similar miRNA profiles suggests that many zygotic miRNAs are maternally inherited [97]. However, miRNome pattern changes immediately after the first embryo division, where 60% of miRNAs are downregulated at the 2-cells stage [47,97]. Moreover, miRNAs are secreted into the extracellular environment by the embryo during the entire free-living stage of development from the 1-cell zygote to blastocyst to ensure cell-to-cell communication for self-organization process of embryonic development [98] (Figure 1). These miRNAs have been demonstrated to circulate in the body in proper packing of EVs required for stable transport [99]. Recent studies of EVs-miRNA profiling reveal specific miRNAs associated with embryo competence (Table 2).

The precision and the timing of such epigenetic processes are pivotal for avoiding developmental defects or embryo death [110,111]. Around 5–6 days post-fertilization, late blastocyst attaches to and invades the endometrial lining of the uterus. This process, known as implantation, marks the beginning of a complex dialogue between the embryo and the mother, an early milestone for pregnancy establishment and maintenance [112]. Implantation necessitates of a finely tuned synchrony between embryo and endometrium [113]. This synchronization occurs during a temporally restricted period (6–12 days post fertilization) known as "window of implantation" (WOI) and ovarian estrogens and progesterone primarily drive this sensitive stage [113,114].

Table 1. Decidua key factors involved in embryo development.

Class	Factor	Role in embryo development	Ref.
Cytokines and Growth Factors	IL-6	Support of trophoblast invasion and adhesion	[74]
	IL-10	Support of trophoblast functions and angiogenesis Promotes maternal-fetal crosstalk	[75,76]
	IL-8	Support of trophoblast invasion and spiral artery transformation	[77,78]
	TNF-α	Support of trophoblast invasion and interactions with maternal immune cells	[79]
	VEGF	Angiogenesis promotion	[80]
	G-CSF	Promote trophoblast invasion and Treg recruitment	[81]
	GM-CSF	Promote embryo growth and immunomodulation	[82]
Nutrient transporters	GLUT-1	Provides glucose to the developing embryo	[83]
	LAT-1	Ensures supply of essential amino acids to the embryo	[84]
Hormones	Progesterone	Regulates trophoblast invasion Creates a receptive environment for the embryo	[85]
	Estrogens	Promote cell growth and survival, modulate immune responses, influence placental development	[86]
	Prolactin	Supports stromal cell differentiation and immune modulation	[87]
miRNAs	let-7 family	Regulates decidualization process	[88]
	miR-290 cluster	Regulates decidualization process, support embryo implantation	[89]
	miR-21	Regulates inflammatory response, inhibit decidual cell apoptosis	[90]
	miR-16	Regulates angiogenesis	[91]
	miR-30c	Supports trophoblast development and endometrial receptivity	[92]

Table 2. Human EVs-derived miRNAs involved in embryo development.

Name	Target	Detection	Function	Ref.
miR-451	Wnt	Follicular fluid	Cell fate determination	[100,101]
miR-214	PTEN	Follicular fluid	Cell growth	[102,103]
miR-145	Abca1	Follicular fluid	Cholesterol levels	[102,104]
miR-454	EPHB4	Follicular fluid	Cell growth, apoptosis	[102,105]
miR-888	E-Cadherin	Follicular fluid	Cell growth	[102]
miR29a-3p	DNMT3A	Blastocoel fluid	Chromatin organization	[106]
miR-26a-5p	DNMT3B	Blastocoel fluid	Chromatin organization	[106]
miR-342-3p	HDAC9	Blastocoel fluid	Chromatin organization	[106]
miR-381	SRSF11	Blastocoel fluid	Stemness	[106,107]
miR-27a	HOXA	Serum	Cell differentiation	[108,109]

During WOI, the endometrium undergoes extensive structural and molecular remodeling. Adhesion molecules, cytokines, growth and transcriptional factors are just a few of endometrium-expressed molecules that collectively allow late blastocyst attachment and trophoblast invasion [115]. They are further supported by the immune tolerance establishment which avoids the rejection of the semi-allogeneic embryo [116]. Any change in the temporal alignment significantly compromises the success of embryo implantation [73], highlighting the need for fine regulated molecular pathways.

Emerging data ascribes to epigenetics an essential role in orchestrating early gestational events. Among epigenetic modifications, DNA methylation is involved in regulating implantation as well as endometrial transition to a receptive state [117,118].

Methylation modulates the expression of endometrial key genes involved in tissue remodeling and immune tolerance [119]. For instance, the hypomethylation of key transcription factors, among all HOXA10, HOXA11, and FOXO1, is critical for establishing a receptive endometrial environment, regulating the transcriptional activity of genes involved in cellular adhesion (e.g., E-cadherin, Integrin β3), immune modulation (e.g., LIF), and hormonal response capability [120].

RNA methylation has recently emerged as a noteworthy epigenetic modification regulating gene expression for endometrial receptivity and embryo implantation [41]. In particular, m6A dynamically controls mRNA stability, splicing, and translation, thereby affecting cell differentiation and tissue remodeling [121]. Actually, the dysregulation of m6A writers (e.g., METTL3) impairs blastocyst attachment and trophoblast invasion [41]. Shifting the focus to the endometrium, m6A allows the expression of genes involved in immune modulation and decidualization (transformation of endometrial stromal cells into highly specialized decidual cells) [41]. A recent study identified the METTL3–EED –YTHDC1 regulatory axis as a linker between m6A and H3K27me3 histone modification, affecting the accessibility of chromatin and the transcription of receptivity-related genes [122]. Moreover, m6A readers (e.g., FXR1) may also interact with DNA demethylation pathways, suggesting an aligned epigenetic landscape [123]. Overall, aberrant m6A signaling has been associated with recurrent implantation failure and reproductive disorders [124].

Likewise, increasing attention has been directed to the role of ncRNAs, which have emerged as master regulators of both embryonic competence and endometrial receptivity, thanks to their ability to fine-tune gene expression [125,126]. These molecules, including miRNAs, lncRNAs, and piRNAs, influence a wide range of biological pathways involved in the complex and synchronized process of embryo implantation, such as immune modulation and tolerance, embryo-endometrium crosstalk, and decidualization [127]. Table 3 reports the main ncRNAs whose up- or down-regulation controls endometrial receptivity and embryo implantation grouped depending on the molecular pathway to which the target genes belong.

For instance, a subset of ncRNAs results upregulated during the WOI and modulates the expression of cell adhesion molecules like E-cadherin and integrin β3 (e.g., miR-192-5p) [130]. Other ncRNAs, such as miR-125b, have been implicated in modulating LIF-STAT3 signaling and immune tolerance, both essential for a receptive environment [132].

Several ncRNAs target the HOX genes encoding for a family of transcription factors involved in the expression of key downstream effectors, including integrins, insulin-like growth factors and cytokines [132,137]. Although less studied, piRNAs are believed to contribute to genomic stability and epigenetic regulation in endometrial cells [153]. Collectively, the intricate network of ncRNAs ensures the precise coordination of molecular events required for successful pregnancy. The epigenetic modulation continues throughout the whole gestation with changes to the fetal and maternal methylome and the specific action of the ncRNA system, ensuring pregnancy maintenance and appropriate termination of gestation [111]. For instance, DNA methylation patterns in the placenta change dynamically to regulate genes involved in nutrient transport and immune tolerance. In the fetus, methylation of genes like *IGF2* is essential for proper growth and development [155,156]. Meanwhile, ncRNAs help pregnancy to reach healthy term by silencing genes that could trigger premature labor and support placental functions (e.g., miR-210) [111,155].

Although outside the primary scope of this review, as gestation progresses the bidirectional communication becomes more complex, involving a dynamic interplay between maternal and fetal growing tissues. In this context, factors deriving from the placenta play a pivotal role in modulating maternal immunological tolerance, vascular remodeling, and metabolic balance to support fetal development and complete pregnancy [157]. Key milestones include the establishment of a functional placental architecture, the regulation of transplacental nutrients and gas exchange, and the fine-tuning of fetal gene expression in response to both maternal and environmental directions [158]. Overall, these interactions are tightly regulated by epigenetic mechanisms that ensure developmental plasticity and at the same time preserve genomic stability [157]. Disruptions in this finely tuned regulation have been associated with pregnancy complications such as intrauterine growth restriction and preterm birth [158].

1.3. *Inter- and trans-generational inheritance*

An additional level of maternal-fetus crosstalk includes the transmission of epigenetic markers and clinical phenotypes from one generation to the next without changes in the primary sequence of DNA ascribing to the whole process a sort of heritability.

Epigenetic inheritance, by investigating the transfer of non-genetic traits or information from one generation (e.g., mother, *F0*) to the next (e.g., fetus, *F1*, and/or offspring *F2-Fn*), is attracting enormous interest in the scientific community

Table 3. Human ncRNAs involved in ZGA, endometrial receptivity, and embryo implantation.

ncRNA Type	Name	Target	Molecular pathway	Function	Ref.
miRNAs	miR-34c	Maternal Bcl2	Cell death	ZGA	[128]
	miR-302/367 cluster	OCT4, SOX2, NANOG	Pluripotency	ZGA	[128,129]
	miR-17	E2F1, PTEN	Cell cycle	ZGA	[128]
	miR-92	E2F1, PTEN		ZGA	[128]
	miR-192-5p	E-chaderin	Cell adhesion	ER	[130]
	miR-491-3p	Integrin β3		ER	[131,132]
	miR-1910-3p	Integrin β3		ER	[131,132]
	miR-183-5p	CTNNA2		ER/EI	[133]
	miR-124-3p	IL-6, IL-11	Immune response	ER	[134]
	miR-21-5p	IL-1β		ER/EI	[135]
	miR-125b	LIF		ER	[132]
	miR-135a	HOXA10	Transcription	ER/EI	[136]
	miR-135b	HOXA10		ER/EI	[132,137]
	miR-145	IGF1R	Cell growth	EI	[132,138]
	miR-27a	IGF1		ER	[132,139]
	miR-449c	IGFBP1		ER	[140]
	miR-17-5p	VEGF	Angiogenesis	ER	[114,141]
	miR-16-5p	VEGF		ER	[142]
lncRNAs	OIP5-AS1	HuR	mRNA stability	ZGA	[143]
	lncRNA-FTX	E-cadherin, Vimentin	Cell adhesion	ER	[144]
	LINC473	FOXO1, HOXA10	Transcription	ER	[126,145]
	lncRNA-RASA3	RASA3	Cell growth	ZGA	[146]
	lncRNA-TCL6	EGFR, AKT		ER	[147]
	lncRNA STAT3-AS	STAT3		EI	[148]
	lncRNA-TCL1	TUNAR		EI	[148]
	TCONS_01729386	VEGF	Angiogenesis	ER	[126,149]
	TCONS_01325501	VEGF		ER	[126,149]
piRNAs	PIWIL2 (HILI)	IL-10	Immune response	EI	[150,151]
	piR-36743	HOXA10	Transcription	ER	[152]
	piR-823	DNMT3B, HSP27		ER	[152]
	piR-55490	AKT	Cell growth	EI	[153]
	uniq_277797	FXR1		EI	[154]
	uniq_271431	DDX6		EI	[154]
	piR-55490	mTOR	Angiogenesis	EI	[153]

Abbreviations: ZGA: zygote-genome-activation; ER: endometrial receptivity; EI: embryo implantation.

due to the broad translational applicability of the underlying knowledge. The mechanistic understanding of the basic processes of transmission and maintenance of environmental information to subsequent generations is of crucial importance also in helping the design of novel epidrug strategies [159–161]. This is because in many instances epigenetic processes may prevail, or strongly cooperate, over the direct genetic trait in the expression of phenotypes or diseases across generations, including protective mechanisms against several complex conditions [162,163].

The concept of heritable "epigenetic-based memory" of a phenotype has been investigated across diverse species. Plant studies revealed that key pathways are regulated via epigenetic modifications in response to environmental and/or biotic stressors to mitigate the trauma effects, maintain and transmit stress memory, or improve resilience [164,165]. Although the epigenetic effectors have long been identified (e.g., DNA methylation, histone acetylation, ncRNAs), and stress-exposed plants appear to be susceptible to epigenetic modifications resulting into transgenerational plasticity, there is still missing knowledge on the dynamics of the epigenetic marks establishment and on their stability [165,166]. Studies in animal models confirm that various epigenetic mediators (e.g., ncRNAs including miRNAs and piRNAs) respond to environment, and that their altered signature in germ cells may impact on offspring phenotype [167,168]. However, in mammals, and in particular in humans, the epigenetic-based inheritance of a phenotype is still a matter of debate, and up to now there is a larger agreement on inter- rather than transgenerational inheritance [168,169]. The interest in understanding the effect on health of offspring after parental exposure is growing, since it represents a novel view in modern biomedicine with strong translational potential [170]. A recent work reviewed the current evidence supporting the epigenetic inheritance of parental exposures in several detrimental contexts such as metabolic diseases, stress and trauma, toxicants, as well as in utero exposures, up to the experience of positive factors, to highlight the contribution of epigenetic traits [171]. Although there is strong evidence of "association," there are still challenges and limitations to ascertain a proper epigenetic-inheritance mechanism in humans, including the demonstration of germline involvement [171].

The maternal effect, that is the in-utero influence of maternal phenotype, and/or genotype, on the phenotype of progenies, independently of their genotypes, belongs to the field of transgenerational epigenetics. During early development in humans, and mammals in general, the intimate role of the mother is the principal reason why she has the greatest chance to shape the developing individual [172]. Starting from zygote conception to delivery, pregnant females have the possibility to weave a mutual exchange of molecular messages with the fetus influencing its development, as well as the health and disease of both [172]. Epigenetic crosstalk between mother and embryo is constantly active and continues even after MZT, which is when it starts to synthesize its own proteins, enzymes, hormones, and ncRNAs, until the late phases of gestation when the mutual cooperation endures.

Although, the most deeply investigated epigenetic marks are methylation and acetylation of DNA or histones, other crucial mechanisms for transmitting information across generations exist, as small ncRNAs, prions, lipids, or microbiome. Interestingly, the crosstalk continues after birth, through tRNA-derived RNAs and miRNAs with gene silencing functions in the mother's milk that can be transmitted from mom to baby and are involved in its immune health and development [172–175].

In general, the biological reason of why organisms transmit non-genetic information across generations is that epigenetic inheritance can transmit past/present environmental information to the descendants, allowing future generations to revert to the ancestral condition when the environmental stressor has passed. Basically, they have been proposed two distinct types of epigenetic transmission in response to the environmental insult based on the persistence and duration of their effect: intergenerational and transgenerational inheritance [173,176].

Intergenerational effects represent any consequence of parental (*F0*) stress on *F1* progeny that either directly acts on or is communicated through *F0* germ cells or developing *F1* embryos in utero. Transgenerational effects are those initiated in the *F0* generation and persisting into *F3* or later (*Fn*) generations. However, effects that started in *F0* and persist in *F2* generation are categorized intergenerational when germ cells of *F1* were in utero at the time of event/stress action and transgenerational if no *F1* germ cells had been formed.

Apart from this general distinction, a great controversy exists on the definition of the maternal-fetus transmission of such non-Mendelian traits. In order to clarify this point and give a clear description of the epigenetic involved mechanisms and transmission we should consider some important details.

When adult mammals are exposed to external stressors, the entire body components, that are somatic cells, germline cells, as well as germline of the fetus in pregnant females, are directly exposed to the potential epigenetic modifications. They all belong to the same generation (*F0*) of exposed individuals and in the successive generations (i.e., *F1* of exposed males or exposed non-pregnant females), observable phenotypes or epigenetic marks cannot strictly presume any kind of inheritance. Similarly, an exposed pregnant female gathers under the same *F0* generation the successive *F1* and *F2*. Then, we consider both these scenarios as intergenerational effect (commonly described as intergenerational inheritance), that is the transmission and maintenance of a same observable epigenetic mark previously acquired at the time of *F0* exposure. Applying the same rational, transgenerational inheritance can be found in *F2* or later *Fn* of exposed males or non-pregnant females, and in *F3* or later *Fn* of exposed pregnant females (Figure 2).

For the reasons above described, it seems that a true epigenetic inheritance is difficult in mammals, and virtually impossible in humans, also considering that DNA methylation is globally reduced twice in each generation closely after fertilization and during the developing PGCs as detailed above. Accordingly, flaws in *DNMTs* genes, as *DNMT1* for methylation maintaining, or *DNMT3A* and *DNMT3B* for de-

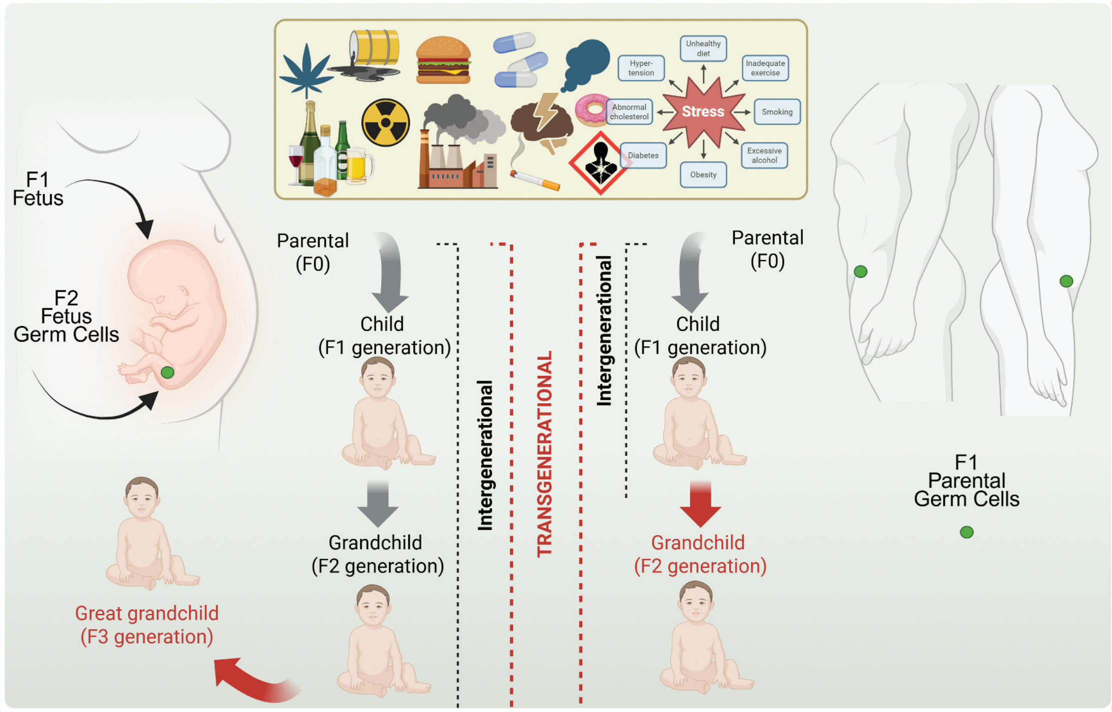

Figure 2. Intergenerational and transgenerational epigenetic inheritance. If a gestating mother (F0, left) is directly exposed to stressors, exposure may affect also the developing fetus and his/her developing germ line (green), resulting in intergenerational effect (possible inheritance) in F1 and F2 generations. F3 generation is the first non-directly exposed and an observed phenotype could be the result of transgenerational epigenetic inheritance. If a male or a non-pregnant female (F0, right) are directly exposed to stressors (for female exposure would occur before conception), germ cells may also be affected influencing F1 generation. F2 generation would be the first not directly exposed and appearance of the phenotype in F2 could be the result of transgenerational epigenetic inheritance. Created in BioRender. Tisato, V. (2025) https://biorender.com/e2brw4i.

novo methylation, or in *TETs* genes, as *TET1*, *TET2* and *TET3*, involved in DNA demethylation, by affecting (re)-establishment or erasing of the epigenetic marks in the specific (hemi)-genome may contribute to epigenetic inheritance in mammals [177]. In humans, any environmental induced epimutation to be considered as transgenerationally inherited should evade the epigenetic reprogramming in the PGCs and in the developing embryo after fertilization, subsequently they should be transmitted to the offspring, and persist in unexposed individuals [178]. This challenge has started a long-standing debate about the possibility of inheriting acquired traits, which has been strongly questioned since the Lamarckian and Darwinian eras. Another challenge is the lack of consensus in the definition of transgeneration in humans. A recent paper in 2024 called the question and proposed five essential elements and criteria to confirm transgeneration [179]: (i-ii) *inheritance of phenotypes* (i) *or epigenetic marks* (ii) *by the first unexposed generation induced by environmental factor(s) applied to the F0 generation;* (iii) *inheritance of the same epimutation(s) across generations;* (iv) *gene expression changes in subsequent generations;* (v) *germ cells tested in each generation* [179]. Although restrictive, they do not rule out transgenerational inheritance in humans, but they make it difficult to detect in research studies. On the other hand, also small ncRNAs, particularly tRNA-derived small RNAs (tsRNAs), and miRNAs have been proposed as emerging mediators of environmental information transmission through gametes in mammals. In detail, sperm-delivered sncRNAs may transmit paternally acquired traits to offspring, modulate zygotic gene expression and influence early embryonic development, broadening the range of actors involved in the process [180,181].

Although most studies on epigenetic inheritance mainly consider DNA methylation [178,182] or ncRNA [183,184], few have examined histones or multiple processes. Accordingly, previous studies demonstrated a role for ncRNAs in RNA-directed DNA methylation in several different systems [185–187]. Similarly, altered DNA methylation, ncRNAs, and differential histone retention sites and histone modifications suggested crucial interactions and integrations between the different epigenetic processes [188,189]. As an example, histones can be modified by ncRNAs and chromatin remodeling can start the transition from active euchromatin and gene expression to inactive heterochromatin [190]. Even though evidence is available on histone retention in sperm and its impact on the embryo [191,192], the role of specific epigenetic processes in histone retention has not been reported. Finally, environmental exposures promoting transgenerational epigenetic inheritance of sperm histone retention have been described [188,189,193]. Interestingly, a model of *C. Elegans*, to translate into human obesogenity, investigating how lipid accumulation leads to signals that pass across generations, authors demonstrated that such signals might be transgenerationally transmitted to progeny [194]. Authors identified histone H3K4me3 as a contributing actor in obesity, though the function of DNA methylation and ncRNAs remains unclear.

Summarizing, DNA methylation, ncRNAs, and histone modifications mutually integrate in the occurrence of epigenetic transgenerational inheritance.

Furthermore, whereas transgenerational epigenetic inheritance has been widely documented in plants, worms, and fruit flies, analogous evidence in mammals remains debated, though several investigations in mice models support some evidence [179,195]. For instance, growth at elevated temperature in *C. Elegans* or in *D. Melanogaster* activates the expression of a subset of endogenous repetitive elements, normally repressed, which may require several generations to be fully restored [196,197]. On the contrary, in humans to confirm epigenetic changes we need to detect them at least up to F3 mainly because of the two waves of epigenetic reprogramming.

Other factors that may influence epigenetic inheritance in mammals are some "confounding factors" such as a direct maternal contribution due to non-optimal in utero conditions, as well as paternal influences mainly due to aberrant components of the seminal fluid. Moreover, postnatal effects, lifestyle or genetic predispositions, may act as the main origin of the observed modified epigenetic or phenotypic trait in the progeny.

For the reasons above described the mother's womb and the in-utero microenvironment are of crucial importance for a correct embryo epigenetic development and future newborn health. Accordingly, there are sensitive crucial phases of life during which the maternal effect strongly may impact on the baby health and development. In detail, all this may potentially be transmitted and have an impact on behavior, cognition, and immune system development of the future individual [198–200]. Globally, from zygote conception to the age of two years this frame includes a critical window called the first 1000 days of life, where an appropriate development of fetus is put in place to allow the child an healthy and epigenetically updated development [201].

As reported by the Genetic/Epigenetic Mother/Child Dyad Study (GEMCDS), offspring might epigenetically suffer the maternal in utero milieu during fetus development and several complex diseases as leukemia, blastoma, or neurodevelopmental disorders can appear during childhood life [202–204]. An extreme consequence of abnormal epigenetic maternal environment can even lead to early pregnancy loss by altering blastocyst implantation or embryo development disturbing the perfect endometrial-embryo synchronization as described above [205,206]. However, the restricted number of investigations in humans is the limiting step, nevertheless it may be bypassed by the presence of several animal studies supporting the transmission of acquired traits across generations [207–209]. Finally, to definitely ascribe and demonstrate epigenetic-mediated mechanisms in human inheritance, there is the need of a rigorous methodology and the systematic investigation of several generations as recently proposed [210,211].

In conclusion, transgenerational epigenetic inheritance in humans is limited but possible, and a fine tuning and mutual synergy between genetics and epigenetics may be the true balance in determining this action. By complementing data from GWAS with EWAS may help us in identifying on the one hand the specific trajectory of epigenetic inheritance and on the other hand the relative contribution of genome and epigenome in transgenerational mechanisms [4].

Regardless of the apparent molecular transfer of epigenetic messages, the direct effect of the mother to the baby, by womb milieu and/or after birth, acts as the first epitreatment the mother directly fix on her progeny according to her own coping toward environment.

1.4. Curable epigenome: is there a role for epidrugs in reproductive medicine?

The term "curable epigenome" refers to the possibility of targeting alteration of the epigenome to correct acquired epigenetic marks associated with pathological phenotypes, which may be either the cause of the development or progression of several diseases, such as cancer, metabolic diseases, and neurodegenerative disorders [161,212–214]. The discovery that complementary epigenetic-modifying enzymes may be useful to target and reverse epigenetic modifications, has opened the road for suitable epidrugs applications toward those proteins involved in epigenetic regulation [215]. Although they were initially developed mainly for the treatment of tumors, such as azacitidine (the first FDA approved for the treatment of myelodysplastic syndrome [216]), interest in their therapeutic potential has increased significantly over time in numerous branches of medicine. In particular, the identification of specific associations between epigenetic marks and male/female infertility, of the role of epigenome in the plasticity of embryo development, and of epigenetic signatures in reproductive-related disorders such as endometriosis [217–219], have increased the relevance of epigenetics as therapeutic target and of epidrugs as innovative therapeutic tool.

Based on their enzymatic function and target, epidrugs can be classified into: DNA methyltransferase inhibitors (DNMTi), histone deacetylases inhibitors (HDACi), and histone methyltransferase inhibitors (HMTi) [220,221]. DNMTi include agents such as 5-azacitidine and decitabine that have been initially developed for the treatment of hematological malignancies. These, by interfering with DNA methylation (e.g., hypomethylating actions) lead to gene expression changes and potential differentiation of myeloid cells. DNMTi inhibit the activity of DNMTs by acting as nucleoside or non-nucleoside analogues, causing overall global DNA demethylation and consequent reactivation of pathologically silenced genes (e.g., tumor suppressor genes in cancer cells) [222,223]. In reproductive medicine, DNMTi may target aberrant hypermethylation associated with defects in gametogenesis or embryonic development that contribute to unsuccessful reproduction, restoring a physiological methylation pattern. HDACi, such as vorinostat (SAHA) or valproate, can block the activity of histone deacetylases, that remove acetyl groups from histones leading to a reduction in transcriptional activity. This inhibitor action makes chromatin more accessible to transcription factors and promotes gene expression [224,225]. In reproductive medicine, HDACi can be used to improve the quality of gametes or embryos, increasing the transcription of genes essential for their correct development, counteracting in turn the negative effects of environmental stressors on chromatin [226]. HDACi may act as potential therapeutic tool in the treatment of ovarian or endometrial dysfunctions [227]. Similarly, HMTi can modulate histone methylation by inhibiting HMTs enzymes that add methyl groups to specific lysine or arginine residues on histones. The overall effect depends on the specific location and number of methylation sites, and these processes may result in activation or global repression of gene transcription [228]. In this perspective, several substances, such as inhibitors of EZH2 (an HMT involved in the methylation H3K27me3), have been shown to be able to modulate gene expression and for this reason, they are deeply investigated for clinical use [225]. Due to its complex and multiple roles in gene expression, modulation of histone methylation may efficiently tune epigenetic defects improving in turn global reproductive health [229].

The potential of epidrugs in reproductive medicine includes their use to improve the quality of spermatozoa and oocytes, both *in vivo* and *in vitro*. Gametogenesis is a very complex process and sensitive to stress and environmental factors which can induce epigenetic abnormalities. For this reason, low doses of DNMTi or HDACi could be used to correct possible alterations, allowing the production of competent gametes for successful fertilization, improving morphology, motility, DNA integrity, and embryonic development [230–232]. In confirmation of this, it has been demonstrated that an inadequate intake of folates and vitamins B6 or B12 (considered natural epidrugs) is associated with anovulation and fertility reduction [233]. As regards embryogenesis, the use of folic acid and vitamin B12 during pregnancy has been widely accepted for years with the aim of improving cell replication, especially in periods of active cell duplication, maintaining the correct epigenetic setting (i.e., faithful DNA replication and stability) and consequently increasing the chance of healthy offspring [234–236]. ART procedures, such as controlled ovarian stimulation and prolonged embryo culture, may in turn induce epigenetic alterations in embryos [237,238]. A recent study aimed at fulfilling the gap in knowledge of ovarian stimulation or blastocyst culture with adverse health outcomes or imprinting disorders in offspring did not reveal strong negative results. By EWAS of blastocysts obtained after superovulation of young or adult mice compared with blastocysts derived from non-stimulated mice, authors found a minimal effect of ovarian stimulation on the DNA methylation but a greater impact of in vitro growth and maturation of oocytes [239].

Theoretically, the addition of epidrugs in embryo culture media at precise timing could mitigate possible negative effects by promoting physiological epigenetic reprogramming. This could lead to increased implantation capacities, reduced risk of epigenetic abnormalities in embryos and ultimately increased full-term pregnancy rates. Hypotheses are widely supported by studies that have correlated the intake of folic acid and vitamins B6 and B12 with a better quality of oocytes and embryos and with a greater probability of successful pregnancies [233]. Even in the case of transgenerational transmission of pathological epigenetic phenotypes, epidrugs could have the potential to erase or correct aberrant epimodifications, interrupting the chain of transmission of possible pathological phenotypes [240]. Moreover, among the acquired conditions that affect the individual epigenome, it is important to consider the effects on sperm or oocyte of cancer survivors after chemotherapy cycles. Theoretically, acquired modifications of the gamete epigenome can be transmitted to the offspring, although cryopreservation of gametes before treatment may represent an opportunity to be considered while waiting for effective and safe treatments with epidrugs [241–244].

In recent years, the potential of epidrugs has been reported for the treatment of female reproductive disorders, including polycystic ovary syndrome (PCOS) and endometriosis. PCOS is a heterogeneous condition characterized by a clear epigenetic component, mainly alterations in DNA methylation and presence of histone modifications in oocytes, granulosa cells, and endometrial tissues that overall result in altered gene expression. Epidrugs may improve these altered gene expression profiles by acting on insulin sensitivity, inflammation, or ovarian steroidogenesis [245]. Endometriosis, a chronic inflammatory disease, is characterized by the presence outside the uterus of endometrial tissue with altered DNA methylation pattern and aberrant histone modifications, which promote growth, survival, and invasiveness of endometrial lesions and may serve as a target for new therapeutic strategies complementary/alternative to current ones [246].

Beyond gametes quality, proper early embryonic development is also essential for a successful pregnancy and the production of healthy offspring. As above reported, we are describing a complex and finely regulated process in which alterations of key pathways may result in perturbation of embryo implantation, development, and viability. It has to be noted that although epidrugs in relation to embryo development has mainly focused on the potential teratogenic effects of compounds, the potential of targeting epigenetic marks as a new strategy to improve early embryonic development, particularly in ART, is attracting more interest. Several preclinical studies report on the use of epidrugs to correct alterations in the embryonic epigenome and improve embryo quality and competence. For example, exposure to HDACi during *in vitro* culture of bovine embryos allowed efficient embryo survival after vitrification and reduced abnormal expression of paternal imprinted genes, suggesting that HDACi can reduce the negative effects on embryonic epigenome [247]. In the same line, Shao et al. analyzed the impact of HDACi (e.g., MGCD0103 and T247) in mouse embryo development reporting effect on ZGA via improved epigenetic regulation, supporting the potential use of HDACi in reproductive medicine [248].

Regarding DNA methylation during embryogenesis, while those mediated by TET proteins do not appear to be essential for gastrulation (i.e., TET knockout embryos exhibit normal gastrulation), overall DNA methylation by DNMTs is critical with embryos lacking DNMTs that do not gastrulate, unlike those with partial deficiency [249]. Consistently, analysis of the expression of DNMTs in the chorionic villi of pregnant women demonstrated their role in placenta, with DNMT3B showing the highest levels. Conversely, in the chorionic villi of those women with early embryo growth arrest, DNMT3A protein was under-expressed. From this, it is deduced that inefficient maintenance of methylation, due to reduced DNMT3A levels, could cause abnormal embryonic development in early pregnancy [250]. Similarly, DNMT3B resulted crucial for embryonic development in a murine model and its absence led to Wnt and Hedgehog pathways deregulation and affected embryo development [251].

Bromodomain and Extra-Terminal Domain (BET) family proteins activate the expression of key genes such as *Nanog*, *Otx2*, and *Sox2*, and are essential for the formation and maintenance of the epiblast in preimplantation mammalian embryos [252]. In this line, BET protein inhibition (e.g., via JQ1), or bromodomain-containing proteins (BRD) deficiency, may significantly reduce *NANOG* expression. BRD inhibitors have been investigated in the modulation of oligodendrocyte progenitor cell differentiation, showing differential effects depending on the BRD target [253]. Their predominant role in early embryonic development relies on pluripotency and specific maintenance at the inner cell mass and epiblast lineages. Understanding how these proteins involved in epigenome preservation act on specific pathways and genes will enable the development of targeted approaches against infertility and developmental diseases. Table 4 shows epidrugs with biological targets investigated in diseases treatment and embryo development.

Despite their great potential, the study of epidrugs in reproductive medicine is still in its early stages and presents several challenges that need to be overcome before considering possible clinical use. First, it is necessary to improve specificity and reduce off-target effects. Current epidrugs, in fact, may lack the specificity needed to intervene on such delicate and complex processes as gametogenesis and embryonic development. Since they target a wide range of cellular processes, they may lead to unwanted or unpredictable side effects [262]. In the same line, establishing the correct drug dosage and the optimal schedule of administration are mandatory tasks [263]. An incorrect dosage or timing could not only be ineffective but also cause irreversible damage or unwanted epigenetic alterations with severe long-term consequences. Furthermore, it is certainly not of secondary importance to minimize their cytotoxicity, especially for those cells in active replication, and identify the best delivery system [262,264]. Finally, a deeper understanding of the "normal" epigenome patterns of both gametes and embryonic cells is essential to overcome the current limited knowledge in this field, lessening the validation of interventions aimed at restoring optimal "physiological" epigenome conditions.

Certainly, the curable epigenome represents an exciting prospect also for human reproductive medicine. The growing understanding of the important role epigenome has in fertility, gametogenesis, and embryo development allows to identify new potential targets for precise therapeutic interventions. Epidrugs may have a considerable potential to improve the quality of gametes and optimize the efficacy of ART to treat specific reproductive diseases.

1.5. Future perspective

Epigenetics is a rapidly developing and expanding field that is shedding light on the mechanisms underlying a wide range of biological processes. Decoding epigenetic landmarks related to health and disease has therefore the enormous potential of changing both human disease prevention and treatment [265]. In the field of human reproduction, selective and specific epigenetic marks need to be established; exact timing and spatial windows have to be identified during gametogenesis, as well as in the early stages of embryogenesis, pregnancy establishment and maintenance. Although our knowledge on the role of the specific epigenetic signatures is significantly increased, the consequence of their dysregulation mainly relies on preclinical animal models while the outcome of embryonic and long-term human development still needs to be deeply investigated.

Table 4. Epidrugs tested for urogenital system disease treatment and embryo development improvement.

Epidrug(s)	Target	Class	Application	Ref.
KSK64, LAK31, MPK409, Romidepsin, Quisinostat	HDACs	HDAC inhibitors	Treatment of urological tumors, cisplatin-resistant germ cell tumors	[254]
ASK44, ASK58, ASK62, JQ1	BET proteins	BET inhibitors		
LAK-FFK11, LAK129, LAK-HGK7	HDACs BET proteins	HDAC/BET inhibitors		
OTX015	BET proteins	BET bromodomain inhibitor	Ovarian cancer treatment	[255]
Vitamin C	Tet enzymes	Essential cofactor for TET protein activity	Influencing DNA methylation patterns and gene expression in ESC	[256]
Guadecitabine (G)	DNMTs	Nucleoside analogue, DNA hypomethylating agent	Recurrent platinum-resistant ovarian cancer	[257]
Decitabine	DNMTs	DNMT inhibitor	Treatment of male fertility	[258]
Vorinostat	HDACs	HDAC inhibitor		
GSK-J4	KDM6A/KDM6B demethylases	Histone demethylase inhibitor	Testicular germ cell tumors	[259]
5-aza	DNMTs	DNMT inhibitor	Cisplatin resistance in germ cell tumors	[260]
5-aza	DNMTs	DNMT inhibitor	Improvement of endometrial receptivity	[261]
GSK343	EZH2 enzyme	Histone methyltransferase inhibitor	Treatment of endometriosis	[229]
Scriptaid	HDACs	HDAC inhibitor	In vitro development of bovine embryos	[247]
MGCD0103 and T247	HDACs	HDAC inhibitor	Effect on early embryonic development (transcriptome level)	[248]

Abbreviations: HDACs: Histone deacetylases; BET: Bromodomain and Extra-Terminal Domain; Tet: Ten-Eleven Translocation; DNMTs: DNA methyltransferases; 5-aza: 5-aza-2′–deoxycytidine.

Advances in epigenetics understandings are therefore rising crucial questions and implications. The role of epigenetic mechanisms in translating environmental signals (including negative experiences and trauma), highlights potential inter- or trans-generational inheritance of epigenetic marks that may influence health and disease in the offspring [266]. Although a clear causal association between exposure to stressors and transgenerational inheritance in humans is still to be completely certified, a recent report calls for consideration [210].

In the same line, the growing utilization of ART mainly due to a trend toward delayed childbearing and low fertility needs to be considered. Although ART procedures do not seem to have a significant effect on specific epigenetic changes [267], the sensitivity of the embryonic epigenome to environmental factors needs to be monitored in dedicated longitudinal studies to reveal the establishment of potential persistent epigenetic dysregulations in ART-conceived offspring [237,239,268].

Finally, as far as our understanding of the epigenetic mechanisms governing the mother-fetus crosstalk, it is to take into account that the same epigenetic marks may represent targets for potential therapeutic interventions. The theoretical principle underlying the use of epidrugs infers the existence of a "correct" epigenome that should be restored. In this line, the basic epigenetic drug discovery/development/repurposing has been until now mainly based on phenotypic observations [269,270]. This assumes that we know what the healthy phenotype is but we do not fully know what the "normal" epigenome is [271]. The increasing insights on epigenetic mechanisms underlying specific pathophysiological contexts and precise developmental stages is currently supporting a proper target-based approach [269]. Of note, epidrugs design needs also to consider the intrinsic flexibility of the epigenome and the reversibility of epigenetic marks, as well as the role of environment as key driver in the acquisition and maintenance of specific signatures [269,272]. Although this approach is still in its infancy, insights coming from other clinical contexts highlight the use of epidrugs to revert epigenetic disrupted processes and promote personalized treatments as stand-alone approaches or in combination with standard therapies [215].

Author contributions

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Giuseppina Peluso: Investigation, Writing – review and editing. Fabio Scarpellini: Investigation, Writing – original draft, Writing – review and editing. Veronica Tisato: Conceptualization, Resources, Writing – original draft, Writing – review and editing, Visualization, Investigation, Supervision, Funding acquisition.

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