Review

Vitamin B₁₂ and Semen Quality

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Abstract: Various studies have revealed the effects of vitamin B₁₂, also named cobalamin, on semen quality and sperm physiology; however, these studies collectively are still unsummarized. Here, we systematically discuss and summarize the currently understood role of vitamin B₁₂ on semen quality and sperm physiology. We searched the Web of Science, PubMed, and Scopus databases for only English language articles or abstracts from September 1961 to March 2017 (inclusive) using the key words “vitamin B₁₂” and “cobalamin” versus “sperm”. Certain relevant references were included to support the empirical as well as the mechanistic discussions. In conclusion, the mainstream published work demonstrates the positive effects of vitamin B₁₂ on semen quality: first, by increasing sperm count, and by enhancing sperm motility and reducing sperm DNA damage, though there are a few in vivo system studies that have deliberated some adverse effects. The beneficial effects of vitamin B₁₂ on semen quality may be due to increased functionality of reproductive organs, decreased homocysteine toxicity, reduced amounts of generated nitric oxide, decreased levels of oxidative damage to sperm, reduced amount of energy produced by spermatozoa, decreased inflammation-induced semen impairment, and control of nuclear factor-κB activation. However, additional research, mainly clinical, is still needed to confirm these positive effects.

Keywords: vitamin B₁₂; cobalamin; semen quality; sperm

1. Introduction

Vitamin B₁₂ (α-(5, 6-dimethylbenzimidazolyl) cobamidcyanide), also named cobalamin since it contains cobalt in the core of its molecular structure, is one of eight known B vitamins [1]. These vitamins are water-soluble and are essential for normal human growth, development, and metabolism [2]. In essence, vitamin B₁₂ is synthesized by bacteria or archaea as they contain the required enzymes to assemble this molecular complexation [3]. Animal products such as meat, fish, and dairy products are proven food sources of vitamin B₁₂ [4].

Vitamin B₁₂ is involved in the metabolism of almost all cells in the human body as it is required for DNA synthesis, as well as amino acid and fatty acid metabolism [5]. Therefore, a wide symptomatic spectrum is related to vitamin B₁₂ deficiency ranging from fatigue and depression to severe anemia and memory loss [6–8].

In many cases, vitamin B₁₂ deficiency is associated with gastric achlorhydria (absence or reduced hydrochloric acid in the gastric secretions), resulting in decreased availability of intrinsic factor, a protein facilitates the absorption of vitamin B₁₂ in the ileum [9]. Biochemically, vitamin B₁₂ is considered as a coenzyme for methionine synthase enzyme [10,11]. This enzyme is required to synthesize methionine from homocysteine to complete the S-adenosylmethionine (SAM) cycle [12]. In this cycle, the critical step is the conversion of SAM to S-adenosylhomocysteine, which results in the methylation of the main functional macromolecules/molecules in the human body such as DNA, RNA, neurotransmitters, lipids, proteins, and amino acids [11,12].
In the last decade, various research studies worldwide have reported low levels or deficiency in vitamin B\textsubscript{12} among the studied populations [13–15]. As a consequence, B\textsubscript{12} supplementation has been recommended in many occasions as a key contribution to the maintenance and enhancement of population health [13,15,16]. Vitamin B\textsubscript{12} deficiency was found to be multifactorial, and most of time it was related to malabsorption, malnutrition, or drug induced causes [17,18].

Since the beginning of the 60s, many studies (clinical and non-clinical) have investigated the effect of vitamin B\textsubscript{12} on semen quality; this effect, however, has yet to be understood and summarized. This review systematically discusses and summarizes the up-to-date impact of vitamin B\textsubscript{12} on semen quality and sperm physiology. To accomplish this, we searched the Web of Science, PubMed, and Scopus databases for only English language articles or abstracts from September 1961–March 2017 using the key words “vitamin B\textsubscript{12}” and “cobalamin” versus “sperm”. Furthermore, certain relevant references were included to support the empirical and mechanistic discussions.

2. Effect of Vitamin B\textsubscript{12} on Sperm Parameters

2.1. Human Studies

2.1.1. Positive Effects

In humans, it has been found that vitamin B\textsubscript{12} is transferred from the blood to the male reproductive organs, which emphasizes a substantial role of vitamin B\textsubscript{12} in spermatogenesis, and hence in semen quality [19,20]. Supporting studies have shown that plasma vitamin B\textsubscript{12} concentrations are lower in infertile men compared to fertile [21,22]. The positive effects of vitamin B\textsubscript{12} on sperm parameters (e.g., count, motility, morphology, sperm DNA) have been investigated in various studies. Table 1 presents a summary of the human studies done on vitamin B\textsubscript{12} and its derived compounds, and their reported positive effect on sperm count.

Table 1. Effect of vitamin B\textsubscript{12} or its derived compounds on human sperm count.

| Affecter Dose and Duration | Population Effect on Sperm Parameters Reference |
|---------------------------|-----------------------------------------------|
| Vitamin B\textsubscript{12} + (Orally) (25 µg) B\textsubscript{12} + (0.25 mg) stilbestrol Daily, for 4 months. Oligozoospermic patients; (n = 23) (+) Sperm count [23] |
| Methylcobalamin 1500 µg/day 4–24 weeks Infertile men, excluding azoospermia (+) Sperm count [24] |
| Methylcobalamin + Clomiphene Citrate (Clomid) (1500 µg/day) B\textsubscript{12} + (25 mg/day) Clomid 12–24 weeks Infertile men, excluding azoospermia (+) Sperm count [25] |
| Methylcobalamin 6000 µg/day 16 weeks Oligozoospermic patients (+) Sperm count [22] |
| Mecobalamin 1500, 6000 µg/day 12 weeks Oligozoospermic patients (+) Sperm count [26] |
| Vitamin B\textsubscript{12} + Other Antioxidants 1 µg/day 3 months Infertile men (+) Sperm count [27] |
| Methylcobalamin 1500 mg/day >3 months Patients with idiopathic oligozoospermia or normozoospermia (+) Sperm count [28] |

(+) : increase of parameter.

In 1984, Isoyama et al. [24] showed that methylcobalamin administered at 1500 µg/day to infertile, but not azoospermic, subjects, enhanced sperm motility by about 50% of cases after eight weeks of administration. Long-term treatment (>3 months) with methylcobalamin at 1500 µg/day increased sperm motility in patients with idiopathic oligozoospermia or normozoospermia [28]. In addition, the study by Boxmeer et al. [19] demonstrated a correlation between sperm count and vitamin B\textsubscript{12}
concentration in seminal plasma. A recent study by Gual-Frau et al. [27] showed that infertile men with varicocele administered multivitamin including vitamin B$_{12}$ at 1 µg/day, for 3 months, had lower sperm DNA fragmentation by about 22.1%.

Since 2000, a number of studies proposed vitamin B$_{12}$ as a candidate therapy to recover or enhance semen quality. Sinclair [29] proposed vitamin B$_{12}$ as a nutritional therapy that improved semen quality, mainly sperm count and motility. In 2006, vitamin B$_{12}$ was suggested as one of the candidate drugs to manage male infertility due to its positive effects on sperm parameters, particularly sperm count [30]. In 2013, an oral antioxidant treatment including vitamin B$_{12}$ was found to improve sperm vitality, motility, and DNA integrity [31]. Such evidence has allowed workers in the field to recommend the use of this antioxidants therapy prior to any assisted reproduction procedure (e.g., in vitro fertilization, intrauterine insemination), given that such intervention increases the success rate of fertilization.

Despite the food sources of vitamin B$_{12}$ being well-known (e.g., red meat, fish), only a few nutritional studies focusing on this area have been published. A study on an Indian population has shown that lactovegetarians from azoospermic, oligozoospermic, and normozoospermic subjects had mean values of seminal plasma vitamin B$_{12}$ activity that were lower than the corresponding mean values in non-vegetarian subjects [32]. In the same study, azoospermic subjects were found to have lower levels of vitamin B$_{12}$ when compared to oligozoospermic and normozoospermic [32]. However, vitamin B$_{12}$ values in seminal plasma revealed no association with the sperm content of the corresponding semen in both oligozoospermic and normozoospermic subjects [32]. Another study on lactovegetarians supported the above findings by showing that these people had markedly lower levels of seminal vitamin B$_{12}$ compared to non-vegetarians, while hydroxocobalamin treatment did not enhance the semen quality of oligozoospermic men with a low seminal content of vitamin B$_{12}$ [33].

In general, amongst the infertile groups, infertile patients with varicocele have a higher proportion of sperm with damaged DNA [27,34]. A recent study by Gual-Frau et al. [27] showed that the integrity of sperm DNA in grade-I varicocele men can be improved by certain forms of oral multivitamin/antioxidant therapy, including vitamin B$_{12}$.

### 2.1.2. Negative Effects

Only a few studies have presented the blunted effect of vitamin B$_{12}$ on semen quality. A clinical trial in 1973 by Halim et al. [35] did not reveal a significant response of vitamin B$_{12}$ therapy on semen quality. In this study, among 16 infertile patients injected weekly with cyanocobalamin for six weeks, only one patient had an improvement in sperm count, which was increased from 1 to 10 million mL$^{-1}$. According to the authors, this patient may have suffered from vitamin B$_{12}$ deficiency [35], while a more likely reason is that the result reflects the spontaneous variation in sperm concentration, which is sometimes observed in some individuals. Another study by Farthing et al. [36] did not find an obvious correlation between vitamin B$_{12}$ levels and semen quality. Furthermore, a study by Chen et al. [37] demonstrated an insignificant difference in seminal vitamin B$_{12}$ concentrations between fertile and infertile men (44 fertile vs. 176 fertile).

### 2.2. Rodent Studies

#### 2.2.1. Positive Effects

Methylcobalamin at 1000 µg/kg (six times a week for 5–10 weeks) caused a marked increase in sperm count in oligozoospermically induced male rats [38]. In addition, oligozoospermic mice orally administered mecobalamin at 1.0 mg/kg/day for 10 weeks had a higher sperm count and more motile sperm, as well as lower sperm abnormalities compared with those of the control [39]. Moreover, methylcobalamin injected subcutaneously at 0.5 mg/kg (5 times per week) protected against ethylene oxide-induced testicular damage in male Wistar rates (higher sperm count, lower sperm abnormalities, and higher epididymis weight) [40]. Furthermore, methylcobalamin (1 mg/kg)
protected against X-ray-induced testicular damage in male mice (higher sperm count motility and diameter of seminiferous tubules) [41].

Vitamin B_{12} deficiency significantly reduced the testes weight and induced clear morphological alterations in the testicular tissue of the B_{12}-deficient mice [42]. Cimetidine, a member of the histamine-2 receptor (also called beta-blockers) antagonists family, was found to induce abnormal changes in the seminiferous tubules in the testis, which consequently negatively affects spermatogenesis, and hence semen quality [43]. B_{12} supplement was found to soften the detrimental effect of cimetidine on spermatogenesis and restore the number of Sertoli cells in adult male rats [44]. A recent study by Beltrame and Sasso-Cerri [45] revealed that vitamin B_{12} supplementation was able to recover cimetidine-induced sperm concentration.

2.2.2. Negative Effects

Male rats fed a B_{12}-deficient diet by pair-feeding for 100 days had atrophy in their seminiferous tubules and impaired spermatogenesis [46]. It has been suggested that dietary vitamin B_{12} deficiency affects both developing (damage to germ cells and sperm maturation) and growing (lower sperm count and morphology, but not motility) male rats [47]. In 2007, Watanabe et al. [47] showed that vitamin B_{12} deficiency during gestation and lactation phases affected the germ cells and led to spermatocyte destruction in the F1 male rats, which consequently reduced the quantity of the produced sperm.

2.3. In Vitro Studies

The addition of vitamin B_{12} at 2.50 mg/mL to bovine semen in vitro increased sperm motility, sperm velocity, and the number of plasma membrane-intact sperm compared to the control [48]. Bovine sperm cryoprotective medium supplemented with vitamin B_{12} at 2.50 mg/mL could reduce the freezing-thawing induced oxidative damage to sperm (e.g., higher catalase and glutathione reductase activities) [48]. Vitamin B_{12} at 2 mg/mL increased sperm parameters (viability, motility, progressive motility, and normality) of Dallagh rams in vitro in both pre- and post-freezing conditions [49].

3. Mechanistic Studies

Vitamin B_{12} supplementation was found to be associated with marked histopathological improvements in the male reproductive system. For example, methylcobalamin at 1000 µg/kg (six times a week for 5–10 weeks) caused a marked increase in the diameter of the seminiferous tubules as well as sperm count in oligospermically induced male rats [38]. In addition, oligozoospermic mice orally administered mecobalamin at 1.0 mg/kg/day for 10 weeks had a higher diameter of seminiferous tubules [39].

An in vivo system study conducted by Oh et al. [50] found that the transport of vitamin B_{12} in adult Leydig cells of the testes was mediated by the transmembrane protein amnionless, a protein that directs the endocytosis of cubilin, a receptor for the vitamin B_{12}-intrinsic factor complex [50].

Only a few studies have presented the effects of vitamin B_{12} on gonadal function. Isoyama et al. [24] showed that infertile men administered methylcobalamin at 1.5 mg/day for 4–24 weeks had unchanged serum levels of testosterone, luteinizing hormone, or follicle-stimulating hormone [24].

Low levels of vitamin B_{12} in the body reduce the catalytic activity of methionine synthase to synthesize methionine from homocysteine. This reduction leads to the accumulation of homocysteine in the plasma, also called hyperhomocysteinemia (\(\sim>15 \mu\text{mol/L}\)) [51]. Hyperhomocysteinemia has been found to be associated with various health problems, including reproductive disorders. For example, Ebisch et al. [52] demonstrated a significant inverse association between embryo quality following in vitro fertilization with intracytoplasmic sperm injection treatment and the total homocysteine concentration in seminal plasma. A recent in vitro study revealed a significant correlation between sperm parameters such as motility and count and thiol concentrations [53]. Such
evidence suggests a possible homocysteine toxicity to sperm, which may negatively affect sperm parameters, as a result of reduced level of bodily vitamin B12.

Homocysteine toxicity is mostly due to the reactive chemical structure of homocysteine, which contains a sulfhydryl group (thiol group) at one end and a carboxyl group at the other end. Chemically, the sulfur in the thiol group is very nucleophilic and can attack other molecular electrophiles [54].

One important set of homocysteine reactions in the body is the oxidation of thiol groups between homocysteine molecules and cysteine residues in other proteins to form disulfide bonds (also called disulfide bridges) [55,56]. Another set of bodily reactions of homocysteine is N-homocysteinylation. In this reaction, homocysteine thiolactone, an active cyclic thioester in which the carboxyl group is condensed with the sulfhydryl group, acylates the free amino groups of protein lysine residues [57]. The integration of homocysteine molecules with any given protein may significantly affect its functional domain, and therefore the entire protein function [58].

In the body, hyperhomocysteinemia was found to inhibit nitric oxide synthase pathways, which reduces the amount of nitric oxide produced [59]. Given that nitric oxide synthase is present in human spermatozoa [60], and that nitric oxide is crucial for adequate sperm motion [61], it is acceptable that vitamin B12 deficiency may reduce sperm function through hyperhomocysteinemia-induced nitric oxide depletion.

Increased levels of reactive oxygen species in human semen has been found to increase oxidative injury to sperm, which negatively affects sperm quantity and quality [62]. Studies have revealed a negative correlation between vitamin B12 concentration and levels of reactive oxygen species in semen [37,63]. Accordingly, decreased levels of vitamin B12 may reduce semen quality as a result of increased accumulation of reactive oxygen species in semen and in reproductive organs.

The study by Hu et al. [48] indicated that the antioxidant activity of vitamin B12 prevents sperm membrane lipid-peroxidation during stress conditions such as freezing–thawing practices. Adding an appropriate amount of vitamin B12 into the freezing extender could prevent the generation of oxygen radicals, which decrease the damaging effect of lipid-peroxidation to sperm membranes, and ultimately improve sperm motility and viability [48].

In fact, a number of studies have revealed a powerful antioxidant activity for vitamin B12. Thiolatocobalamin was found to act as potent but benign antioxidant at pharmacological concentrations [64]. It was found that administration of vitamin B12 at 0.63 µg/kg/day for 30 days in combination with folic acid significantly reduced the arsenic-induced oxidative injury in rat pancreatic tissues [65]. In addition, cobalamin was found to protect against superoxide-induced cell injury in human aortic endothelial cells [66]. Moreover, in 2014, using the chemiluminescence method, Boyum et al. [67] showed that vitamin B12 had a significant reducing aptitude, which is the main chemical property of antioxidants.

Creatine is naturally synthesized in the human body from the amino acids arginine and glycine [68]. In the first step of synthesis, these two amino acids are combined to produce guanidinoacetate [68]. Next, the latter is methylated, using SAM as the methyl donor, to produce creatine [68,69]. Given that vitamin B12 is crucial in synthesizing methionine [10], which is the precursor of SAM, the amount of SAM produced, and hence the amount of creatine, is affected by low levels of vitamin B12. In human spermatozoa, adenosine triphosphate (ATP) is generated from the chemical shuttle between creatine and creatine phosphate using creatine kinase [70]. Accordingly, it can be suggested that vitamin B12 deficiency alters sperm function by affecting the rapid buffering and regeneration of ATP.

Systemic inflammation was found to be associated with low sperm count, abnormality of sperm morphology, and impaired sperm motility [71]. Evidence strongly suggests that vitamin B12 and transcobalamins supplementation may be beneficial in the management of systemic inflammatory response syndrome in some patients [72]. Such evidence suggests that vitamin B12 could be beneficial to decrease inflammation-induced semen impairment.
Studies have shown that, during testicular stress, Sertoli cell nuclear factor-κB proteins, transcription factors considered to be main regulators of the stress and immune responses, exert pro-apoptotic effects on germ cells, which consequently affect the number of sperm produced [73,74]. Vitamin B₁₂ supplementation was found to be useful in controlling these transcription factors [72], thus avoiding the excessive germ cell death and hence sperm loss.

4. Conclusions and Future Perspectives

Thus far, the mainstream published studies (clinical, in vivo, or in vitro) present the positive effects (approximately 23 studies) of vitamin B₁₂ on semen quality in primarily increasing sperm count and secondarily enhancing sperm motility and reducing sperm DNA damage, though there are still a few in vivo system studies (three studies) that have deliberated some adverse/blunted effects. As a result, vitamin B₁₂, typically at the normal or therapeutic doses, is vital for adequate semen quality.

The favorable effects of vitamin B₁₂ on semen quality may be due to increased efficacy of male reproductive organs, decreased homocysteine toxicity, increased amount of nitric oxide produced, decreased accumulation of reactive oxygen species, reduced energy production by spermatozoa, decreased inflammation-induced semen impairment, and control of nuclear factor-κB activation.

However, further research—primarily clinical—is still necessary to confirm these favorable effects. At present, our laboratory is running a clinical study that uses different bioanalytical methods, such as flow cytometry, to standardize the favorable and unfavorable concentrations of vitamin B₁₂ for human spermatozoa.

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