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Role of Epigenetic changes in Reproductive Inflammation and Male Infertility

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ABSTRACT

Male infertility is a public health problem affecting one in twenty couples globally. This multifactorial reproductive health issue is a consequence of testicular failure, ejaculatory dysfunction, and altered sperm characteristics caused by a combination of (epi) genetic, environmental, and lifestyle factors. Abnormal epigenetic changes have been proposed as an important causative factor for infertility in men. Abnormal DNA methylation, histone modification, altered non-coding RNAs have been well documented as in pathological conditions such as oligospermia, azoospermia, asthenospermia and tetratospermia in males. Additionally, chronic inflammations in the male genital tract have long been linked with infertility, possibly via affecting the sperm epigenome or its surrounding microenvironment. This review article summarizes the relationship between epigenome, inflammation, and its contribution to male infertility.

Keywords: Spermatogenesis, Epigenetics, Inflammation, Oxidative stress, Male infertility

INTRODUCTION

Infertility is defined as inability of sexually active couples to achieve pregnancy even after a year of unprotected, regular sexual intercourse. Being a complex multifactorial problem, male infertility is affecting ~ 7% of the males and about 15% of couples globally. Both genetic and epigenetic factors are reported to contribute to infertility in general. Approximately 50% of infertility cases are contributed by males, affecting one in twenty men of reproductive age group.[1] Chronic illness, obesity, infection, inflammation, epigenetic errors, lifestyle choices and environmental factors play a pivotal role in the genesis of infertility in males. The term "epigenetics," coined by C.H. Wardington, refers to reversible changes in gene expression without any changes in DNA sequences and are inherited via mitotic and meiotic divisions.[2]. In addition to genetic changes, male infertility cases have reported to harbour abnormal DNA methylation of genes belonging to reproduction pathways. Therefore, understanding the genes and pathways regulated by epigenetics and its contribution to male infertility is a promising area of research. It is now clear that, like other diseases and disorders, aberrant DNA methylation, altered expression of non-coding RNAs, and histone tail modifications play a critical role in the genesis and establishment of male infertility.[3] Thus, this review article summarizes the current knowledge of epigenetic dysregulation and underlined mechanisms in male infertility.

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COMPLEXITY OF SPERMATOGENESIS

Spermatogenesis is a highly organized process of development of sperm which involves both mitotic and meiotic divisions, differentiation, maturation, and capacitation. The production of spermatids from spermatogonia takes place in seminiferous tubules. This process happens continuously from puberty to old age within the coiled seminiferous tubules of testis. Development of spermatozoa starts with the formation of spermatogonia in the germinal epithelium, followed by their progressive transformation into primary and secondary spermatocytes and then to mature spermatozoa. Development of spermatids into sperm takes place in epididymis by a process called maturation[4]. Further, spermatogonia undergo continuous mitotic divisions to form primary spermatocytes.[5] Two meiotic divisions will take place in primary spermatocytes resulting in the formation of secondary spermatocytes followed by haploid spermatids. Epigenetic changes such as DNA methylation, histone modifications play a crucial role in the regulation of spermatogenesis (Figure 1). All these processes are highly regulated through post-transcriptional modification and repair.[4]

ETIOLOGY OF MALE INFERTILITY

Male infertility is a consequence of testicular failure, ejaculatory dysfunction, and altered sperm characteristics caused by a combination of (epi) genetic, environmental, and lifestyle factors. The congenital factors of male infertility include cryptorchidism, congenital absence of vas Deference, anorchia, and genetic abnormalities (Kallmann syndrome, Y chromosome microdeletions, Klinefelter syndrome, and mutations in Hypothalamus-pituitary-gonadal axis).

Testis trauma, testicular torsion, inflammation (orchitis, epididymitis), exogenous factors (drugs, irradiation), systemic diseases, varicocele, erectile dysfunction are some of the acquired factors linked with male infertility.[6] The gene sequencing and re-sequencing studies have shown that defects and abnormal functioning of the genes related to endocrinopathies, metabolism, antioxidants, meiosis, cell cycle, development and differentiation, sperm function and spermatogenesis contributes to male infertility.

The environmental factors such as pharmaceutical and industrial by-products, pesticides, herbicides, and phthalates promotes oxidative stress and interrupts the hypothalamo–pituitary–gonadal axis(Table 1).[7] This disruption is reported to hinder the release of gonadotrophin-releasing hormone with the consequent inhibition to the release of Follicle stimulating hormone and Luteinizing hormone. Environmental endocrine toxicants such as Bisphenol A are shown to promote male infertility through inducing hormonal imbalance, oxidative DNA damage leading to epigenetic modification in sperm cells. Chronic inflammation and epigenetic aberrations are the leading cause for the incidence of infertility in male.[8] This article is a comprehensive review focussing epigenetic changes and inflammation axis and its contribution to male infertility.

ROLE OF EPIGENETICS IN MALE INFERTILITY

The causative factors for male infertility are diverse in nature which may be initiated by epigenetic influences such as chromatin remodelling during protamination, abnormal sperm DNA methylation, post translational histone modifications and alteration in non-coding RNA expression.[27] The association of epigenetic dysregulation in male infertility is supported by

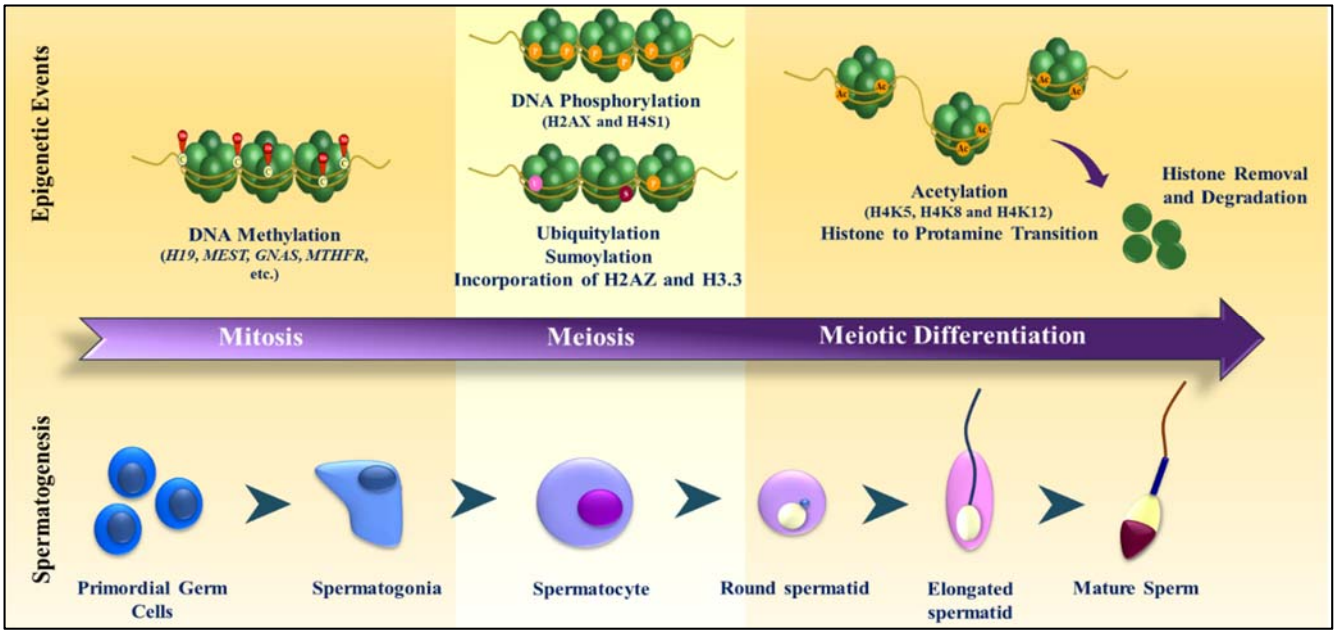

Figure 1: Different stages of spermatogenesis with the associated epigenetic events. In primordial germ cells, DNA methylation by DNMT3L helps to silence transposable elements in order to inhibit its propagation. Later, spermatogonia develop into haploid spermatids through a series of mitotic and meiotic divisions. Global nuclear remodelling occurs in haploid round spermatids, followed by the appearance of a testis-specific linker histone variant H1T2, which in turn leads to chromatin condensation. Hyperacetylation of nuclear histones occurs during the histone-protamine exchange process, which will shortly disassemble and then replaced by transition proteins such as TP1 and TP2 (transition proteins). Replacement of transition proteins by protamines happens at the final stage of spermiogenesis.

Table 1: Effect of Endocrine disrupting chemicals on male reproductive system

Environment toxic metabolites	Industry or occupational group	Effect on reproductive system
Phthalates[9]	Cosmetics, shampoo and soaps, plastics, medical tubing and medication coatings, paints	• Decreases testosterone level and Results in down regulation of genes associated with steroidogenesis • Decreased concentration, normal morphology and motility
Dibutyl phthalate (DBP) [10]	Resins and polymers, adhesives, lacquers, varnishes and printing inks	• Reduced testosterone production in rodents Induces Leydig cell aggregation (LCA) in neonatal marmosets
Di(2-ethylhexyl) phthalate (DEHP) [11]	Plasticizer in polymer products (footwear, shower curtains and toys, medical devices and commercial/ industrial uses)	• In utero exposure resulted in abnormal seminiferous cord formation and multinucleation of spermatogonia in cryptorchid boys
Bisphenol A[12]	Polycarbonate and epoxy resins	• Increases the risk of cryptorchidism and decreases semen quality • Reduced normal sperm morphology and motility.
Dioxins[13]	By-products of manufacturing processes including smelting, chlorine bleaching of paper pulp including herbicides and pesticides	• Induces morphologically abnormal sperm and low linear motility
Polychlorinated biphenyls (PCBs) [9]	Carbonless copy paper and in heat transfer fluids. dielectric and coolant fluids in electrical apparatus,	• Reduces sperm count, motility, and normal morphology
Pesticides[9,14]	Agriculture, gardening, greenhouse work	• Decreased normal sperm morphology, count, volume and motility
Organo-phosphates[9]	Agriculture and in household applications as pesticides	• Decreased semen volume and increased pH,
Alkyl-phenols[15]	Industrial surfactants. emulsifiers for polymerization, as detergents and pesticides	• Increases ROS production, apoptosis, DNA fragmentation and decreases cell viability, spermatozoa MOT and viability
Nonyl-phenol[16]	Nonylphenol is the breakdown product of the surface-active agent, nonylphenol ethoxylate. Used in the domestic cleaning, industrial and institutional cleaning, and also textiles and leather processing	• Cause a toxic effect on both the testis and epididymis and increase the testicular adverse intracellular accumulation of ROS, increase the number of apoptotic cells in testes, and eventually causes of male infertility • Induces male infertility via a negative impact on spermatogenesis and sperm quality
Cadmium[17]	Alloys, pigments, coatings, stabilizers and rechargeable nickel-cadmium (Ni-Cd) batteries,	• Affects human male reproductive organs/system and deteriorates spermatogenesis, especially sperm motility, semen quality and hormonal synthesis/release
Methyl-mercury[18]	Naturally occurring element that is found in air, water and soil. released into the environment from volcanic activity, weathering of rocks and human activity	• Damages on male reproductive functions which may be attributed to the reduction in serum testosterone levels
Arsenic[19]	Wood preservatives, pharmaceuticals, and applications in the metallurgical, glass-making, mining and semiconductor industries.	• Cause steroidogenic dysfunction, disrupts the process of meiosis and post-meiotic stages of spermatogenesis and decreased sperm counts, sperm motility and testicular weight
Aluminium (Al) (Al sulphate, Al nitrate, Al chloride) [2]	Cans, foils, kitchen utensils, window frames, beer kegs and aeroplane parts.	• Cause focal necrosis of the testes, shrinkage of the tubules and spermatogenic arrest at the primary spermatocyte or spermatogonial stages • Decreases in testicular and spermatid counts, and significant decreases in epididymal sperm counts • Caused a decrease in sperm motility, viability, testosterone level and enhancement of free radicals and alterations the activities of 17-ketosteroid reductase, CAT and GST, and GSH content
Lead[21]	Ammunition industry, pottery industry, lead smelting, Battery industry, foundries, and some other metal industries	• Altering the reproductive hormonal axis and the hormonal control on spermatogenesis, • Reduce male fertility by decreasing sperm count and motility,
Carbon disulfide[22]	Viscose rayon industry	• It is a toxin of the male reproductive system resulting in abnormal coital behaviour and decreased sperm counts
Ethylene glycol ethers and their acetates[23]	Silk screen printing, Electronics industry, photography, dyeing, other industries	• Affect testicular function in man and reduced semen quality
Toluene[23]	Shoe industry, painting, laboratory work	• Decreases in the weights of the epididymis and spermatic count
Nitrous oxide[24]	Hospital, dental and veterinary personnel	• Induce toxicity as well as decrease forward progressive motility in human spermatozoa and also cause sperm damage
Paraben[25]	Chemicals used as preservatives in cosmetics and body care products, including Cream, lotions and deodorants.	• Hormone mimicking activities • Reduce synthesis of testosterone
Triclosan[25]	Anti-bacterial and antifungal chemical used in soaps, toothpaste and plastic products such as kitchen chopping boards	• Hormone mimicking activities • Reduce synthesis of testosterone
Triclocarban[25]	Used as an anti-bacterial in personal care products such as	• It has sex hormone disrupting properties

	soaps	
Diesel fuel Exhaust[25]	Used as a fuel in many vehicles	• Disrupts androgen action • Suppresses testicular function in male rats.
Tobacco smoke (Polycyclic aromatic hydrocarbons (PAH) [25]	It includes active or passive smoking	• Blocks androgen synthesis • Testicular dysfunctions • Low sperm count • Sperm abnormalities
Heat, Ionizing radiation, Non-ionizing radiation, microwaves, electromagnetic fields[26]	Occupational exposures in home or industry as well as the mobile phone users.	• Decrease in the level of FSH may negatively affect spermatogenesis. • Decreases the level of testosterone • Radiation-induced increased oxidative stress • Decreased testicular size and insufficient testosterone production • Damage sperm production and deleterious effects on sperm parameters

many studies whose finding suggests that abnormal DNA methylation in imprinted and reproduction associated genes may lead to idiopathic male infertility.[28]

Chromatin remodelling as a risk for male infertility

During the process of spermatogenesis, the histones proteins are substituted by arginine-rich protamines regulated by multistep epigenetic mechanism. Evidently, the fundamental role driven by unique sperm epigenome implies their importance and necessity in normal spermiogenesis. During protamination, increased histone acetylation allows DNA topoisomerase to unwind the DNA followed by replacement of histones with TP1 and TP2. These proteins help in histone removal and subsequent replacement of protamines (P1 and P2)[29]. In mouse spermatozoa, protamines are characterized by 11 post translational modification sites for acetylation, phosphorylation and methylation. It has been shown that improper protamine transcripts processing and irregular P1 and P2 proportions may affect semen quality and can contribute to male infertility[30]. The patients having low testicular volume displayed with lower compactness of the chromatin and poor quality of sperms. The compactness of sperm chromatin was found to be impaired due to overweight, obesity and infections in male accessory organs. Further, poor quality of sperm and less chromatin compactness has been associated with reduced testicular volume possibly leading to male infertility.[31]

DNA Methylation during spermatogenesis

DNA Methylation is a process of addition of a methyl group to the 5th position of cytosine in a CpG dinucleotide by DNA methyltransferases. DNA methylation is vital for X-chromosome inactivation, genomic imprinting, organization of the chromatin into an active and inactive state, and governing tissue-specific gene expression. One of the critical epigenetic changes associated with male infertility is aberrant DNA methylation in sperms and genes associated with spermatogenesis[32]. The development of germ cells is a highly regulated process that is initiated during the development of the foetus and is regulated by epigenetic modification. The male germ cells have reported to possess distinct and unique methylated loci than the somatic tissues.[33] Interestingly, the sperm epigenome is closely similar to embryonic stem cells. Moreover, abnormal methylation of imprinting genes is linked to defective spermatogenesis, thereby contributing to infertility in males. For example, semen DNA methylation analysis showed that 14.4% of infertile men harboured defective H19 locus methylation.[34]

Further, oligozoospermic males showed hypomethylation of H19 locus, especially in cases with ejaculation volumes of $<10\times10^6$/ml. Simultaneous hypo and hypermethylation of imprinted locus are reported in idiopathic infertile males[35]. These data suggest that aberrant DNA methylation of imprinted locus may contribute to oligoasthenoteratozoospermia and oligozoospermia. Furthermore, infertile males also show lower 5-methylcytosine levels when compared with fertile control samples. A meta-analysis by Santi et al. 2017, showed that altered methylation of *H19, MEST, and SNRPN* are strongly associate with male infertility. Interestingly, the study by Sarkar et al. 2019, showed that the methylation profile of the blood DNA of infertile males is significantly different from control samples. This data provided an opportunity to develop blood-based biomarkers for the diagnosis of male infertility.[36] Another study showed that the methylation level of *MEST, GNAS*, and *LINE1* is linked with sperm concentration. *MTHFR* promoter hypermethylation was correlated with low motility, poor morphology, and low sperm count. This data suggests that defect in folate pathway may affect male sexual development and might contribute to male infertility.[32] Qin et al. 2019, investigated the global methylation level in oligospermia and asthenozoospermia cases in a Chinese population. Their study showed that abnormal DNA methylation along with risk factors, might contribute to different types of sperm disorders.[35] A study by Rahiminia and co-workers in 2018 showed that men with oligoasthenoteratozoospermia showed unusual chromatin/DNA integrity, sperm parameters, increased global DNA methylation and upregulated DNMT expression. The defective methylation in imprinting genes was linked with sperm abnormalities, especially sperm motility. For instance, asthenozoospermic males showed diminished DNA methylation in imprinted genes such as *MEST, GNAS*, and *FAM50B* when compared to control.[37]

Interestingly, the DNMT level in germ cells is also crucial for normal spermatogenesis. The *DNMT1* knockdown has been reported to induce apoptosis of sperm germ cells. In addition to this, *DNMT3a, DNMT3b* knock out in male mice caused infertility due to defective methylation patterns in imprinted genes such as *H19, IGF2*. Further, treatment with a demethylating agent such as 5-aza 2'- deoxycytidine leads to defective germ cells, which are directly associated with abnormal sperm morphology and reduced sperm count[38]. Altogether, these studies indicate that errors in methylation patterns or epimutation in imprinting genes and non- imprinting genes can contribute to male infertility by inducing abnormal spermatogenesis.

Histone variant signature and male infertility

Histone modification has a significant effect on controlling the sperm gene expression and are brought about by specific enzymes. Disruption of histone modification, therefore, plays a vital role in the onset of various clinical conditions leading to male infertility. For example, the defects in histone modification in azoospermia, oligospermia, or teratozoospermia. In addition to histone modification, histone variants expressed specifically during spermatogenesis helps in histone to protamine replacement[31]. The histone H1 variants, namely H1T, H1T2, and HILS1, are essential for the normal spermatogenic process, histone to protamine transition, sperm elongation as well as maintenance of normal sperm head. Further, H1t2 mutant males are infertile and showed abnormally elongated spermatids. The defective production of Mst77F has shown to produce defective sperm heads and subsequent infertility in drosophila model. Th2a and Th2b are testes specific H2B variants expressed in mice.[39] The Th2a and Th2b knockout mice exhibited male infertility. The H2a.b knockout male mouse displayed the production of abnormal spermatozoa and clogging of seminiferous tubules and was sub fertile. Th2b is a testis-specific histone H2B variant. The male mouse having mutant Th2b were infertile due to defects in sperm elongation. H3.1, H3.2, H3.3, H3T, and H3.5 are testes specific H3 variants. The depletion of some of these H3 variants is reported to produce abnormal spermatozoa resulting in male infertility. Moreover, testes specific H3 variant encoding genes *H3F3B* is critical for chromatin reorganization and histone to protamine transition. *H3F3B* deficient mouse was completely infertile. Many clinical conditions leading to male infertility also show the depletion of H3 histone variants.[40] For example, non-obstructive azoospermia (NOA) show significantly reduced levels of H3.5.

Abnormal Histone Modifications in male infertility

Histones undergoes several post-translational modifications affecting chromatin conformation thereby, controlling gene expression. Histone modification such as acetylation, phosphorylation, methylation, and ubiquitination play an important role in histone to protamine transition[41]. Abnormal histone modification can contribute to sperm abnormalities and subsequent male infertility. Spatial distribution of Histone H4 acetylation was observed during spermatogenesis, and it plays a crucial role in the histone-protamine transition. Expression of H4K5ac, H4K8ac, and H4K12ac are observed only in spermatogonia, pre-leptotene spermatocytes and elongating spermatids, whereas H4K16ac expression was detected only in elongating spermatids. Studies have shown the importance of H4 acetylation in destabilization and remodelling of nucleosomes. Nucleosomal incorporation of H4K16ac prevents compact chromatin fibre formation and also impacts chromatin forming cross-fibre interactions.[40] All these observations reveal the significance of H4 acetylation in the modulation of a higher order of chromatin structure and the facilitation of histone-protamine transition. In round and elongating spermatids, components of mammalian NuA4 complex, such as TIP60 and EPC1, are found to be colocalized in the periphery of the nucleus near acrosome. The reduction in the levels of TIP60 or EPC1 can affect histone hyperacetylation and histone replacement during sperm formation[42]. SIRT1 also has an essential role in histone acetylation. The higher proportion of abnormal spermatozoa and reduced sperm count was observed in *SIRT1* knockout mice. In *SIRT1* null spermatids, the absence of TP2 in the nucleus and diminished levels of H4K5, H4K8 and H4K12 were found to cause chromatin condensation defects.[43] Expression of PA200 is high in male testis and interruption in its level can lead to defective spermatogenesis. PA200 can recognize the acetylated histones through its bromodomain-like module and can lead to their degradation in ubiquitin independent manner.[44]

Histone phosphorylation is one of the critical post-transcriptional modifications observed during spermatogenesis. In germ cells, phosphorylation of Ser139 residue of histone H2AX plays a crucial role in meiotic recombination and male sex chromosome inactivation. TSSK6 phosphorylate H2AX during spermiogenesis process.[39] The targeted deletion of TSSK6 in mice causes impairment in spermatozoa motility and morphology, which finally leads to infertility. In chromatin compaction and histone accessibility, H4S1 phosphorylation is essential, suggesting its important role in histone replacement during sperm development.[45]

Dynamic regulation of histone methylation was observed in testis with the detectable levels of histone methyltransferases and demethylases during spermatogenesis. H3K9 and H3K27 methylation are found to be linked with the repressed configuration of chromatin, wherein, H3K4 methylation and acetylation are involved in the facilitation of open chromatin configuration.[31] Few studies have revealed the modulation of histone to protamine transition through histone methylation. PYGO2 is localized in the nucleus of elongating sperm recognizes H3K4me3. Downregulation of *PYGO2* can influence *TNP* and *PRM* gene expression and thereby causes abnormal chromatin condensation leading to sterility in males. SETD2, a histone methyltransferase, catalyses H3K36me3, and its knock out in mouse germ cells leads to abnormal sperm development. Impaired activation of *TNP* and *PRM* genes and complete loss of H3K36me3 are the consequence of *SETD2* disruption[46]. Loss of JHDM2A, an H3K9me2/1-specific demethylase, results in defective post-meiotic condensation of chromatin.[47]

The ubiquitination of target proteins is found to modulate numerous cellular events such as autophagy, protein degradation and DNA damage response. Enrichment of ubiquitinated H2A and H2B was found in spermatocytes and elongating spermatids. RNF8 (an E3 ubiquitin ligase) promotes the recruitment of DNA damage response factors on the affected sites by catalysing the ubiquitination of H2A. Alterations in the level of RNF8 leads to defective histone-to-protamine replacement and thus causing the developmental anomalies in spermatids.[48] Ubiquitinated H2A and H2B were reduced in RNF8 null mice with a drastic drop in H4K16ac in testes. Studies have reported the importance of ubiquitinated H2A and H2B for the efficient recruitment of the MOF acetyltransferase complex, which is, in turn, responsible for acetylation of the H4K16 in the chromatin. In humans and mice,

Table 2: Epigenetically regulated genes which are involved in different stages of spermatogenesis and the resulting consequence of their dysregulation

Stages	Genes Hypomethylated	Genes Hypermethylated	Histone modification and variants	Histone modification defects	Clinical Condition	References
Primordial Germ Cells	*IGF2, PLAGL1, SOX2, LEFTY, PRDM14, GTL2, NANOG, KLF4*	*PEG3, MTHFR, DLK1, HRAS, KCNQ1, RHOX*	H3.3	Loss of LSD1/KDM1, loss of TNP1 and P1 expression	OAT, TT, sterility	50,51,52,53,54,55 56,57,58,59,60
Spermatogonia	*H19*	*DAZL, MEST, PEG1 P16, LINE1*	H3K4me3, H3K9me1/2/3, H4K5/8/12ac	Abnormal P1/P2 ratio	OAT, OZ	50,61, 62,
Spermatocyte	*DIRAS3, GNAS*	*DAZL, RASGRF1, MEST, SNRPN*	H3T, H3K9me1/2/3, H3K36me3	Reduction of H3K4 methyl transferase	OAT, OZ	50, ,59,60, 63
Spermatid	*H19*	*DAZL, RASGRF1, RASGRF1, JAMC*	H3T, Th2B, H2A.B, H2AL2, TH2A, HILS1, H1T2 UbH2A, UbH2B H3K36me3, H3K79me3, γH2AX	Hyperacetylated histone H4, Disruption of JHDM2A, crotonylation	OAT, OZ	60 ,63,64,65,66, 62,67
Spermatozoa	*H19, IGF2, PEG3, GNAS*	*RPS6KA2, PAX8, ZAC, NTF3, SFN, CREM, FAM50, PRM1, PRM2*	5% histone and 95% protamine	Higher ratio of H2B:protamine ratio, loss of TSSK6 blocks γH2AX	AT, OS	50,68,69,70,71, 60

***Oligoasthenoteratozoospermia (OAT), Asthenozoospermia (AT), Oligozoospermia (OZ), Teratozoospermia (TT),**

HIWI and MIWI proteins mutations in the conserved destruction box (D-box) lead to RNF8 stabilization, due to defected histone ubiquitination and impaired of histone-to-protamine transition which finally associated with male infertility. PHF7, a novel H2A ubiquitination E3 ligase, is located specifically in the nuclei of elongating spermatids. Aberrant PHF7 expression results in a higher proportion of abnormal spermatozoa and reduced sperm count and thus leading to infertility in the male mouse.[49] Epigenetically regulated genes which are involved in different stages of spermatogenesis and its possible contribution to male infertility is tabulated in Table 2.

Noncoding RNAs (ncRNAs)

Noncoding RNAs (ncRNAs) are a class of regulatory RNAs used by cells to modulate the expression of genes at the transcriptional or post-transcriptional level. These include microRNAs (miRNAs), small-interfering RNAs (siRNAs), endo-small interference RNAs (endo-siRNAs), piwi interacting RNAs (piRNAs) and long noncoding RNAs (lncRNAs).[72] ncRNAs are expressed in male reproductive cells and play a pivotal role in male spermatogenesis. Currently, the functional role of noncoding RNAs in male infertility has been gaining interest due to their enormous role in spermatogenesis.[73]

Azoospermia, oligospermia or asthenozoosperimia have been reported to contribute to male infertility. The abnormal ncRNAs expression is reported during the above described pathological conditions. It has been reported that failure to capacitation of the sperm is one of the risks associated with male infertility. The abnormal expression of ncRNAs can affect the sperm capacitation. For example, HongrES2 is ncRNA expressed in testes. The overexpression of Mil-HongrES2, a spliced product of HongrES2 is reported to affect the capacitation via downregulation of *CES7*. Spermatogonia and early stage spermatocytes shows the expression of Narcolepsy candidate-region 1 gene (NLC1-C). The lower expression NLC1-C is reported to induce apoptosis.[74] Lu and co-workers in 2015 showed the reduced expression of NLC1-C in male infertility cases as opposed to healthy fertile males. These data suggest that large number of ncRNAs are expressed in germline cells which are important for division, differentiation, maturation and capacitation.[75] Study by Wichman et al. 2017, showed expression of specific lncRNAs are important to maintain the sperm count. Further, lncRNAs are important for spermatogenesis and hence can be used as a biomarker for sperm quality.[76] For instance, Dai et al. 2019, showed that lncRNA4667 is critical for spermatogenesis and subsequent fertility in mice. Upregulation of lncRNA Gm2044 and miR-202 is reported in non-obstructive azoospermia. HOTTIP, a lncRNA is linked with male infertility and testicular cancer. Long non-protein coding RNA, NLC1-C, has a role in initial stages of spermatogenesis, which get downregulated in maturation arrested infertile men.[77] NLC1-C gets accumulated more in the nucleus and downregulates miR-383, and miR-320a transcripts lead to the onset of testicular carcinoma. Studies have shown that Tslrn1 (testis-specific long noncoding RNA 1) an X-linked lncRNA in knockout mice impacted spermatozoa count and considered as a potential biomarker for infertility. Furthermore, HOTAIR expression gets reduced in asthenozoospermic and oligoasthenozoospermic males; the study also suggests that sperm dysfunction due to elevated

Table 3: Post transcriptional modifications mediated through small non-coding RNAs in male infertility conditions.

ncRNA type	Sample	Detection method	Differential ncRNA in Male Infertility		Clinical Condition	Ref
			Upregulated	Downregulated		
miRNA	Testicular tissues	Microarray	miR-193a-5p, miR-193a-3p, miR-554, miR-423-3p, miR-557, miR-210, miR-23a, miR-491-3p, miR-371-5p, miR-374a, miR-744, miR-654-5p, miR-129-5p, miR-663, miR-638, miR-572 and miR-302a	miR-17-92 and miR-371/2/3 clusters, miR-1, miR-181a, miR-9, miR-221, miR-145, miR-383, let-7f, let-7i, let-7f-2, miR-19a, miR-29c, miR-30a, miR-20b, miR-30d, miR-34b, miR-652, miR-92a and miR-449a	Non-obstructive azoospermia	82
	Seminal plasma	TaqMan quantitative RT-PCR	miR-7-1-3p, miR-141 and miR-429	-	Non-obstructive azoospermia	83
	Testes	Real Time PCR	-	miR-383	Non-obstructive azoospermia	84
	Seminal plasma	Real time PCR	miR-34c-5p, miR-146b-5p, miR-374b, miR-122, miR-509–5p, miR-181a, and miR-513a-5p	-	Asthenozoospermia	85
	Spermatozoa	Microarray and Real time PCR	miR-30a, miR-26a, miR-200a, miR-429, miR-193b, miR-363, miR-29a, miR-1274a, miR-141, miR-24, miR-428 and miR-99a	miR-34b, miR-1973 and miR-122	Asthenozoospermia	85
	Spermatozoa	Microarray and Real time PCR	miR-141, miR-26a, miR-193b, miR-200c, miR-200a, miR-29a, miR-429, miR-363 and miR-99a	miR-34b, miR-449a, miR-34c-5p, miR-15b, miR-122, miR-16, miR-19a and miR-1973	Oligoasthenozoospermia	86
	Sperm	Microarray and Real time PCR	miR-101-5p, miR198, miR-32-3p, miR-16-1-3p, miR-5095p, miR-6163p, miR-34a5p, miR-7705p, miR-380-5p, miR-1305, miR-605	miR-151-5p, miR-132-5p, miR-935, miR-125a-3p, miR130b-5p, miR320b, miR-195-5p and miR-191-3p	Teratospermia	87
	Sperm	Real time PCR	-	miR-28-5p, miR-449a, miR148b, miR-19b-3p 3p and miR106b-5p	Teratospermia	88
	Seminal plasma	Real Time PCR	-	miR-424/322	DNA damaged sperm	68
piRNA	Seminal plasma	Real Time PCR	-	piR-31068, piR-31925, piR-43771, piR-43773 and piR-30198	Azoospermia	87
	Seminal plasma	Real Time PCR		piR-31068, piR-31925, piR-43771 and piR-43773	Asthenozoospermia	87
	Testicular tissues	Microarray and Real time PCR	piR-20830, piR-4731, piR-6254, piR-419, piR-7152, piR-7548, piR-14195, piR-5026, piR-11482, piR-17765, piR-17102, piR-4484, piR-17260, piR-17098, piR-20511, piR-5802, piR-19121, piR-2510, piR-4745, piR-11873	-	Non-obstructive azoospermia	89
lncRNA	Testicular germ cells	Microarray	475 and 163 up regulated lncRNAs in maturation arrest and hypospermatogenesis respectively	757 and 2370 downregulated lncRNAs in maturation arrest and hypospermatogenesis respectively	Maturation arrest and Hypospermatogenesis	75

reactive oxygen species.

miRNAs are a class of regulatory RNAs crucial for fine-tuning the expression of protein-coding genes. miRNAs are abundantly expressed in testes and is required for normal spermatogenesis process. For example, short RNA transcriptome analysis identified the expression of 775 miRNAs and 20121 piRNAs in normal testes, implying the abundance and complexity of small ncRNAs in testis.[78] let-7 and its family members, miR-34c-5p, miR-103a-3p, miR-202-5p, miR-508-3p, and miR-509-3-5p were abundantly present in normal testis which regulates gene transcripts involved in meiosis regulation, spermatogenesis, apoptosis of germ cell, development of testis and p53-related pathways. Further, the importance of ncRNAs in spermatogenesis has clearly evidenced in *DICER1* gene knockout, and conditional knockout of *DROSHA* and *DICER* which leads to the impaired spermatogenesis and the reduction of spermatocytes in mice[79]. Essential protein-coding genes associated with the multistep spermatogenesis process are abnormally expressed due to aberrant expression of miRNAs and are linked with infertility in males. For example, a study by Wang and co-workers showed that abnormal expression of 7 candidate genes (miR-34c-5p, miR-122, miR-146b-5p, miR-181a, miR-374b, miR-509-5p, and miR-513a-5p) to contribute to azoospermia and asthenozoospermia. Interestingly, the lower expression of these 7 miRNAs contributed to azoospermia, while higher expression has contributed to asthenozoospermia.[79]

Wu et al. in 2012 showed that miR-19b and let-7a are overexpressed oligozoospermia in comparison with fertile males.[80] Abnormal miRNA expression may contribute to spermatogenic failure. For example, seminal plasma analysis

identified the upregulation of miR-141, miR-429, and miR-7-1-3p in non-obstructive azoospermia. In semen, upregulation of hsa-miR-429 and downregulation of hsa-miR-34b, hsa-miR-34c-5p, and hsa-miR-122 are reported in subfertile and non-obstructive azoospermia cases. miR-27a is overexpressed in the semen of asthenoteratozoospermia cases.[79] These data clearly suggest that the abnormal expression of miRNAs may contribute to subfertility and infertility in males.

miRNAs are associated with the regulation of the multi-step spermatogenesis process. For example, regulation of the primordial germ cell (PGC) by miR-290-295 cluster. The reduced expression of the miR-290-295 cluster leads to abnormal specification in mice. MiR-430 also regulates PGC migration and germ cell formation through *CXCR7* and *SDF1A*[81]. A miRNA expression microarray study showed dysregulation of a large number of miRNAs in oligoasthenozoospermic cases (50 overexpressed; 27 downregulated) compared to normal men (42 overexpressed; 44 downregulated). Recent studies have suggested that miRNA expression profiling can be used as a diagnostic marker for male infertility. For instance, the miR-122-3p and miR-141-5p expression analysis in seminal plasma can be used for the diagnosis of idiopathic asthenospermia. Further, sub-fertile males with oligoasthenozoospermic showed higher expression of miR-765 and miR-1275 and lower expression of miR-15a. Additionally, miR-141 has been involved in erectile dysregulation. A recent study showed the dysregulation of piRNAs in male infertility, especially in asthenozoospermia and azoospermia cases.[22] Taken together, these data clearly demonstrate the importance of ncRNA in male infertility and its potential diagnostic application. Summary of post transcriptional modifications mediated through small non-coding RNAs in male infertile conditions are listed in Table 3.

Impact of Inflammation on Male Infertility

Infection and inflammation of the urogenital tract are critical factors associated with male infertility.[90] Inflammation, a process through which the body responds to tissue damage, brings plasma molecules, and leucocytes to infection sites. During acute inflammation, there is increased blood flow, enhanced capillary permeability to larger serum molecules, and increased migration of leukocytes to the affected area.[90] The inability to eliminate infectious agents may result in chronic inflammation, which is characterized by the accumulation and activation of lymphocytes, macrophages, and other immune cells and concomitant stimulation of cytokines. Impaired functionality of male accessory gland, dysregulated spermatogenesis, and blockage for sperm transport can lead to diminished semen quality and may contribute to male infertility.[91]

A variety of factors and process linked with inflammation in the male reproductive system includes obstruction in the ejaculatory duct, epididymitis, urethritis, infection, varicocele, testicular torsion, leucocytospermia, obesity and excessive usage of tobacco and alcohol. The epididymis is the part of the male reproductive system that connects vas deference and testes. Inflammation of epididymis is known as epididymitis, which originates mostly through infection, with bacteria such as *Neisseria gonorrhoeae, Chlamydia trachomatis, Escherichia coli* and many more. Chronic epididymitis may further lead to scrotal swelling, pain and hematuria.[92] Urethritis is characterized by urethral itching and burning sensation during urine discharge. Gonococcal and chlamydial infections are the typical causative factors for urethritis. Chronic urethritis can lead to abnormal narrowing of the urethra, epididymo-orchitis followed by impaired testicular function and hence associated with reduced fertility in males.[92] Testicular torsion is a consequence of an abnormality in supportive tissue, which permits the testis to twist within scrotum leading to severe swelling. Torsion causes testicular damage by squeezing the blood vessels of the testis.[93] Abnormal enlargement and bending of spermatic veins that channels blood from testicle to abdomen are termed as varicocele. Approximately 15-20% of men with fertility issues are diagnosed with varicocele worldwide. Increased seminal ROS, diminished antioxidants, reduced blood flow in the spermatic veins leading to an elevation in nitric oxide, and cytokines is proposed to create an adverse environment for developing sperm and may contribute to male infertility.[9]

Leucocytospermia

A higher level of seminal leucocytes was observed in 10-20% infertile men globally. Leucocytes being the primary source of ROS in semen, produces a thousand times more ROS than normal spermatozoa.[94] Macrophages and polymorphonuclear granulocytes are the most common leucocytes in the ejaculate. Macrophages releases ROS, proteases, and neutrophil chemotactic factors upon activation to fight against infection [95]. This, in turn, produces higher amounts of NADPH, whose oxidation eventually generates superoxide anions. Elevated oxidative stress, in turn, activates chemokines such as IL-6, CXCL5, IL-8, and CXCL8, creating an imbalance between seminal ROS and antioxidant levels resulting in oxidative stress-induced infertility.[90]

Infection

Viruses, bacteria, protozoa, and other microbial infections in the male genital tract can impart negative effects on male fertility. The inflammatory response triggered as a result of microbial infection in the genital tract can impair spermatogenesis and can also obstruct the seminal tract. In response to infection, the levels of granulocytes and polymorphonuclear leucocytes were elevated, resulting in increased oxidative stress and impaired sperm production.[96] *Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum, Mycoplasma sps, Escherichia coli,* HIV, Hepatitis B, and C viruses are the most common infectious agents causing infertility in male. Common microorganisms causing male genital tract infections and their manifestations are summarized in Table 4. *N. gonorrhoeae*, upon infection, activate NOD receptors, which in turn activates complement proteins, cytokines, chemokines, TLR2, and TLR4 (cytosolic pattern recognition receptors on cell surfaces).[97] *Chlamydia trachomatis* is one of the most predominant sexually transmitted

bacterial infection. Three theories have been proposed to explain the mechanism behind chlamydia mediated infertility in males. The first theory states that infection will result in tissue damage and subsequent activation of IL1, which will further activate macrophages and polymorphonuclear WBCs to induce TNF-α, IFN-γ, IL6, IL8, and IL10. This cascade by activating oxidative stress contributes to infertility. The second mechanism explains the induction of excessive ROS through interaction between the CD14 receptor and LPO present in high concentration on the sperm membrane, where the third mechanism proposes the generation of anti-sperm antibodies [96]. *Treponema pallidum,* being a causative organism of syphilis, activates macrophages and dendritic cells through CD14 and TLR1 and TLR2 dependent pathways. Studies have shown the involvement of IL1, IFN-γ, and IL2 in the clearance of *T. pallidum*. Abnormal elevation in the levels of these cytokines results in harmful inflammation leading to oxidative stress-induced DNA damage and apoptosis. Infection with *Mycoplasma genitalium* and *Mycoplasma hominis* alters sperm morphology and motility and increases DNA damage via inflammation-induced ROS production.[98] ~65%–80% of cases of type I and II prostatitis are due to *Escherichia coli* infection. Recruitment of leucocytes caused by *E. Coli* infection induces neutrophilic production of ROS. It causes a reduction in sperm motility by activating proinflammatory cytokines such as IL6, which directly affects the sperm cell membrane.[99] Accumulation of leucocytes, monocytes, and macrophages is observed in men with symptomatic HIV infection. In men with chronic Hepatitis B infection, a higher concentration of IL18 in semen is reported. Elevated levels of IL18 can further trigger natural killer cells to release INF-γ.[100] Infection with Hepatitis C virus increases NO and TNF-α levels. Consequent activation of polymorphonuclear leukocytes and lymphocytes can produce ROS via NOX2 and lead to a drop in mitochondrial membrane potential in spermatozoa. Oxidative stress-induced by Hepatitis C infection results in decreased sperm motility, increased DNA damage, and apoptosis.[101]

Obesity

Several studies have shown the association of obesity with infertility in men.[101] Obesity is characterized by the immoderate accumulation of white adipose tissue or body fat to the extent that it can potentially affect life expectancy and health. Individuals with Body Mass Index exceeding 30kg/m^2 are considered obese. Numerous studies have proved a negative correlation between BMI and sperm motility. Chavarro et al in 2007, have shown reduced sperm count, concentration, and ejaculate volume in men having BMI of more than 25kg/m^2 than in men with normal body mass index. Male obesity is proposed to alter sperm morphology and total motile sperm count[114,115]. Ample evidence supports the association between obesity and chronic inflammation of the male reproductive tract[116]. Obesity can result in a systemic inflammatory response which in turn influence semen quality and parameters. Altered adipokine secretion as a result of elevated visceral abdominal fat, can generate reactive oxygen species and can damage DNA

Table 4: Infectious agents responsible for inflammation mediated infertility in male

Disease and pathogen	**Affecting area and Manifestations**
Bacteria Chlamydia[102] *Chlamydia trachomatis*	Epididymis • Epididymis, orchitis, prostatitis and urethritis which leads to testicular atrophy, canalicular damage and obstructive azoospermia
Gonorrhea[96] *Neisseria gonorrhoea*	Reproductive tract, urethra • Causes urethral strictures and epididymo-orchitis
Syphilis[103,93,104] *Treponema pallidum*	Semen, epididymis • Syphilitic epididymitis can cause obstruction of the epididymis • Obstruction of the epididymis • Causes inflammation leading to OS-induced sperm DNA damage and apoptosis
Prostatitis[99,101] *Escherichia coli*	Sperm membrane • Generates ROS • Diminishes sperm membrane integrity • Decreases sperm motility, membrane integrity
Urethritis, cervicitis and endometritis, salpingitis and pelvic inflammatory disease[105,106] *Mycoplasma homini*	Urogenital tracts • Cause genital diseases (urethritis, prostatitis, rarely orchitis) and change in motility, morphology, and fertilization potential in human spermatozoa
Chorioamnionitis, pelvic inflammatory disease, urethritis, prostatitis, epididymitis, and infertility[106] *Ureaplasma urealyticum*	Urogenital tracts • Cause of nonchlamydial, non-gonococcal urethritis (NGU) in men
Virus Herpes [107,108] Herpes simplex virus (HSV)	Male genital tract • Reduction in the proliferative activity of spermatogonia. • Meiosis block and increase in apoptosis of germ cells
Bluetongue[109] Bluetongue virus (BTV)	Testicles • Testicular degeneration and azoospermia
Sexually transmitted disease[104] Human immunodeficiency virus	Semen • Associated with infectious semen and the risk of virus transmission. • HIV in the male reproductive tract are infected leukocytes.
Hepatitis (Sexually transmitted disease) [101] Hepatitis B	Semen • Defective spermiogenesis and decreased fertilisation rates
HCV infection[104]	Sperm

Hepatitis C	• Chronic inflammation • Decreases sperm motility • Increases DNA damage and apoptosis
Viral orchitis[110,111,106] Mumps virus	Epididymis • Viral infections may cause epididymitis. • Persistent focal inflammation in human testis • Affect androgen production and cause atrophy of seminiferous tubules
Chronic orchitis[112] Coxsackie, Epstein-Barr, varicella, influenza	Testis • Chronic orchitis • Disrupt spermatogenesis and irreversibly alter both sperm number and quality
Genital tract infections (Sexually transmitted diseases) [104] Human papillomavirus	Genital tract, Sperm • Low capacity for fertilization, abnormal count, production of anti-sperm antibodies and; in particular, reduction of motility
Genital tract infections (Sexually transmitted diseases)[104] Human cytomegalovirus (HCMV)	Sperm, Genital tract • Viruses can attach to the surface of spermatozoa and present in the epididymis, deferent duct, prostate or in seminal vesicles • Virus can infect immature germ cells and cause gametotoxic effect • HCMV impaired the sperm parameters
Parasite Nongonococcal urethritis[113,104] *Trichomonas vaginalis*	Urethritis, Epididymitis • Nongonococcal urethritis and are contribute to male infertility • Trichomonal cytopathogenicity in men and balanoposthitis, epididymo-orchitis, prostatitis and possible infertility • Reduces the serum function
Fungai Epididymitis[110] *Candida albicans*	Epididymis • Ascending canalicular infection
Epididymitis[110] *Histoplasma capsulatum*	Epididymis • Ascending canalicular infection

integrity of sperm through recruitment of proinflammatory WBCs and higher NADPH oxidase activity. Further, enhanced NADH and $FADH_2$ levels were observed in men with a high-fat diet. These reducing agents can promote electron leakage and can also form superoxide radicals. Removal of these radicals by superoxide dismutase generates H_2O_2, which will further promote systemic ROS generation, thereby imparting a deleterious effect on spermatogenesis.[124] Studies using rodent models have found the expression of TNF-α in adipocytes. Association of NLRP3 (NOD-like receptor family pyrin domain containing-3), an inflammasome, with obesity-induced inflammation, has been reported recently. Elevated levels of cytokines, such as IL-6 and TNF-α, were observed in the seminal plasma of obese men. Adipose fibroblasts harbour aromatase enzyme, which readily converts testosterone to estradiol. This conversion not only reduces testosterone levels but also increases serum estradiol with the concomitant decline in inhibin B level in serum. Thus, obesity in men adversely affects fertility by altering these endocrine pathways, elevated seminal ROS, and also by causing physical manifestations like erectile dysfunctions and sleep apnea.[101,118]

Excess intake of Tobacco and Alcohol

Increased levels of oxidative stress markers and seminal ROS are observed in the case of tobacco users. Smoking can raise ROS in semen by 107% and also can enhance leucocyte accumulation by 48%. Recruitment of proinflammatory leucocytes eventually elevates seminal ROS, which will, in turn, affect sperm quality and spermatogenesis.[119] Smoking induced oxidative stress can produce oxidized DNA base adducts (such as 8-hydroxy-2'-deoxyguanosine), thus affecting chromatin integrity in sperm. Decreased sperm viability, motility, and morphological anomalies are the consequences of tobacco usage. Smoking not only affects sperm development but also can cause varicocele, compromised accessory gland functions, epididymitis, erectile dysfunctions, and changes in the hypothalamic-pituitary-gonadal axis.[120,121]

Further, tobacco can generate many carcinogens and mutagens such as benzopyrene, polycyclic aromatic hydrocarbons, dimethylbenzanthracene, and radioactive polonium through a series of chemical reactions and most of these toxicants are known to promote chromosomal aberrations in spermatozoa and thus reducing the success rate in ART (Assisted Reproductive Technologies).[120,122] Alcohol consumption can affect male fertility by modifying sperm shape, count, size, and motility. When compared to non-drinkers, alcohol consumers are observed to have significantly higher serum lipid peroxide and reduced antioxidant levels. Alcohol can generate highly reactive free radicals by impairing the metabolic pathways and hence interferes with the antioxidant defence system of the body. Smoking and excessive drinking not only affects sperm quality and hormone levels but also alters DNA methylation patterns of imprinted genes.[101,123]

Interplay between Inflammation, Oxidative Stress and Epigenetic Modifications in Male Infertility

Inflammation and epigenetics are closely linked. Several genetic and environmental factors such as diet, chemicals, infection, and personal lifestyle triggers inflammation and epigenetic modifications that may contribute to male infertility.[39,92] Studies have shown that epigenetic changes can induce inflammation and vice versa. Environmental factors, together with genetic susceptibility and epigenetic factors, can

induce physiological inflammation.[124] The failure to resolve physiological inflammation is reported to induce pathological inflammation leading to various pathological conditions. It is now clear that a variety of external factors can bring about the establishment of abnormal epigenetic alteration leading to the development of a pro-inflammatory phenotype contributing to male infertility. The abnormally high levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF–α), interleukin–1 alpha (IL–1 α), and interleukin 1 beta (IL–1 β) in the male reproductive system can severely affect the sperm production.[93] Further, inflammation can also induce oxidative stress by inducing sperm DNA damage impairs sperm function. Intake of high-fat diet induces alterations at ROS level, sperm DNA fragmentation, and reduced sperm capacitation in mice.[125] Furthermore, aging also was shown to be associated with fragile DNA, chromatin fragmentation, and meiotic errors in cell division during spermatozoa development.[126] The epigenetic marks in sperm DNA and reproduction-related genes confers possible risk for male infertility. The repro-toxicants are endocrine disruptors with the ability to alter the epigenetic pattern at the post-transcriptional level, and these modifications contribute to inheriting change in upcoming generations through germ cells. The various inflammatory and infectious agents cause spermatozoal dysfunction through oxidative stress. On the other hand, certain antibiotics can induce oxidative stress, and especially antiretrovirals used for treating viral infections can reduce fertility.[125]

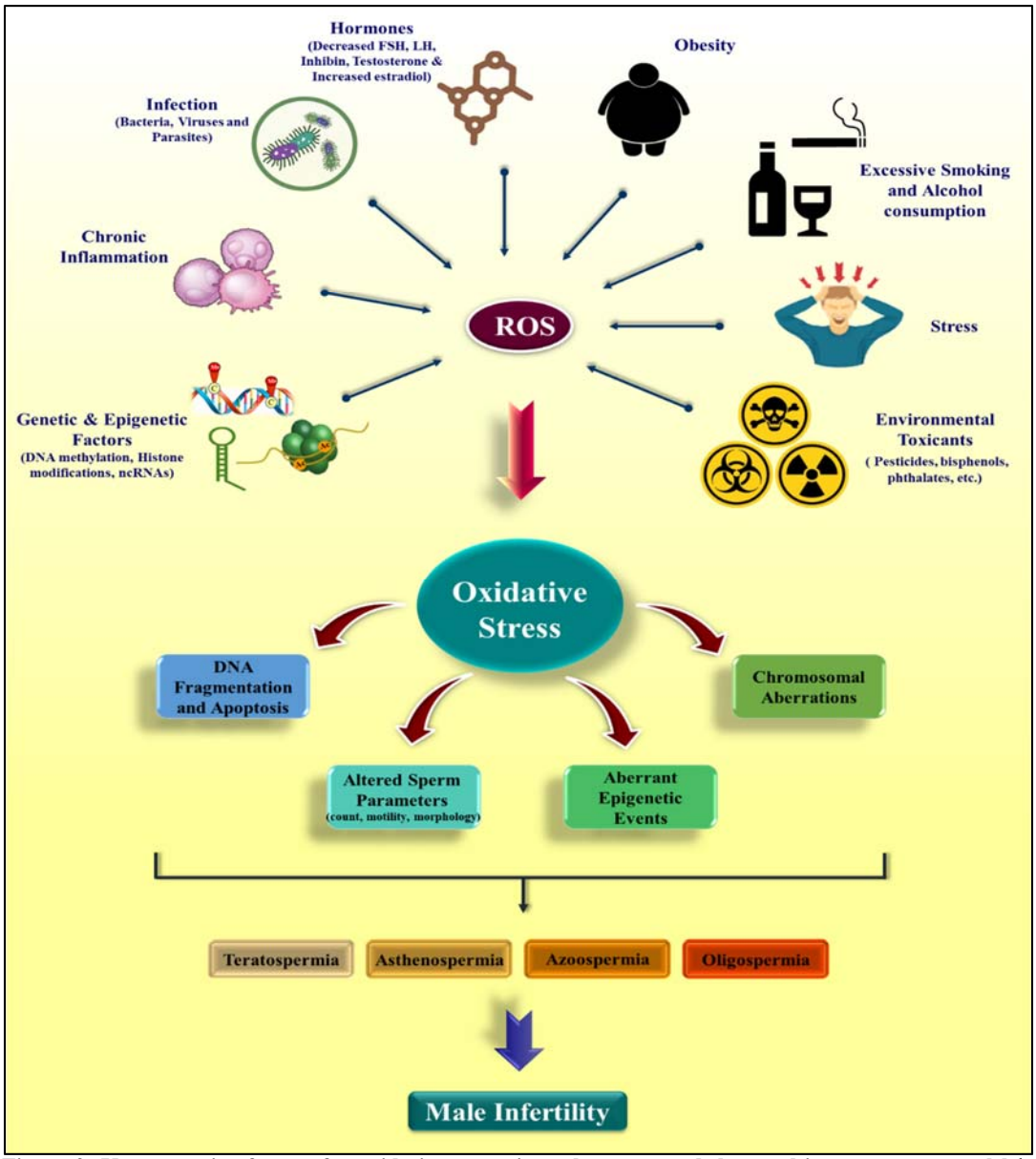

Figure 2: Key causative factors for oxidative stress in male sperm and the resulting consequences. Male infertility is a multifactorial reproductive health issue affecting 1 in 20 men of reproductive age group. Genetic and epigenetic factors (DNA methylation, histone modifications and ncRNAs), chronic inflammation, infection (Bacteria, virus, fungi and parasites), hormones (decreased FSH, LH, inhibin, testosterone and increased estradiol), obesity, excessive smoking and alcohol consumption, psychological stress and environmental toxicants (pesticides, bisphenols, phthalates, etc.) are the major contributors of ROS, which eventually generates oxidative stress in spermatozoa. Elevated OS promotes DNA fragmentation and apoptosis, alterations in sperm parameters, aberrant epigenetic changes and chromosomal aberrations and will finally results in male infertility.

The non-communicable diseases like obesity have a strong correlation with infertility due to hormonal imbalance, which subsequently increases the secretion of pro-inflammatory cytokines and ROS, which can cause sperm DNA fragmentation and epigenetic modifications. Certain lifestyle changes, pharmacotherapy, and treatment with aromatase inhibitors can help to overcome obesity-induced male infertility.[127] The development of assisted reproductive techniques (ARTs) leads to epigenetic changes that may alter the normal gene imprinting processes.

The studies have shown that protamination is a crucial mechanism to ensure the functional capability of sperms. Certainly, abnormal protamination could result in inadequate condensation and fragmentation of sperm DNA, which directly predisposed to oxidative stress. The external factors like heat, radiation, and metals increase leucocyte count, oxidative stress, and decrease circulating antioxidants and show direct defects like immature spermatogenesis, sperm structural distortions, and apoptosis in testis.[39,125]

A study by Schütte et al. 2013, is one of the earliest and comprehensive studies providing evidence for the association between aberrant DNA methylation, inflammation, and male infertility. Using Illumina HumanMethylation27 BeadChips showed the hypermethylation of genes associated with spermatogenesis pathway genes and hypomethylation of genes belonging to inflammation and immune response-related genes[128]. This study clearly suggests the important role of aberrant DNA methylation inactivation of inflammation pathway and its contribution to male infertility. Very interestingly, a study by Spiess et al. 2007, showed that the transcript level of inflammatory pathway genes was elevated in infertile males. These data suggest the role of inflammatory pathway activation by epigenetic dysregulation in male infertility.[129]

Epigenetic changes play key role in gonadal sex determination and testes development. External agents such as steroidal factors can induce epigenetic transgenerational phenotypes via male germ line reprogramming. Further, male infertility patients show DNA methylation defects either in DNA methylation machineries or at imprinted loci strongly suggesting the link between epigenetic dysregulation and male infertility[130]. Inflammation and oxidative stress are implicated in male reproductive ageing. Increased paternal ageing is associated with changes in reproductive hormones bringing about epigenome alteration in reproductive system. Reproductive tract inflammation can induce oxidative stress leading to the establishment of altered epigenome which in turn is an important contributor of male infertility. The inflammation due to exogenous or endogenous agents can lead to increased ROS production and oxidative stress. Consistent and abnormally high levels of ROS can induce epimutation in the DNA and may lead to establishment aberrant DNA methylations and are implicated in infertility in male[130]. Studies have also shown the association between elevated OS and enhanced DNMT expression.[131,132] Based on the available literature we propose that epigenetic changes are one of the critical events in clinical conditions associated with male infertility. In this direction, more work needs to be undertaken.

CONCLUSION

Varieties of anomalies ranging from epigenetic errors to physical aberrations, to psychological abnormalities have been implicated in male infertility (Figure 2). Despite the availability of numerous treatment options, the success rate in combating infertility issues is still low. Epigenetic studies will provide a handy platform for a better understanding of intricate interaction between environmental factors with the genome in causing such abnormalities and actionable targets for diagnosis and treatment. Studying the epigenetic pattern of defective spermatozoa could help to explore the underlying mechanism causing male infertility. All possible interventions which can reduce the substantial consequence of inflammation (oxidative stress, DNA damage, and apoptosis) will not only improve male reproductive health but also enhance the success rate of ART. There is a need for further functional studies to unravel the secrets of epigenetic patterns in sperm because the knowledge of sperm epigenetics is a prerequisite for the diagnosis and prognosis of male infertility.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest for this article.

ABBREVIATIONS

ART- Assisted Reproductive Technologies
BMI – Body Mass Index CD- Cluster of Differentiation
CES7 – Carboxylesterase 7
CXCL 5/8 - C-X-C Motif Chemokine Ligand 5/8
CXCR - C-X-C chemokine receptor
DNA- Deoxy Ribonucleic Acid DNMT- DNA Methyl Transferase
EPC1 – Enhancer of Polycomb homologue 1
FADH2 - Flavin adenine dinucleotide
FAM50B - Family with Sequence Similarity-50 Member B
GNAS - Guanine nucleotide binding protein, alpha stimulating
hsa – Homo sapiens
H1T2 - Histone H1-Like Protein
H2AX – H2A Histone family member X
H3K4 – Methylation at 4th lysine residue of histone H3
H3K9 - Methylation at 9th lysine residue of histone H3
H3K27me3 - Tri-methylation at the 27th lysine residue of histone H3
H3K4me3 - Tri-methylation at the 4th lysine residue of histone H3
H4K5ac – Acetylation at 5th lysine residue of histone H4
H4K8ac - Acetylation at 8th lysine residue of histone H4
H4K12ac - Acetylation at 12th lysine residue of histone H4
H4K16ac - Acetylation at 16th lysine residue of histone H4
H4S1 – Phosphorylation at 1st Serine residue of histone H4.
HIls1 - Histone H1-like protein in spermatids 1
HILS1 - Spermatid-specific linker histone
HIV – Human Immunodeficiency Virus
HOTAIR – HOX transcript antisense RNA

HOTTIP - HOXA distal transcript antisense RNA
IGF2- Insulin Like Growth Factor 2
IL-1α/6/8 - Interleukin–1 alpha/6/8
JHDM2A - JmjC-domain-containing histone demethylase 2A
LH- Luteinizing Hormone
LINE1 - Long interspersed nuclear elements 1
lncRNA – long noncoding Ribonucleic Acid
LPO – Lipid Peroxidase MI – Male Infertility
MEST - Mesoderm-specific transcript
miRNA – microRNA MIWI – Piwi protein in mice
Mst77F - Male-specific transcript 77F
MTHFR – Methylene Tetrahydrofolate Reductase
ncRNA – noncoding Ribonucleic Acid
NLC1-C - narcolepsy candidate-region 1
NLRP3 - NOD-like receptor family pyrin domain containing-3
NO – Nitrogen Oxide NOA – Non-Obstructive Azoospermia
NOD - Nucleotide-Binding Oligomerization Domain
NOX2 – NADPH Oxidase 2
NuA4 – Nucleosome Acetyl transferase of H4
OS – Oxidative Stress PA200 – Nuclear Proteasome Activator 200
PGC - Primordial germ cell PHF7 - PHD Finger Protein 7
PRM – Protamines piRNA – piwi-interacting Ribonucleic Acid
PYGO2 – Pygopus Homologue 2
RNF8 - E3 ubiquitin-protein ligase
ROS – Reactive Oxygen Species
SIRT1 - Sirtuin 1
sdf1a - stromal cell-derived factor 1a
SETD2- SET domain–containing 2
SNRPN - Small nuclear ribonucleoprotein-associated protein N
Th2a/b - Testes specific H2B variants
TIP60 – Tat Interactive Protein 60
TLR - Toll Like Receptor
TNF-α - Tumor Necrosis Factor-alpha
TNP – Transition Nuclear Proteins TP- Transition proteins
Tslrn1 - Testis-specific long noncoding RNA 1
TSSK6 - Testis Specific Serine Kinase 6

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