UNIVERSA MEDICINA

pISSN: 1907-3062 / eISSN: 2407-2230

Univ Med 2026;45:XX

DOI: https://doi.org/10.18051/UnivMed.2026.v45.XX

REVIEW ARTICLE

Role of global deoxyribonucleic acid methylation and molecular hypoxia changes in male infertility

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Cite this article as: Abdul Jabbar ZR, Al-Jumaily RMK. Role of deoxyribonucleic acid methylation and molecular hypoxia changes in male infertility. Univ Med 2026;45:XXX

Date of first submission, January 24, 2026
Date of final revised submission, February 20, 2026
Date of acceptance, March 6, 2026

ABSTRACT

Infertility is recognized as a major global health issue and has become one of the primary challenges in reproductive health; its prevalence and incidence are steadily increasing worldwide. Recent research has highlighted the critical role of epigenetic dysregulation as a direct factor affecting male infertility. The objective of this review is to investigate the relationship between epigenetic modifications (specifically 5-methylcytosine and 5-hydroxymethylcytosine) and essential male reproductive outcomes, including the spermatogenesis process, testicular function, and semen quality parameters. Furthermore, this study explores the influence of micronutrients such as vitamin B_{12}, homocysteine metabolism, and antioxidant capacity on DNA methylation and genomic stability. Significantly, this review establishes a novel link between infertility and cellular hypoxia focusing on the modulation of hypoxia-inducible factor (*HIF-1α* and *HIF-2α*) as a potential therapeutic strategy. Additionally, this work introduces the overlooked association between *HIF-2α* and infertility, offering novel insights since current findings regarding *HIF-2α* in male infertility are remarkably limited. A literature search was conducted on PubMed, ScienceDirect, Springer Link, Oxford Academic and Nature databases for sources published in English from 2015 to 2025, using the following keywords: male infertility, DNA methylation, hypoxia, *HIF-1α*, and immunity. Our work investigates the interruption of inflammatory cytokines and immunological markers by linking them with molecular factors, micronutrients and hypoxia pathways. This approach provides an integrated foundation for advanced diagnostic and therapeutic strategies. Ultimately, understanding these molecular signatures provides ways to enhance reproductive outcomes and improve success rates of assisted reproductive techniques.

Key words: Male infertility, DNA methylation, vit. B_{12}, homocysteine, hypoxia, *HIF-1α, HIF-2α*), interleukin-41

Abbreviations:
5-hmC : 5-hydroxymethylcytosine
5-mC : 5-methylcytosine
ASA : anti-sperm antibodies
BAX : Bcl-2-associated X protein
Bcl-2 : B-cell lymphoma 2
DNA : deoxyribonucleic acid
DNMTs : DNA methyltransferases
FSH : follicle-stimulating hormone
Hcy : homocysteine

HIF-1α : hypoxia-inducible factor-1 alpha
HIF-2α : hypoxia-inducible factor-2 alpha
HRE : hypoxia-response element
ICSI : intracytoplasmic sperm injection
IFN-γ : interferon-gamma
IL-1β : interleukin-1 beta
IL-4 : interleukin-4
IL-10 : interleukin-10
IL-17A : interleukin-17A
IL-18 : interleukin-18
IL-41 : interleukin-41
LH : luteinizing hormone
MTHFR : methylenetetrahydrofolate reductase
NRF-1 : nuclear respiratory factor 1
PGCs : primordial germ cells
ROS : reactive oxygen species
SNRPN : small nuclear ribonucleoprotein polypeptide N gene
TLRs : toll-like receptors
TNF-α : tumor necrosis factor-alpha
VEGF : vascular endothelial growth factor
B_{12} : vitamin B_{12} (cobalamin)
WHO : World Health Organization

INTRODUCTION

Infertility is a significant public health problem and has become one of the leading challenges in reproductive health. Globally, infertility affects between 48 million couples and 186 million individuals annually; approximately one in eight couples of reproductive age is affected, with a 20% contribution from male factors.[1] Recent studies have reported the prevalence of male infertility to be about 56 million people worldwide. The total combination of female and male factors contributes an additional 30%.[2]

The high prevalence of infertility may be linked to numerous factors, including occupational hazards, smoking, stress, war, lifestyle, and genetics.[3] Aging has a significant effect on male sexual function including sperm parameters, fertility state, reduced sperm capacity, prolonged period to conception, and increased rates of miscarriage.[4]

According to the World Health Organization (WHO) there are two types of infertility: primary infertility meaning that a woman has never conceived after one year without using contraception; and secondary infertility is the inability to conceive in a couple who had previously achieved at least one successful conception. Generally, infertility may be related to either male or female reproductive systems or both partners.[5] Recently, the term subfertility has been used for couples failing to conceive despite maintaining frequent unprotected sexual activity and who do not fit into the infertility diagnostic criteria.[3,6] Male subfertility is more prevalent and poorly understood in most couples; therefore, intracytoplasmic sperm injection (ICSI) may solve the problem despite poor semen quality. [7] Causes of male infertility vary widely including varicocele, endocrine dysfunction and environmental pollutants.[8]

This article discusses the molecular mechanisms underlying male infertility, specifically focusing on epigenetic modifications and hypoxic stress. The following sections will outline the roles of DNA methylation, vitamin B_{12} and immunological markers, providing the necessary background to understand their impact on male fertility.

METHODS

In this review paper, a total of approximately 600 articles published in English in the last 10 years (from 2015 to 2025) were retrieved from Science Direct, PubMed, Springer Link, Oxford Academic, and Nature databases using the following keywords: Male Infertility, DNA Methylation, Hypoxia, *HIF-1α*, and immunity. Initially, 600 articles were found to match the inclusion criteria, but eventually, only 80 articles

were selected and analyzed. Articles were removed due to duplication, lack of access and irrelevance to the molecular mechanisms discussed (Figure 1).

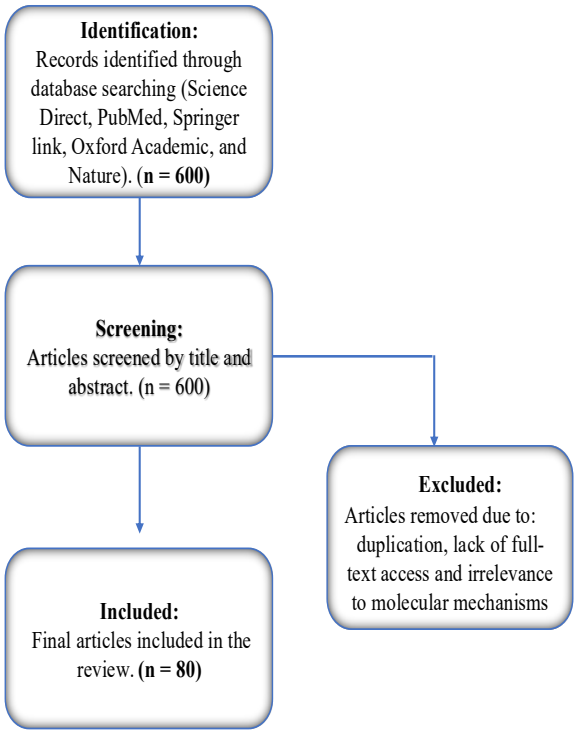

Figure 1. Flow diagram of the literature search and study selection process

Epigenetics and DNA methylation

Because biological life is based on genetic interactions in living organisms and different mechanisms of disease onset have emerged, arising from regulatory disorders, the behavior of cells is regulated by the expression of proteins, both by transcription and translation processes that are controlled by epigenetics.[9] The term "epigenetics" describes the mechanism of regulating genes without altering the DNA sequence, including DNA methylation, histone modifications and microRNA regulation.[10,11] It has been found that the DNA modifications influence gene expression and cell activity, providing an interaction between the genetic and the environmental factors.[12]

Epigenetic processes play a crucial role in understanding the mechanisms of many human diseases, using screening technology for the CpG sites of the whole genome. These technologies provide a new viewpoint on methylation-based biomarkers that may be applied to the prognoses of many diseases.[13,14]

DNA methylation is the main player in the epigenetic process; it is an epigenetic change that occurs when a methyl group (CH3) is added to a specific region within the promoter, mainly at the cytosine CpG dinucleotides. It comes in two forms: hypermethylation and hypomethylation. The enzymes DNA-methyltransferases (DNMTs) and DNA demethylases are responsible for adding or removing the methyl group, respectively.[9,15]

Subsequently, it was revealed that methylated cytosine may be oxidized to 5-hydroxymethylcytosine (5-hmC) or further to 5-formyl cytosine and 5-carboxylcytosine, which action is catalyzed by 2- oxoglutarate- dependent dioxygenases. The compound 5-hmC acts as a biological marker and plays an important role in controlling biological processes within the living cell.[13] The highest levels of 5hmC are observed in the brain tissue and primordial germ cells (PGCs), whereas demethylation patterns of 5-hmC are seen in embryonic cells, causing an epigenetic reset and the restructuring of the 5mC landscape through the development of specific tissues.[16] However, the 5-hmC level in sperm cells is four times lower than in other somatic cells or tissues.[17] This reduction is biologically unique and may represent a protective approach to maintain methylation stability and prevent defective inheritance during the primary stages of embryonic development.[18]

Relation of DNA methylation with infertility

Male infertility has been closely linked to DNA methylation, therefore developing strategies for treatment requires an understanding of the mechanisms of abnormal sperm DNA methylation patterns.[19] Environmental factors and lifestyle choices, such as diet, smoking and alcohol consumption are the main causes of abnormal condensation patterns of chromatin in sperm which are related to male infertility.[10] Distinct DNA methylation patterns were found in the spermatozoa compared to other somatic cells and specific sperm-methylation sites, and are directly associated with controlling the functional capacity of the germ cells. [20] There are appropriate control and regulation of DNA *de novo* methylation and demethylation mechanisms, which are required for normal sperm function. A high methylation pattern was seen at the promoter region of developmental genes in the sperm genome.[21]

Global 5- methylcytosine may be used as an indicator for spermatogenesis and testicular function, in which normal spermatogenesis is

associated with high methylation levels, while low methylation levels are related to defective spermatogenesis.[22] This process has been linked to mutations in the methylenetetrahydrofolate reductase gene (MTHFR), which is involved in folate-mediated methyl group metabolism, where these mutations are more likely to lead to the development of infertility.[23] Furthermore, there is an association between hypermethylation of the *MTHFR* gene promoter and methylation abnormalities in the paternal imprinted gene H19 of sperm DNA from infertile males with both normal and defective semen parameters. These findings reveal that *MTHFR* hypermethylation-induced inactivation causes defective methylation of sperm at imprinted loci that hinders fertilization.[10]

In addition, hypermethylation of the imprinted small nuclear ribonucleoprotein polypeptide N gene (*SNRPN* gene) has been related to impaired sperm motility, sperm abnormalities, aberrant gene expression and a lower percentage of morphologically normal sperm in the oligospermia group compared to normospermia.[24,25]

There usually is a strong association between poor semen quality and a higher frequency of hydroxymethylcytosine (5-hmC). High levels of 5-hmC-positive spermatozoa are linked to negative outcomes regarding semen quality, such as poor morphology and reduced motility. On the other hand, 5-hmC is positively correlated with DNA fragmentation of sperm, which is associated with fertility impairment.[18] Moreover, many studies have mentioned the role of DNA methylation on spermatogenesis and confirmed the role of these mechanisms as prognostic markers for male infertility.[10] **Figure 2** explains the effect of defective DNA methylation on male reproductive outcomes.

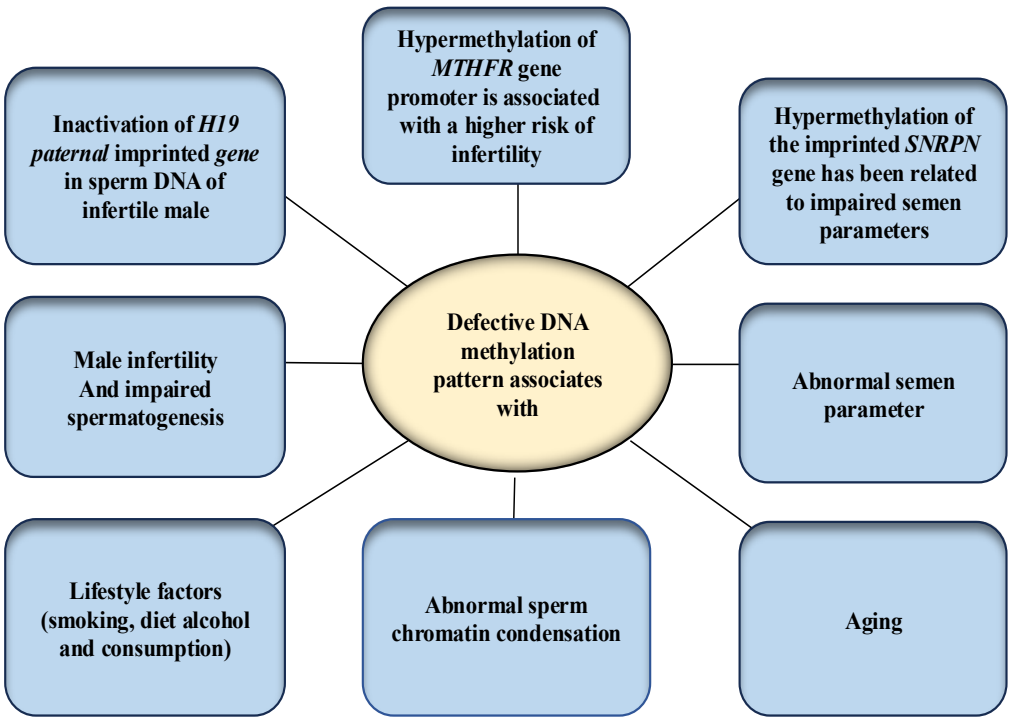

Figure 2. The effect of DNA methylation and epigenetic modifications patterns on the male reproductive outcomes. The diagram shows the interplay between environmental factors (lifestyle, aging, diet, and smoking) and the methylation patterns of the genes [e.g., methylenetetrahydrofolate reductase (MTHFR), small nuclear ribonucleoprotein polypeptide N (SNRPN), and H19]. Abnormal methylation patterns are associated with impaired semen parameters, abnormal chromatin condensation, and an increased risk of infertility

Role of vitamin B_{12} and homocysteine on DNA methylation and infertility

Cobalamin, also known as vitamin B_{12}, is the cobalt-containing corrin ring cofactor that is essential for two enzymes in higher animals, namely L-methylmalonyl-CoA mutase and methionine synthase.[26] It is a water-soluble vitamin obtained exclusively from animal food sources and plays a vital role in the synthesis and repair of DNA and in regulating the nervous

system.[27] Many studies focus on the role of methyl-donating nutrients (vitamins B_6, B_{12}, and folic acid) in increasing DNA methylation levels and reducing the risk of cancer development. [28] It has been found that intake of micronutrients including zinc and B vitamins decreases sperm DNA damage and fragmentation, and enhances sperm nuclear maturation along with other clinical outcomes.[29] Moreover, a low serum vitamin B_{12} level is associated with infertility through impaired spermatogenesis, impacting androgenic hormones and increasing the risk of testosterone deficiency.[30]

On the other hand, homocysteine (Hcy) is a sulfhydryl group containing amino acid that requires two metabolic pathways for its degradation, in the presence of vitamins B_6, B_{12}, and folic acid. It plays a central role in folate and methionine metabolism by supporting the process of methylation-dependent epigenetic modification.[31] Methionine is the primary source of Hcy in the human body. Under high levels of methionine, the transulfuration mechanism transforms Hcy into cysteine. Conversely, homocysteine is remethylated to methionine when the methionine ratio is low in the presence of vitamin B_{12} as a cofactor and methionine synthase.[32] A high level of Hcy is associated with many diseases, therefore, B vitamin treatment can control plasma Hcy levels, which are considered a biomarker for disease prognosis and a guide for disease prevention.[33] It was reported that Hcy levels are inversely related to fertility outcomes in which a high level of Hcy is associated with increased oxidative stress, the release of inflammatory cytokines and decreased DNA methylation.[34] Furthermore, a high plasma homocysteine level is associated with many diseases, including infertility in which infertile patients showed significantly higher serum Hcy levels compared to normospermic individuals, which is inversely linked to sperm motility and concentration.[35] However, more studies are needed to fully understand the linkage between these studied parameters.

Effect of hypoxia on male infertility

Hypoxia is defined as a decrease in arterial oxygen partial pressure and oxygen levels that lead to tissue oxygen deficiency. There are two types of hypoxia, pathological and environmental hypoxia. [36] The former occurs in response to low partial pressure such as at high altitudes, whereas the latter occurs due to pathological factors mainly, including varicocele. However, both of them have a negative association with male infertility, forming aberrant sperm morphology, impaired sperm motility, and decreased sperm count.[37] Moreover, hypoxia adversely alters male reproductive functions such as spermatogenesis, testicular steroidogenesis and other reproductive outcomes. It is also associated with many diseases including myocardial infarction, cancer, cerebral ischemia and infertility.[38] Hypoxia has an adverse effect on normal spermatogenesis processes. Under low oxygen levels oxidative stress is generated in the testes, impacting the physiological processes of the Leydig cells, leading to decreased testosterone production, and impaired sperm development.[39]

The primary regulator that controls oxygen detection and adaptation at the cellular level is known as hypoxia inducible factor (HIF), which is a heterodimeric transcription factor consisting of the HIF-1α or *HIF-2α* and *HIF-1β* (ARNT) subunits. These subunits stimulate genes involved in oxygen consumption, erythrocyte formation, angiogenesis, and mitochondrial metabolism and control other processes that promote oxygen homeostasis.[40] Under normal oxygen conditions, the *HIF1-α* subunit is cleaved and broken down by the proteasome in the cytoplasm. Conversely, the *HIF1-α* subunit combines in the nucleus with the *HIF1-β* subunit under low oxygen levels. Subsequently, the complex joins with hypoxia response elements to activate genes related to the cell cycle, cell death, glucose metabolism and transport.[41]

Hypoxia-inducible factor 1-alpha responds rapidly to low oxygen levels and influences cell physiology either by activating cell survival or apoptosis based on the cell type and the duration of oxygen deficiency.[42] Furthermore, HIF-dependent mechanisms affect various epigenetic processes which involve DNA methylation, histone acetylation and modify the expression of hypoxia-responsive genes in cells.[40] Hypoxia-inducible factor 1-alpha is a crucial hypoxia factor that can be utilized to predict testicular apoptosis, and can act as a clinical indicator to predict the degree of apoptosis in spermatozoa resulting from testicular dysfunction.[42]

Interestingly, HIF-1α responds to acute hypoxia in which its protein levels generally peak between 4 and 8 hours and then gradually decline until becoming undetectable between 18 and 24 hours, whereas HIF-2α response to chronic hypoxia relatively stabilizes later and plays an

important role during long-term hypoxia.[43] Despite the fact that *HIF-1α* and *HIF-2α* have identical DNA binding and dimerization domains, their transactivation domains are different. Another difference is that the expression of *HIF-2α* is more restricted to particular tissues, whereas that of HIF-1α is more general.[44] Acute exposure to hypoxia increases testosterone production via enhancing voltage-gated L-type calcium channels, steroidogenic enzymes, and inducing autophagy, while chronic exposure to hypoxia suppresses the hypothalamic-pituitary-testicular axis, by reducing steroidogenesis.[38] In addition, a significant decrease in luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone with downregulation in nuclear respiratory factor-1 (NRF-1), has been observed, which is needed to enhance testicular steroidogenesis in males living at high altitudes.[45]

Hypoxia-inducible factor 2-alpha has been demonstrated to control several aspects of angiogenesis, such as cell division, migration, blood vessel maturation, and metastasis. Therefore, *HIF-2α* is an essential modulator of both pathological and physiological angiogenesis.[42] However, there are few findings regarding the association of *HIF-2α* and male infertility, especially in varicocele cases, while *HIF-1α* response is more extensively studied. **Figure 3** shows the role of hypoxia on the male reproductive function in both acute and chronic conditions.

Oxidative stress and the protective role of antioxidants in male infertility

One of the main causes of male infertility is oxidative stress, which represents an imbalance between the body's antioxidant systems and the generation of reactive oxygen species (ROS) produced as byproducts of cellular metabolism during the electron transport process in the mitochondria.[46,47] Reactive oxygen species cause significant cellular damage when generated under stress conditions, such as exposure to toxins, environmental stressors or infections. Subsequently they attack the sperm plasma membrane, altering its integrity and fluidity. [48] Reactive oxygen species can be generated from both exogenous and endogenous processes, while low levels of ROS are essential for sperm physiological processes, such as the acrosomal reaction and sperm–oocyte fertilization. High amounts of these free radicals cause an imbalance with the antioxidant system. These factors damage sperm biological functions and reduce male fertility outcomes.[48,49]

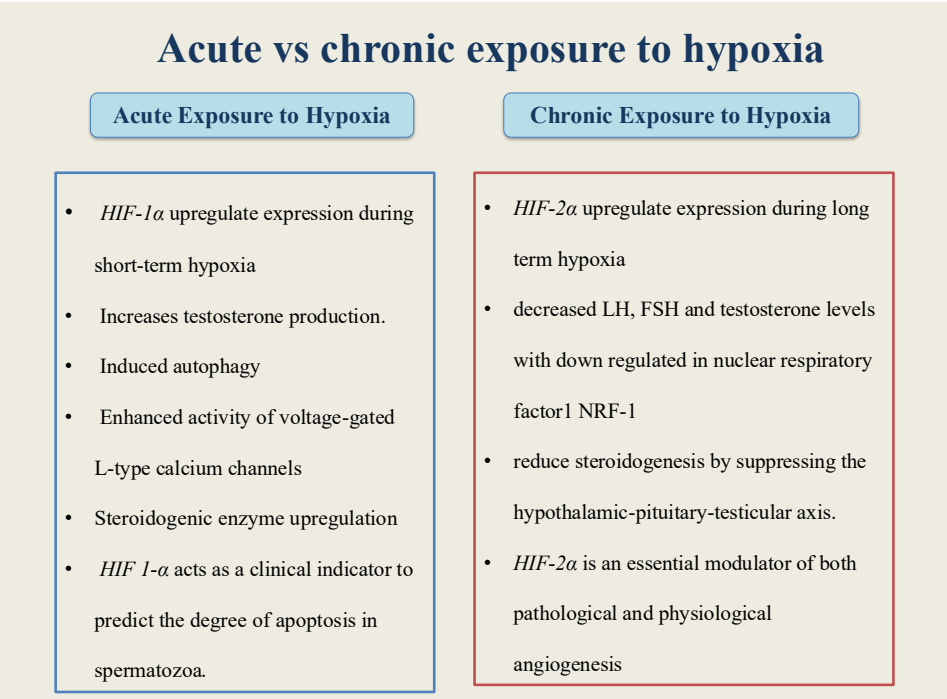

Figure 3. Mechanisms of acute and chronic hypoxia in male infertility. The chart compares differential roles of hypoxia-inducible factors. Acute hypoxia primarily upregulates HIF-1, causing increased apoptosis and ROS generation. Conversely, chronic hypoxia involves HIF-2 triggering which leads to significant hormonal imbalance and long-term pathological angiogenesis via downregulation of nuclear respiratory factor 1 (NRF-1)

Under hypoxic conditions, the cells suffer from excessive ROS production, mainly hydrogen peroxide (H_2O_2), which affects sperm function and motility and induces oxidative DNA damage.[36] Hypoxic stress is linked to increased mutagenesis, impairment of DNA repair mechanisms, and adverse effects on embryo development and offspring physiology. In addition, DNA damage generated (from reoxygenation) causes breaks in DNA strands.[50]

On the other hand, there are two types of antioxidants: enzymatic and non-enzymatic. The enzymatic antioxidants include catalase, superoxide dismutase and glutathione reductase, whereas non-enzymatic antioxidants are mainly acquired from supplements and foods which include vitamins, carotenoids, metals, carnitines and cysteines. Both types of antioxidants reduce ROS formation and eliminate free radicals.[51] Antioxidants decrease sperm cell ROS levels and enhance semen quality; moreover, supplementation with zinc, selenium, coenzyme Q10 (CoQ10), and folate, has a positive effect on semen quality, raising the sperm concentration as well as total and progressive motility.[52] Antioxidants act as a driver to protect DNA methylation patterns by controlling chromatin remodeling mechanisms and epigenetic enzymes on one hand, and by lowering the oxidative stress levels on the other hand.[53] An imbalance between antioxidant levels and oxidative stress causes aberrant DNA methylation and DNA fragmentation, subsequently effecting cellular gene expression.[54] **Figure 4** provides an illustration of the effect of ROS-induced oxidative stress and the protective role of antioxidants on the spermatozoa.

Immunological marker related to male infertility

Understanding the mechanism of immune cell responses to different diseases helps in finding successful ways to treat male infertility. The immune response is characterized by the release of immune factors, an increase in the numbers and types of immune cells, and the secretion of cytokines.[55] Cytokines are molecules generated and secreted mainly by immune cells in response to infectious pathogens or severe damage, and regulate the inflammatory response by managing its intensity and nature and modulating its interactions between the cells of the immune system.[56] Cytokines bind to target cell receptors and promote intracellular signaling, controlling a wide range of biological processes within cells, including tissue repair, inflammation, and physiological regulation.[57]

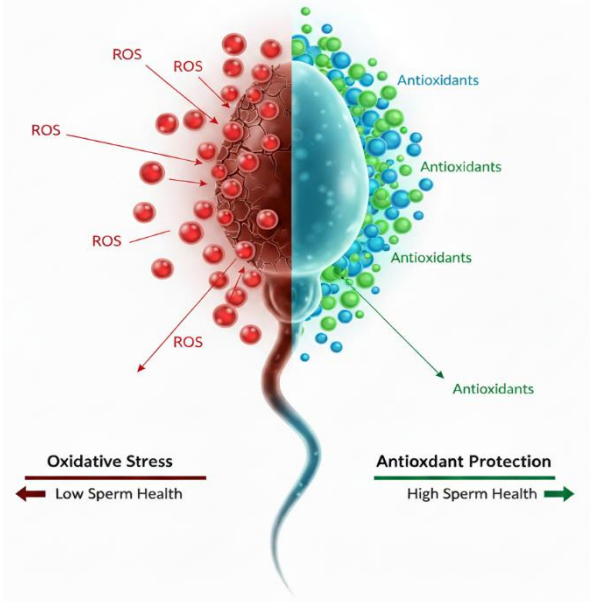

Figure 4. The balance between oxidative stress and antioxidant protection in the sperm health. Excessive ROS levels cause low sperm health, while the antioxidant system maintains cellular integrity and high sperm health

Leukocytospermia is a reliable sign of infection in the male urogenital tract, even though a variety of pathogens, including bacteria and viruses, can damage the spermatogenetic process and have an adverse effect on sperm activity and motility, thereby reducing fertilization capacity. In addition, recruitment of leukocytes to the male genital tract can impact fertility through direct cellular interactions, agglutination, the release of cytokines and reactive oxygen species (ROS), ultimately leading to genital tract dysfunction.[55,58] Recent studies show that a significant increase in the levels of anti-inflammatory cytokines, such as IL-4 and IL-10, appears to be linked to successful pregnancies. Conversely, pregnancy-threatening cytokines associated with high levels of tumor necrosis factor-alpha (TNF-α) and interferon-γ (IFN-γ) can suppress fetal growth and development.[59]

There is a significant correlation between high levels of leukocyte counts, inflammatory cytokines and oxidative stress with male infertility. An increase in IL-17A, IL-1β and IFN-

γ levels is associated with impaired sperm activity, increased ROS generation and the promotion of sperm apoptosis.[60] Overexpression of IL-1β and IL-18 along with NOD-like receptor protein 3 (NLRP3) inflammasome activation leads to mitochondrial dysfunction, excessive ROS production, lipid peroxidation, and sperm DNA fragmentation, which impair spermatogenesis and cause aberrant testicular function.[61]

Interleukin-41 (IL-41) is a newly discovered cytokine associated with many diseases, particularly infertility. It has anti-inflammatory properties, enhances metabolism, modulates immunity, regulates fat metabolism, and plays a role in inflammatory injury and autoimmune diseases.[62] It acts as immunomodulatory cytokine in autoimmune diseases by controlling cytokine release via macrophages; its levels are upregulated by factors such as exercise, inflammation and cold exposure.[63]

A recent study showed that IL-41 levels were significantly higher in infertile male patients compared to the fertile group. These findings suggest that IL-41 might be a novel biomarker for male infertility diagnosis.[57] In addition, its levels were positively linked to high white blood cell counts in semen, therefore IL41 is considered a potential biomarker for detecting male infertility.[62] However, there are limited findings regarding the relation of IL41 with male infertility and no study has yet explored its role in specific infertile groups, such as those with azoospermia and oligospermia. **Table 1** A summary of interleukins related to male infertility.

Anti-sperm antibodies

Immunological infertility is a type of infertility caused by the presence of specific autoantibodies known as anti-sperm antibodies (ASA); a low prevalence is reported and estimated at approximately 2.6–6.6% in infertile males.[64] Anti-sperm antibodies are immunoglobulins that react with sperm antigens and hinder their ability to fertilize; they also affect other sperm biological functions associated with the impairment of male fertility.[65] Furthermore, oxidative stress affects sperm motility parameters resulting in poor ejaculate quality by promoting ASA synthesis through two routes: firstly, by causing hormonal disturbances**,** and secondly, by increasing the number of dead and damaged sperm. This leads to clumping and agglutination of the spermatozoa, hindering their ability to migrate through the cervix to the site of fertilization.[66]

Although the mechanism of ASA development remains unclear, it is known that the blood-testis barrier, made up of closely packed cells linked together, prevents immune cells from penetrating the seminiferous tubule lumen.[67] The ASA are most likely to form due to traumatic disruption or developmental issues with the blood-testis barrier that otherwise shields the novel sperm antigens away from immune cells.[68]

Table 1. Summary of interleukins related to the male reproductive functions

Interleukin	Relation to male infertility	Properties and function
IL-4, IL-10	Increased levels are associated with successful pregnancies outcomes	Anti-inflammatory cytokine
TNF-α	High levels are associated with pregnancy-threatening and suppression of fetal growth	Inflammatory cytokine that can suppress fetal growth and development
IL-17A, IL-1β and IFNγ	Levels are associated with impaired sperm activity, increase ROS generation and promote sperm apoptosis	Inflammatory cytokines
IL-18, IL-1β	Mitochondrial dysfunction, excessive ROS production, lipid peroxidation, DNA fragmentation of the sperms	Inflammatory cytokine that that can impair spermatogenesis and cause aberrant testicular function
IL-41	levels were significantly higher in infertile males; considered a novel biomarker for the diagnosis ~~the~~ male infertility	An anti-inflammatory and immunomodulatory cytokine that regulates immunity and metabolism

Note : IL-4 : Interleukin-4; IL-10 : Interleukin-10; TNF-α: Tumor necrosis factor-alpha; IL-17A : Interleukin-17A; IL-1β : Interleukin-1 β; IFNγ : Interferon-gamma**;** Il-18 : Interleukin-18; IL-41: Interleukin-41

However, the complex structure of the human cells presents a challenge in different research areas; the concept of ASA induced-immunological infertility is not unique, mostly because human semen cells are inherently heterogeneous.[67] In addition, ASA testing provides unclear medical significance and uncertain variable indications due to threshold values that define a positive ASA result. Therefore, the treatment of ASA patients lacks clarity and this test is not recommended for couples undergoing intracytoplasmic sperm injection (ICSI).[66]

The impact of hypoxia on the immune response

One of the important aspects of the body's response to hypoxic stress is inflammation. Tissues experience hypoxia when they are injured, infected or during ischemia, anemia and exposure to high altitudes.[69] Hypoxia directly causes sperm DNA fragmentation, increases oxidative DNA damage and promotes cellular apoptosis through activation of the P53 pathway as well as the release of inflammatory cytokines, such as TNF-α and activation of Toll-like receptors (TLRs).[41] Toll-like receptors are distinct pattern recognition receptors that are found in immune and nonimmune cells such as decidual cells and trophoblasts. They play a vital role in innate immunity by triggering the expression of genes related to inflammatory responses.[70] The expression of TLRs is influenced by the phases of pregnancy, aberrant DNA methylation patterns and histone modifications, which directly influence fertility status and embryonic development.[71]

On the other hand, a significant increase in the levels of cytokines, such as TNF- α, IL37, IL-6, and IL-18, and in oxidative stress, is observed in varicocele patients in response to hypoxia. Increased oxidative stress (ROS) is associated with permanent testicular injury and aberrant spermatogenesis. In addition, elevated TNF- α causes changes in mitochondrial function, increases NO generation and stimulates ROS production.[72] Remarkably, TNF-α is strongly associated with both angiogenic and apoptotic markers. It increases the expression of Bcl-2-associated X protein (BAX) and decreases the expression of B-cell lymphoma 2 (Bcl-2), while exhibiting a synergistic effect with HIF-1α to enhance vascular endothelial growth factor (VEGF) synthesis under hypoxic conditions.[73] Furthermore, there is a significant association between hypoxia and high sperm autophagy levels through the activation of the HIF-1α pathway and glycolysis-gluconeogenesis pathways. However, alternative therapies for treating the male infertility will become available if the causal relationship between hypoxia and infertility is further demonstrated.[36,74] **Figure 5** illustrates the relation between hypoxia and immunity, showing how HIF-1 targeting of inflammatory pathways can inform therapeutic strategies for male infertility.

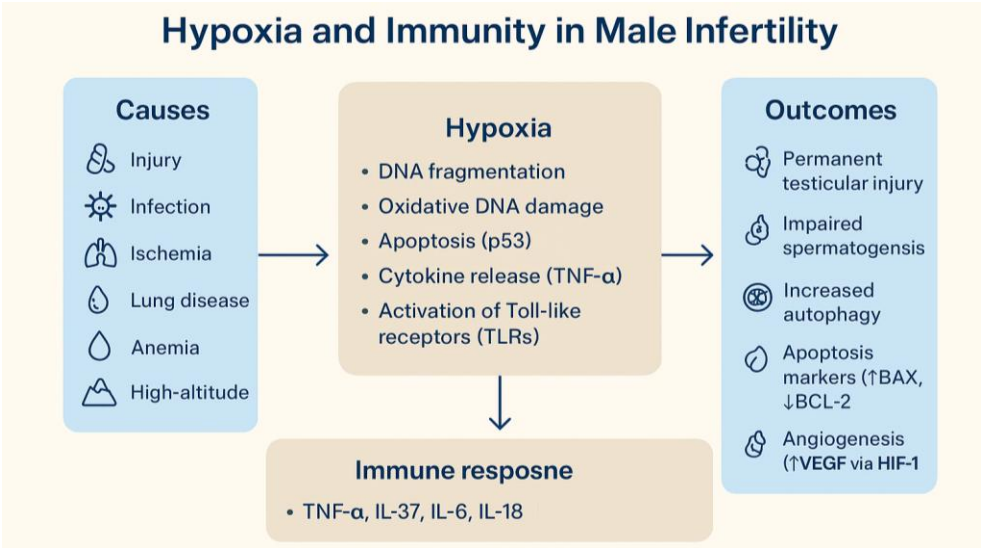

Figure 5. Pathophysiology of hypoxia and immunity. This infographic chart explains how the causes of hypoxia can induce inflammatory pathways that impair spermatogenesis and damage testicular functions

Relationship between hypoxia and DNA methylation in male infertility

Hypoxia has a potential effect on DNA methylation via changes in metabolic pathways and influences the transcriptional control of epigenetic enzymatic activity. [75] Additionally, low cellular oxygen levels affect genomic stability, causing mutagenesis and reducing the efficiency of DNA repair pathways. However, the effects of hypoxia on DNA repair mechanisms are complex, and varied, influencing processes, including transcriptional, translational, post-translational and epigenetic mechanisms.[76]

It was found that the underlying chromatin patterns specific to each cell type control which HIF-1α target genes are accessible and activated in response to acute hypoxia.[77]

Remarkably, HIF-1α binds to a hypoxia-response element (HRE) sequence 5′-RCGTG-3′ adjacent to the target gene. HIF-1-dependent gene regulation is intrinsically sensitive to cytosine methylation by DNA methyltransferases (DNMTs). [78] Hypoxia-inducible factors (HIFs) are attracted to genes that are actually expressed in normoxic cells. It has been reported that HIF-1α binding to the erythropoietin promoter is responsive to DNA methylation, which controls the HIF complex's accessibility to the 5'-RCGTG-3' core sequence within the hypoxia-response element (HRE).[79] Moreover, DNA methylation may inhibit hypoxia-responsive gene activation, demonstrating a bilateral association between epigenetic control and hypoxia.[75] Defective DNA methylation patterns induced by prolonged exposure to hypoxia are considered the main cause of male infertility particularly in idiopathic cases. Understanding these molecular insights will help in investigating these underlying alterations via epigenetic modulators or antioxidant strategies for treatment.[80]

CONCLUSION

In this review, we discussed the evidence of alterations in DNA methylation patterns and pathological hypoxia which adversely impact the sperm parameters, reproductive hormone secretion, testicular functions, and overall male reproductive outcomes. Moreover, excessive generation of oxidative stress directly affects the physiological activity of the sperm, while *HIF-1α* and *HIF-2α* expression mediates the suppression of steroidogenesis and spermatogenesis, processes representing the primary mechanisms of male infertility. Additionally, IL-41 is closely associated with male infertility and might serve as a novel prognostic marker for diagnosing infertility. Since infertility is a growing global concern, further studies on the specific roles of IL-41 and *HIF-2α* are essential to identify more effective therapeutic options for management.

Conflicts of Interest

The authors declare no conflict of interest. The research was conducted using the authors' resources and institutional support as a part of academic duties. The funders had no role in the design of the study and in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Acknowledgment

The authors extend their sincere gratitude to Professor Dr. Rakad Mohammed Khamas AL-Jumaily for the invaluable intellectual guidance of this review article. The authors affirm that this work was completed solely through personal effort, utilizing self-acquired resources and accessing digital literature databases. The authors also acknowledge the institutional support provided by the **University of Baghdad, College of Science, Department of Biology**, for providing an effective academic environment to conduct this article.

Author Contributions

ZRAJ: Acquisition of data, analysis and interpretation of data (Group 1); Drafting the article (Group 2); Final approval of the version to be published (Group 3). RMKA: Conception and design (Group 1); Critical revision of the article (Group 2); Final approval of the version to be published (Group 3). All authors have read and approved the final manuscript.

Funding

There was no **external funding** from a commercial or private entity. The work was conducted as part of the authors' academic responsibilities at the University of Baghdad, and the authors fully funded the publication process.

Data Availability Statement

All data generated or analyzed in this review are included in this published article and its supplementary information files, where applicable.

Declaration of AI Usage in Scientific Writing

The authors confirm that they did not use any AI tools or Large Language Models in the generation of the content, text, or references of this manuscript. The entire written content is based on the authors' original literature review and synthesis. AI Use in Figures: Two illustrative figures (Figure 3 and Figure 4) were utilized with the assistance of AI image generation tools. **Figure 3** (the effect of ROS levels and antioxidants on the sperm) was created using AI image generation tool for purely visual representation of a complex concept. **Figure 4** (relation of hypoxia and immunity show how HIF-1 targeting inflammatory pathway can suggest therapeutic strategies for the male infertility.) was originally designed by the authors as a text in PowerPoint and then converted into an infographic format using an AI tool. The use of AI was strictly limited to the visualization and conversion of the authors' original concepts and visual designs, and it did not generate any data or scientific text.

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