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Research Progress on the Effects of Methylmalonic Acid on Male Reproductive Function

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ABSTRACT

Methylmalonic acid (MMA), a sensitive marker of vitamin B_{12} deficiency, exerts multifaceted adverse effects on male reproductive function when accumulated. This systematic review examines how MMA leads to reduced sperm count, decreased motility, and DNA damage by inhibiting mitochondrial complex II, inducing oxidative stress, and disrupting one-carbon metabolism; causing Leydig cell atrophy, impaired testosterone synthesis, and compromised Sertoli cell function; and disrupting endocrine regulation within the hypothalamic-pituitary-testicular axis. Mechanistically, MMA induces reactive oxygen species (ROS) production, ATP synthesis inhibition, inflammatory responses, and epigenetic modifications, forming a pathological cascade of "energy crisis–oxidative damage–apoptosis." Clinical observations indicate that MMA levels are negatively correlated with sperm quality and testosterone levels, and supplementation with vitamin B_{12} can effectively reverse the associated damage. It is recommended that MMA monitoring be incorporated into the screening system for male infertility etiology, and that early nutritional intervention be implemented for high-risk populations to improve male reproductive health.

KEYWORDS

Methylmalonic acid; Male infertility; Mitochondrial dysfunction; Oxidative stress; Vitamin B_{12}

1. INTRODUCTION

Methylmalonic acid (MMA) is a key intermediate in the metabolism of branched-chain amino acids, odd-chain fatty acids, and cholesterol; its normal metabolism depends on methylmalonyl-CoA transposase, which uses vitamin B_{12} (in the form of adenosylcobalamin) as a coenzyme [1]. In cases of vitamin B_{12} deficiency (due to inadequate intake or absorption disorders) or hereditary methylmalonic acidemia (MUT gene mutation), MMA metabolism is impaired, leading to its accumulation in the blood, urine, and tissues, which serves as a core pathological marker for these conditions [2]. Serum MMA levels serve as a sensitive indicator for assessing vitamin B_{12} status and are more indicative of subclinical deficiency than total vitamin B_{12}; its accumulation is also closely associated with metabolic diseases such as chronic kidney disease and diabetes, contributing to multisystem damage through pathways including oxidative stress and epigenetic modifications [3]. However, the impact of MMA on male reproductive function has not yet been systematically elucidated. Male fertility depends on testicular spermatogenesis and regulation by the hypothalamic-pituitary-gonadal (HPG) axis [4], while disruptions in the metabolic microenvironment can interfere with germ cell function and hormone secretion via the blood-testis barrier or local inflammation [5]. Although the reproductive toxicity of homocysteine (Hcy), a precursor of MMA has received attention [6], the role of MMA as a downstream product remains controversial. Only a few studies

suggest an association with oligoasthenozoospermia and testicular dysfunction; specific mechanisms (such as oxidative stress, apoptosis, and hormonal regulation) and cross-species evidence require further integration. This review aims to summarize the metabolic characteristics of MMA and the patterns of its accumulation in disease states; focusing on male reproduction, it systematically analyzes the effects of MMA on sperm quality, testicular structure, and HPG axis hormones (testosterone, LH/FSH); and explores its potential mechanisms to provide new perspectives for the diagnosis and treatment of metabolic male infertility.

2. BASIC PHYSIOLOGICAL FUNCTIONS OF METHYLMALONIC ACID IN THE MALE REPRODUCTIVE SYSTEM

2.1. Overview of the Male Reproductive System

The male reproductive system consists of the testes, epididymis, vas deferens, accessory glands, and external genitalia, with the testes serving as the central organs for spermatogenesis and androgen synthesis [7]. Spermatogenesis is a highly complex process of cellular differentiation involving three stages: mitosis of spermatogonia, meiosis of spermatocytes, and spermatid differentiation, ultimately resulting in mature sperm. This process relies on the unique microenvironment of the seminiferous tubules in the testes, including the blood-testis barrier formed by Sertoli cells, testosterone secreted by Leydig cells, and an optimal local temperature and nutrient supply. Spermatogenesis is precisely regulated by the hypothalamic-pituitary-gonadal axis [4]. The hypothalamus pulsatile releases gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [8]. FSH primarily acts on Sertoli cells, promoting the synthesis of androgen-binding protein (ABP) and inhibin to regulate the microenvironment of the seminiferous tubules; LH, in turn, stimulates interstitial cells to synthesize testosterone [9]. Testosterone is converted into the more potent dihydrotestosterone (DHT) within the Sertoli cells, which, by binding to androgen receptors (AR), regulates key steps such as spermatogonial proliferation, meiosis, and sperm morphogenesis [10]. Furthermore, gap junctions between Sertoli cells and germ cells form a "metabolic coupling," ensuring the energy and nutrient supply for spermatogenesis [11]. MMA is a key intermediate in the propionate metabolic pathway; it is converted into succinyl-CoA by methylmalonic acid coenzyme A transposase (MCM) and enters the citric acid cycle to provide energy. Due to its high metabolic activity, testicular tissue is extremely sensitive to energy supply. Studies indicate that MCM is expressed in both testicular supporting cells and interstitial cells, suggesting that MMA metabolism may be involved in the energy regulation of spermatogenesis [12]. Under normal physiological conditions, serum MMA levels are maintained at 0.07–0.27 μmol/L, and this homeostasis depends on an adequate supply of vitamin B_{12} as a cofactor for MCM. In the absence of vitamin B_{12}, MMA metabolism is impaired, leading to its accumulation, which may affect testicular function by interfering with energy metabolism and inducing oxidative stress. This metabolic characteristic lays the theoretical foundation for understanding the association between MMA and male reproductive function.

2.2. Potential Distribution of MMA in the Reproductive System

As a small-molecule dicarboxylic acid (molecular weight 118.09 Da), MMA exhibits significant organ specificity in its distribution within the body. Animal studies have demonstrated that MMA accumulates in urine and liver in models of vitamin B_{12} deficiency or methylmalonyl-CoA mutase (MCM) deficiency, and it can also be significantly accumulated in testicular tissue [3]. Its physicochemical properties (low molecular weight, high water solubility, and low protein binding) enable it to readily cross biological membrane barriers, reaching high concentrations in the seminiferous tubule microenvironment. As the final carrier of testicular secretions, semen reflects the metabolic state within the testes; the detection of MMA in semen suggests that it can directly

influence the microenvironment of sperm maturation. The blood-testis barrier (BTB), composed of tight junctions between supporting cells and specialized peribasement membrane structures, serves as a key anatomical foundation for maintaining the stability of the spermatogenic microenvironment [13]. However, the BTB provides limited barrier function against small molecules such as MMA. Studies indicate that polar small molecules with a molecular weight <500 Da primarily cross the BTB via passive diffusion or carrier-mediated transport [14]. As a dicarboxylic acid, MMA may enter the testis via the following pathways: (1) passive diffusion: direct passage through the supporting cell membrane driven by a concentration gradient; (2) organic acid transporters: such as the OAT (organic anion transporter) family expressed in supporting cells, which can actively uptake MMA from the circulation; (3) paracellular pathway: entry into the glandular lumen during periodic openings of the BTB (e.g., during the sperm morphogenesis phase). Once inside the spermatogenic epithelium, MMA can directly affect spermatogenic cells at various developmental stages. Its toxic mechanisms include: inhibition of mitochondrial respiratory chain complex II, disruption of energy metabolism in the tricarboxylic acid cycle, induction of oxidative stress, and secondary excitotoxicity. Because spermatogenic cells are highly sensitive to energy supply during meiosis and morphogenesis, MMA accumulation can lead to impaired spermatogenesis, manifested as reduced sperm count, decreased motility, and DNA damage. This distribution pattern provides a pathophysiological basis for understanding MMA-related male infertility.

3. EFFECTS OF METHYLMALONIC ACID ON SPERM PARAMETERS

3.1. Sperm Count, Density, and Morphology

MMA, a marker of mitochondrial dysfunction [15], is closely associated with impaired spermatogenesis when elevated. In animal models with vitamin B_{12} deficiency or methylmalonic acid coenzyme A transposase (MCM) deficiency, MMA accumulation significantly inhibits sperm production [16]. Watanabe et al. found that male rats with vitamin B_{12} deficiency during pregnancy and growth exhibited significantly reduced sperm counts, along with a large number of acrocephalic, short-tailed, and amorphous sperm; the proportion of abnormal sperm reached as high as 14.4% (acrocephalic) and 4.8% (short-tailed) [17]. This effect is directly related to MMA's inhibition of the mitochondrial respiratory chain and disruption of energy metabolism, as spermatogenic cells have extremely high ATP requirements during meiosis and sperm morphogenesis. Population studies have further validated the association between MMA and sperm parameters. A comparative study by Rehman et al. involving 186 men with normal semen parameters and 88 infertile men found that serum MMA levels were significantly negatively correlated with total sperm count, motility, and morphology [18]. Multivariate regression analysis revealed that vitamin B_{12}, folate, and MMA were all significantly associated with total sperm count, suggesting that elevated MMA is an independent risk factor for infertility [19]. The underlying mechanism involves MMA's inhibition of mitochondrial complex II, leading to increased apoptosis in spermatogenic cells, while simultaneously disrupting the metabolic coupling function of supporting cells and affecting the homeostasis of the spermatogenic microenvironment. MMA-induced sperm morphological abnormalities primarily manifest as head deformities (amorphous, conical) and tail defects (short tail, curled tail). These changes stem from the toxic effects of MMA on cytoskeletal proteins (such as tubulin) and DNA damage caused by oxidative stress [20]. In a vitamin B_{12} deficiency model, arrest in spermatogenesis primarily occurs during the meiosis of spermatocytes, a stage in which cells are most sensitive to energy deprivation and oxidative stress. Supplementation with methylcobalamin partially reverses these damages, suggesting that the clearance of MMA is crucial for maintaining normal sperm morphology [21].

3.2. Sperm Motility and Mobility

Sperm motility is highly dependent on ATP generated by mitochondrial oxidative phosphorylation [22]. As an endogenous inhibitor of complex II (succinate dehydrogenase) in the mitochondrial respiratory chain, MMA can directly block electron transport and ATP synthesis. In conditions of vitamin B_{12} deficiency or methylmalonic acid coenzyme A mutase (MCM) deficiency, MMA accumulation leads to an "energy crisis" in spermatogenic cells and mature sperm, manifested as a significant decline in motility.Evidence from animal studies: Watanabe et al. found that in male rats with vitamin B_{12} deficiency, sperm motility rates (path velocity and linear velocity) decreased to 20–40% of those in the control group, and this impairment was more severe in individuals exposed to the deficient environment during the embryonic stage, suggesting that MMA's interference with mitochondrial function has developmental toxicity [17]. The mechanism involves MMA inhibiting Complex II, disrupting the tricarboxylic acid cycle, and reducing the production of NADH and $FADH_2$, ultimately leading to insufficient energy supply to the dynein arms of the sperm flagellar axoneme. MMA also exacerbates sperm membrane lipid peroxidation by inducing reactive oxygen species (ROS) production. The sperm plasma membrane is rich in polyunsaturated fatty acids and is extremely sensitive to oxidative stress [23]. ROS attack leads to decreased membrane fluidity and collapse of the mitochondrial membrane potential, creating a vicious cycle of "energy deficiency–oxidative damage" [24]. Clinical studies show that MMA levels in the semen of infertile men are significantly negatively correlated with sperm motility. Supplementation with vitamin B_{12} (methylcobalamin 1500 μg/day) can increase sperm motility by 50% within 8 weeks, indirectly confirming the importance of MMA clearance in improving sperm motility [25].

3.3. DNA Integrity and Chromosomal Abnormalities

MMA causes sperm DNA damage by inducing the production of reactive oxygen species (ROS) and disrupting mitochondrial function. As an endogenous inhibitor of complex II of the mitochondrial respiratory chain, MMA blocks electron transport, leading to electron leakage and the accumulation of superoxide anions, which trigger a cascade of oxidative stress reactions. Sperm DNA is highly condensed and has limited repair capacity, making it extremely sensitive to oxidative damage and prone to single-strand breaks (SSBs) and double-strand breaks (DSBs) [26]. MMA accumulation resulting from vitamin B_{12} deficiency affects DNA methylation patterns by interfering with one-carbon metabolism [27]. Elevated MMA levels are accompanied by homocysteine (Hcy) accumulation, which reduces S-adenosylmethionine (SAM) synthesis, leading to low sperm DNA methylation. This epigenetic alteration can affect imprinted gene expression and the meiosis process, increasing the risk of aneuploidy. Animal studies have shown that sperm DNA integrity is significantly reduced in rats with vitamin B_{12} deficiency, and supplementation with methylcobalamin can partially reverse this damage [28]. MMA-induced oxidative stress can attack spindle microtubules, disrupting chromosomal segregation during meiosis and leading to the formation of aneuploid sperm. Clinical studies have revealed that the sperm DNA fragmentation index (DFI) in infertile males is positively correlated with serum methylmethanesulfonate (MMA) levels, accompanied by an increased proportion of abnormal morphology sperm [29]. Furthermore, MMA weakens sperm's ability to repair oxidative damage by inhibiting the activity of DNA repair enzymes, thereby exacerbating chromosomal instability and the risk of genetic material transmission.

4. EFFECTS OF METHYLMALONIC ACID ON TESTICULAR FUNCTION

4.1. Morphological Changes in Testicular Tissue

MMA accumulation is closely associated with structural damage to testicular tissue. In animal models of vitamin B_{12} deficiency, testicular weight was significantly reduced; histological observations revealed atrophy of the seminiferous tubules, aplasia of the spermatogenic epithelium, and absence of spermatocytes. Experiments in rats have shown that long-term B_{12} deficiency leads to a reduction in seminiferous tubule diameter and a significant decrease in the number of sperm within the tubule lumen; in some tubules, only supporting cells (Sertoli cells) remain, resulting in changes resembling "sertoli cell-only syndrome" [30]. Leydig cells, as the primary site of testosterone synthesis, are extremely sensitive to MMA-induced oxidative stress and energy metabolism disorders. Morphologically, this manifests as reduced cell volume, decreased lipid droplets in the cytoplasm, and blurred mitochondrial cristae. Functional studies show that MMA accumulation inhibits 3β-hydroxysteroid dehydrogenase activity, leading to reduced testosterone synthesis and, consequently, weakening its regulatory role in the spermatogenesis process [31]. Furthermore, damage to Leydig cells disrupts paracrine signaling with the seminiferous tubules, exacerbating the degeneration of the spermatogenic epithelium [32]. Morphological changes in Sertoli cells primarily manifest as cytoplasmic vacuolization, disruption of tight junctions, and impaired blood-testis barrier integrity [33]. By inhibiting mitochondrial ATP synthesis, MMA interferes with the metabolic support function of Sertoli cells, leading to a decline in their nutritional supply and phagocytic capacity. Electron microscopic observations reveal dilated endoplasmic reticulum and fragmented Golgi apparatus in Sertoli cells [34]. These changes directly impair their structural support and nutritional supply to spermatogenic cells, ultimately leading to the detachment of spermatogenic cells and the formation of multinucleated giant cells.

4.2. Impaired Function of Leydig and Sertoli Cells

MMA directly interferes with testosterone synthesis by inhibiting mitochondrial function in Leydig cells. As an endogenous inhibitor of complex II of the mitochondrial respiratory chain, MMA blocks electron transport and reduces ATP production; however, key steps in testosterone synthesis—such as cholesterol side-chain cleavage and 17α-hydroxylation—rely on an adequate energy supply [35]. A study by Panah et al. involving 303 infertile men found that serum vitamin B_{12} levels were independently positively correlated with total testosterone ($\rho = 0.19$, $P = 0.001$); the risk of testosterone deficiency in the low-B_{12} group was 2.3 times higher than in the high-B_{12} group (OR = 0.44, 95% CI: 0.22–0.87), indirectly confirming the association between MMA accumulation and decreased testosterone [36]. The accumulation of homocysteine (Hcy) accompanying elevated MMA levels can further disrupt the function of the hypothalamic-pituitary-gonadal axis. Elevated Hcy damages pituitary gonadotropin secretion through oxidative stress, reducing the pulsatile frequency of LH and FSH, thereby creating a "low gonadotropin-low testosterone" vicious cycle [37-38]. Furthermore, damage to Leydig cells reduces testosterone synthesis and impairs regulation of the spermatogenesis process [39]. Concurrently, impaired Sertoli cell function leads to reduced secretion of androgen-binding protein (ABP), further lowering the concentration of active testosterone in the spermatogenic microenvironment [40]. As the "nurse cells" of spermatogenic cells, impaired Sertoli cell function directly affects spermatogenesis [41]. MMA inhibits glycolysis and lactate production in Sertoli cells, cutting off the primary energy source for spermatogenic cells; simultaneously, the disruption of tight junctions leads to increased permeability of the blood-testis barrier, allowing harmful substances to infiltrate the spermatogenic epithelium and exacerbating germ cell damage. This dual impairment of metabolic support and barrier function ultimately results in reduced sperm count, decreased motility, and morphological abnormalities.

4.3. Apoptosis and Oxidative Stress

As an endogenous inhibitor of Complex II (succinate dehydrogenase) in the mitochondrial respiratory chain, MMA blocks electron transport, leading to electron leakage and the massive production of superoxide anions (O_2^-). In the absence of vitamin B_{12} or in the presence of methylmalonic acid coenzyme A mutase (MCM) deficiency, MMA accumulation causes mitochondrial membrane potential collapse and impaired ATP synthesis, creating a vicious cycle of "energy crisis–oxidative stress" [42]. Due to its high metabolic activity and abundant mitochondrial content, testicular tissue is extremely sensitive to MMA-induced oxidative damage. Excessive ROS triggers spermatogenic cell apoptosis through multiple pathways: (1) Mitochondrial pathway: ROS-induced DNA damage activates p53, which subsequently upregulates the pro-apoptotic protein Bax, promoting the release of cytochrome C and activating the caspase-9/3 cascade [43]; (2) Death receptor pathway: ROS activates the Fas/FasL signaling pathway, directly initiating apoptosis via caspase-8 [44]. A rat model of vitamin B_{12} deficiency showed a significant increase in apoptotic cells within the seminiferous tubules, with spermatogonia and spermatocytes being the most sensitive, ultimately leading to atrophy of the spermatogenic epithelium and reduced sperm count [45]. MMA accumulation is accompanied by impaired glutathione (GSH) synthesis and decreased antioxidant enzyme (SOD, GPX) activity, weakening the testis's redox buffering capacity. Oxidative stress further activates PINK1/Parkin-mediated mitochondrial autophagy; however, MMA simultaneously inhibits this pathway, leading to impaired clearance of damaged mitochondria and exacerbating cell apoptosis [46]. This "oxidative damage–repair failure" mechanism represents the core pathological mechanism underlying MMA-associated testicular dysfunction.

5. EFFECTS OF METHYLMALONIC ACID ON ENDOCRINE REGULATION

5.1. The Hypothalamic-Pituitary-Glandular (HPG) Axis

MMA accumulation indirectly affects hormone secretion in the hypothalamic-pituitary-testicular (HPG) axis by disrupting mitochondrial function and one-carbon metabolism [47]. As an endogenous inhibitor of complex II of the mitochondrial respiratory chain, MMA blocks electron transport, leading to reduced ATP synthesis; however, the pulsatile release of GnRH from hypothalamic neurons is highly dependent on energy supply [48]. Under conditions of energy crisis, the function of KNDy neurons (Kisspeptin/Neurokinin B/Dynorphin) is impaired, leading to abnormalities in GnRH pulse frequency and amplitude, which in turn affect pituitary gonadotropin secretion [49]. MMA damages pituitary gonadotropin-secreting cells by inducing oxidative stress, thereby reducing their responsiveness to GnRH. Concurrently, elevated homocysteine (Hcy) levels associated with MMA accumulation can further inhibit gonadotropin synthesis, creating a "low gonadotropin-low testosterone" vicious cycle. Animal studies have confirmed that serum LH and FSH levels are significantly reduced in rats with vitamin B_{12} deficiency, and their response to GnRH stimulation is attenuated [50]. At the testicular level, MMA directly inhibits Leydig cell mitochondrial function, thereby reducing testosterone synthesis. A state of low testosterone weakens negative feedback inhibition on the hypothalamus and pituitary gland, which theoretically should promote GnRH and gonadotropin secretion; however, MMA-induced neuronal energy metabolism disorders render this compensatory mechanism ineffective, leading to overall dysfunction of the HPG axis. This pattern of disorder characterized by the "interplay of primary and secondary factors" is a hallmark of MMA-related male endocrine imbalance.

5.2. Mechanisms of Hormone Signaling Pathways

As an endogenous inhibitor of complex II of the mitochondrial respiratory chain, MMA reduces ATP production by blocking electron transport, thereby indirectly inhibiting energy-dependent signaling cascades. The cAMP/PKA pathway serves as a central hub for the regulation of reproductive hormones, and its activation depends on ATP-driven adenylate cyclase [51]. The energy crisis induced by MMA reduces cAMP synthesis, weakens PKA-mediated phosphorylation and activation of the steroid synthesis acute regulatory protein (StAR), and ultimately inhibits testosterone synthesis. MMA, which shares a structural similarity with succinic acid, acts as a ligand to activate the G protein-coupled receptor SUCNR1 (GPR91) [52]. SUCNR1 is expressed in both testicular interstitial cells and supporting cells; its activation elevates intracellular Ca^{2+} via the Gq/11-PLC-IP3 pathway, which should promote testosterone synthesis. However, as a partial agonist, MMA competitively inhibits the full activation of SUCNR1 by succinic acid, leading to desensitization of the signaling pathway and disruption of hormone synthesis. Furthermore, MMA activates the MAPK/ERK pathway via SUCNR1, inducing the release of inflammatory factors and further impairing Leydig cell function [53]. The PI3K/AKT pathway regulates cell survival and metabolic adaptation [54]. After MMA accumulation inhibits mitochondrial function, it indirectly suppresses mTORC1 by activating AMPK, thereby weakening the cell-protective effects mediated by the PI3K/AKT pathway. In spermatogenic cells, this inhibition exacerbates apoptosis sensitivity; in Sertoli cells, it reduces their ability to provide nutritional support to spermatogenic cells. This synergistic inhibition across multiple pathways constitutes the molecular basis for MMA-induced testicular dysfunction.

6. MOLECULAR MECHANISMS AND SIGNALING PATHWAYS

6.1. Oxidative Stress

As an endogenous inhibitor of Complex II (succinate dehydrogenase) in the mitochondrial respiratory chain, MMA blocks the electron transport chain, leading to electron leakage and triggering the massive production of superoxide anions (O_2^-). In the absence of vitamin B_{12} or in the presence of methylmalonic acid coenzyme A mutase (MCM) deficiency, MMA accumulation causes mitochondrial membrane potential collapse and impaired ATP synthesis, creating a vicious cycle of "energy crisis–oxidative stress." Due to its high metabolic activity and abundant mitochondrial content, testicular tissue is extremely sensitive to MMA-induced oxidative damage. MMA-induced ROS attack the polyunsaturated fatty acids abundant in the sperm plasma membrane, triggering a lipid peroxidation chain reaction and generating toxic aldehydes such as 4-hydroxy-2-nonenal (4-HNE) [55]. These products further modify DNA bases, leading to single-strand breaks (SSBs) and double-strand breaks (DSBs). Concurrently, MMA inhibits the activity of glutathione peroxidase (GPX) and superoxide dismutase (SOD), thereby weakening endogenous antioxidant defenses. Antioxidants such as vitamin E can dose-dependently alleviate MMA-induced oxidative damage and apoptosis, confirming the central role of ROS in MMA-induced reproductive toxicity [56]. This mechanism provides a theoretical basis for the clinical use of B-complex vitamins and antioxidants to improve MMA-related male infertility.

6.2. Mitochondrial Dysfunction

MMA inhibits mitochondrial energy metabolism through multiple targets. As an endogenous inhibitor of complex II (succinate dehydrogenase) in the mitochondrial respiratory chain, MMA blocks electron transfer from succinate to coenzyme Q, thereby reducing proton gradient-driven energy production. Concurrently, MMA inhibits ATP synthase (complex V) activity, directly blocking ADP phosphorylation. In spermatogenic cells and sperm, this dual inhibition leads to a sharp decline in ATP production, failing to meet the energy demands of energy-dependent processes such as flagellar motility and the acrosome reaction. MMA and its co-metabolites (2-methylcitrate,

malonate) induce a significant decrease in mitochondrial membrane potential (ΔΨm) and trigger the opening of the mitochondrial permeabilization transition pore (mPTP) [57]. The opening of the mPTP leads to mitochondrial swelling, cytochrome C release, and activation of the apoptotic cascade [58]. In testicular tissue, this process manifests as increased spermatocyte apoptosis and loss of sperm motility. Sperm motility is highly dependent on mitochondrial oxidative phosphorylation. Following MMA-mediated inhibition of Complex II, the tricarboxylic acid cycle is disrupted, and the production of NADH and $FADH_2$ is reduced, leading to insufficient ATP supply to the kinesin arms of the flagellar axoneme. Animal studies have shown that in vitamin B_{12}-deficient rats, sperm motility rates decrease to 20–40% of those in the control group, and this impairment is dose-dependent on MMA levels [59]. This energy metabolism disorder is the core mechanism by which MMA causes a decline in sperm motility.

6.3. Inflammatory Response

MMA accumulation activates a local inflammatory response in the testis by disrupting mitochondrial function and energy metabolism. MMA inhibits respiratory chain complex II, leading to reduced ATP production and prompting a metabolic reprogramming of immune cells from an oxidative phosphorylation-dependent to a glycolysis-dependent pro-inflammatory phenotype. This shift activates key pathways such as HIF-1α, mTOR, and NF-κB, upregulating the secretion of pro-inflammatory factors including IL-6, TNF-α, and IL-1β, thereby establishing a state of chronic low-grade inflammation within the testis. Inflammatory factors compromise the integrity of the blood-testis barrier (BTB) through multiple mechanisms: ① Inhibiting the expression of tight junction proteins (e.g., occludin, claudin-11); ② increasing vascular permeability and promoting the infiltration of neutrophils and macrophages. ROS and proteases released by immune cells further damage the spermatogenic epithelium, leading to apoptosis and sloughing of spermatogenic cells.Inflammatory factors induce spermatogenic cell apoptosis via the Fas/FasL pathway and the mitochondrial pathway [60]. TNF-α activates the p38 MAPK and JNK pathways, inhibits the expression of steroid synthases, and reduces testosterone levels [61]. Concurrently, factors such as IL-6 disrupt the metabolic support functions of Sertoli cells, reducing the supply of lactate and neurotrophic factors, thereby exacerbating the energy crisis in the spermatogenesis process [62]. This vicious cycle of "metabolic dysfunction–inflammation activation–reproductive damage" constitutes the core pathological mechanism of MMA-related male infertility.

6.4. Epigenetic and DNA Methylation Changes

MMA accumulation indicates vitamin B_{12} deficiency and one-carbon metabolism disorders. As a key cofactor in the methionine cycle, B_{12} deficiency leads to the accumulation of 5-methyl-tetrahydrofolate (5-methylTHF), forming a "folate trap" that impedes methionine synthesis and reduces the production of S-adenosylmethionine (SAM) [63]. SAM is the sole methyl donor for DNA, RNA, and histone methylation; its depletion directly leads to abnormal epigenetic modifications during spermatogenesis [64]. Reduced SAM levels result in DNA hypomethylation, manifested as decreased 5-methylcytosine (5mC) levels and the de-repression of repetitive sequences (such as LINE-1) [65]. Arsenic exposure models demonstrate that similar metabolic disturbances lead to hypomethylation of histones and LINE-1 DNA, upregulate transposon expression, induce defects in DNA double-strand break (DSB) repair, and cause meiosis arrest, ultimately triggering spermatocyte apoptosis [66]. Supplementation with folate and B_{12} restores SAM and 5mC levels, preventing spermatogenesis defects, thereby indirectly confirming the epigenetic toxicity of MMA-associated metabolic dysregulation [67]. Abnormal sperm DNA methylation patterns can be transmitted to offspring, affecting embryonic development and metabolic programming [68]. Vitamin B_{12} deficiency alters the expression of imprinted genes (such as H19/IGF2) by interfering with histone modifications like H3K4me3, thereby increasing the risk of metabolic diseases in offspring [69]. This

epigenetic reprogramming represents a potential long-term consequence of MMA-related male infertility, highlighting the importance of early metabolic intervention.

7. CLINICAL RELEVANCE AND CURRENT RESEARCH STATUS

7.1. Association Between Abnormal MMA Levels and Male Infertility

MMA is the most sensitive indicator of vitamin B_{12} status; the normal reference range is <0.271 μmol/L, and levels >0.271 μmol/L indicate B_{12} deficiency. In the field of male reproductive health, the association between elevated MMA and infertility is receiving increasing attention. Although epidemiological studies directly examining the link between MMA and male infertility are limited, the association between B_{12} deficiency and elevated MMA levels has been well established. The Office of Dietary Supplements explicitly states that B_{12} deficiency can lead to reproductive system symptoms, including infertility. In the population undergoing assisted reproductive technology (ART), Zhang et al. found that serum MMA levels were negatively correlated with the rate of high-quality embryos, suggesting that elevated MMA may reduce fertility success rates by affecting gamete quality [70]. This study, which included 216 IVF-ET patients, demonstrated a significant decline in embryo quality in the group with elevated MMA levels. Elevated MMA leads to abnormalities in sperm concentration, motility, and morphology through mechanisms such as mitochondrial dysfunction, oxidative stress, and DNA damage (see Chapters 3 and 6). Clinical observations indicate that elevated MMA is often accompanied by homocysteine (Hcy) accumulation, with both factors synergistically impairing spermatogenesis. Although some studies have not found a significant correlation between vitamin B_{12} and semen parameters, given that MMA is a more sensitive marker of functional deficiency than vitamin B_{12} levels alone, future research should directly evaluate the dose-response relationship between MMA and sperm quality to clarify its value in the diagnosis of male infertility.

7.2. Clinical Observations and Case Reports

Currently, clinical observational data directly assessing the relationship between MMA and male reproductive function primarily come from studies on vitamin B_{12} deficiency. A cross-sectional study by Rehman et al. involving 274 men (186 with normal sperm parameters and 88 with abnormal parameters) showed that serum MMA levels were significantly negatively correlated with total sperm count, motility, and morphology [71]. Multivariate regression analysis indicated that for every 1-unit increase in MMA, the prevalence of infertility decreased by 74% (suggesting that elevated MMA is associated with fertility protection; this result may reflect a compensatory mechanism for B_{12} deficiency), but the independent negative correlation between MMA and sperm parameters remained statistically significant ($P < 0.05$). The study also found that testosterone levels were significantly positively correlated with vitamin B_{12} and folate ($r = 0.326$, $P < 0.001$), while elevated MMA, as a marker of B_{12} functional deficiency, indirectly reflects impaired testosterone synthesis. In the context of B_{12} deficiency, MMA accumulation is accompanied by elevated homocysteine (Hcy) levels; together, they synergistically inhibit Leydig cell mitochondrial function and reduce testosterone synthesis efficiency. This explains the clinically observed negative correlation between elevated MMA and decreased testosterone levels. Although case reports directly addressing MMA are rare, clinical observations of B_{12} supplementation provide indirect evidence. In patients with B_{12} deficiency and elevated MMA, treatment with methylcobalamin resulted in significant improvements in sperm motility and restoration of testosterone levels. For example, in a case of a male with infertility due to long-term B_{12} deficiency and elevated MMA, sperm motility increased by 50% and serum MMA returned to normal after 8 weeks of treatment with 1500 μg/day of methylcobalamin. These cases support the use of elevated MMA as a marker of reversible reproductive dysfunction.

8. FUTURE RESEARCH DIRECTIONS

Current research on MMA and male reproductive function is still in its early stages; future efforts should focus on deepening exploration in three key areas: molecular mechanisms, clinical translation, and diagnostic tools. At the basic research level, although it is known that MMA affects spermatogenesis by inhibiting mitochondrial complex II, inducing oxidative stress, and disrupting one-carbon metabolism, its specific molecular targets (such as the regulation of SUCNR1 receptor expression in testicular cells) and downstream signaling cascades (cAMP/PKA, MAPK/ERK pathway) have not yet been fully elucidated. Single-cell sequencing and metabolomics technologies must be employed to construct a molecular network map of MMA-induced damage, thereby identifying key intervention points. In terms of clinical translation, existing studies have primarily focused on indirect evidence of vitamin B_{12} deficiency, and there is a lack of prospective randomized controlled trials specifically targeting MMA levels. Future clinical trials should be designed to target MMA reduction, evaluating the effects of methylcobalamin supplementation or metabolic interventions on sperm parameters and fertility outcomes, while also examining the influence of individual factors, such as MTHFR gene polymorphisms, on the response to intervention. Regarding the development of diagnostic tools, while MMA serves as a sensitive marker of B_{12} functional status, a comprehensive evaluation system integrating MMA with traditional semen parameters, sperm DNA fragmentation indices, and reproductive hormones has yet to be established. Large-scale cohort studies are needed to explore the association between MMA and multidimensional reproductive health indicators, and to develop a composite biomarker scoring system based on MMA to enhance the accuracy of diagnosing the causes of male infertility. Furthermore, given the close association between MMA and chronic diseases such as metabolic syndrome and diabetes, exploring MMA monitoring strategies for men with comorbid metabolic disorders may provide new avenues for reproductive health management.

9. CONCLUSION

Current evidence indicates that MMA accumulation exerts significant adverse effects on the male reproductive system through multiple targets and pathways. At the sperm level, MMA induces an energy crisis and oxidative stress by inhibiting mitochondrial complex II, leading to reduced sperm count, decreased motility, increased DNA fragmentation rates, and morphological abnormalities; At the testicular tissue level, MMA causes Leydig cell atrophy and impaired testosterone synthesis, while simultaneously disrupting the metabolic support function of Sertoli cells and the integrity of the blood-testis barrier, ultimately triggering spermatocyte apoptosis and degeneration of the spermatogenic epithelium; at the endocrine regulatory level, MMA interferes with the function of the hypothalamic-pituitary-testicular axis, exacerbating hormonal imbalances. The core mechanism underlying these damages lies in MMA's inhibition of mitochondrial energy metabolism and the resulting oxidative stress, inflammatory responses, and epigenetic modifications. Given the close association between elevated MMA levels and vitamin B_{12} deficiency, and the fact that B_{12} supplementation can effectively reverse related reproductive damage, clinically, monitoring of MMA levels should be strengthened in high-risk men (such as long-term vegetarians, patients with gastrointestinal absorption disorders, and those with infertility). A serum MMA level <0.271 μmol/L should be adopted as a reference threshold for metabolic health, and early nutritional intervention strategies targeting MMA should be explored to improve male reproductive health and fertility outcomes.

This study systematically elucidates the pathogenic mechanisms of methylmalonic acid (MMA) (including induction of mitochondrial dysfunction, oxidative stress, disruption of one-carbon metabolism, and apoptosis induction), and provides detailed evidence of its adverse effects on the male and female reproductive systems (such as decreased sperm quality in males, impaired testosterone synthesis, HPT axis dysfunction, and reproductive stress injury in females). It further

clarifies the clinical value of MMA in male infertility screening and the significance of early vitamin B12 intervention, offering theoretical foundations for the diagnosis and treatment of reproductive-related diseases.

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DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationshipsthat could have appeared to influence the work reported in this paper.

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