

# Epigenetic Modifications, A New Approach to Male Infertility Etiology: A Review

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## Abstract

Recent studies have indicated that epigenetic alterations are critical for normal function and development of spermatozoa during the fertilization process. This review will focus on the latest advances in epigenome profiling of the chromatin modifications during sperm development, as well as the potential roles of epigenetic mechanisms in the context of male infertility. In this review, all data were collected from published studies that considered the effect of epigenetic abnormalities on human spermatogenesis, sperm parameters quality, fertilization process, embryo development and live births. The database PubMed was searched for all experimental and clinical studies using the Keywords “epigenetic modifications”, “male infertility”, “spermatogenesis”, “embryo development” and “reproductive function”. Post-translational modifications of histone, DNA methylations and chromatin remodeling are among the most common forms of epigenetic modifications that regulate all stages of spermatogenesis and fertilization process. Incorrect epigenetic modifications of certain genes involved in the spermatogenesis and sperm maturation may be a main reason of male reproductive disorder and infertility. Most importantly, abnormal patterns of epigenetic modifications or transgenerational phenotypes and miRNAs expression may be transmitted from one generation to the next through assisted reproductive techniques (ART) and cause an increased risk of birth defects, infertility and congenital anomalies in children. Epigenetic modifications must be considered as a one of the main factors of unexplained male infertility etiology. Due to high risk of transmitting incorrect primary imprints to offspring, there is a need for more research into epigenetic alterations in couples who benefit of ART support.

**Keywords:** Epigenetics, Male Infertility, Reproductive Function, Sperm

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## Introduction

Human infertility is now considered as a serious health problem affecting 15% of couples throughout the world (1). Male infertility factors contribute to about 50% of all infertile cases (2). It is a multifactorial syndrome that can be caused by numerous factors (Fig.1). Anatomical or structural defects such as varicocele and obstructive problems, ejaculatory failures, genital tract infections, hyperviscous semen, endocrinological disorders, impaired spermatogenesis, chromosomal abnormalities, and immunologic problems are the most common reasons for male infertility (3-5). Environmental or occupational factors such as smoking or opiate using, exposure to pesticides or radiation can influence gamete health and subsequently, male fertility (6). Males with normal sperm parameters, but unexplained cause are defined as idiopathic infertile men. Several factors such as oxidative stress caused by the massive production of reactive

oxygen species (ROS), are categorized as the most common causes of this type of male infertility. It seems that ROS accompanied with sperm DNA fragmentation, gene mutations, and genetic disorders (7). The molecular genetic abnormalities rate in the male infertility etiology are notable, ~15-30%, that affect various physiological processes such as steroidogenesis, sperm production and development, maturation, function and quality. Knowing the molecular genetic factors, related genes and potential regulatory mechanisms may provide better understanding of this disease pathogenesis.

Chromosomal abnormalities, point mutations in single genes such as cystic fibrosis transmembrane receptor (*CFTR*) gene, microdeletions of the Y chromosome, mitochondrial genome (mtDNA) mutations, and imprinting disorders are the most important causes of the molecular genetic basis of infertility in men (8, 9). Also, mutations and polymorphisms in different genes,

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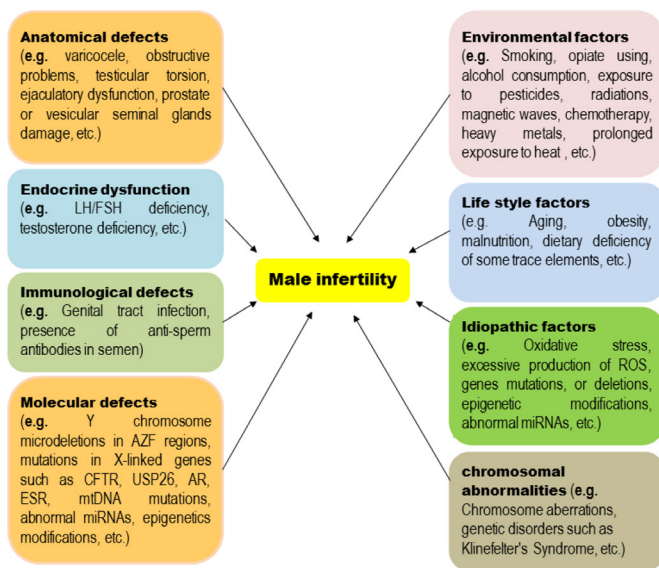
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which may adversely affect sperm production and male fertilization ability, can be associated with severe infertility in men (9). For instance, deletions of the Y chromosome, particularly azoospermia factor A, B and C regions (AZFa, AZFb and AZFc), are the most common causes of Y-linked male infertility which leads to severe damage in spermatogenesis (10). Additionally, deletions in various autosomal and X-linked genes such as androgen receptor (*AR*), and ubiquitin-specific protease-26 (*USP26*) genes, which are considered as critical genes of normal testis development and spermatogenesis, can be associated with male fertility disorder (11). Therefore, any changes such as translocations, inversions, mutations, deletions or insertion and etc. in the related chromosomes and/or genes can change the normal function of the male reproductive system and result in severe damage to male fertility.



**Fig.1:** A schematic for the most common causes of male infertility. Male infertility is a multifactorial syndrome that is caused by multiple factors. Anatomical defects, endocrine deficiency, immunological defects, molecular defects and chromosomal aneuploidy, as well as environmental exposure to toxicants and lifestyle are the most common causes of male infertility. Unexplained infertility may be resulted from idiopathic factors such as oxide stress, over expression of ROS and unknown mutations. ROS; Reactive oxygen species, LH; Luteinizing hormone, FSH; Follicle-stimulating hormone, and AZF; Azoospermia factor.

Recent studies have provided new insight into the role of a class of non-coding RNAs, micro RNAs (miRNAs), in the sperm production, development and fertilization (12, 13). A growing number of evidences have shown that dysregulation of various specific miRNAs, especially miR34a-c, can cause impaired spermatogenesis and male infertility (12, 14). Epigenetic modifications are the other significant factors that regulate spermatogenesis and male fertility. Epigenetics regulates gene regulation without any changes in the DNA sequence. These modifications include methylation of DNA, post-translational histone modifications and chromatin rearrangement (Fig.2) (15). Regulation of gene expression by epigenetic modifications is crucial for normal spermatogenesis. Recent studies reveal that several genes in the testicular cells are regulated through

the epigenetic process, that indicates the critical role of epigenetic mechanism in spermatogenesis, sperm development or maturation, fertilization process, as well as male fertility (16). Therefore, there is a growing interest in determining whether epigenetic modifications serve their key regulatory role. In the following sections, the association of epigenetic alterations with abnormal spermatogenesis and male infertility, as well as their significance in assisted reproduction will be reviewed.



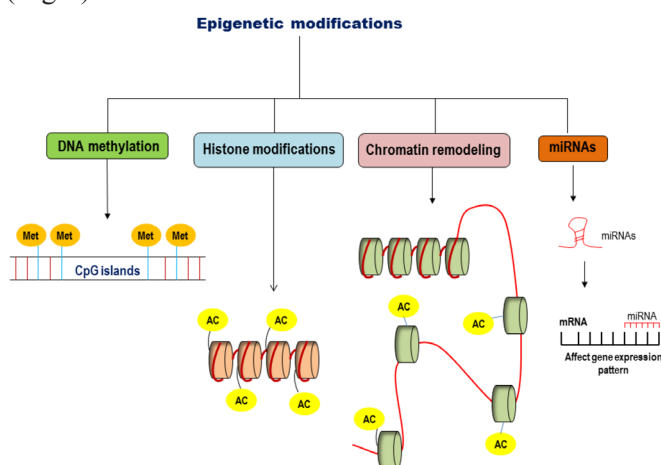
**Fig.2:** The regulatory function of epigenetic modification in all stages of male reproduction. Epigenetic modifications (EPs) are involved in nearly all stages of the reproductive system, including, testicular cell development, germ cell differentiation, steroidogenesis, spermatogenesis, sperm maturation, fertilization, and embryo development.

### Molecular basis of epigenetic alterations

The molecular mechanisms of epigenetic processes come from covalent modifications of protein and DNA components of chromatin. It consists of a range of modifications, including methylation of DNA, post-translational modifications of the core histone, and chromatin remodeling (17). These modifications are processed by three different enzymes, i. A writer that adds epigenetic marks on DNA and histone, ii. A reader that recognizes epigenetic marks, and iii. An eraser responsible for removing these modifications (18).

Epigenetic alterations regulate gene expression by changing the chromatin structure and DNA accessibility. These modifications are extremely important for the development and differentiation of all cells, especially spermatozoa. Recent studies have illustrated that epigenetic modifications affect expression of various genes which are responsible for the development of the male reproductive system, sperm production, and male sexual behavior (19). This indicates that incorrect modifications of DNA can cause abnormal male sexual development and reproductive dysfunction (20). Epigenetic modifications not only control the process of spermatogenesis, but also they modulate the process of sperm maturation, sperm-egg interaction, fertilization, and embryo development. The interesting chemical changes of the DNA molecule, such as chromatin remodeling, methylation of DNA, histones post-translational modifications, and gene

expression regulation by miRNAs are the molecular basis of epigenetic information during these processes (Fig.3).



**Fig.3:** The basic molecular mechanisms of epigenetic modifications. DNA methylation, histone modifications, chromatin remodeling and miRNA biogenesis are the main mechanisms of epigenetic modifications that regulate many physiological processes in various cells. DNA methylation occurrence in the CpG islands regulates expression of various spermatogenesis genes. Histone acetylation that reversibly occurs at lysine residues within the N-terminal tail increases the propensity for gene transcription and also modulates chromatin remodeling. Also, miRNAs biogenesis is regulated by epigenetic modifications such as DNA methylation. It plays a significant role in the numerous physiological processes, particularly spermatogenesis, sperm maturation and fertilization process.

### Role of DNA methylation/demethylation in the male infertility

DNA methylation is the most common type of DNA alteration in the somatic cells. It occurs frequently on the cytosine-phosphate-guanine (CpG) islands that have been located upstream of nearly 40% of mammalian genes. Here, methylation occurs in a C5 position of cytosine residues to create 5-methyl cytosine. In this process, the methyl group is originated from S-adenosyl methionine. Hypermethylation of DNA can suppress transcription by preventing transcription factors binding or inducing several transcriptional repressors binding (20). DNA methylation is controlled by several classes of DNA methyltransferases (DNMTs), including DNMT1, DNMT3a and DNMT3b, that serve as writers (21). All DNA methyl transferases serve as writers. DNMT enzymes regulate both initiation and maintenance of methylation marks.

DNA demethylation can occur through passive or active mechanisms. Active DNA demethylation occurs when a methyl group is removed independently during DNA replication, while we encounter passive DNA demethylation when newly synthesized DNA strands are replicated by DNMT1 (3). DNA demethylation is another process that prevents transmission of inappropriate epigenetic marks to the future generations. Despite of DNA demethylation importance in the development process, its action mechanism is unclear (22).

CpG regions methylation/demethylation, one of the

important processes of gene expression regulation, affects transcription factors binding and then represses expression of imprinted and developmental genes, while its deficiency may result in some disorders (21). Correct DNA methylation plays critical role in the various essential cellular processes such as X- chromosome inactivation and chromatin stability (23).

Recent evidence has indicated that correct methylation of DNA is essential for fertility and embryo development. Also, abnormal DNA methylation pattern has been reported in spermatozoa of idiopathic infertile men with poor semen quality (24). Some studies demonstrated that there is a stable single CpG methylation pattern in sperm of fertile men, while in the subfertile men show significantly modified pattern (25). Interestingly, there is a correlation between DNA methylation level and sperm motility, sperm chromatin and DNA integrity (26, 27). For example, promoters' hypermethylation of genes, including *MTHFR*, *IGF2*, *H19*, *PLAG1*, and *SNRPN* has been shown to be correlated with poor sperm quality and increased risk of infertility. Houshdaran et al. (28) found that hypermethylation of DNA, especially in the promoter regions of *PLAG1*, *PAX8*, *DIRAS3*, *MEST*, *SFN*, *NTF3*, and *HRAS* genes, can decrease counts, motility and normal morphology of sperm. Khazamipour et al. (29) reported hypermethylation of *MTHFR* promoter in nearly 53% of the men with non-obstructive azoospermia, while they observed no hypermethylation of *MTHFR* promoter in the men with obstructive azoospermia. Similarly, Wu et al. (30) found that the *MTHFR* gene promoter hypermethylation in human spermatozoa causes idiopathic infertility. These findings indicate that *MTHFR* hypermethylation is an important epigenetic abnormality which can contribute to male infertility. A more recent study has revealed that hypermethylation of *SOX30* gene causes its silencing, impaired spermatogenesis and non-obstructive azoospermia in mice (31).

Abnormal methylation of the promoter regions of *H19* and *MEST* genes can be associated with impaired spermatogenesis and oligozoospermia (32). Hammoud et al. (33), compared CpG methylation of *LIT1*, *SNRPN*, *MEST*, *H19*, *PLAG1*, *PEG3*, and *IGF2* genes in spermatozoa of fertile and infertile men. Methylation of DNA in spermatozoa of infertile patients was significantly modified for all genes except *IGF2*. Furthermore, a significant relationship was observed between impaired spermatogenesis and hypermethylation of these six genes. Boissonnas et al. (34), found that H19 was considerably hypomethylated in men with teratozoospermia and oligoasthenoteratozoospermia. Another study indicated that *MEST* hypermethylation has more adverse effects on sperm quality than *H19/IGF2-ICR1* hypomethylation. In this study, Rajender et al. (35) compared hypermethylation of *MEST* with hypomethylation of H19/IGF2.

The X-linked reproductive homeobox (*RHOX*) gene



clusters, are essential for normal spermatogenesis, germ cell survival and male fertility. These Homeobox genes are exclusively expressed in the reproductive system. A recent study has introduced *ROHX* cluster functions as an appropriate biomarker for idiopathic male infertility. This study also revealed that hypermethylation of *RHOX* cluster can cause severe abnormalities in the sperm parameters (36). It is also assumed that hypermethylation of DNA may suppress *RHOX* gene clusters transcription. Incorrect DNA methylation in the promoter region of the deleted-in-azoospermia-like (*DAZZL*) gene has been shown to be associated with impaired spermatogenesis and sperm dysfunction (37). It is estimated that approximately 10% of men with impaired spermatogenesis suffered from *DAZZL* deletion (38). Recently it has been reported that decreased expression of *DNMTs* genes is associated with azoospermia and infertility (39). Hammoud et al. (33), revealed a substantial relationship between modified methylation of *H19*, *LIT1*, *SNRPN*, *MEST*, *PEG3*, *PLAGL1*, and *IGF2* genes in human sperm and male infertility. In another study, DNA methylation at *MEST* gene was considerably correlated to increased level of FSH, decreased testicular volume and resulted in oligozoospermia (40). Also, it has been found that abnormal methylation reaches to about 41% in the sperm cells of patients who undergoing ART (41). Decreased level of DNA methylation in the *H19*-ICR has been reported that is negatively correlated with sperm count. A significant decrease of DNA methylation in the *H19* gene was found in testicular sperm of azoospermic men in comparison with fertile men (42). In another study by Vieweg et al. (25), methylation of sperm DNA in normozoospermic men exhibited low standard deviation, while a higher variability in the percentage of DNA methylation was observed in the promoters of subfertile men. These data suggest that DNA methylation abnormalities in the human sperm impact on sperm parameters quality and male infertility.

### Histone modifications Role in male infertility

Nucleosome, fundamental unit of chromatin, includes a short turn of DNA that wrapped around a histone octamer, two copies of each H2A, H2B, H3, and H4. This organization provides a rigid structure to chromatin. Histone modification is a covalent post-translational change on the lysine rich tail of any of the histone proteins, particularly H3 and H4 histones (43). Histone modifications serve as a key regulator for activation and inactivation of various genes. This reversible process is controlled by acetyltransferases, deacetyltransferases, methyltransferases and demethylases enzymes (44). Different types of post-transcription or modifications may occur by targeting the N-terminal tail of histones via acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, glycosylation, butyrylation, citrullination, and propionylation (Table 1) (45). However, histone acetylation, methylation and phosphorylation are

the most frequently events during sperm development, spermatogenesis, fertilization and embryo development.

**Table 1:** Different types of histone modifications

Modification types	Residue (s)
Acetylation	Lysine
Methylation	Lysine/Arginine
Phosphorylation	Serine/Threonine
Ubiquitylation	Lysine
Sumoylation	Lysine
ADP-Ribosylation	Lysine
Glycosylation	Serine/Threonine
Butyrylation	Lysine
Propionylation	Lysine

Histone modification is a key regulator of mitosis and spermatogenesis. Some studies have found that abnormal histone modifications during spermatogenesis may result in severe damage to sperm development and male fertility (35). The whole-genome of the male germ cells is entirely reorganized during the post-meiotic differentiation of spermatids into spermatozoa. While the core of the spermatid extends and condenses, most somatic histones are replaced progressively by protamines. Protamines are small basic sperm-specific nuclear proteins which are responsible for haploid genome condensation. This crucial process, tight packaging, protects sperm genome against side effects of physical and chemical agents during fertilization process (46). The exact molecular mechanisms of histone replacement by protamines during the spermatogenesis process have been not well-understood. Recent evidence has indicated that epigenetic modifications of histones are probably the most important regulators for histones/protamines exchange in the human spermatozoa (47). However, histone/protamine exchange may differ in species because of the different amounts of protamine in mature mammalian sperm.

Histones acetylation, a reversible process, takes place at certain lysine residues on the amino-terminal ends of the core histones, which neutralizes the positive charge on the histones tails and therefore, decreases their affinity for DNA and provides open chromatin structure for transcription (48). In other hand, histone acetylation enhances the transcription level, such as acetylation of histones H3K4 (H3K4ac) and H3K39 (H3K39ac) (49). Histone acetyl transferases (HATs) and histone deacetylases (HDACs) are key regulators of histone acetylation/deacetylation (50). While HDACs suppress gene expression, HATs activity enhances gene expression. In other words, deacetylation is associated with gene expression suppression, but acetylation is associated with genes overexpression. Also, Histone acetylation throughout the human spermatogenesis has been reported. Increased level of histone H4 hyperacetylation (Hypac-H4) has been shown in spermatogonia, spermatocyte and also,

spermatids (44). Histone H4 hyperacetylation facilitates interaction of transcription factors with the chromatin. Hypac-H4 in spermatids increases accumulation of bromodomain protein, which in turn triggers nuclear reorganization. Recently, Oikawa et al. (51), have revealed a significant decrease in the number of spermatogonia cells with Lys12ac-H4 or Hypac-H4. Several studies considered the relationship between Hypac-H4 and impaired spermatogenesis (47). A significant reduction in the number of spermatids with Hypac-H4 can be found in men with spermatogenesis deficiency and spermatid arrest. Shirakata et al. (44), demonstrated that hypac-H4 may trigger histones replacement by protamines during spermatogenesis. They also found different specific patterns of histone H4 modification during each stage spermatogenesis. For example, H4K8ac, H4K12ac, H4K5ac, H4K20me2 and H4me were increased in preleptotene spermatocytes, while H4me3 was decreased. These data suggest a significant association between histone H4 modifications and gene expression patterns during spermatogenesis process.

Recent evidence has identified several post-translational modifications, including S42 and K49 acetylation on protamine-1 (PRM1) and K64 acetylation on protamine-2 (PRM2). Although acetylation of histones is associated with genes transcription, sperm cells are transcriptionally inactive. Therefore, it can be hypothesized that histone acetylation displays an epigenetic mark which is transmitted from spermatozoa to oocyte. This event regulates expression of various genes involved in embryo development (25).

Histone methylation is another type of histone modifications which can be associated with activation or repression of chromatin state. For example, histone H3 trimethylation at lysine 36 (H3K36me3) and 4 (H3K4me3) are often associated with an open chromatin structure for active transcription, while H3 trimethylation at lysines 9 (H3K9me3) and 27 (H3K27me3) can cause transcriptional repression (25). A histone methyltransferases (HMTases) involved in methylation of histone that is generally associated with genes suppressing. Recent studies have shown different patterns of histone methylation in human spermatozoa. Therefore, abnormal methylation of histones can cause severe damages to spermatogenesis process and incorrect epigenetic programming in human spermatozoa (51).

Also, enzyme histone demethylases (HDMs), a member of the Jumonji protein family, involve in the histone demethylation. Recently, scientists have reported a tight link between the activity of JHMD2A (Jumonji C-terminal containing histone demethylase 2A) histone demethylase and spermatogenesis (47). JHMD2A regulates the expression of protamine 1 (*Prm1*) and transition nuclear protein 1 (*Tnp1*), which are necessary for condensation and proper packaging of sperm chromatin. A recent study has found histone H3 lysine 4 hypermethylation (H3K4me) or H3 lysine 27 (H3K27me) hypermethylation in sperm

of infertile individuals compared to fertile subjects (25).

Histone phosphorylation, another kind of epigenetic modifications, is associated with transcription process activation and plays an important role in chromatin rearrangement during spermatogenesis (44). Also, Histone phosphorylation can regulate several biological events, including mitotic/meiotic chromosome condensation, activation and inactivation of genes transcription, and double-strand DNA breaks (DSB) repair (47). Phosphorylation of histones is also involved in regulation of meiotic replication, chromatin remodeling and compaction in the nucleus of spermatozoa. Therefore, defects in histone phosphorylation can be associated with sperm dysfunction and male infertility problems.

### Chromatin remodeling role in male infertility

Chromatin remodeling is the dynamic rearrangement of chromatin structure in which several protein complexes such as a SWI/SNF, ISW1 and MI-2 proteins change the nucleosomes location and structure in an ATP-dependent process (52). During the chromatin remodeling process the condensed chromatin of spermatozoa transmits imprinting and epigenetic information to the embryo (53). Miller et al. (54), showed that incorrect DNA packaging in the mice spermatozoa causes infertility. Correct DNA packaging is essential for normal spermatogenesis because approximately 85% of the histones are substituted by protamines during the spermatogenesis process (55). During the initial stages of spermiogenesis, histones are hyperacetylated and undergo other epigenetic modifications. Nucleosomes are progressively disassembled in the final stage of spermatogenesis and then replaced by TNPs and eventually protamines.

Protamines incorporation into the chromatin of spermatozoa induces DNA condensation, which is critical for sperm production and migration. Phosphorylation/dephosphorylation of protamines is a key mechanism regulating interaction between them and DNA. Phosphorylation of protamines by a protein kinase A (PKA) increases their affinity or binding to DNA. Also, protamines dephosphorylation occurs concomitantly with the maturation of nucleoprotamine. Mutations in calcium/calmodulin-dependent protein kinase 4 (CaMK4), which is involved in phosphorylation of protamine 2, causes impaired spermiogenesis and consequently, male infertility (56). In the mature spermatozoa, DNA is packaged densely with protamines during the fertilization process, while the maternal genome is densely packaged with histones. Therefore, the highly condensed structure of nucleoprotamine must be unpacked and rearranged into a nucleosomal structure during the fertilization process. Incorrect epigenetic modifications during each stage of these processes can lead to severe male infertility. Given the critical roles of PRM1 and PRM2 in normal sperm function and fertilization process, the haploinsufficiency of these proteins can be associated with decreased amount of the respective protein, which in turn increases the

percentage of sperm with abnormal chromatin structure and damaged DNA (35). Additionally, it has been shown that the optimal proportion of PRM1 to PRM2 (PRM1/PRM2 ratio), which is critical for normal fertilization, is tightly regulated (57). It has been indicated that the PRM1/PRM2 ratio in the fertile individuals ranges from 0.8-1.2 and deviation from this ratio may negatively affect sperm quality, sperm DNA integrity and increase the risk of infertility in men. Men with an increased PRM1/PRM2 ratio are characterized by poor quality of sperm and reduced fertilizing capacity. Aberrant H4 acetylation has been shown to be correlated to impaired spermatogenesis (42). Sonnack et al. (58), observed that a decreased level of histone H4 acetylation in men with infertility problems is significantly associated with impaired spermatogenesis. Histone H4 hyperacetylation, which decreases the affinity of histone to DNA, is essential for histone to protamine transition (35). Hyperacetylation of histone H4 is also found in the infertile men with Sertoli cell-only syndrome (SCOS) (59).

### Genetic imprinting role in male infertility

Genetic imprinting is an epigenetic process in which the expression of alleles occurs in a parent-of-origin-specific manner. This process is a consequence of changes in methylation of CpG islands in one allele. Genetic imprinting determines the origin of genes expression from the parental or maternal genomes, a process which is critical for embryo development (41). There are multiple loci imprinting in human. For example, human spermatogonia from the fetal period are often unmethylated at H19 differentially methylated regions (DMR), while spermatogonia from the testis are highly methylated in DMR (35). Several imprinted genes such as the paternally imprinted *GTL2* and *H19* genes were previously considered in men with infertility problems (60). The relationship between reduced methylation of the paternal *IGF2/H19* imprinting control region 1 (ICR1) and *GTL2* imprints in spermatozoa of men with disturbed spermatogenesis indicates the importance of genetic imprinting during spermatogenesis. In a study, Poplinski et al. (61), demonstrated that fertile individuals have high levels of *IGF2/H19*-ICR1, while methylation of *MEST* shows a significant decrease. They also showed that hypermethylation of *IGF2/H19*-ICR1 and *MEST* has a correlation with low sperm counts. These data indicated that imprinting deficiencies in the *IGF2/H19* ICR1 and *MEST* can be associated with idiopathic male infertility. Therefore, aberrant *MEST* methylation can be considered as a strong indicator of sperm quality. Recent evidence has indicated that ART techniques such as intracytoplasmic sperm injection (ICSI), *in vitro* fertilization (IVF) and round spermatid injection (ROSI) can enhance the prevalence of imprinting defects and also, negatively affect human embryonic development through selecting immature sperm cells which may not have proper imprints or global methylation (3). More importantly, ART techniques may transmit epigenetic modifications

to the next generation and cause the birth of children with a higher risk of infertility and other congenital abnormalities. Therefore, more efforts are necessary to optimize current ART techniques. Additionally, selection of appropriate procedures needs careful evaluation. Since epigenetic modifications can be maintained throughout the human lifespan and can potentially be transmitted to the next generations, long-term follow-up and health evaluation of ART offspring are essential to provide more robust clinical evidence (62).

### miRNAs role in the male infertility

Epigenetic modifications may also extend on mechanisms, including non-coding RNA such as micro-RNAs (miRNAs). Non-coding RNAs, especially miRNAs and PIWI interacting RNAs (piRNAs), consist the most contents of RNAs in a cell and regulate gene expression at different levels. Also, miRNAs negatively regulate the expression pattern of various genes at the post-transcriptional level. Recent investigations confirmed the role of miRNAs in the regulation of development, differentiation, and progression of various diseases. Expression pattern alterations of these RNAs in the sperm cells can be associated with severe abnormalities of these cells and next generations (12). In an experimental study, Skinner et al. (63), demonstrated that alteration in the DNA methylation and ncRNA of sperm by Dichloro Diphenyl Trichloroethane (DDT) can be transferred and inherited in the next generation.

### Epigenetic regulation of sperm development

Epigenetic modifications control all steps of fertilization from testicular cells development to embryo development (Fig.2). The initial stage of reprogramming event takes place in the developing gonads. The testes of all mammals involve all stages of spermatogenesis from germ cells to mature spermatozoa. In the normal germ cells, epigenetic alterations are reversible and cause changes in the various genes expression whenever it is necessary. Epigenetic modifications are highly active in developing germ cells, where sperm and oocyte are equipped with an appropriate epigenetic information before embryonic development (15). Germ cells undergo a series of dramatic morphological alterations, as well as specific and extensive chromatin remodeling during their differentiation into mature spermatozoa (64).

Spermatozoa are highly specialized cells, which are produced during spermatogenesis from germ cells. It is a highly conserved process that regulates by extensive cellular, epigenetic and chromatin modifications. Sperm cells transfer the genetic information from one generation to the next and are thus critical for maintenance of life. Genetic and epigenetic mechanisms, especially unique gene expression patterns, histone modifications and DNA methylation, have important functions in the regulation of germ-cell development (45). Meiosis, a process in which a germ cell is divided to four gametes, is associated with



histone modifications. After meiosis division, germ cells undergo several chromatin rearrangements, especially histone replacement with protamine.

Dynamic epigenetic alteration is central for differentiation of mammalian sperm; however, the mechanism of these modifications is still unknown. Spermatogenesis is a unique process which is precisely regulated by epigenetic modifications and results in the formation of haploid spermatozoa with the fertilization ability. The process of spermatogenesis is accompanied by chromatin rearrangement from a nucleosomal histones-based structure to a protamines-based structure. Mitotic amplification, meiotic, and post-meiotic phase (also known as spermiogenesis) are the critical phases of spermatogenesis. Replication and differentiation of spermatogonial stem cells into primary spermatocytes is the initial phase of spermatogenesis process. In meiosis, the primary spermatocytes are developed into haploid secondary spermatocytes through genetic recombination. During the haploid stage of spermatogenesis, round spermatids are differentiated into spermatozoa (65). In this process, histone H4 hyperacetylation causes nucleosome disassembly, DNA breaks and incorporation of non-allelic variants of histones (43). Histones are largely exchanged by transition proteins and then by the protamines PRM1 and PRM2. Recent evidence has indicated that ~99% of histones in the mice mature spermatozoa and ~85% of histones in the human sperm are exchanged by PRM1 and PRM2. Human sperm protamines are phosphorylated at several specific sites, including PRM1S11, PRM2S59 and PRM1S9; however, the exact mechanism of this phosphorylation is still unclear. These proteins also undergo further post-translational modifications during sperm maturation and transition in the epididymis. During this process, protamines form disulfide bonds and zinc bridges which stabilize the sperm chromatin structure (57). The relationship between protamines and DNA results in significant structural and molecular remodeling which subsequently compacts sperm DNA in the nucleoprotamine toroidal structures. The protamine-DNA interaction not only facilitates the normal morphology and hydrodynamic shape of the sperm head, but also protects the paternal genome against physical and chemical damages.

During the fertilization process, oocyte and spermatozoa fuse to form a single totipotent cell called zygote which can produce a new organism. Although sperm chromatin is extensively rearranged, histones are associated with the paternal genome postfertilization process and contribute to zygotic chromatin. The highly compacted sperm DNA is decondensed from its transcriptionally inactive state and expands to active state found in the paternal pronucleus. In the zygote, the paternal histone proteins might then serve as a template for newly synthesized histones during replication. In postfertilization, the programmatic sperm DNA packaging can therefore deliver epigenetic information to the oocyte and the zygote (53).

The patterns of DNA methylation are first acquired

during gametogenesis. A growing number of evidences has illustrated the importance of DNA methylation for male germ cell development in a mouse model. It has been shown that gene targeting of enzymes responsible for DNA methylation leads to loss of germ cells methylation, meiosis deficiency and male infertility (42). A recent study has demonstrated that mono-, di- and trimethylations of different histones, H3K9, H3K4, and H3K27, tightly regulate temporal expression and correct progression of spermatogenesis (44). Methylation level of H3K4 is enhanced in the spermatogonial stem cell. Reduced methylation level of histone H3K4 decreases the activity of Mll2 (an H3K4 methyl transferase) which in turn declines the number of spermatocytes. This suggests the important role of H3K4 methylation in differentiation of spermatocyte from the stem cell stage (64). Conversely, the methylation of histones H3K9 and H3K27 in the stem cell is low, but its level increases during meiosis. Several lines of studies indicated the association between the methylation of histone H3K9 and euchromatin, heterochromatin and sex chromosomes in the late pachytene stage. However, the H3K9 methylation level decreases during the completion of meiosis, which is associated with an increase in H3K4me levels.

## Conclusion

The advent of new technologies has revealed differential expression patterns of various genes in testicular cells and spermatozoa which opened a new window to male infertility etiology. Epigenetics alterations of DNA, histones, chromatins and miRNAs are significant factors that regulate spermatogenesis and impact fertility. These modifications not only involve in the regulation of somatic and germ cells development, but also control all stages of spermatogenesis process. Epigenetic alterations also control the correct expression patterns of various genes during the spermatogenesis and subsequently, fertilization. In other words, epigenetic alterations are necessary for the correct expression of various genes in normal conditions. Although ART technologies such as ICSI and IVF have provided a good opportunity for most infertile couples to have their own child, the abnormal epigenetic information transmission to the next generation may associate with increased risk of infertility and other health problems.

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## Authors' Contributions

A.B.H., I.L., H.Sh, H.I.; Were contributed to data collection and evaluation, drafting and statistical analysis, and manuscript writing. E.T.M.; Was contributed to manuscript writing, study concept and design, drafted or provided critical revision of the manuscript. A.Sh.; Was responsible for overall supervision. All authors read and

approved the final manuscript.

## References

- Aitken RJ. Oxidative stress and the etiology of male infertility. *J Assist Reprod Genet.* 2016; 33(12): 1691-1692.
- Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol.* 2015; 13: 37.
- Tahmasbpour E, Balasubramanian D, Agarwal A. A multi-faceted approach to understanding male infertility: gene mutations, molecular defects and assisted reproductive techniques (ART). *J Assist Reprod Genet.* 2014; 31(9): 1115-1137.
- Beigi Harchegani A, Rahmani H, Tahmasbpour E, Shahriary A. Hyperviscous semen causes poor sperm quality and male infertility through induction of oxidative stress. *Curr Urol.* 2019; 13(1): 1-6.
- Beigi Harchegani A, Irandoost A, Mirmamniha M, Rahmani H, Tahmasbpour E, Shahriary A. Possible Mechanisms for the effects of calcium deficiency on male infertility. *Int J Fertil Steril.* 2019; 12(4): 267-272.
- Hosseinzadeh Colagar A, Jorsaraee GA, Tahmasbpour Marzony E. Cigarette smoking and the risk of male infertility. *Pak J Biol Sci.* 2007; 10(21): 3870-3874.
- Agarwal A, Roychoudhury S, Sharma R, Gupta S, Majzoub A, Sabanegh E. Diagnostic application of oxidation-reduction potential assay for measurement of oxidative stress: clinical utility in male factor infertility. *Reprod Biomed Online.* 2017; 34(1): 48-57.
- Ambulkar PS, Sigh R, Reddy M, Varma PS, Gupta DO, Shende MR, et al. Genetic risk of azoospermia factor (AZF) microdeletions in idiopathic cases of azoospermia and oligozoospermia in central Indian population. *J Clin Diagn Res.* 2014; 8(3): 88-91.
- Tahmasbpour Marzouni E, Beigi Harchegani A, Layali I. Chromosomal aneuploidies and associated rare genetic syndromes involved in male infertility. *J Men's Health.* 2021; 17(1): 1-11.
- Zhang YS, Dai RL, Wang RX, Zhang ZH, Fadlalla E, Liu RZ. Azoospermia factor microdeletions: occurrence in infertile men with azoospermia and severe oligozoospermia from China. *Andrologia.* 2014; 46(5): 535-540.
- Lo Giacco D, Chianese C, Ars E, Ruiz-Castane E, Forti G, Krausz C. Recurrent X chromosome-linked deletions: discovery of new genetic factors in male infertility. *J Med Genet.* 2014; 51(5): 340-344.
- Harchegani AB, Shafaghathian H, Tahmasbpour E, Shahriary A. Regulatory functions of micrnas in male reproductive health: a new approach to understanding male infertility. *Reprod Sci.* 2018; 1933719118765972.
- Abu-Halima M, Hammadeh M, Schmitt J, Leidinger P, Keller A, Meese E, et al. Altered microRNA expression profiles of human spermatozoa in patients with different spermatogenic impairments. *Fertil Steril.* 2013; 99(5): 1249-1255. e16.
- Heidary Z, Zaki-Dizaji M, Saliminejad K, Khorram Khorshid HR. MicroRNA profiling in spermatozoa of men with unexplained asthenozoospermia. *Andrologia.* 2019; 51(6): e13284.
- Shaoqin G, Zhenghui Z, Xueqian Z, Yuan H. Epigenetic modifications in human spermatozoon and its potential role in embryonic development. *Yi Chuan.* 2014; 36(5): 439-446.
- Cannarella R, Condorelli RA, Mongioi LM, Vignera SL, Calogero AE. Molecular biology of spermatogenesis: novel targets of apparently idiopathic male infertility. *Int J Mol Sci.* 2020; 21(5): 1728.
- Ge SQ, Lin SL, Zhao ZH, Sun QY. Epigenetic dynamics and interplay during spermatogenesis and embryogenesis: implications for male fertility and offspring health. *Oncotarget.* 2017; 8(32): 53804-53818.
- Al About NM, Tupper C, Jialal I. Genetics, epigenetic mechanism. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK532999/>.(11Aug 2021).
- Mani S, Ghosh J, Coutifaris C, Sapienza C, Mainigi M. Epigenetic changes and assisted reproductive technologies. *Epigenetics.* 2020; 15(1-2): 12-25.
- Gunes S, Esteves SC. Role of genetics and epigenetics in male infertility. *Andrologia.* 2021; 53(1): e13586.
- Cisneros FJ. DNA methylation and male infertility. *Front Biosci.* 2004; 9: 1189-1200.
- Jenkins TG, Aston KI, James ER, Carrell DT. Sperm epigenetics in the study of male fertility, offspring health, and potential clinical applications. *Syst Biol Reprod Med.* 2017; 63(2): 69-76.
- Sujit KM, Singh V, Trivedi S, Singh K, Gupta G, Rajender S. Increased DNA methylation in the spermatogenesis-associated (SPATA) genes correlates with infertility. *Andrology.* 2020; 8(3): 602-609.
- Chi F, Zhao M, Li K, Lin AQ, Li Y, Teng X. DNA methylation status of imprinted H19 and KvDMR1 genes in human placentas after conception using assisted reproductive technology. *Ann Transl Med.* 2020; 8(14): 854.
- Vieweg M, Dvorakova-Hortova K, Dudkova B, Waliszewski P, Otte M, Oels B, et al. Methylation analysis of histone H4K12ac-associated promoters in sperm of healthy donors and subfertile patients. *Clin Epigenetics.* 2015; 7(1): 31.
- Montjean D, Zini A, Ravel C, Belloc S, Dalleac A, Copin H, et al. Sperm global DNA methylation level: association with semen parameters and genome integrity. *Andrology.* 2015; 3(2): 235-240.
- Capra E, Lazzari B, Turri F, Cremonesi P, Portela AMR, Ajmone-Marsan P, et al. Epigenetic analysis of high and low motile sperm populations reveals methylation variation in satellite regions within the pericentromeric position and in genes functionally related to sperm DNA organization and maintenance in *Bos taurus*. *BMC Genomics.* 2019; 20(1): 940.
- Houshdaran S, Cortessis VK, Siegmund K, Yang A, Laird PW, Sokol RZ. Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm. *PLoS One.* 2007; 2(12): e1289.
- Khazamipour N, Noruzinia M, Fatehmanesh P, Keyhaneh M, Pujol P. MTHFR promoter hypermethylation in testicular biopsies of patients with non-obstructive azoospermia: the role of epigenetics in male infertility. *Hum Reprod.* 2009; 24(9): 2361-2364.
- Wu W, Shen O, Qin Y, Niu X, Lu C, Xia Y, et al. Idiopathic male infertility is strongly associated with aberrant promoter methylation of methylenetetrahydrofolate reductase (MTHFR). *PLoS One.* 2010; 5(11): e13884.
- Han F, Jiang X, Li ZM, Zhuang X, Zhang X, Ouyang WM, et al. Epigenetic inactivation of SOX30 is associated with male infertility and offers a therapy target for non-obstructive azoospermia. *Mol Ther Nucleic Acids.* 2020; 19: 72-83.
- Li B, Li Jb, Xiao Xf, Ma Yf, Wang J, Liang Xx, et al. Altered DNA methylation patterns of the H19 differentially methylated region and the DAZL gene promoter are associated with defective human sperm. *PLoS One.* 2013; 8(8): e71215.
- Hammoud SS, Nix DA, Hammoud AO, Gibson M, Cairns BR, Carrell DT. Genome-wide analysis identifies changes in histone retention and epigenetic modifications at developmental and imprinted gene loci in the sperm of infertile men. *Hum Reprod.* 2011; 26(9): 2558-2569.
- Boissonnas CC, Abdalaoui HE, Haelewyn V, Fauque P, Dupont JM, Gut I, et al. Specific epigenetic alterations of IGF2-H19 locus in spermatozoa from infertile men. *Eur J Hum Genet.* 2010; 18(1): 73-80.
- Rajender S, Avery K, Agarwal A. Epigenetics, spermatogenesis and male infertility. *Mutat Res.* 2011; 727(3): 62-71.
- Richardson ME, Bleiziffer A, Tuttelmann F, Gromoll J, Wilkinson MF. Epigenetic regulation of the RHOX homeobox gene cluster and its association with human male infertility. *Hum Mol Genet.* 2014; 23(1): 12-23.
- Zhang S, Xu L, Yu M, Zhang J. Hypomethylation of the DAZ3 promoter in idiopathic asthenospermia: a screening tool for liquid biopsy. *Sci Rep.* 2020; 10(1): 17996.
- Kuo PL, Wang ST, Lin YM, Lin YH, Teng YN, Hsu CC. Expression profiles of the DAZ gene family in human testis with and without spermatogenic failure. *Fertil Steril.* 2004; 81(4): 1034-1040.
- Uysal F, Akkoyunlu G, Ozturk S. Decreased expression of DNA methyltransferases in the testes of patients with non-obstructive azoospermia leads to changes in global DNA methylation levels. *Reprod Fertil Dev.* 2019 (ahead of print).



40. Zhang W, Li M, Sun F, Xu X, Zhang Z, Liu J, et al. Association of sperm methylation at LINE-1, four candidate genes, and nicotine/alcohol exposure with the risk of infertility. *Front Genet.* 2019; 10: 1001.
41. Kobayashi H, Hiura H, John RM, Sato A, Otsu E, Kobayashi N, et al. DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm. *Eur J Hum Genet.* 2009; 17(12): 1582-1591.
42. Minor A, Chow V, Ma S. Aberrant DNA methylation at imprinted genes in testicular sperm retrieved from men with obstructive azoospermia and undergoing vasectomy reversal. *Reproduction.* 2011; 141(6): 749-757.
43. Yuen BTK, Bush KM, Barrilleaux BL, Cotterman R, Knoepfler PS. Histone H3.3 regulates dynamic chromatin states during spermatogenesis. *Development.* 2014; 141(18): 3483-3494.
44. Shirakata Y, Hiradate Y, Inoue H, Sato E, Tanemura K. Histone h4 modification during mouse spermatogenesis. *J Reprod Dev.* 2014; 60(5): 383-387.
45. Chioccarelli T, Pierantoni R, Manfredola F, Porreca V, Fasano S, Chianese R, et al. Histone post-translational modifications and circRNAs in mouse and human spermatozoa: potential epigenetic marks to assess human sperm quality. *J Clin Med.* 2020; 9: 640.
46. Patel DP, Jenkins TG, Aston KI, Guo J, Pastuszak AW, Hanson HA, et al. Harnessing the full potential of reproductive genetics and epigenetics for male infertility in the era of "big data". *Fertil Steril.* 2020; 113(3): 478-488.
47. Wang T, Gao H, Li W, Liu C. Essential role of histone replacement and modifications in male fertility. *Front Genet.* 2019; 10: 962.
48. Pan Xy, Li Zx, Wang Zc, Wang Xn, Huang By, Dou Zh, et al. Histone modifications during spermatogenesis and male infertility]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao.* 2014; 36(1): 108-113.
49. Kleiman SE, Bar-Shira Maymon B, Hauser R, Botchan A, Paz G, Yavetz H, et al. Histone H4 acetylation and AZFc involvement in germ cells of specimens of impaired spermatogenesis. *Fertil Steril.* 2008; 89(6): 1728-1736.
50. Parab S, Shetty O, Gaonkar R, Balasinar N, Khole V, Parte P. HDAC6 deacetylates alpha tubulin in sperm and modulates sperm motility in Holtzman rat. *Cell Tissue Res.* 2015; 359(2): 665-678.
51. Oikawa M, Simeone A, Hormanseder E, Teperek M, Gaggioli V, O'Doherty A, et al. Epigenetic homogeneity in histone methylation underlies sperm programming for embryonic transcription. *Nat Commun.* 2020; 11(1): 3491.
52. Giaccone F, Cannarella R, Mongioli LM, Alamo A, Condorelli RA, Calogero AE, et al. Epigenetics of male fertility: effects on assisted reproductive techniques. *World J Mens Health.* 2019; 37(2): 148-156.
53. Balasubramanian D, Tahmasbpour E, Agarwal A. Insights from epigenomics analysis of sperm: sperm development and male infertility. In: Dincer Y, editor. *Epigenetics: mechanisms and clinical perspectives.* 1<sup>st</sup> ed. Nova Science; 2016: 153-174.
54. Miller D, Brinkworth M, Iles D. Paternal DNA packaging in spermatozoa: more than the sum of its parts? *DNA, histones, protamines and epigenetics. Reproduction.* 2010; 139(2): 287-301.
55. Oliva R. Protamines and male infertility. *Hum Reprod Update.* 2006; 12(4): 417-435.
56. Crapster JA, Rack PG, Hellmann ZJ, Le AD, Adams CM, Leib RD, et al. HIPK4 is essential for murine spermiogenesis. *Elife.* 2020; 9: e50209.
57. Nemati H, Sadeghi M, Nazeri M, Mohammadi M. Evaluation of the association between polymorphisms of PRM1 and PRM2 and the risk of male infertility: a systematic review, meta-analysis, and meta-regression. *Sci Rep.* 2020; 10(1): 17228.
58. Sonnack V, Failing K, Bergmann M, Steger K. Expression of hyperacetylated histone H4 during normal and impaired human spermatogenesis. *Andrologia.* 2002; 34(6): 384-390.
59. Dada R, Kumar M, Jesudasan R, Fernandez JL, Gosalvez J, Agarwal A. Epigenetics and its role in male infertility. *J Assist Reprod Genet.* 2012; 29(3): 213-223.
60. Kobayashi H, Sato A, Otsu E, Hiura H, Tomatsu C, Utsunomiya T, et al. Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. *Hum Mol Genet.* 2007; 16(21): 2542-2551.
61. Poplinski A, Tüttelmann F, Kanber D, Horsthemke B, Gromoll J. Idiopathic male infertility is strongly associated with aberrant methylation of MEST and IGF2/H19 ICR1. *Int J Androl.* 2010; 33(4): 642-649.
62. Chen W, Peng Y, Ma X, Kong S, Tang S, Wei Y, et al. Epigenetic effects of assisted reproductive technology in human offspring. *bioRxiv.* 2019: 1-32.
63. Skinner MK, Guerrero-Bosagna C, Haque MM. Environmentally induced epigenetic transgenerational inheritance of sperm epimutations promote genetic mutations. *Epigenetics.* 2015; 10(8): 762-771.
64. Castillo J, Estanyol JM, Ballesca JL, Oliva R. Human sperm chromatin epigenetic potential: genomics, proteomics, and male infertility. *Asian J Androl.* 2015; 17(4): 601-609.
65. Rathke C, Baarends WM, Awe S, Renkawitz-Pohl R. Chromatin dynamics during spermiogenesis. *Biochim Biophys Acta.* 2014; 1839(3): 155-168.