Chapter

Epigenetics in Male Infertility

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Abstract

Male infertility is a complex medical condition, in which epigenetic factors play an important role. Epigenetics has recently gained significant scientific attention since it has added a new dimension to genomic and proteomic research. As a mechanism for maintaining genomic integrity and controlling gene expression, epigenetic modifications hold a great promise in capturing the subtle, yet very important, regulatory elements that might drive normal and abnormal sperm functions. The sperm's epigenome is known to be marked by constant changing over spermatogenesis, which is highly susceptible to be influenced by a wide spectrum of environmental stimuli. Recently, epigenetic aberrations have been recognized as one of the causes of idiopathic male infertility. Recent advances in technology have enabled humans to study epigenetics role in male infertility.

Keywords: Male infertility, Epigenetics, Environmental epigenetics

1. Introduction

Male infertility is a complex condition of which genetic, epigenetic and environmental lifestyle have been identified as major contributing factors [1–3]. In spermatogenesis, which is a complex of multistep differentiation process, millions of mature spermatozoa are daily produced by fertile male. In addition, this process comprises a variety of unique genetic and epigenetic mechanisms that eventually generate haploid sperm, which provides half of the genetic material and epigenetic information that is needed to create a new life upon fertilization. The sperm's differentiation is error prone and problems at all stages of spermatogenesis might contribute to male infertility [4]. It is believed that epigenetic modifications are essential to regulate normal gonadal development and spermatogenesis. This includes the normal distribution of variant epimarks controlling the testis-tissue specific chromatin compaction and the resultant gene expression accordingly. In this regards, several lines of evidence have highlighted the present of abnormal epigenetic marks in somatic and germ testicular tissues that are associated with impaired fertility or poor semen criterions. Hypermethylated of genes involve in PIWI -associated small RNAs (piRNAs) have been observed in testicular tissues of males having various forms of fertility problems [5]. Within this context, studies have reported disrupted epigenetics patterns of cells from different testicular tissues including Leydig [6] and Sertoli cells [7]. Interestingly, in patients with low testicular volume have been reported to have lower chromatin compactness and poor sperm quality [8].

At present, efforts have been concentrated on understanding the potential key role for epigenetic modifications in male reproduction health and the prevention of paternal disease transmission [9]. Epigenetics is defined as molecular factors or processes around DNA that regulate germline activity independent of DNA sequence and are mitotically stable [10]. Epigenetic changes are also a set of factors that affect the expression of genes, but do not affect the DNA sequence. In this chapter, we review the epigenetic marks in normal and abnormal human sperms, the influence of environmental stimuli on germ cells' epigenetic modifications in relation to male infertility, and technologies used for the analysis of epigenetic modifications associated with male infertility.

2. Epigenetic profile in normal sperms

The chromosomes of sperms are arranged in a hairpin-like structure, with the centromeres being confined to the interior of the nucleus and the telomeres being at the periphery [11]. The DNA of sperm is packed with specific, small, basic proteins into a tight, almost crystalline, status that is at least six times more condensed than that in mitotic chromosomes [12]. In sperms, the somatic cell histones are replaced with 90–95% of specific basic nuclear proteins known as protamines, leading to highly packaged chromatin. There are two types of protamines (P1 and P2). The P1 protamine is present in all of the mammalian species while P2 promatine is a family proteins formed by the P2, P3 and P4 components present in some of the mammalian species. Actually, protamines have many functions, such as enabling faster sperm movement and, thus, having the potential to fertilize the oocyte first. In addition, they are involved in the imprinting of the paternal genome during spermatogenesis [13]. In some of the mammalian species, including humans, this replacement involves a set of special proteins, i.e. a group of arginine (R)- and lysine (K)-rich proteins, known as transition nuclear proteins (TPs) [14]. The major TPs are TP1 and TP2. TP1 play an important role in the initiation of chromatin condensation and/or cessation of transcriptional activity during mammalian spermatogenesis [12], while TP2 is closely linked to the two protamine genes [15], suggesting that they arose by gene duplication and might have retained common functions. About 5–10% of nucleohistone component retains within sperm chromatin and provide a means for further epigenetic regulation. Expression of these TPs is presumed to regulate changes in chromatin occurring as part of the condensation process [16].

Along with the proposed role for the sperm epigenome marks in shaping the embryonic development, they also could be used for male's gamete stratification based on the comparison to their normal counterparts. Such marks include histone retention and modification, and protamine incorporation into the chromatin. In addition, DNA methylation and spermatozoal non-coding RNAs appear to play important roles in the epigenetic state of mature sperms. These epigenetic marks may also reveal a historical record of spermatogenesis, future functions in embryogenesis, and fertilization [17].

2.1 Histone modifications

Histone modifications are type of epigenetic marks that can potentially be transmitted from parent to offspring [10]. During mitosis and meiosis, male germ cell DNA is packaged in nucleosomes comprised of histone 2A (H2A), histone 2B (H2B), histone 3 (H3) and histone 4 (H4), all of which are susceptible to covalent modifications, such as methylation, acetylation, ubiquitination and

phosphorylation. Each of these histone chemical modifications works alone or in concert to influence gene repression and/or activation [18]. Histone methylation is controlled by histone methyltransferases (HMTs) which modifies lysine (K) residues of H3 or H4 and can promote gene activation and/or repression [19]. For example, monomethylation, dimethylation and trimethylation modifications of H3K4, H3K9 or H3K27 display tightly controlled temporal expression and ensure proper progression through spermatogenesis [20, 21]. Demethylation in histone is controlled by histone demethylases (HDMs). On the other hand, histone acetylation of lysine residues is dynamically regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), both of which are shown to be essential for spermatogenesis [22]. Histone acetylation relaxes chromatin and promotes polymerase II (Pol II) gene transcription, whereas deacetylation is associated with gene silencing [23]. Acetylation levels on both H3 and H4 are completely removed during meiosis.

Histone phosphorylation occurs at serine (S) residues of all core histones and is generally associated with gene activation [24]. However, H2Ax phosphorylation (also known as gH2Ax) in germ cells confers the formation of Y sex body during spermatogenesis and is a marker for telomere clustering and double stranded breaks [25]. Moreover, ubiquination is another epigenetic mark in histone and its effects are dependent on the core histone modified. For example, ubiquination of H2A associates with transcriptional repression in sperm, whereas mono-ubiquination of H2B is linked to transcriptional activation in sperms [26].

Although most histones are replaced with protamines during the elongating spermatid stage, some of the modified nucleosomes escape the histone to protamine transition and, as a result, are retained in the mature sperm. The retained nucleosomes are enriched at CpG rich sequences that lack DNA methylation. The non-canonical histone H3.3 variant was shown to be abundant and trimethylated at K4 in these nucleosomes, while the canonical histones H3.1 and H3.2 are trimethylated at K27 [27]. Other non-canonical histone variants were reported previously to be present in the retained nucleosomes of the mature sperm; TH2B was observed in humans, whereas H2A-Bbd, H2AL1/L2, and H2BL1 were described in mammalian sperms [28].

2.2 DNA methylation

DNA methylation has been implicated in the development of spermatozoa and early embryos through the regulation of gene expression [29]. DNA methylation process primarily involves the addition of methyl groups to the 5′ carbon at cytosine residues preceding guanine nucleotides. These groups are linked together by phosphate bonds (CpG) utilizing a methyl donor like S-adenosylmethionine. CpG is clustered primarily in short CpG-rich DNA sequences named CpG islands. DNA methylation is catalyzed by a group of enzymes termed as DNA methyl transferases (DNMTs) that target these CpG islands. According to their structure and functions, DNMTs are divided into two major families in mammalian cells: maintenance methyltransferase (DNMT1) and *de novo* methyltransferases (DNMT3a, DNMT3b, and DNMT3L). In addition, DNA methylation can disrupt the process of transcription by inhibiting the binding of the transcriptional factors with the target sites. Also, the methylated cytosine residues act as the site for docking of various methylated DNA-binding proteins (MBD1, MBD2, MBD3, and Mecp2) that are recognized by various histone modifying enzymes like histone deacetylases (HDACs), which in turn can lead to gene repression [30]. Furthermore, methylcytosine can modify and potentially erase DNA methylation [22] by ten-eleven translocation protein 1 (TET1). TET1 belongs to a family of three proteins—namely, TET1, TET2,

and TET3, that catalyze the successive oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) [31, 32].

The proper regulation of DNA *de novo* methylation and demethylation is essential for the normal function of the mature sperm and early embryo [18, 33]. Dynamic erasure and reestablishment of DNA methylation marks is catalyzed by TET dioxygenases and DNA DNMTs, respectively, which are required for the formation of mature sperm as well as for embryogenesis [34]. Methylation analysis in genome-wide studies have demonstrated that the promoters of developmental genes in sperm cells are highly hypomethylated [35]. A previous gene ontology analysis demonstrated that hypomethylation in mature sperm cells promoted developmental transcription and signaling, which is bound by the self-renewal of a network of transcription factors of human embryonic stem cells, including OCT4, SOX2, NANOG, KLF4 and FOXD3 proteins [29]. Moreover, recurrent regions of the sperm genome demonstrate high degrees of methylation, while transposons manifest lower levels of DNA methylation [36–38]. The paternally expressed human gene, MEST/PEG1, remains unmethylated throughout all stages of sperm development in the adult life [39, 40]. By contrast, in male germ cells, H19 gene is methylated prior to meiosis at the spermatogonial stage of development [41]. Moreover, reinitiation of mitotic division of male germ cells during puberty coincides with an upregulation of DNMT1 within the spermatocytes. However, the levels of DNMT1 in spermatocytes are increased during the early stage of meiosis, and reduced in pachytene stage spermatocytes [42]. The DNA methylation loss is then followed by *de novo* DNA methylation throughout all stages of sperm cell maturation, with global CpG methylation levels of 70% in fully mature human spermatozoa, yielding approximately 4% of total cytosines methylated [43]. However, DNMT3L is the only enzyme that is expressed in sperms within a low level [44]. While, DNMT3a expression is upregulated in the testis prior to birth and during early postnatal life, and DNMT3b expression is downregulated during embryonic development and upregulated postnatally [45].

2.3 Non-coding RNA

Regarding the non-coding RNAs as germ cells epigenetic marks, there are several ncRNAs that contribute to the transgenic epigenetics in the developing embryo. Among these are the small non-coding (sncRNA) and the long non-coding RNAs (lncRNAs).

Small ncRNAs are involved in the control of sperm production. Male germ lineages express classes of sncRNAs, including Dicer-dependent microRNAs (miRNAs), tiRNAs (tiny), as well as Dicer-independent PIWI-interacting RNAs (piRNAs) [46]. miRNAs observed in spermatozoa, such as miR-122, has been described to participate in the post-transcriptional down-regulation of the transition protein 2 (TNP2) during spermatogenesis [47]. TNP2, together with transition protein 1 (TNP1), transitionally substitute some of the histones during spermiogenesis. This intermediate step precedes and facilitates protamine replacement that is required to compact the paternal genome into the relatively small sperm head [48]. Piwi-interacting RNAs (26–31 nucleotides) are specifically expressed in the gonads, and are thought to silence transposable elements, especially in the germline, protecting the integrity of the genome. piRNAs are involved in epigenetic inheritance and they mediate their effect through PIWI-proteins, a subfamily of the argonaute family of proteins.

Another kind of sncRNA, known as Piwi-interacting RNAs (piRNAs), are highly expressed in germ cells and required for male fertility [49]. Mechanistically, piRNAs

repress gene expression at both transcriptional level, by promoting *de novo* DNA methylation [50], and post-transcriptional level, by cleaving target transposon mRNAs [51].

Furthermore, lncRNAs is dynamically regulated during male germline development and may function to regulate gene expression at both transcriptional and posttranscriptional levels via genetic and epigenetic mechanisms. In the testis, expression profiles of lncRNAs can be detected at different ages and stages of development [52]. The Y- linked lncRNAs showed higher expression in pachytene spermatocytes. In human mature sperms, an association between lncRNA expression and sperm motility was indicated [53]. Mammals exhibit condensation and remodeling of their chromatin material during late spermatogenesis by omitting excess cytoplasm and replacing histones with protamine for spermatid individualization, leading to a highly compact sperm nucleus [54]. Enhancer-associated lncRNAs participate in transcriptional activation by acting over long distances on distal promoters, associated with protein factors, and by the modulation of chromatin structures [55].

The N6-methyladenosine (m6A) modification is the most prevalent internal RNA modification that has been defined as another important epigenetic and epitranscriptomic marker in eukaryotes [56]. m6A has notable influence on the regulation of gene expression at the post-transcriptional level, animal development, and human diseases [57]. The m6A modification mainly occurs in intragenic regions including coding sequences (CDS), stop codon flanking regions and 3′-UTR, especially the 3′-end of CDS and the first quarter of the 3′-UTR, also near transcription starting sites (TSS) [58], and the modification site is often on the conserved sequence of RRACH (R = A or G; H = A, C, or U) [59]. The m6A modification process is dynamically reversible and regulated by three kinds of regulatory proteins: methyltransferases (writers) such as methyltransferase like protein 3 (METTL3), methyltransferase like protein 14 (METTL14), Wilm's tumor-associated protein 1 (WTAP), Vir-like m6A methyltransferase-associated (VIRMA; also known as KIAA1429), RNA binding motif protein 15 (RBM15), and zinc finger CCCH domain protein 13 (ZC3H13); demethylases (erasers), such as obesity-associated protein (FTO) and AlkB family homolog 5 (ALKBH5); and m6A binding proteins (readers), such as the YTH domain family proteins (YTHDFs) and YTH domain containing protein 1–2 (YTHDC1–2), the insulin-like growth factor 2 mRNA binding proteins (IGF2BPs), heterogeneous nuclear ribonucleoprotein A2B1 (HNRNPA2B1), and eukaryotic translation initiation factor 3 (eIF3) [60].

A number of findings demonstrating the significance of m6A in male fertility and spermatogenesis. Methyltransferases (writers) especially METTL3 and METTL14 are widely expressed in different tissues in mouse and human, and essential for male fertility and spermatogenesis [61]. It found an ablation of *Mettl3* in germ cells severely inhibited spermatogonial differentiation and blocked the initiation of meiosis. Analysis of transcriptome and m6A profiling revealed that gene functioning in spermatogenesis had altered profiles of expression and alternative splicing [62]. Another study has proved that the lack of m6A by germ cell-specific inactivation of *Mettl3* or *Mettl14* results in spermatogonial stem cells (SSC) depletion due to significant changes in translational efficiency (TE) while double deletion of Mettl3 and Mettl14 in advanced germ cells leads to impaired spermiogenesis due to altered TE of m6A-containing transcripts [63].

On the other hand, ALKBH5 as demethylase is expressed in testes. It proved that m6A was increased in male mice with ALKBH5-targeted deletion, and the number of sperm released and incised caudal epididymis was significantly reduced, sperm morphology was abnormal and motility was greatly reduced. The results showed that fertility was impaired due to the abnormal apoptosis and production of a small

number of abnormal spermatozoa during meiosis [64]. Concerning FTO as another demethylase, it was reported that FTO expression alters by using Di-(2-Ethylhexyl) phthalate, and results in an increasing m6A RNA modification, deteriorates testicular histology, reduces testosterone concentration, down-regulates spermatogenesis inducer expression, enhances oxidative stress, and increases testicular cell apoptosis [65]. YTHDC2 is one of m6A binding proteins (readers), that highly expressed in mammalians testes [66]. YTHDC2-knockout mice showed defects in spermatogenesis and the germ cells do not develop to the zygotic stage [67]. The regulation of m6A transcription by YTHDC2 is the key to the success of meiosis in the mammalian germline.

3. Aberrant sperm's epigenetic modifications

Epigenetic modification, especially DNA methylation, plays an important role in determining the differentiation potential of mammalian cells and ensuring the normal development. The methylation changes of a specific gene in the parent sperm cannot directly lead to the defective phenotype of the offspring, but it is undeniable that the methylation of CpG islands affects a series of cascade gene expressions, resulting in abnormal gene crossover network expression [68]. The potential roles of epigenetic processes in the progress of sperm function and male fertilization were studied by many researchers. This includes studies of DNA methylation, histone modifications, chromatin remodeling, and ncRNAs roles in the development of gonads and spermatogenesis [3, 69]. Recently, most of studies have been concentrated on understanding the role of different epigenetics mechanisms in male reproduction health and spermatogenesis. Infertility is the inability of males and females to achieve a pregnancy after 12 months or more by natural means. The development and normal functioning of male reproductive system are thought to be highly sensitive to epigenetic changes. However, the epigenetic modifications underlying male infertility remain unclear [29]. The role of epigenetic modifications in infertility and their impact on male reproduction are summarized in this review.

3.1 Aberrant sperm's DNA methylation patterns

DNA methylation plays critical roles in the regulation of gene expression during the development of mature spermatozoa, which may lead to male infertility [69, 70]. The process of germ cell development is highly organized, starting from early fetal life and is finished in the adulthood. The epigenetic alterations occurring in germ cells, including DNA methylation, are significant for the healthy development of sperm function and for embryonic development [71, 72]. DNA methylation markers have been detected in the spermatogonia stage; therefore, the abnormal DNA methylation patterns observed in infertile men may be due to the failure of re-methylation in spermatogonia or alterations to methylation maintenance in spermatocytes, sperm cells or the mature sperm cell. In addition, abnormal DNA methylation may be associated with the abnormal activation of DNMTs [73, 74]. However, earlier studies on candidate genes have shown a strong association between abnormal semen parameters and aberrant DNA methylation in imprinted, testes-specific and other genes [75–77]. Genome-wide methylation analysis by using 450 K BeadChip on spermatozoal DNA from six infertile and six fertile men to identify DMCs showed that the methylation changes in a number of genes have been correlated with reduced sperm count and motility. Also, the loss of spermatogenesis and fertility was correlated with 1680 differentially-methylated CpGs (DMCs) across 1052 genes [35]. The DNA methylation profile of the

imprinted genes MEST and SNRPN DMRs have been recently investigated by using meta-analysis. Comparisons of sperm DNA methylation aberrations showed that these genes have higher methylation levels in idiopathic infertile men than in fertile men [78]. Among other differentially-methylatyed genes, CRISPLD1 may have a role in cellular adhesion, which is essential for fertilization [79]. Also, poor semen parameters showed to be most affected by H19 hypomethylation [80].

Moreover, many clinical studies have established that germ cells in adult male mice have a unique genome-wide pattern of DNA methylation. In testicular DNA, the level of DNA methylation is known to be high, which may be associated with the hypermethylation of the CpG rich region as compared with somatic tissues [81, 82]. However, a previous analysis demonstrated that altered expression of spermatogenesis genes was associated with abnormal DNA methylation. Interestingly, poor semen parameters in men were associated with these genes defects [29]. These finding are in line with other results that showed that the oligozoospermic men have different DNA methylation profiles as compared with normozoospermic controls [83]. As a result, the specific sperm DNA methylation in mammals is suggested to be essential for spermatogenesis, fertilization and early embryonic development.

DNA methylation profile of gametes is of a particular importance because it is one of the factors that control the expression of imprinted genes that are crucial for embryonic development, fetal growth, and post-natal behavior [84]. In addition, the results of rodent models suggest that DNA methylation may be involved in the pathogenesis of human male infertility through spermatogenesis alteration. DNA methylation is a reversible epigenetic mark and its effects may be reversed by using either demethylating agents, like DNA methyltransferase (DNMT) inhibitors, 5-azacitidine, and 5-aza-20 –deoxycytidine, or methyl donors, like choline, methionine, and folate [85, 86]. However, a new study suggest that sperm methylation is a possible mechanism of age-induced poor reproductive outcomes among couples undergoing infertility treatment and that it can be used to identify the possible candidate genes for mediating the effects [87]. On the other hand, sperm cells from Norwegian red bulls of inferior fertility have less compact chromatin structure, higher levels of DNA damage, and are hypermethylated compared with bulls of superior fertility [88].

The study of Ni et al. showed that the level of TET1–3 expression is pivotal for male fertility and that TET enzymes are successively expressed at different stages of human spermatogenesis [89]. More recently, it was also reported that modifications in 5hmC pattern in sperms are associated with male infertility. In these reports, infertile males were shown to contain higher rate of 5hmC than fertile males and infertility was correlated with defects in sperm morphology and a high sperm DNA fragmentation rate [90]. Recent results also showed that Tet1-deficient mice undergo a progressive reduction of spermatogonia stem cells and spermatogenesis and thus accelerated infertility with age. Tet1 deficiency decreases 5hmC levels in spermatogonia and downregulates a subset of genes important for cell cycle, germ cell differentiation, meiosis and reproduction, such as Ccna1 and Spo11, resulting in premature reproductive aging [91]. However, during spermatogenesis, 5hmC level is changed dynamically and correlated with gene expression, and RNAseq data shows that Tet1 gene is expressed in spermatogonia [92].

3.2 Disrupted sperm's histone marks

Demethylation of histone plays critical roles in the regulation of gene expression during the spermatogenesis, which may lead to male infertility [22]. In normal human sperm, histone modifications and their enrichment patterns suggest a highly regulated epigenetic landscape. However, it has been reported that aberrant histone

methylation and/or acetylation are implicated in the mature sperm in various forms of infertility [17]. Chon and colleagues investigated the histone post-translational modification status in normal and abnormal sperm samples; they found significant histone post-translational modification alterations in abnormal sperm samples compared to those of normal spermatozoa [93]. Sperm histones are involved in chromatin conformation and gene expression through various posttranslational modifications. Modification of sperm histones may influence the large scale demethylation wave after fertilization, altering the expression of offspring genes [68].

Multiple histone variants found in sperms play an essential role throughout spermatogenesis as well as in the mature spermatozoa. Among these, important nuclear proteins are histone 2A and B (H2A and H2B), histone 3 (H3), histone 4 (H4), and the testes variant (tH2B) [94]. The amino terminus of the four core histones (H2A, H2B, H3, and H4) stores a rich source of genetic information. The residues are subjected to enzymatic reactions to produce posttranslational modifications, which transmit epigenetic information transgenerationally. H3 at lysine 4 (K4) is specifically methylated by Set9, which is related to the activation of gene transcription [95]. La Spina et al. [96] investigated the acetylation status of H3K4Ac and H4K5Ac and the methylation profile of H3K4Me3, H3K4Me, H3K9Me2, H3K79Me2 and H3K36Me3 in abnormal and normal human sperm samples. The authors found partial heterogeneous modifications of histones and the existence of H3K4Me1, H3K9Me2, H3K4Me3, H3K79Me2 and H3K36Me3 marks in normal spermatozoa [96].

Several studies have shown that some novel Histone posttranslational modifications (HPTMs) are associated with sperm maturation disorders and dysgenesis. These abnormalities are closely connected with the bromodomain of BRDT, which can recognize histone Kac and recruit transcription complexes from chromatin to promote specific gene expression [97]. However, defects in either the replacement or the modification of histones might result in azoospermia, oligospermia or teratozoospermia, which leads to male infertility [98]. In general, deviations in the sperm histone code have been associated with sperm incompetency and decreased fertility [29]. For example, the role of dimethylation of lysine K4 on histones H3 (H3K4me2) is well-studied in sperm abnormalities. It has been detected at the promoters of transcriptionally active housekeeping genes and indispensable genes that play an important role in the development the spermatogenesis processes [99]. Also, studies in TH2A/TH2B double knockout male mice indicate its role in chromatin compaction and male fertility [94].

3.3 Sperm's chromatin remodeling and non-coding RNA

Chromatin remodeling is the dynamic modification of chromatin architecture to allow access of condensed genomic DNA to the regulatory transcription machinery proteins, and thereby control gene expression. Chromatin remodeling is a landmark event in spermatogenesis, during which transition of nucleohistone to nucleoprotamine takes place in male germ cells. It is initiated by histone hyperacetylation, followed by replacement of somatic histones with testis specific histone variants [98]. However, Sperm chromatin reorganization is an important process that allows spermatozoa to pack huge amounts of DNA into a small nucleus [3]. Patankar et al. [94] revealed that chromatin compaction is positively correlated with sperm- motility, concentration, viability and transcript levels of PRKAG2 and CATSPER B. Also, the authors found that the altered expression of TH2B associated genes in infertile individuals with sperm chromatin compaction defects indicates involvement of TH2B in transcriptional regulation of these genes in post meiotic male germ cells. This altered transcriptome may be either a consequence or a cause of abnormal

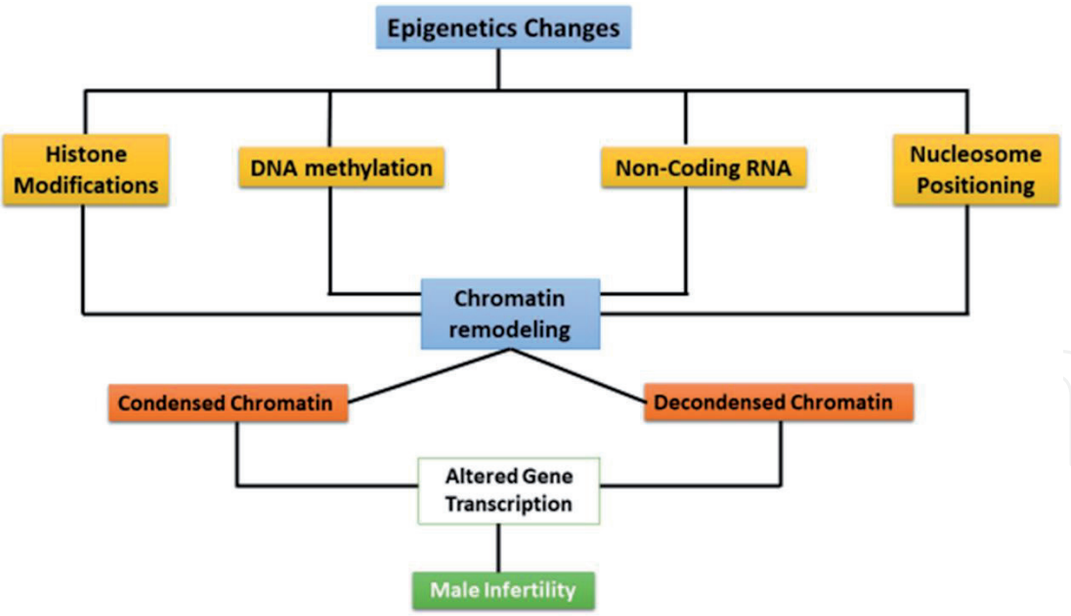

Figure 1.
A schematic diagram summarizing the main epigenetic changes associated with male infertility.

nuclear remodeling during spermatogenesis [94]. Indeed, spermatogenesis offers a unique process to study mechanisms of chromatin remodeling. However, this process is currently understudied and still poorly understood, mainly due to the complexity of the process itself and lack of in vitro experimental systems for studying it [100].

Non-coding RNAs (ncRNAs), as a mark in the epigenetics of germ cells, can be classified into two master groups according to their length as small non-coding and long non-coding RNAs (lncRNAs). However, many potential lncRNAs have been identified in male germ cell development and male infertility, but till date only few have been functionally characterized by gene specific studies [101]. Zhang et al. undertook sequencing to identify lncRNAs that differ in motile and immotile human sperms. While 9879 lncRNA genes (13,819 lncRNA transcripts) showed differential expression between motile and immotile sperms, three lncRNAs, i.e. lnc32058, lnc09522, and lnc98497, showed specific and high expression in immotile sperm in comparison to normal motile sperm [53]. Short ncRNAs can be grouped into three major classes called miRNAs, siRNAs and piRNAs. However, piRNAs are only expressed at the pachytene stage in spermatocytes and in round spermatids. These 30 nucleotide-long piRNAs are involved in sperm maturation and interact with the piwi proteins. Germ line mutations of piwi have been found to prevent piwi ubiquitination and degradation in patients with azoospermia [3, 102]. Understanding the mechanisms underlying non-coding RNA is particularly important in order to develop therapeutic strategies for male genital system diseases caused by abnormal sperm. Studies on the mechanisms underlying the regulation of non-coding RNA during spermatogenesis are still in their initial stages. Numerous issues remain, such as transgenerational inheritance of human epigenetic genes and the association between non-coding RNA and other epigenetic factors (**Figure 1**).

4. Environment and epigenetic in male infertility

The global environment has changed over time as a consequence of industrialization and the progressive accumulation of synthetic pollutants. Such compounds can present in every manufactured product with which humans have contact including cosmetics, food items and containers, packaging materials, toys,

agrochemicals. Some of these pollutants take the form of endocrine disruptors that could act with others to alter the ecological balances in natural populations and affected human health and associated with increased incidence of reproductive disease [103–105].

The development and normal functioning of male reproductive system are thought to be highly sensitive to environmental contaminants exposure/insults and metabolic status that could adversely affect sperm's number, quality and the reproductive health of the subjected individuals. In line with the thought that the epigenome is more vulnerable than the genome for such environmental insults, a large number of studies have investigated the role of epigenetic modifications in shaping endocrine functions and their potential influence on spermatogenesis. Due to the protracted period of replication and cell division along the continuous cycles of mitosis and meiosis in adult males spermatogenesis, it is thought that the accumulation of environmentally induced epigenetic are much greater in males than in females [106]. In addition to studies that highlighted dynamical reaction of sperm epigenome to a wide range of environmental and lifestyle stressors [105, 107]. Sperm epigenome is believed to be affected by a large number of biological factors (including aging, obesity, diet, endocrine disruptors and disease), environmental exposures (such as smoking, alcohol, medications, air pollutions, toxic waste socioeconomic stress) and life style (i.e. exercise intervention). These factors might contribute to primary sources of the increased male factor infertility and decline in seminal parameters [108]. In comparison to the reproductive system's genome, its cellular epigenetic landscape shows a high degree of plasticity, and thus it is more susceptible to be influenced by the environment insults.

Indeed, the different critical timeframes of spermatozoa development represent windows of susceptibility for epigenetic errors to occur and aberrations potentially induced by environmental insults, possibly affecting fertility and embryonic competence. In an attempt to address the question whether genetic predisposition or environment have significant impact on an individual being infertile, earlier monozygotic twins studies have concluded that socioeconomic environment seemed to influence relative magnitude and pattern with certain genetic background [109]. Based on the findings which emerged from the statistical analysis of heath survey on 1795 Vietnamese male twin cohort, "factors unique" to individual twins could influence to their infertile state more prominently than additive genetic or the common environment effects [110].

Considering both the high rate of unexplained male infertility (up to approximately 50%, [111]) and the lower sensitivity (15%) of semen analysis in predicting infertility [112], the epigenetic disruptions induced by environmental is hoped to contribute in a deeper understanding of male reproductive problems causality and evaluating forms of therapeutic strategies to counteract male infertility. Thus it is believed that environmental epigenetics is considered as the primary molecular actions involve in the increased male infertility and decline in seminal parameters [108]. In both animal model and human studies, a number of defined toxicants and other environmental stimuli exposures have reported to promote testis effects and alter the epigenetic marks that affect spermatogenesis and conferring poor sperm parameters and male infertility. It is expected that sperm differentiation anomalies are attributed to influence of epigenetic aberrations including histone modification and abnormal DNA methylation in imprinted and reproduction associated genes [113].

A large number of studies have shown that exposures to environmental factors, either synthetic or natural origins such as toxicants or nutrition, can have influence on testis biology and male fertility. However, the vast majority of environmental factors is believed to not induce alterations in the DNA sequence but more likely to

produce epigenetic alteration. Such environmentally disrupted epigenetic modifications are thought to generate phenotypic variation that includes the induction of disease such as subfertility and imprinting disorders [114]. In this regard, long term exposure of adult rat to butyl-paraben, previously known to affect male rodent reproductive parameters, including testosterone levels and sperm production, resulted in increased DNA methylation changes in the sperm [115]. In respect to the influence on imprinted gene, prenatal exposure to ethanol has also been shown to induce decreased spermatogenesis and loss DNA methylation of the imprinted gene *H19* in mice model studies [116]. Decreased DNA methylation of the *H19* has been suggested to be involved in human male infertility [117, 118]. Another interesting observation relates to exogenous follicle stimulating and luteinizing hormones to immature rats caused epigenetic changes represented by hypomethylation of seminiferous tubular and Leydig cells [119]. Studies have also shown that the epigenetic marks of spermatogenesis are dynamic and can be modulated by micronutrients environment interactions [120]. A number of different environmental toxicants, that take the form of endocrine disruptors, have been shown to promote exposure-specific alterations in the F3 generation sperm epigenome (DNA methylation). These pollutants include the influence of pesticides, insecticide such as DEET [121], hydrocarbon mixture (e.g. jet fuel [122]), dioxin [123] . The findings from aforementioned cited studies suggest that such effects could also induce transgenerational epigenetic phenotypes of inheritance disease and sperm epimutations.

4.1 Epigenetic mechanisms are sensitive to environment and lifestyle: response to antioxidant stress and life style influence as proof of principle

4.1.1 Antioxidant stress

The epigenetic profile of sperm cell has also shown to be influenced by oxidative stress. In this regard, environmental endocrine toxicants such as Bisphenol A are shown to promote male infertility through oxidative DNA damage leading epigenetic modifications in sperm cells mediated by hormonal imbalance [124]. Studies have shown a negative correlation between sperm DNA methylation and sperm DNA fragmentation, as well as for seminal reactive oxygen species (ROS) production. Interestingly, antioxidant supplementation appears to have the potential to reduce DNA damage and normalize sperm DNA methylation in infertile subjects [125]. ROS can lead to chronic inflammation and the latter is closely related to epigenetics as epigenetic aberrations can induce inflammation and vice versa. Various external factors that confer aberrant epigenetic alteration are thought to develop a pro-inflammatory phenotype that contributing to male infertility [126]. Sperm morphological anomalies associated aberrant DNA methylation patterns have been reported experimental rat exposed to uranium [127].

4.2 Life style

A further support to the notion that the main factor driving epigenetic remodeling is induced by external influences came from studies focused on the influence in life style. Modulating of life style factors has shown to influence the sperm's epigenetic landscape by inducing aberrant DNA methylation marks. Of interest, endurance training intervention for relatively short period, 3 months, has resulted in altering the methylation patterns of genes related to the development of the central nervous system, neurogenesis, neuron differentiation and linked to

numerous diseases such as schizophrenia and Parkinson's disease [128]. Similar finding was reported by Dokin and colleagues when losing weight induced through gastric-bypass give rise to dynamic changes in the epigenome of human spermatozoa in genes implicated in the central control of appetite under such environmental pressure [129].

In respect to the investigation of the potential influence of nutrition on the sperm epigenome, mice fed low protein diet induced substantial (~30%) increase in methylation at an intergenic CpG island ~50 kb upstream of Ppara [130]. Additionally, prenatal undernutrition can alter DNA methylation in the sperm of adult offspring at regions resistant to zygotic reprogramming such as in long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). Studies have concluded that utero undernutrition profoundly disrupt DNA remethylation during germline reprogramming, and contributing to germline hypomethylation [131]. Similarly, high fat diet (HFD) alters sperm cells epigenome of sperm cells reprograming, thereby affecting metabolic tissues of offspring throughout two generations [132]. Through such effect, HFD can modulate spermatogenesis via increasing chromatin compaction and affecting the transcription profile of the spermatozoid [133]. Within this context, HFD-induced obesity provides a link with disease related phenotypes in male infertility, i.e. the impaired quality of spermatozoa from obese men, and transgenerational epigenetic modifications [134]. Interestingly, an altered signature of piRNA, that is known to cause epigenetic changes, was identified in sperms from obese men and rats than their lean counterparts [132]. In line with this evidence, the expression pattern of piRNAs is significantly changed in SPZ when lean and healthy individuals subjected to endurance training highlighting the potential effect of lifestyle on the dynamic expression of sperm [135].

The daily consumption of nutrients and micronutrients represents a source of methyl and acetyl groups that are necessary for the epigenetic dynamics and thus epigenetic modifications targeting spermatogenesis markedly affected by diet depletion of specific nutrients. In this regards, human sperm quality showed to be enhanced by a dietary supplementation with folate [136] and vitamin D [137]. The latter is known to have a key role in the epigenetic routes as genes involved in the vitamin D signaling pathway have several CpG islands in their promoters that can be methylated [138]. Paternal folate-deficient diet is able to induce malformations in the offspring with relevant altered methylation in sperm DNA [139]. Further support to these observations came from studies that linked male infertility onset to the mutations of Mthfr gene, a key enzyme in folate and methionine metabolism [140, 141]. Accordingly, a growing area of research exploring agents able to modulate methyltransferase and histone deacetylase enzyme activities, as nutraceutical agents, has been of an increasing interest.

Considering the dramatic shift in the eating habits, higher calorie intake and the ingestion of highly processed foods and animal products of modern societies, thus, dietary modulation of epigenetic information in spermatogenesis remains an interesting issue currently under investigation with the aim of better understanding the mechanisms underlying transgenerational transmission of environmental conditioning and of evaluating forms of therapeutic strategies to counteract male sub/infertility.

Understanding the role of environmentally driven gametes' epigenetic changes driven by environmental stimuli and their potential effects on the next generations' phenotype could be considered in future risk assessments of male infertility and could add with the developing of important preventing and treating infertility strategies. **Table 1** summaries some of the reported sperm quality anomalies and epigenetic alterations in respect to environmental stimuli exposure.

Chemicals	Use	Influence on male reproductive parameters	Impact on sperm's epigenome	References
Atrazine	Herbicide	Affects meiosis, spermatogenesis and reduces sperm output in mice following in utero exposure	Global decrease of H3K4me3 and transcription dysregulation	[142]
p,p'-DDE	Organochlorine pesticide DDT	Impairs testis histology and male fertility	Igf2 hypomethylation in sperm and hypomethylation of the H19-imprinted gene spermatogonia impairment in prepubertal and pubertal rats.	[143, 144]
Vinclozolin	fungicide	Causes transgenerational sperm epimutations. Effects sperm motility, counts, daily sperm production [145].	Alterations in sperm DNA methylation, non-coding RNA expression, and histone retention	[146, 147]
Carbendazim and chlorothalonil		Impaired spermatogenesis of pubertal mice via estrogen receptor (ER) signaling modulation	Disturbance of the global DNA methylation and histone methylation observed in mice	[148, 149]
Zearalenone	A mycotoxin produced by Fusarium	Acts via the ER signaling pathway to impair mouse spermatogenesis by producing elevated DNA double stranded breaks and decreasing the number of spermatogenic cells	Promote a global decrease in DNA methylation, an increase in the methylation of histone marker H3K27 and a decrease of estrogen alpha in the testis of pubertal CD1 mice exposed to a dose lower	[150]
PCB Arochlor 1254	Industrial compounds used in electrical equipment & building materials	Impaired Sertoli cells by decreasing the expression of both follicle-stimulating hormone receptor (FshR) and androgen receptor (AR);	Increase the protein levels of the enzymes DNMT1, DNMT3ab, DNMT3l leading transcriptional gene repression observed in the Sertoli cells	[151]
Bisphenol A (BPA)	Production of polycarbonate plastics and epoxy resins	Induced spermatogenesis dysfunction and reduced sperm quality.	Alter methylation of the ER alpha promoter and enhance the expression of the enzymes DNMT3a and DNMT3b at both transcript and protein levels in adult rat testis, a global increase of both genome-wide and locus-specific methylation in these spermatocytes.	[151, 152]
DEHP	Plasticizer diethylhexyl phthalate	Significantly disrupt spermatogenesis in mice exposed to DEHP	number of differentially methylated regions across the genome showing differences towards FVB/N mice strain	[153]

Chemicals	Use	Influence on male reproductive parameters	Impact on sperm's epigenome	References
Melatonin	Insomnia and improving sleep medication	Helps maintain a normal spermatogenesis and male fertility	Increase of histone methyltransferase ESET abundance, besides diminishing apoptosis and the global increase of H3K9me3.	[154]
Fat -HFD	A food source	HFD-induced DNA modifications in gametogenesis HFD-modulated spermatogenesis, leading to DNA hyper-compaction in SPZ.	Induce specific histone marks variation in mature sperm, especially through H3K9me3 and H3K27me3.	[130]

Table 1.
Sperm epigenetic alterations upon exposure to some of the studied hazardous environmental substances/lifestyle stressors and their associated influence on male reproductive parameters.

5. Conclusions and future prospective

Over all, studies have revealed the effects of several epigenetic factors on infertility in men, including histone modification, defects in chromatin-modifying complexes, and methylation modification in promoters of various genes. At present, the available treatments do not account for all infertile men, and this is especially important for idiopathic infertility. Regarding the epigenetic role in male infertility, recognizing epigenetic mechanisms enables us to develop new epidrugs that can be used in the treatment of infertility in near future. Understanding the role of environmentally driven epigenetic changes in gametes on the phenotype of the offspring constitutes not only a fascinating biological question on its own but also represents a moral obligation for the health of future generations. Due to this, the main focus of recent epigenetic research would be to focus on discovering new factors involved in altering chromatin state and further looking at its involvement in diseased and normal tissue.

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