ANNUAL REVIEWS

Annual Review of Genomics and Human Genetics

Epigenetic Regulation and Risk Factors During the Development of Human Gametes and Early Embryos

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Annu. Rev. Genom. Hum. Genet. 2019. 20:21–40

First published as a Review in Advance on March 27, 2019

The *Annual Review of Genomics and Human Genetics* is online at genom.annualreviews.org

https://doi.org/10.1146/annurev-genom-083118-015143

Keywords

epigenetic regulation, human gametes, human preimplantation embryos, imprinting, infertility, assisted reproductive technology, ART

Abstract

Drastic epigenetic reprogramming occurs during human gametogenesis and early embryo development. Advances in low-input and single-cell epigenetic techniques have provided powerful tools to dissect the genome-wide dynamics of different epigenetic molecular layers in these processes. In this review, we focus mainly on the most recent progress in understanding the dynamics of DNA methylation, chromatin accessibility, and histone modifications in human gametogenesis and early embryo development. Deficiencies in remodeling of the epigenomes can cause severe developmental defects, infertility, and long-term health issues in offspring. Aspects of the external environment, including assisted reproductive technology procedures, parental diets, and unhealthy parental habits, may disturb the epigenetic

reprogramming processes and lead to an aberrant epigenome in the offspring. Here, we review the current knowledge of the potential risk factors of aberrant epigenomes in humans.

1. INTRODUCTION

In eukaryotic cells, linear double-stranded DNA is packed with octamer histone proteins in the nucleus. Various chemical modifications on DNA and histone residues affect the accessibility of DNA regions, providing a fine machinery to regulate gene expression in a spatiotemporal pattern. Growing evidence supports the idea that proper epigenetic reprogramming is essential for gametogenesis and early embryo development (4, 41, 81, 105). Current knowledge about early mammalian development has come mainly from studies of mice because of their easy accessibility and fast breeding and, more importantly, because using mice involves fewer ethical issues. However, due to species-specific differences, such as developmental timing and cell lineage specifications, findings from experiments with mice cannot be directly extrapolated to human embryos (72). Constrained by ethical issues, technical limitations, and the scarcity of human embryo resources, early work on human embryo development focused on examining embryo morphology, identifying factors that can improve in vitro culture, and profiling the expression of a few specific genes and proteins. The past several years have seen the development and implementation of low-input and single-cell sequencing in this field. These techniques have enabled researchers to profile the transcriptomes, DNA methylomes, and chromatin accessibility of human gametes and early embryos in single cells with single-base resolution (33, 39, 63, 85, 102, 103, 106, 108), paving the way for dissection of the molecular regulation of human embryo development.

In this review, we focus mainly on recent progress in understanding the epigenetic dynamics in human gametogenesis and early embryo development. Globally, epigenetic reprogramming is susceptible to external factors, such as assisted reproductive technology (ART) procedures and the health status and lifestyles of the parents. An aberrant epigenome passed to the offspring will cause severe health problems in the fetus or in later life. Here, we summarize current knowledge of risk factors for an aberrant epigenome.

2. THE DYNAMIC EPIGENOME DURING GAMETOGENESIS AND EARLY EMBRYOS

In mammals, global epigenetic reprogramming occurs during germline development and early embryo development (8, 41, 61, 90, 91, 105). A much clearer picture of these processes has been developed in mouse models. Recently, with the development of low-input and single-cell epigenome sequencing techniques, such as single-cell bisulfite sequencing (scBS-seq) (16), single-cell chromatin overall omic-scale landscape sequencing (scCOOL-seq) (63), assay for transposase-accessible chromatin using sequencing (ATAC-seq) (103), and DNase I hypersensitive site sequencing (DNase-seq) (33), we are now able to detect dynamic changes in the DNA methylation and chromatin accessibility landscape genome-wide during human gametogenesis and early embryo development. Among the different molecular layers of epigenetic marks, the 5-methylcytosine modification at CpG dinucleotides is the most extensively studied epigenetic mark in both mouse and human species (41). Two major waves of genome-wide DNA methylation reprogramming occur during embryo development: The first takes place during the early germ cell development of parental embryos, and the second happens during the preimplantation development of the next generation's embryos.

2.1. The Dynamic Epigenome During Primordial Germ Cell Development

In humans, the development of the germ cell begins with the specification of primordial germ cells (PGCs) at the perigastrulation embryo stage (approximately embryonic weeks 2–3). PGCs are the precursors of sperm and oocytes. When a human embryo develops to the fourth week, PGCs begin to migrate from the yolk sac wall to the hindgut endoderm and finally reach the developing gonads. During the migration stage, PGCs rapidly proliferate. After sexual determination, they proceed on different paths to differentiate into oocytes or sperm, depending on the sex of the embryo. At week 10, female gonadal PGCs asynchronously enter meiotic arrest in prophase I and remain quiescent in the developing ovary until puberty. Male gonadal PGCs enter mitotic quiescence at this time and do not initiate meiosis until puberty.

In humans, the first wave of genome-wide DNA demethylation in PGCs takes place before human embryonic weeks 4–7 (61, 95). This is because at this time, the migratory PGCs in the hindgut have already exhibited a lower level of DNA methylation than adjacent somatic cells (34, 37, 38, 90). After approximately 10–12 weeks of gestation, the global DNA methylation levels of PGCs reach their lowest point, at which point the entire genome is nearly devoid of DNA methylation, with only 6–7% (median level) residual methylation left in the whole genome. To our knowledge, this is the lowest DNA methylation level that occurs in the human genome regardless of cell type. The global low methylation levels are maintained through embryonic weeks 17–19 in human PGCs, suggesting that the global reestablishment of DNA methylation occurs later (37, 38). The exact lowest methylation level and the time points vary among studies from different groups, which may result from different strategies of isolating samples and preparing libraries, different analysis pipelines, or biological differences in human samples. Nonetheless, the DNA demethylation dynamics found in mouse PGCs during embryonic days 10.5–13.5 and those found in human PGCs during developmental weeks 5–19 are consistent across studies (95), which implies that the clearance of DNA methylation during germline development is a fundamental and highly conserved process in mouse and human species.

The 5-methylcytosine modification at CpG dinucleotides is established by de novo DNA methyltransferases, including DNMT3A, DNMT3B, DNMT3C, and DNMT3L, whereas DNMT1 and its cofactor UHRF1 are responsible for duplicating the methylation pattern to the newly synthesized DNA strand at the replication fork to maintain the DNA methylation level during cell replication. During the extensive DNA methylation erasure stages in both human and mouse PGCs, neither DNA methylation maintenance enzymes (e.g., UHRF1) nor de novo methylation enzymes (e.g., DNMT3A and DNMT3B) are expressed, which could result in replication-coupled DNA demethylation while PGCs proliferate (53, 75). Moreover, recent evidence has suggested that the active demethylation mediated by TET1 and TET2 that converts 5-methylcytosine to 5-hydroxymethylcytosine also contributes to the global DNA demethylation, especially for the imprinting regions in early PGC development (43). Therefore, both passive dilution and active demethylation mechanisms contribute to this extensive erasure of the parental DNA methylation memory.

DNA methylation is important for regulating gene expression, repressing retrotransposon activity, and maintaining genome stability. The global clearance of DNA methylation observed in human PGCs leads to a great loss of DNA methylation in various regions, including CpG islands, transcription start sites, gene bodies, intergenic regions, repeats, and even most imprinting regions, which leads to the levels of DNA methylation in PGCs being the lowest among all the known human methylomes (34, 37, 38, 90). However, the transcriptomes of PGCs are relatively stable in general, with only several hundred genes' expression significantly changed when the global DNA methylation is removed from the PGC genomes, although a small subset of genes

involved with late germ cell development and genome defense are upregulated and correlate with the removal of DNA methylation at their promoters. These results suggest that the key purpose of demethylation is to clear the parental epigenetic memory.

Despite the global DNA demethylation in PGCs, evolutionarily young and potentially hazardous retrotransposons—such as intracisternal A particles (IAPs) in mice and the nonautonomous, hominid-specific SINE/variable number of tandem repeats/Alu (SVA) retrotransposons in humans—remain relatively highly methylated, which might contribute to their repression (37). Notably, there are also some repeat-poor regions that can escape from global demethylation, including those located at enhancers, CpG islands, promoters, and gene bodies, which may represent hot spots of transgenerational epigenetic inheritance.

Hypomethylation at the promoter regions is usually associated with gene activation. When global demethylation happens during human PGC development, how is gene expression repressed? Histone covalent modifications are important carriers of epigenetic information and play important roles in regulating gene expression and embryo development. Genome-wide studies in cell lineages have revealed the general relationship between various histone modification marks and gene expression. Overall, methylation on histone H3 lysine 4 (H3K4) is usually associated with permissive promoters and enhancers, whereas methylation on H3K9 and H3K27 is usually associated with repressive regions. Recent results have revealed that global chromatin modification is reorganized during early PGC development in both human and mouse species. Repressive H3K27 trimethylation (H3K27me3) is enriched during the course of PGC development, and H3K9me3 is retained predominantly at pericentric heterochromatin, which could contribute to the repression of gene expression in the globally hypomethylated genomes of PGCs (82, 95). These marks have also been implicated in the repression of retrotransposons in mouse PGCs. In addition to DNA methylation and histone modifications, the accessibility of key regulatory DNA elements is directly associated with the transcription regulation of gene expression. Thus, establishing proper chromatin accessibility is essential to spatiotemporally controlling gene expression.

Nucleosome occupancy and methylation sequencing (NOMe-seq) is built on the ex vivo methyltransferase activity of the M.CviPI enzyme, which can artificially methylate the cytosines of the CpG sites in open chromatin regions but keep the cytosines of the CpG sites in closed chromatin regions unmethylated (38, 57, 88). Therefore, NOMe-seq can simultaneously dissect chromatin accessibility and endogenous DNA methylation. By using this technique, Guo et al. (38) found that the open chromatin of the germline-specific genes or retroelements is established and the corresponding genes are upregulated in both human and mouse PGCs, suggesting evolutionary conservation of the reprogramming of the epigenome and functional modulation of chromatin accessibility during PGC development. More importantly, the chromatin states of evolutionarily younger subfamilies of repeat elements such as Alu and LINE1 (long interspersed nuclear element 1) tend to be less accessible than their evolutionarily older counterparts in human PGCs. Together, these results suggest that, during the global DNA demethylation, human PGCs are likely to maintain a higher level of DNA methylation, less accessible chromatin state, and more enriched repressive histone marks in the evolutionarily younger and probably more active and hazardous transposable elements than the counterparts to repress their activity, thereby better safeguarding genome integrity.

2.2. The Dynamic Epigenome During Spermatogenesis and Oogenesis

Following PGC development, the gonadal PGCs progress to spermatogenesis in male embryos and oogenesis in female embryos. However, because of the difficulties in obtaining samples from

late-gestation fetal gonads, the dynamic epigenomes during spermatogenesis and oogenesis in human have not yet been explored (41).

2.2.1. Spermatogenesis. The developmental stage from male PGCs to the mature spermatozoa is a key time of epigenetic reprogramming (11, 32, 78). Through spermatogenesis, DNA methylation and histone modifications undergo huge changes. In mice, the establishment of DNA methylation in early sperm progenitors (prospermatogonia) lasts from embryonic days 15.5–18.5 to the termination of meiotic pachytene after birth. In sperm, the promoters of germ cell–specific genes that are involved in spermatogenesis are hypomethylated, whereas the promoters of pluripotent and somatic tissue-specific genes are hypermethylated (28, 58, 70). Many sites outside genic regions and CpG islands are also differentially methylated between sperm and somatic cells, and these sites might play other significant roles in spermatogenesis (74). For instance, sites at centromeric and intergenic regions are necessary for the structure of chromatin when male germ cells undergo meiosis and spermatogenesis (73, 107). Histone modification appears to be essential in male germ cell development, especially in spermatogenesis. During spermatogenesis, extensive chromatin remodeling occurs, which includes the replacement of histones by transition proteins and then the replacement of transition proteins by protamines. The replacements package the DNA into the sperm head, which reduces the sperm size. In human sperm, 5–15% of histones remain bound to the genome (60).

Since epigenetics plays a critical role during male germ cell development, perturbations may cause abnormal reproductive outcomes. In particular, many studies have focused on methylation defects at imprinted gene loci. The depletion of DNMT1 in embryos could lead to abnormal phenotypes, including the disordered biallelic expression of imprinted genes, expression of normally silenced IAP sequences, and ectopic X-chromosome inactivation (62, 76, 97). In humans, mutations in DNMT3B cause an autosomal recessive genetic disorder known as ICF (immunodeficiency, centromere instability, and facial anomalies) syndrome (104). DNMT3L-deficient mice had male germ cells that lacked methylation of most repetitive elements, leading to abnormal transcription in early germ cells as well as hypomethylation of paternally methylated imprinted loci (6, 56, 99).

Chromatin structure and modifications play an important role in the final stages of spermatogenesis. When round sperm develop into mature sperm, histones are replaced by protamines, which can help sperm DNA avoid damage and mutagenesis (78). The normal ratio of protamine to histone in sperm is important to male fertility (112): Infertile males have higher ratios than fertile males do. Cryopreservation can affect chromatin integrity—that is, a low temperature can influence chromatin decondensation (30).

In the past, RNA in sperm has been regarded as a carryover or contamination from other cells. Recently, growing evidence has suggested that this RNA is required for important stages of spermatogenesis and epigenetic inheritance (80). Sperm transcriptomes differ between fertile and infertile males (50). Differentially expressed small RNAs between highly motile and less motile sperm are related to apoptosis and spermatogenesis alteration (10). In addition to the differentially expressed RNA, modifications on these RNAs also contribute to male fertility and epigenetic inheritance, such as N^6-methyladenine. Germ cell–specific inactivation of the N^6-methyladenine RNA methyltransferase gene *Mettl3* or *Mettl14* causes loss of N^6-methyladenine and dysregulates the translation of transcripts required for the proliferation and differentiation of spermatogonial stem cells, thereby impairing spermatogenesis (65).

2.2.2. Oogenesis. Mammalian oogenesis is a gradual and complex process of dynamic differentiation (**Figure 1**). Oocytes eventually give rise to a fully functioning organism through a series of

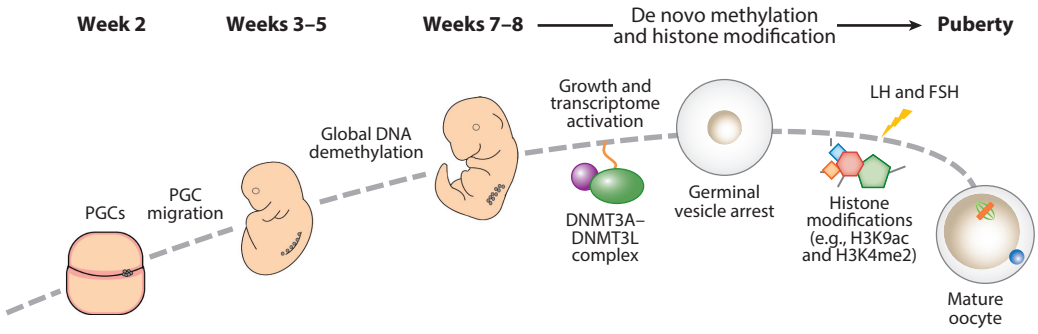

Figure 1

Epigenetic reprogramming during oogenesis. Human primordial germ cells (PGCs) are specified in the posterior epiblast at week 2 and then migrate through the hindgut to the developing genital ridges during weeks 3–5. Before weeks 4–7, the first wave of global DNA demethylation occurs, including significant loss of methylation at CpG islands. During puberty, under the stimulation of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), the oocytes finish meiosis I and go through meiosis II. During this procedure, with the help of the DNMT3A–DNMT3L complex and histone modifications such as histone H3 lysine 9 acetylation (H3K9ac) and histone H3 lysine 4 dimethylation (H3K4me2), the oocytes establish DNA methylation and prepare for subsequent biological processes, such as fertilization and embryo development.

processes, including oogenesis, maturation, fertilization, and early embryo development. The volume of an oocyte is much larger than that of a sperm. In addition to genetic information, oocytes carry the material and energy reserves (nutrients) of the embryo and developmental instructions. All of the factors necessary to complete oocyte maturation, meiosis, fertilization, and early development are produced in the early stages of a transcriptionally active oocyte.

Oocyte maturation undergoes a series of pathways. In the fetal ovaries, the PGCs migrate to the surface of the gonadal ridge and develop into oogonia. When the oogonia divide by mitosis and enter meiosis I, they develop into primary oocytes. After birth, mammalian oocytes are arrested at the germinal vesicle stage in ovary. These oocytes need the stimulation of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) to finish meiosis I and go through meiosis II.

After global DNA demethylation in early PGCs, female germ cells remain hypomethylated and maintain meiotic arrest throughout the rest of embryonic development. The establishment of DNA methylation in oocytes is accompanied by the growth of follicles and is largely completed during the germinal vesicle stage in mice. Unlike the highly methylated sperm, DNA methylation in the oocyte genome is highly enriched at the transcribed gene bodies, with almost no methylation at intergenic regions and nontranscribing gene bodies, forming partially methylated domains (39, 85). By using single-cell whole-genome bisulfite sequencing, Yu et al. (109) explored dynamic DNA methylation during human oocyte maturation. They found that, although CpG methylation has been largely established at the germinal vesicle stage, localized changes, especially in the regions with high CpG densities, continue into later development. By contrast, as the oocyte matures, non-CpG methylation in the genome gradually accumulates, and this methylation establishment process is generalized, without particular genomic regions or functional features (109). Genetic studies in mice have shown that Dnmt3A and its cofactor Dnmt3L (forming the Dnmt3A–Dnmt3L complex) are essential for reestablishing DNA methylation in oocytes, and depletion of Dnmt3A and Dnmt3L resulted in a global reduction in both CpG and non-CpG methylation in oocytes (84). However, the functional role of DNA methylation in the oocyte is not very informative. Loss of DNA methylation in the oocyte has no impact on its growth, maturation, or fertilization. Nonetheless, embryos conceived from those oocytes die by embryonic day 10.5, largely because of a deficiency of methylation at maternally imprinted regions (55).

Besides global de novo methylation, different histone modification patterns have been observed during mouse oocyte maturation. As oocytes grow, H3K9 acetylation (H3K9ac), H3K18ac, H4K12ac, and H4K5ac as well as H3K4me2, H3K4me3, and H3K9me2 increase gradually. And with the oocyte meiotic maturation, chromatin histones undergo widespread deacetylation (17). Deletion of the histone deacetylase genes *HDAC1* and *HDAC2* can block oocyte maturation and decrease overall transcriptional activity (69). Knocking down *MLL2* (mixed lineage leukemia 2), which encodes the methyltransferase of H3K4, causes abnormal spindles in mouse oocytes, but these oocytes are still able to grow to full size through meiosis (1). In general, these studies indicate the necessity of histone modification during oocyte maturation. The various posttranslationally modified forms of histones could promote maturation during oocyte development. Disruption of histone modifications leads to defective chromosome condensation and segregation, delayed maturation progression, and even oocyte aging.

More recently, low-input chromatin immunoprecipitation sequencing (ChIP-seq) has revealed a noncanonical broad pattern of H3K4me3 and H3K27me3 during oogenesis (18, 67, 111, 113). H3K4me3 is usually distributed at active promoters in somatic cells and embryonic stem cells. However, during oogenesis, noncanonical broad H3K4me3 accumulates at unmethylated intergenic regions, overlapping with partially methylated domains in oocytes (102). Surprisingly, the accumulation of noncanonical H3K4me3 seems to be required for silencing genome-wide transcription during oocyte maturation, as several groups found that, when they removed these noncanonical H3K4me3 marks in oocytes, the transcription activity increased. Actually, the H3K27me3 also seems to be unusual in oocytes. This noncanonical H3K27me3 broadly accumulates at partially methylated domains during oogenesis and has been suggested to function in imprinting maternal genes during early embryo development (48).

2.3. Dynamic Epigenome During Early Embryo Development

After global erasure has occurred and the epigenome has been reestablished during gametogenesis, fully differentiated mature gametes need to acquire the totipotent ability to generate a fully new individual. Along with the first several cleavages of zygotes, early embryos experience another drastic global epigenetic reprogramming to restore the totipotency and further specify the inner cell mass and trophectoderm fates. Over the last several years, substantial progress has been made in basic aspects of embryo development research to elucidate the epigenetic change. A large number of epigenetic techniques have been used to achieve a fine-grained analysis at the genome-wide level.

During the development of human preimplantation embryos, there are three waves of global demethylation: one 10–12 hours after fertilization, one from the late zygote stage to the four-cell stage, and one from the eight-cell stage to the blastocyst stage (114). The major wave of genome-wide demethylation is complete at the four-cell stage, and DNA methylation reaches its lowest level in the blastocyst stage. It has been thought that the entire process of preimplantation development is accompanied exclusively by the genome-wide demethylation process. However, recent studies have shown that there are actually two strong waves of de novo DNA methylation during the interval of DNA demethylation, indicating that the global DNA methylation reprogramming is in fact a dynamic balance between strong genome-wide demethylation and local remethylation (**Figure 2**). More importantly, regions that gain methylation are strongly enriched for evolutionarily younger subfamilies, such as the Alu and LINE1 retroelements, which are transiently active during preimplantation development. De novo methylation of these regions may play roles in repressing their transcriptional activity and protecting genome stability during zygotic genome activation (ZGA) (85, 114).

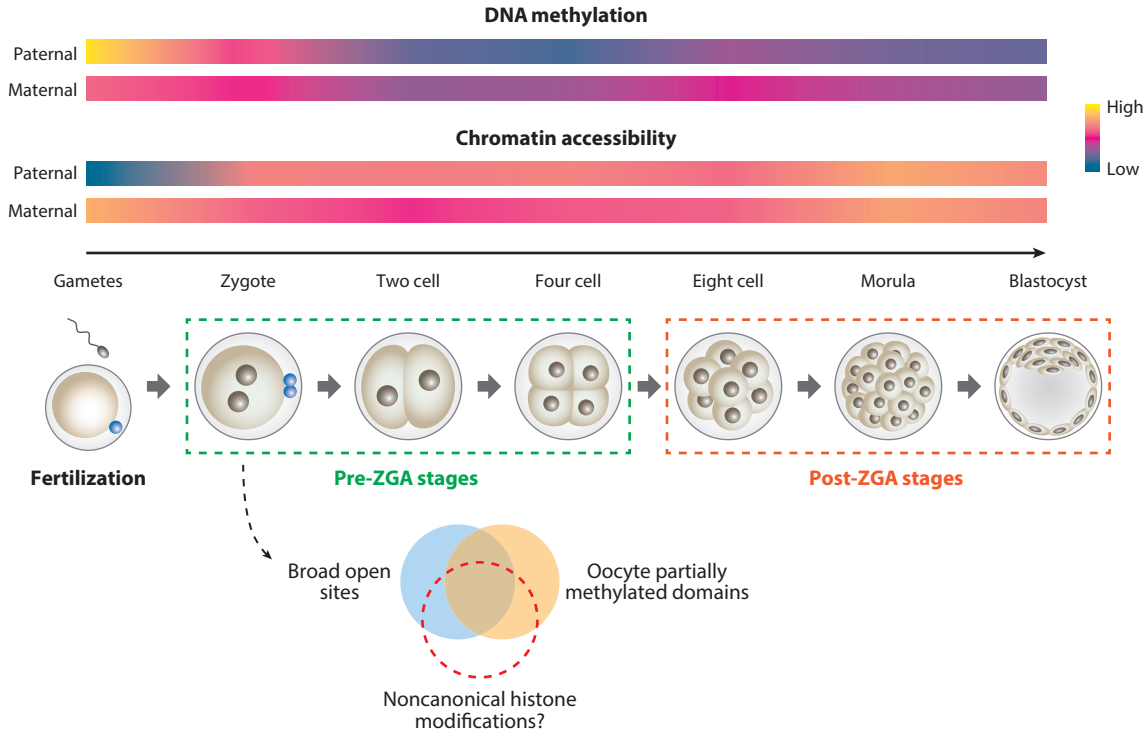

Figure 2

Dynamic epigenomes of human preimplantation embryos. After fertilization, the DNA methylation of the paternal and maternal genomes is quickly erased and dynamically balanced between strong genome-wide demethylation and local de novo methylation. The major wave of genome-wide demethylation is complete at the four-cell stage, and DNA methylation reaches its lowest level in the blastocyst stage. Imprinted genes evade global DNA demethylation throughout preimplantation development. Accompanied by global DNA demethylation, chromatin accessibility is gradually established. After fertilization, the overall accessibility of the two parental alleles quickly becomes similar. In humans, zygotic genome activation (ZGA) takes place from approximately the four-cell stage to the eight-cell stage. In pre-ZGA embryos, unique broad open chromatin is highly enriched at oocyte partially methylated domains.

In fully differentiated gametes, the DNA methylation level in the sperm genome (median of ~82%) is much higher than that in the oocyte genome (median of ~55%) (39, 85, 114). Upon fertilization, the paternal genome is demethylated much faster than the maternal genome at the zygote stage. After the two-cell stage, the DNA methylation in the paternal genome is lower than that in the maternal genome throughout human preimplantation and postimplantation development and in both embryonic and extraembryonic lineages. However, the functional significance of such parent-specific methylation remains unclear, as the majority of DNA methylation differences between parental genomes (except for known imprinting control regions) do not seem to contribute to allele-specific gene expression in the blastocyst stage (39, 114).

In addition to the global erasure of DNA methylation in human preimplantation embryos, the highly dynamic landscape of chromatin accessibility in human early embryos has been recently revealed (33, 63, 103) (**Figure 2**). Mapping the open regions of chromatin during embryonic development can help us to identify key regulatory elements and key transcription factors and to explore the relationship between chromatin open state changes in a dynamic transcriptome network. There are several powerful tools to interrogate genome-wide chromatin accessibility, including ATAC-seq, DNase-seq, and NOMe-seq. To explore the chromatin accessibility of human early

embryo development, all of these techniques have been optimized to minimize the usage of input cells. In the past few years, low-input (ATAC-seq and DNase-seq) and single-cell (scCOOL-seq and single-cell NOMe-seq) techniques have been developed that can overcome the limited resources of human gametes and early embryos and dissect the dynamic landscapes of chromatin accessibility.

In the gamete stage, the chromatin of the oocyte is much more accessible than that of the sperm, as expected given that the sperm genome is tightly packaged by protamine. After fertilization, the overall accessibility of the two parental alleles quickly becomes similar, suggesting that the drastic allele-specific chromatin accessibility reprogramming occurs at this stage (63). During human preimplantation embryo development, the gradually established promoter chromatin accessibility is usually associated with the corresponding gene expression levels (63). The major ZGA takes place at the eight-cell stage in human embryos. Consistent with transcriptomic results, human preimplantation embryos could cluster into two distinct classes—those in pre-ZGA stages (from the zygote stage to the four-cell stage) and those in post-ZGA stages (from the eight-cell stage to the blastocyst stage)—based on the promoter open chromatin states, suggesting that the most dramatic chromatin remodeling occurs at the ZGA stage (103). Moreover, inhibition of RNA polymerase II–mediated transcription by α-amanitin in human zygotes significantly impairs the transition of chromatin states between pre-ZGA and post-ZGA stages, indicating a transcription-dependent mechanism of establishing the post-ZGA chromatin accessibility landscape (63, 103).

Although the time point of ZGA is different between mouse and human embryos, the unusual widespread open chromatin in these species has a similar pattern in pre-ZGA embryos (36, 63, 102, 103). In pre-ZGA embryos, accessible chromatin preferentially enriches at CpG-rich promoters and DNA hypomethylated domains in human oocytes. After ZGA, a large fraction of pre-ZGA distal regions are lost when accessible chromatin is established at putative regulatory elements (such as enhancers). Whether the unusual broad open chromatin in pre-ZGA embryos contributes to transcription regulation remains unclear. So far, there is no clear evidence supporting the idea that these open chromatin regions have roles in regulating minor ZGA gene expression in human embryos (103). By contrast, broad open chromatin in mouse pre-ZGA embryos, which overlaps with noncanonical broad maternal H3K4me3 and hypomethylated regions of oocytes, has been proposed to act as chromatin harbors that facilitate docking or sequestering transcription resources when the genome is silenced (102, 103). Whether this is the case in human embryos should be tested in the future.

Due to the limited resources of human early embryos, genome-wide histone modifications and the functional importance of dynamic epigenomes have not been tested in human samples. Genome-wide study of histone modification marks relies on ChIP-seq, which usually requires millions of cells to enrich enough specific regions, depending on the different genome enrichment of histone marks. Collecting millions of female gametes and early mammalian embryos is a great challenge even in mice. Therefore, early studies of female gametes and early mammalian embryos mainly used immunofluorescence staining until the recent development of low-input ChIP-seq techniques [such as small-scale TELP-assisted rapid ChIP-seq (STAR ChIP-seq) (111), ultra-low-input ChIP-seq (ULI-ChIP-seq) (67), and micro-ChIP-seq (18)], which require only hundreds of cells. However, collecting hundreds of cells remains difficult for human oocytes or early embryos. Nonetheless, several groups have explored different histone marks (including H3K4me3, H3K9me3, H3K27me3, and H3K27ac) in mouse gametes and early embryos (18, 67, 98, 111). These pioneering studies not only depicted the landscapes of different histone modifications during early mouse embryo development but also, more importantly, revealed the specific contributions of noncanonical H3K4me3 and H3K27me3 to oogenesis and early embryo development, highlighting the necessity of genome-wide study of histone modifications.

3. IMPRINTING AND DISEASE

Genomic imprinting is a specific epigenetic phenomenon in which some alleles that were inherited from both parents express only a maternal or paternal copy of the genes and silence the other copy, which leads to a monoallelic expression pattern. If a certain imprinted gene exhibits inhibition at a maternal allelic locus but not at the paternal allelic locus, it is called a maternally imprinted gene; conversely, one that exhibits inhibition at a paternal allelic locus but not at the maternal allelic locus is called a paternally imprinted gene. The imprinted genes from a paternal or maternal source have specific sites and patterns. The presence of genomic imprinting was first confirmed in 1991 by the deletion of *Igf2* (insulin-like growth factor 2) in mice. The paternal *Igf2* knockout mice showed growth deficiency and could no longer express Igf2; however, the maternal *Igf2* knockout mice were phenotypically normal (21). Nearly 150 imprinted genes have been found in mice, half of which have functions in humans (77). Most imprinted genes gather in clusters and are controlled by the imprinting control element, also known as the imprinting control region or imprinting center.

Monoallelic gene expression is controlled by asymmetrically marked epigenetic modifications, which are inherited from the parental germ cells. DNA methylation is the canonical mark to imprint genes and can be passed through generations. During gametogenesis, the DNA methylation of imprinting regions from the previous generation is globally erased and reestablished in a manner that depends on the sex. After fertilization, the imprinting regions evade the genome-wide DNA demethylation, are maintained through early development, and are further passed to somatic cells. Several proteins have been identified in mice, including Pgc7/Stella, Zfp57, Trim28, Uhrf1, and Dnmt1 (3); these proteins are essential for maintaining the DNA methylation of imprinting regions during early embryo development. More recently, the development of low-cell-input epigenetic sequencing techniques has enabled the identification of H3K27me3 as a noncanonical maternal imprinting mark, which mediates the repression of maternally imprinted genes (48). However, whether other repressive histone modification marks, such as H3K9me3 and H2AK119 ubiquitination (H2AK119ub), could contribute to the establishment of parent-of-origin imprinting remains unexplored.

It has been well established that imprinted genes not only have an important influence on embryo or placental development before birth but also play an important role after birth. Studies of the postpartum stage have shown that imprinted genes affect a wide range of biological processes in adulthood, and imprinted genes appear to be key regulators in life. Twelve imprinted disorders have been well defined to date. Apart from Birk–Barel mental retardation, precocious puberty, maternal uniparental disomy of chromosome 20 syndrome [upd(20)mat], and Schaaf–Yang syndrome, the eight other imprinted disorders are all related to the aberrant regulation of DNA methylation: transient neonatal diabetes mellitus, Angelman syndrome, Prader–Willi syndrome, Beckwith–Wiedemann syndrome, Silver–Russell syndrome, pseudohypoparathyroidism, Kagami–Ogata syndrome, and Temple syndrome (86).

4. ENVIRONMENT, EPIGENETICS, AND DISEASE

4.1. Assisted Reproductive Technologies

ARTs are the common clinical treatments for infertility and have helped millions of couples to have their own children. In vitro fertilization (IVF) is the dominant form of ART, and the use of it with another technique, preimplantation genetic diagnosis, can help identify embryos that are free of inherited mutations, which consequently prevents the transmission of inherited genetic diseases. Although the use of IVF with preimplantation genetic diagnosis has shown some

promising results, the success rate of IVF remains relatively low, as it has a high frequency of implant failure or incomplete pregnancy even with embryos that were selected based on static morphological assessment and developmental kinetics. Moreover, a growing amount of evidence suggests that ARTs may cause an abnormal epigenome in the offspring and increase the risk of long-term health issues (9, 19). Although this technology has been used by many infertile couples, its safety and adverse influences on future generations raise a variety of concerns. Indeed, the entire process of traditional ART procedures, including superovulation, fertilization, and preimplantation embryo development, occurs in the time window of drastic global epigenetic reprogramming, when the embryonic epigenome may be more vulnerable to external changes. IVF, in vitro maturation, intracytoplasmic sperm injection, frozen embryo transfer, preimplantation genetic diagnosis, and preimplantation genetic screening technologies involve environmental factors such as in vitro temperature, chemical factors such as different culture media and cryoprotectants, and physical factors such as artificial intracellular injection and biopsies, which may disturb the natural developmental process of germ cells and embryos, especially in their epigenetic aspects (93).

Over the past few decades, clinical studies have revealed an increased incidence of maternal complications after ARTs (49). An increased frequency of birth defects—including low birth weight (79), congenital malformations (45), and cardiovascular, musculoskeletal, and endocrine abnormalities—has been reported as well (27). Moreover, imprinting disorders such as Angelman syndrome and Beckwith–Wiedemann syndrome have been associated with ARTs (7). Several human epidemiology studies have found an association between ART procedures and a high frequency of imprinting syndromes, although the prevalence of the disorders remains extremely low (47, 101). Additionally, imprinted gene defects may cause a series of adverse pregnancy outcomes, including an increased rate of spontaneous abortion (68).

A large number of basic research studies have shown that ART may induce unimprinted gene dysregulation and imprinting deletions, which could lead to imprinted genomic diseases in offspring (9, 13, 15, 20, 102). As early as 1998, a study of primate models produced by intracytoplasmic sperm injection found an asynchronous remodeling of chromatin decondensation of the male pronuclei (42, 46). In recent years, imprinted genes that were affected by ART have been discovered. Fernandez et al. (29) and Goel et al. (35) reported that ART may cause pseudohypoparathyroidism type 1B. Han et al. (40) reported that one case of Prader–Willi syndrome was associated with ART. These reports suggest that ART may increase the risk of imprinted disorders in the offspring. In addition, many studies have shown that the culture media used in IVF cycles can affect implantation and pregnancy rates because they affect the quality of the embryo (31). By comparing the effects of superovulation, IVF, and in vitro maturation on imprinted genes in mouse blastocysts, Chen et al. (14) found that superovulation had a greater impact on the genome and *Grb10* DNA methylation level, as well as on *Grb10* and *H19* expression, than IVF and in vitro maturation did. Nonetheless, some studies have shown that ART did not increase the risk of abnormal expression of *H19*, *IGF2*, and *SNRPN* and DNA methylation (51). Tang et al. (89) showed that abnormalities of imprinting genes in sperm after intracytoplasmic sperm injection did not have a significant effect on progeny gene imprinting. Derakhshan-Horeh et al. (22) showed that verification of embryos from day 3 did not affect the methylation level in the *H19/IGF2* region. Therefore, whether imprinting disorders are directly related to ART remains controversial.

Ventura-Juncá et al. (92) have suggested that analyzing the epigenetic profiles of children and adults born by IVF could reveal the link between epigenetic alterations in early developmental stages and pathologies in adult life. Scientists have already done a large amount of research on the epigenetics of newborn babies conceived naturally and those conceived via IVF and have

integrated almost all of the imprinted differentially methylated regions (DMRs). In a study that examined the effects of ART on the stability of DNA methylation at DMRs in twins conceived by IVF, Li et al. (64) found that IVF-conceived twins had slightly increased levels of epigenetic variability in the H19/IGF2 DMR and KvDMR1 in their umbilical cord blood, whereas naturally conceived twins showed no significant differences. They also found that the H19 DMR in the placenta and buccal epithelium was hypomethylated in IVF-conceived individuals.

ART procedures may have adverse impacts on the global epigenetic reprogramming during gametogenesis in early embryo development. No epigenetic marks other than DNA methylation have been explored so far. H3K27me3 has been revealed as a maternal imprinting mark independent of DNA methylation in mice. Whether ART causes imprinting disorders by interrupting proper H3K27me3 reprogramming needs further investigation.

4.2. Other Factors: Parental Diseases and Lifestyle

While the potential adverse epigenetic effects of ART on humans remain uncertain, it seems that almost every step involved in ART can disturb the epigenome to some degree. However, subfertile patients themselves may be an important reason for changes in the fetal epigenome. Litzky et al. (66) reported that IVF operation did not increase the risk of placental imprinting gene errors, but the parents' subfertility did.

4.2.1. Parental diseases. Many studies have found that parental diseases affect fetal health in different ways. Studies have shown that some diseases, such as polycystic ovary syndrome, can be inherited by the next generation. Although the specific mechanism of heredity is not clear, it is considered to be related to epigenetic regulation. In addition, studies have shown that metabolic diseases may cause an abnormal intrauterine environment and epigenome modifications in the fetus, leading to metabolic disorders and even diseases in adulthood. Tumors and cancers can be transmitted to the next generation by susceptible genes, and recent studies have started to pay attention to epigenetic influences.

Chronic metabolic disorders, such as obesity, can affect fetal health in both the short term and the long term (for a review, see 12); some of these disorders can even be inherited by the next generation (for a review, see 44). The mechanisms have been widely studied in both animal models and humans. With the increasing prevalence of obesity among pregnant women, the number of studies on the impact of maternal obesity on the health of offspring has grown dramatically in recent years. Maternal obesity may affect metabolic disorders in offspring, such as insulin resistance, which can lead to diabetes (44). It may also affect vascular dysfunction, which can lead to cardiovascular diseases (23). The pathogenesis of these diseases includes mainly the effect of epigenetic inheritance on fetal adipose tissue (5), the pancreas, muscle tissue, the liver, the vasculature, and brain development (44). Paternal obesity could also influence the offspring epigenome through sperm, causing prediabetes in the next generation (100).

Polycystic ovary syndrome, a complex endocrine disorder that is widely believed to be transmitted to subsequent generations (94), can cause infertility in women of reproductive age. Results from animal model studies show that excess androgen can affect the maternal intrauterine environment, which can then induce epigenetic changes in the fetus that lead to health problems, such as reproductive and metabolic dysfunction, in adulthood (24).

4.2.2. Parental lifestyle. In 1990, the British epidemiologist David Barker proposed the Barker hypothesis, which suggests that malnutrition during pregnancy is one of the important origins of early adult heart and metabolic disorders (2). He believes that the intrauterine environment

permanently shapes body structure, function, metabolism, and the possibility of disease in adulthood. With the establishment of the developmental origins of health and disease (DOHaD) concept, Barker's hypothesis is now widely accepted and has been verified by many studies (96).

During pregnancy, the lifestyle and habits of the parents (e.g., diet and smoking) may greatly affect the health of the fetus through epigenetic regulation. High-fat and low-protein diets have been reported to cause changes in the epigenetic regulation of the fetus, and some of them may even cause diseases such as metabolic disorders in the fetus (for reviews, see 71, 83, 87). A well-known example is that children born during the Dutch famine at the end of World War II had poorer glucose tolerance than children born the year before the famine. A low-protein diet during gestation is also associated with increased incidence of diabetes and growth defects in the next generation (110). A high-fat diet could affect not only the F2 generation (25) but also the F3 generation (26) by passing an aberrant methylated promoter of the growth hormone secretagogue receptor to the offspring.

Unhealthy habits may also affect later generations; for example, smoking and alcohol consumption have been regarded as risk factors that alter epigenetic regulation. A father's alcohol consumption could affect offspring mental development, not through DNA methylation in the sperm but rather through an RNA-mediated mechanism or altered chromatin remodeling in imprinting genes (59). Maternal ethanol exposure could cause aberrant DNA methylome regulation and lead to potential health issues, such as postnatal growth restriction and craniofacial dysmorphology (54). Furthermore, maternal smoking during pregnancy could generate differential methylation across the genome, which leads to potential health concerns in the offspring (52).

Epigenetics can sense and respond to external changes in order to adapt to environmental changes. However, during the development of gametes and early embryos, global epigenetic reprogramming is susceptible to external factors, such as ART procedures and the health status and lifestyles of the parents (**Figure 3**). An aberrant epigenome passed to the offspring can lead to health problems in the fetus or in later life. The trait gained from the parents might then be inherited by the next several generations even though they might not be exposed to the same environmental factors. A better understanding of the regulation of human epigenetic reprogramming during gametogenesis and early embryo development may contribute to the development of strategies to prevent or clinically intervene in infertility and other diseases.

5. CONCLUDING REMARKS

In the past several years, low-input epigenetic sequencing techniques have greatly increased our knowledge of epigenetic dynamics during gametogenesis and early embryo development at different molecular layers. From germ cells to embryos, two waves of global DNA methylation reprogramming occur, accompanied by global chromatin reorganization. DNA methylation has been the most extensively studied epigenetic mark during gametogenesis and early embryo development. It plays many important roles in these processes, including repressing the activity of transposable elements and imprinting genes, and aberrant DNA methylation reprogramming causes infertility or health issues in offspring.

Chromatin accessibility is another layer of epigenetics. Properly established chromatin accessibility is directly linked to the generation of a cell type–specific transcriptome network. Open chromatin is associated with putative transcription factor binding sites, which could help to identify the hierarchy of transcription factors. For example, with the help of motif enrichment analysis of open chromatin regions in human eight-cell embryos, Oct4 has been identified as a key regulator during ZGA (33).

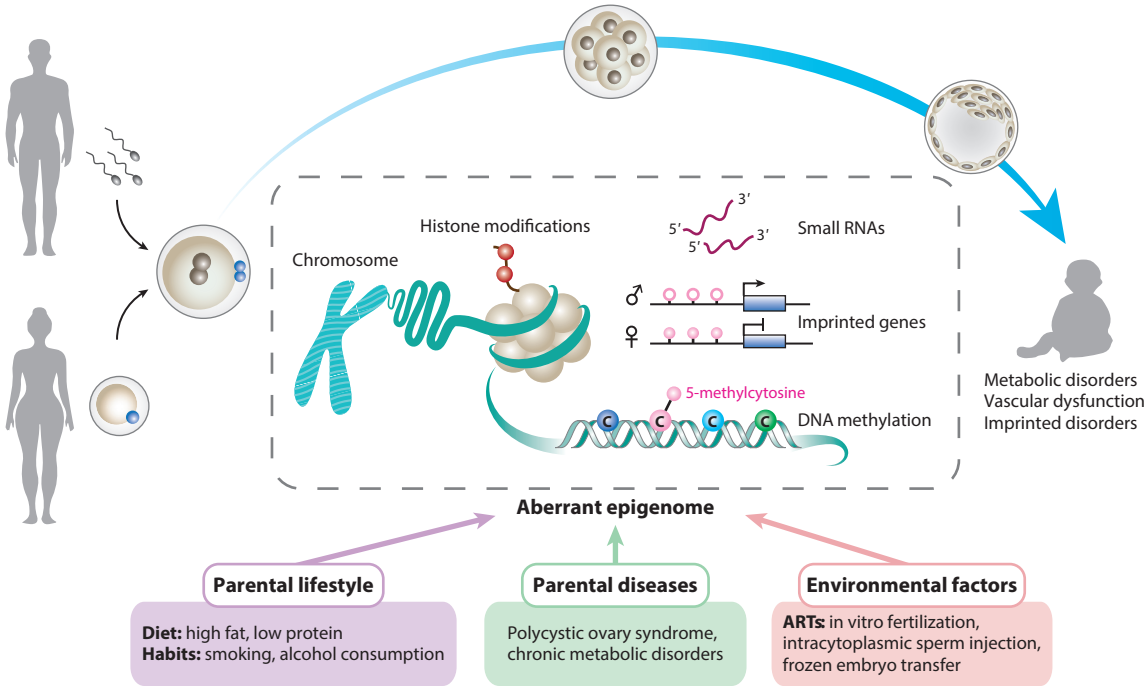

Figure 3

Potential risk factors and aberrant epigenomes. Epigenomes are vulnerable to external changes, especially during global epigenetic reprogramming in gametes and early embryos. Parental lifestyle, parental diseases, and use of assisted reproductive technologies (ARTs) may have adverse impacts on the epigenomes of offspring, transmitted through small RNAs and 5-methylcytosine. The imprinted genes are important epigenetic phenotypes in mammals that are regulated by DNA methylation and histone modification. Disturbance of proper epigenome reprogramming could cause imprinted genes to be misregulated and increase the risk of long-term health issues, such as metabolic disorders, vascular dysfunction, and imprinted disorders.

Although ChIP-seq has been optimized to minimize the required number of cells (several hundred) for the analysis of genome-wide histone modifications, such experiments have not been done in human oocytes and early embryos. Studies from mouse oocytes and early embryos have provided novel insights into histone modifications during early embryo development. For instance, during oogenesis, noncanonical broad H3K4me3 is gradually established. Interestingly, this noncanonical H3K4me3 seems to contribute to the genome-wide transcription silencing in oocytes and pre-ZGA embryos. In addition, maternal H3K27me3 has been revealed as a noncanonical imprinting mark independent of DNA methylation. The genome-wide dynamics of H3K4me3 and H3K27me3 in human early embryos remain unknown. As the required number of cells is further reduced, we would like to see these techniques applied in human oocytes and early embryos in the near future. Fully understanding the dynamics of different epigenetic molecular layers during gametogenesis and early embryo development could provide a basis to improve ART procedures and ultimately prevent infertility.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize to all those whose work we did not highlight owing to space restrictions. We thank Jim Duncan for editing the manuscript and an anonymous reviewer for constructive comments. We also thank our lab members Yi Qu, Long Jin, Ming Yang, Xixi Liu, Yidong Chen, Xinyi Ma, Cong Zhang, Fan Zhai, Qingyuan Qin, and Qianying Guo for their critical input and comments on this review. This work was supported by the Foundation of National Natural Science of China (grants 31522034, 81521002, 81730038, and 31701261). Y.W. was supported in part by a postdoctoral fellowship at the Peking-Tsinghua Center for Life Sciences.

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