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Shelley M. Vanderhout
University of Toronto

Matineh Rastegar Panah
University of Toronto

Bibiana Garcia-Bailo
University of Toronto

Patricia Grace-Farfaglia
Sacred Heart University, grace-farfagliap@sacredheart.edu

Konrad Samsel
University of Toronto

See next page for additional authors

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Authors

Shelley M. Vanderhout, Matineh Rastegar Panah, Bibiana Garcia-Bailo, Patricia Grace-Farfaglia, Konrad Samsel, Judith Dockray, Keith Jarvi, and Ahmed El-Sohemy



Nutrition, genetic variation and male fertility

Shelley M. Vanderhout¹, Matineh Rastegar Panah¹, Bibiana Garcia-Bailo¹, Patricia Grace-Farfaglia², Konrad Samsel¹, Judith Dockray³, Keith Jarvi³, Ahmed El-Sohemy¹

¹Department of Nutritional Sciences, University of Toronto, Toronto, ON, Canada; ²Department of Nutritional Sciences, University of Connecticut, Waterbury, CT, USA; ³Murray Koffler Urologic Wellness Centre, Department of Urology, Mount Sinai Hospital, Toronto, ON, Canada

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Correspondence to: Ahmed El-Sohemy. 1 King's College Circle, Room 5326, Toronto, ON M5S 1A8, Canada. Email: a.el.sohemy@utoronto.ca.

Abstract: Infertility affects nearly 50 million couples worldwide, with 40–50% of cases having a male factor component. It is well established that nutritional status impacts reproductive development, health and function, although the exact mechanisms have not been fully elucidated. Genetic variation that affects nutrient metabolism may impact fertility through nutrigenetic mechanisms. This review summarizes current knowledge on the role of several dietary components (vitamins A, B₁₂, C, D, E, folate, betaine, choline, calcium, iron, caffeine, fiber, sugar, dietary fat, and gluten) in male reproductive health. Evidence of gene-nutrient interactions and their potential effect on fertility is also examined. Understanding the relationship between genetic variation, nutrition and male fertility is key to developing personalized, DNA-based dietary recommendations to enhance the fertility of men who have difficulty conceiving.

Keywords: Micronutrients; reproduction; fertility; nutrition; genetics; nutrigenomics; nutrigenetics

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Introduction

Infertility is defined by the World Health Organization as “a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse” (1). Although the causes of infertility are often complicated and difficult to identify, health and lifestyle factors affect the ability of both men and women to reproduce. In men, abnormalities in sperm count, morphology and motility all have an impact on successful fertilization (2). Oxidative stress, which is an imbalance between oxygen-derived free radicals and antioxidants leading to cell damage, has also been identified as a factor which may affect sperm quality and fertilization potential (3).

Nearly 50 million couples worldwide experience infertility (4,5). According to the 2009–2010 Canadian Community Health Survey, estimates of the prevalence of infertility in Canada range from 10% to 15.5% of

couples (6). Male factor infertility accounts for 20–30% of total cases, with an additional 20–30% being due to a combination of male and female factor infertility (7).

There is mounting evidence to suggest a relationship between various dietary components and fertility. While adherence to a prudent diet appears to be protective for fertility in both men and women, results from randomized controlled trials (RCTs) on the effect of specific antioxidant and micronutrient supplements are less consistent (8–10). The inconsistent results from supplementation studies may be due, in part, to genetic differences in absorption, distribution, metabolism and excretion of specific dietary components. However, little research to date has examined how genetic variation modifies the relationship between diet and fertility. Ultimately, knowledge of an individual's genotype could lead to the development of tailored nutritional recommendations to optimize fertility. The present review summarizes the evidence on the relationships

Table 1 Overview of the potential impact of genetic variation in micronutrient metabolism on male fertility

Micronutrient	Gene and SNP	Impact
Retinoic Acid	<i>BCMO1</i> : rs11645428 (11)	Meiosis I/II and post meiotic spermatid development (12,13)
Vitamin B ₁₂	<i>FUT2</i> : rs602662 (14)	Sperm count, quality and motility (15)
Vitamin C	<i>GSTT1</i> : insertion or deletion (16)	Semen volume, concentration, sperm count, morphology and motility (17)
Vitamin D	<i>CYP2R1</i> : rs10741657 (18); <i>GC</i> : rs2282679 (18,19)	Sperm motility and morphology (20); sex hormone binding globulin (SHBG) (21)
Vitamin E	<i>CYP4F2</i> : rs2108622 (22); <i>SCARB1</i> : rs11057830 (22); <i>APOA5</i> : rs12272004 (22)	Acrosome reaction (23); sperm morphology (24)
Folate	<i>MTHFR</i> : rs1801133 (25)	Sperm density and morphology (26)
Choline	<i>CHDH</i> : rs12676 (27,28); <i>PEMT</i> : rs4646343 (29); <i>PEMT</i> : rs7946 (29)	Sperm motility (27,28)
Betaine	<i>CHDH</i> +432: rs12676 (30); <i>PEMT</i> -744: rs12325817 (30)	Spermatogenesis (31)
Iron	<i>TMPRSS6</i> : rs4820268 (32); <i>TFR2</i> : rs7385804 (33); <i>HFE</i> : rs1800562 (34); <i>SLC17A1</i> : rs17342717 (35); <i>HFE</i> : rs1799945 (34); <i>TF</i> : rs3811647 (34)	Spermatogenesis (36); sperm volume, density, motility and morphology (37); excess leads to oxidative DNA damage (38)
Calcium	<i>GC</i> : rs7041 (39); <i>GC</i> : rs4588 (39)	Sperm maturation (40), motility (41), morphology (42), and overall function (43,44)

between specific dietary components (micronutrients, macronutrients, and other food bioactives) and fertility in men. The potential effects of genetic variation on these relationships are discussed (summarized in *Tables 1,2*).

Micronutrients and male fertility

Vitamin A

Vitamin A plays an important role in both male and female reproductive health. Vitamin A supports the immune system, which mediates reactive oxygen species (ROS) activity and thus protects the gonads and reproductive tissues from oxidative stress (68). Vitamin A circulates in two main forms in the body: beta-carotene (inactivated) and retinol (activated). Retinoic acid, another vitamin A metabolite, may play a role in male fertility via its influence on regulation of sperm morphology and concentration (*Figure 1*) (68-70).

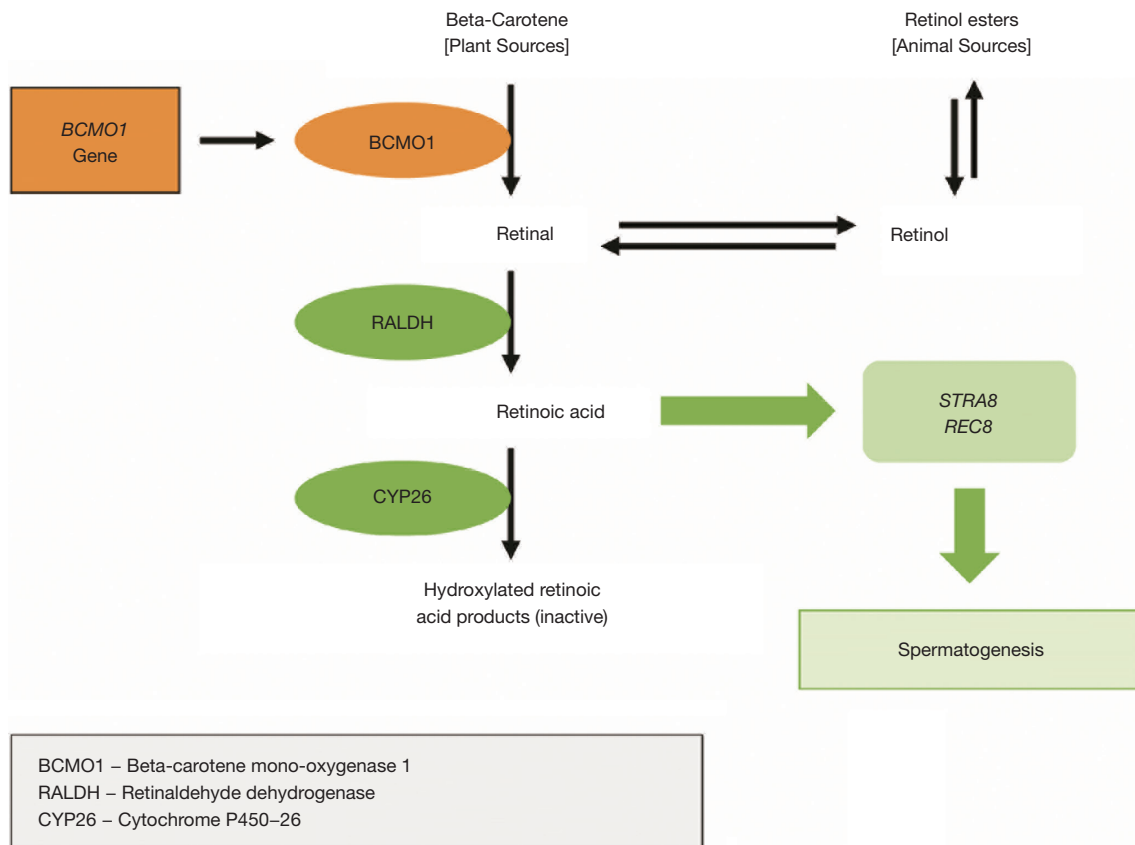
Retinoic acid induces spermatogenesis during early development and throughout childhood (71). Higher serum retinol has been observed in men with normozoospermia compared to those with oligospermia, asthenozoospermia, and azoospermia (68). With a deficiency of retinoic acid,

the blood-testis barrier (BTB) is compromised by halting meiosis I/II and post-meiotic spermatid development, which occurs behind the immunological BTB (12,13). Vitamin A deficiency damages the seminiferous epithelium of the epididymis, prostate, and the seminal vesicle, which results in the termination of spermatogenesis (71). Despite vitamin A deficiency leading to early cessation of spermatogenesis, one recent study found that long-term chronic excessive intake of vitamin A impairs sperm production, morphology, motility and viability in mice (72).

The beta-carotene mono-oxygenase 1 (*BCMO1*) enzyme, which is encoded by the *BCMO1* gene, converts circulating beta-carotene to retinol (*Figure 1*). It has been shown that about 70% of individuals possess the GG variant of the *BCMO1* (rs11645428) gene (73), which is associated with inefficient conversion of beta-carotene to retinol (11). Two genes that play a role in spermatogenesis in humans, *STRA8* and *REC8*, are both induced by retinoic acid (74). Sequence variants in genes encoding tight junction proteins that fortify the BTB have yet to be identified. Further studies are needed to identify all proteins involved in vitamin A metabolism, sperm development and vitality. Additional human studies exploring toxicity of excessive vitamin A intake on male fertility parameters are also needed.

Table 2 Overview of the potential impact of genetic variation in metabolism of macronutrients and other food bioactive ingredients on male fertility

Dietary component	Gene and SNP	Impact
Ratio of unsaturated to saturated fat	<i>TCFL2</i> : rs7903146 (45,46)	Improper ratio can lead to obesity, insulin resistance, negatively impacting semen quality (47,48)
Omega-3	<i>NOS3</i> : rs1799983 (49); <i>FADS1</i> : rs174547 (50); <i>FADS2</i> : rs2727270, rs498793 (51,52)	Sperm motility; membrane fluidity; sperm concentration (53,54)
Saturated fat	<i>APOA2</i> : rs5082 (55)	Elevated BMI; sperm count, concentration, motility, morphology (56,57)
Sugar	<i>GLUT2</i> : rs5400 (58)	Sperm motility and count (59)
Fiber	<i>TCF7L2</i> : rs12255372 (60)	Low fiber diet can lead to insulin resistance and type 2 diabetes, negatively impacting spermatogenesis, sperm maturation (61)
Gluten	<i>HLA</i> : rs2395182, rs7775228, rs2187668, rs4639334, rs7454108, rs4713586 (62)	Androgen resistance (63); sperm morphology and motility (64)
Caffeine	<i>CYP1A2</i> : rs7662551 (65)	Sperm motility, count and morphology improved at low amounts (66); testosterone levels and sperm volume impaired on high amounts (67)

**Figure 1** Vitamin A metabolic pathway. Beta-carotene mono-oxygenase 1 (BCMO1) converts circulating beta-carotene to retinal (aldehyde), the aldehyde form is then converted into retinoic acid. Retinoic acid induces *STRA8* and *REC8* expression, which aids in spermatogenesis.

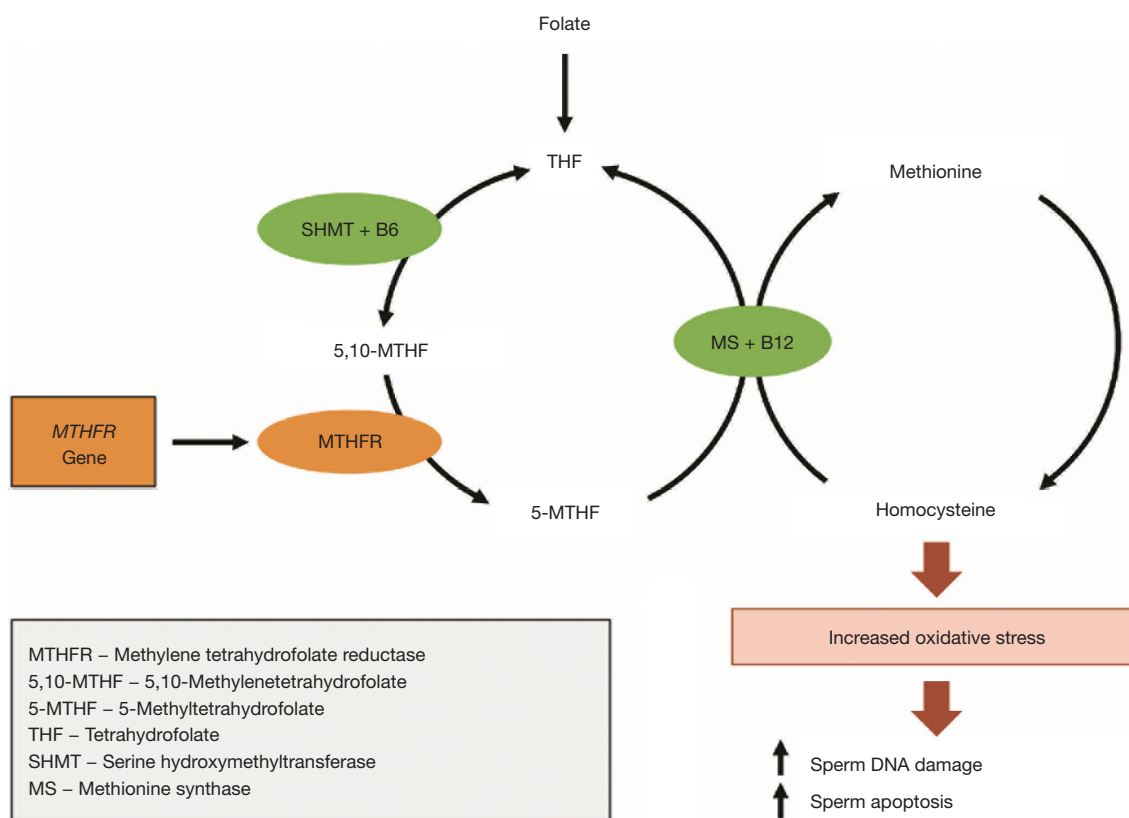


Figure 2 Folate metabolic pathway. Methylene tetrahydrofolate reductase converts 5,10-methylenetetrahydrofolate, an intermediate in the folate metabolic cycle, to 5-methyltetrahydrofolate (5-MTHF). 5-MTHF is a methyl group donor in the conversion of homocysteine to methionine.

Vitamin B₁₂

Vitamin B₁₂, in combination with folate, plays an integral role in the remethylation cycle of homocysteine (Hcy) to methionine (Met) (Figure 2) (75). Circulating homocysteine concentrations have been positively associated with oxidative stress (76). Vitamin B₁₂ acts as a cofactor to the enzyme methylene-tetrahydrofolate-reductase, which catalyzes the remethylation of homocysteine to methionine (77). Hyperhomocysteinemia, a condition associated with poor *in-vitro* fertilization outcomes and reduced sperm concentration, quality (DNA fragmentation index) and motility (78-80), is most often attributable to low folate and vitamin B₁₂ levels (80).

In addition to vitamin B₁₂ obtained from food, the stability of human plasma concentrations depends on gut *de novo* microbial biosynthesis and transport (81). In humans, the fucosyltransferase 2 (FUT2) enzyme is regulated by the FUT2 gene and is responsible for vitamin B₁₂ homeostasis

and transport throughout the body. Variation in the FUT2 gene is associated with differing levels of circulating vitamin B₁₂. Indeed, the GG variant of FUT2 (rs602662) is linked to lower plasma vitamin B₁₂ (14) and has been observed in about 50% of the population (82). Since dietary vitamin B₁₂ is only naturally derived from animal food sources, vegetarians and vegans are at an increased risk of vitamin B₁₂ deficiency if supplementation does not occur, and this risk is even greater among those with the GG genotype for FUT2 rs602662 (14). The relationship between vitamin B₁₂ intake and fertility may, therefore, be partly mediated by the FUT2 genotype.

Circulating vitamin B₁₂ levels also correlate with sperm concentration, motility, morphology and sperm DNA damage (15). Low plasma levels of vitamin B₁₂ have been associated with male infertility (83). Few studies have reported on the role of dietary B₁₂ on semen parameters in humans (80,84). Recent *in vitro* evidence has shown that

B₁₂ insufficiency resulting in hyperhomocysteinemia creates a toxic environment which reduces both sperm count and motility (85). Overall, the available evidence emphasizes the importance of vitamin B₁₂ in male reproductive health through its role in Hcy metabolism and nucleic acid synthesis.

Vitamin C

Vitamin C, or ascorbic acid, has various physiological functions including aiding in tissue and hormone development, acting as a co-factor for enzymes and reducing oxidative damage via its role as a powerful antioxidant (86). Enzymes called glutathione S-transferases, or GSTs, are responsible for maintaining the glutathione-ascorbic acid antioxidant cycle. The GSTT1 enzyme aids in the conversion of dehydroascorbic acid into ascorbic acid, and is encoded by the glutathione S-transferase T1 gene (*GSTT1*). Variation in this gene, specifically, possessing two copies of the deletion variant of *GSTT1*, which has been observed in 20–40% of the population, depending on ethnicity (35), can impair GSTT1 enzymatic activity (87). *GSTT1* genotype influences the relationship between dietary intake of vitamin C and circulating ascorbic acid. Individuals homozygous for the deletion variant of *GSTT1* are at a much greater risk of being deficient when they do not meet the recommended daily allowance (RDA) for vitamin C (16). Results from a small (N=24) two-week intervention providing a high fruit juice and vegetable antioxidant-rich diet in healthy males and females with varying glutathione-S-transferase (GST) genotypes showed evidence of an increase in GST activity in the *GSTT1*+ group compared to baseline and the *GSTT1*- group (16). There was evidence that plasma vitamin C levels in the *GSTT1* deletion group were different from baseline, while there was no difference in total antioxidant capacity or retinol levels. Dietary intake did not differ between groups, leading to the conclusion that response to the diet varied by genotype. Therefore, it may be especially important for individuals who are homozygous for the deletion variant of *GSTT1* to meet the vitamin C RDA for both overall and reproductive health.

Vitamin C intake has been positively associated with healthy semen parameters, as dietary intake influences seminal ascorbic acid concentrations (88). Seminal ascorbic acid concentrations can measure up to 10 times that of serum ascorbic acid (89) and have been shown to protect against oxidative damage to sperm DNA when sufficient (90). Ascorbic acid may account for 65% of seminal antioxidant

activity (91) and affects the integrity and structure of sperm by promoting an environment where sperm can thrive, develop and reproduce, minimizing structural and functional flaws (86). Despite some studies showing no benefit of vitamin C to sperm quality (92–94), other studies including RCTs have supported a positive association between vitamin C intake, serum and semen ascorbic acid concentrations, and healthy semen parameters including semen volume, and sperm concentration, number, motility and morphology, and overall fertility (95). Higher dietary vitamin C intake has been linked to reduced sperm DNA damage (90,96–98) and low levels of seminal vitamin C have been associated with higher sperm DNA fragmentation in infertile men (99).

Variation in the *GSTT1* gene influences susceptibility to deficiency of vitamin C (16). Despite the evidence for beneficial effects of vitamin C on male fertility, consuming an excess amount of vitamin C poses the risk of promoting oxidative stress by acting as a pro-oxidant (100). Further research is needed to determine concentrations at which this effect occurs (101).

Vitamin D

Vitamin D has a well-known role in bone health through modulation of calcium metabolism as well as a variety of other proposed functions such as immune and inflammatory modulation and reproductive system regulation (102,103). Vitamin D can be obtained through the diet and through endogenous production after exposure to sunlight (104). To exert its biological functions, both dietary and endogenous vitamin D must be activated to 1,25-hydroxyvitamin D by the enzyme vitamin D 25-hydroxylase (105), which is regulated partly by the *CYP2R1* gene (18). Activated vitamin D is transported throughout the body by the vitamin D binding protein (DBP), which is encoded by the *GC* gene (18). Certain variants of both genes impact functioning of both vitamin D 25-hydroxylase and DBP, such that individuals who possess the GG or GA variant of *CYP2R1* (rs10741657), and/or the GG variant of *GC* (rs2282679), together prevalent in over 80% of the population (106,107), have an increased risk of lower circulating vitamin D (18,19).

The biologically active 1,25-hydroxyvitamin D binds to target genes via the vitamin D receptor (VDR), triggering their transcription and subsequent physiological effects (108). The VDR and the 25-hydroxyvitamin D metabolizing enzyme 1 α -hydroxylase (*CYP27B1*) have active roles in sperm motility. *CYP24A1* expression has also been

identified as a differential marker for sperm quality (109). VDRs are present in the testicular Leydig cells, epididymis, prostate, seminal vesicles and sperm (110), suggesting a need for vitamin D in such tissues for spermatogenesis and sperm maturation (109,111). Vitamin D modulates cholesterol and triglycerides in sperm head membranes, which are important for the protection of sperm DNA, and may potentially have an impact on sperm viability, motility, and fertilization capacity (110,112). Vitamin D's role in maintaining calcium homeostasis may also contribute both to motility and to the acrosome reaction, which potentiates fertilization (20,113,114). Human studies analyzing circulating vitamin D and semen parameters are less common than rodent models, but some have shown that serum 25-hydroxyvitamin D concentrations correlate positively with sperm motility and normal morphology (20) as well as circulating testosterone levels (115-117). However, there appears to be a U-shaped relationship between serum vitamin D and androgen concentrations, where both deficiency and excess may be associated with adverse reproductive outcomes (118).

In a RCT investigating the effects of dietary supplementation on sperm motility in men with idiopathic oligoasthenozoospermia, eighty-six participants were divided into two equal groups and given 200 IU vitamin D and 600 mg calcium daily, or 100 mg vitamin E and 100 mg vitamin C daily for 3 months (119). There was evidence that sperm count per ejaculate increased in the calcium/vitamin D group, while there was no evidence of a difference in the lower dose supplementation group from baseline. The calcium/vitamin D group achieved pregnancy in 16.3% of cases, compared to 2.3% in the vitamin E/vitamin C group. Another RCT of 300 infertile men in Denmark investigated the effect of 1,400 IU vitamin D and 500 mg calcium daily for 5 months on male infertility by analyzing the relationship between serum 25-hydroxyvitamin D concentration with ionized calcium on testicular function (120). The baseline characteristics suggested that infertile men with vitamin D deficiency had fewer motile sperm, lower sex hormone binding globulin (SHBG) and a lower testosterone/estradiol ratio. Men with serum 25-hydroxyvitamin D >75 nmol/L [considered sufficient (121)] had higher sperm motility compared to men with serum 25-hydroxyvitamin D <25 nmol/L [considered deficient (121)]. However, the study showed that there was no evidence of an association between vitamin D supplementation and sperm parameters (morphology, motility and DNA fragmentation) between the two study groups or in comparison to baseline within

each group. The noted difference in hormone profiles were higher free testosterone and estradiol and lower SHBG levels in men with serum 25-hydroxyvitamin D <25 nmol/L. Furthermore, higher spontaneous pregnancy rates were seen in couples with the male in the vitamin D/calcium supplementation group. Lower calcium intake levels were associated with higher sperm motility and a more favourable hormone profile (21).

A study compared serum 25-hydroxyvitamin D with serum 1,25-dihydroxyvitamin D levels in males with fertility and infertility (122), and results showed no evidence of an association between serum 25-hydroxyvitamin D concentrations and semen parameters, but there was evidence of an association between higher 1,25-dihydroxyvitamin D concentrations and improved sperm motility and total sperm count in infertile men. This suggests 1,25-dihydroxyvitamin D concentration may potentially have a stronger association with sperm parameters in comparison to circulating 25-hydroxyvitamin D.

Vitamin E

Vitamin E, or alpha tocopherol, is a vital antioxidant in the cell membrane supporting reproductive function in men. It has been observed that there is inter-individual variability in the metabolism of α -tocopherol in humans and mice fed vitamin E-enriched diets (123). Bioavailability of vitamin E varies by age, gender, absorption, catabolism and the presence of other nutrients, such as vitamin K (124). Single nucleotide polymorphisms (SNPs) associated with variation in vitamin E status encode for cytochrome P450 4F2 (*CYP4F2*), a plasma membrane receptor for HDL which is involved in tissue uptake (*SCARB1*), and genetic variance in *APOA5* (22).

In men, sperm membranes are composed of cholesterol, phospholipids, and polyunsaturated fatty acids (125). Appropriate distribution and proportion of these components are integral to the function and vitality of sperm. As an antioxidant and protector of sperm membrane lipids, vitamin E is important in promoting motility and proper morphology of sperm (24), as well as fertilization within the acrosome reaction (23). Dietary vitamin E correlates with both serum and seminal alpha-tocopherol levels (126,127), which are positively related to fertility (128) and normal sperm parameters (68). RCTs have shown improved sperm motility and reduced lipid peroxidation in sperm as a result of vitamin E and selenium supplementation (126,127), which was also associated with

greater chance of clinical pregnancy (24). Further research is needed on the combination of genetic factors that predicts vitamin E status and its effect on semen parameters and fertility.

Folate

Folate, or vitamin B₉, is best known for its preventative effects on the development of neural tube defects (NTDs) in a fetus during the early stages of pregnancy (129). The methylene tetrahydrofolate reductase (MTHFR) enzyme is one of the enzymes involved in converting folate to 5-methyltetrahydrofolate, which is a methyl group donor in the conversion of Hcy to Met (Figure 2) (130). The *MTHFR* gene regulates MTHFR enzyme activity (131). A common variant of the gene is the 677TT genotype, which affects approximately 40% of the population (132), is associated with reduced capacity to methylate DNA, lower levels of circulating folate and higher serum Hcy concentrations (25).

Low levels of serum and seminal folate can result in high levels of Hcy, which may induce oxidative stress, sperm DNA damage and apoptosis lowering sperm counts (78,79,133). Though some studies have shown no relationship between folate intake and semen quality (134-137), others suggest a positive effect of folate intake on male reproductive health. Folate supplementation has been positively associated with lower sperm injury (98), higher sperm density, normal sperm morphology (80), overall higher semen quality (26) and negatively associated with infertility (84). RCTs have shown that folate supplements increase sperm concentration (136,138) and motility (139) among men.

The *MTHFR* gene has been associated with fertility in men. Specifically, the 677TT genotype has been associated with infertility (140), azoospermia (80,141-143), sperm DNA fragmentation, and spontaneous abortion (144). Low sperm concentration related to possession of the 677TT genotype can be improved with folate supplementation (134). Thus, adequate folate intake may play a part in offsetting the effect of *MTHFR* genotype on semen parameters. A study investigating the relationship between Hcy and *MTHFR* SNP carriers emphasized the importance of analysis in couples experiencing infertility is important. The study concluded that physiological doses of 5-MTHFR are more effective than high doses of folic acid in reducing Hcy levels and improving the methylation process for *MTHFR* SNP carriers (145).

Choline

Choline is an essential nutrient found in a variety of foods in the diet, with high concentrations found in animal-based products such as liver, eggs, and wheat germ (146). It is present in dietary sources in both hydrophilic and lipophilic forms, which affect absorption and metabolism within the body (147). Despite being an essential nutrient, intake for women and men frequently falls below the recommended adequate intake level (148). Choline is an important source of one carbon (1C) units for DNA methylation (149) and is critical for regulation of gene expression as well as for the biosynthesis of lipoproteins and membrane phospholipids (150). Single nucleotide polymorphisms in the choline metabolizing gene choline dehydrogenase (*CHDH*) are associated with greater risk for infertility. It has been reported that men with a genetic variation in *CHDH* (rs12676) had reduced sperm motility or asthenospermia (27,28). SNPs in folate and choline metabolizing genes increase the need for dietary choline by favoring phosphatidylcholine (PC) biosynthesis via the cytidine diphosphate-choline pathway (29,151). As phosphatidylethanolamine N-methyltransferase (*PEMT*) catalyzes the synthesis of choline, *PEMT* variants rs4646343 and rs7946 also affect endogenous phosphatidylcholine homeostasis (29).

Betaine

Betaine is a naturally occurring amino acid found in high concentrations in seafood and whole wheat products (152). This nutrient functions to protect cells from environmental stressors, including differences in osmolarity and temperature, and is a methyl donor in the Hcy-Met cycle (152). Betaine consumption in animal studies have been shown to raise sperm density and improve spermatozoa quality, with these effects occurring on a timespan shorter than one spermatogenic cycle (153).

Circulating betaine concentration depends on both folate and choline metabolism, and betaine status can be impacted by polymorphisms through two pathways: choline dehydrogenase, *CHDH* (+432 G→T; rs12676) and phosphatidylethanolamine N-methyltransferase (*PEMT*; -744 G→C; rs12325817) genes (30). Studies of *MTHFR* knockout mice have found a beneficial effect of betaine on spermatogenesis (31), and in folate deficiency, both betaine and choline supplementation prevent DNA

hypomethylation (154). In humans, the consumption of a varied and balanced diet with adequate supply of both choline and betaine may mitigate loss of activity in the CHDH pathway on fertility (155).

Iron

Iron is an essential nutrient for the maintenance of healthy red blood cells, oxygen transport in the blood, immune function and free radical homeostasis (156). Deficiency can lead to impaired immune function and compromised oxygen availability to bodily tissues (156); however, iron in excess can act as a pro-oxidant and cause iron overload, in which iron deposits in tissues, impairing their function (157).

Iron is essential to ejaculate fluidity and maintaining sperm pH within a functional range (158). In addition, Sertoli and Leydig cells are sources of ferritin, an iron transport protein, to developing sperm. Ferritin also protects testicular tissue (159,160). Furthermore, mitochondrial redox reactions create ATP, which support spermatogenesis and contribute to sperm motility, but require oxygen to occur (161,162). Iron deficiency anemia, a manifestation of low serum iron, results in reduced circulatory oxygen transport and therefore creates a hypoxic environment to the testes (36). Individuals with health conditions leading to low serum iron such as sickle cell disease often have compromised fertility; men with the condition are known to have reduced ejaculate volume, sperm density, motility, and morphology (37). However, it is difficult to attribute compromised fertility parameters to low serum iron alone.

Though dietary iron relates to individual iron status, individual variation may in part be controlled by genes that regulate iron absorption and transport in the body, specifically *TMPRSS6*, *TFR2* and *TF* genes. The transmembrane protease, serine 6 (*TMPRSS6*) gene codes for matriptase-2, a protein that influences hepcidin, which controls iron absorption at the gut epithelium. The transferrin receptor 2 (*TFR2*) gene regulates the TFR2 protein that aids in iron transport across cell membranes, and the transferrin (*TF*) gene codes for transferrin, which carries iron in the blood. Variation in each of these genes can cause reduced functioning of the proteins they code for, which collectively can impact individual risk for low iron status (32-34). Individuals with variants of these genes that increase risk of iron deficiency may require higher dietary iron, or supplemental iron.

Conversely, genetic variation in the human hemochromatosis,

or *HFE*, and sodium-dependent phosphate transport protein 1 (*SLC17A1*) genes can alter coding of HFE and SLC17A1 proteins, which also influence gut absorption of iron and risk of iron overload (33,35,163). Iron overload poses risk for detrimental effects to the male reproductive system. Excess iron levels in seminal plasma have been associated with teratozoospermia and decreased motility, with the proposed mechanism being increased levels of reactive oxygen species leading to lipid peroxidation (164). Additionally, high levels of testicular iron are associated with impaired spermatogenesis (159), as well as direct damage to sperm (165). Iron deposits in the pituitary gland can lead to lower levels of testosterone (166). Further, any antioxidant in high quantities can exhibit pro-oxidative effects. Iron can cause oxidative damage to sperm DNA (38), and impair spermatogenesis and fertility in excess (167,168). Those possessing genetic variants that increase risk of iron overload are advised not to over-consume iron rich foods and should avoid combining iron- and vitamin C-containing foods as the presence of an acid helps keep iron in its more soluble ferrous form (Fe^{2+}) (169). Additionally, individuals at risk for iron overload may be recommended to make dietary changes from sources of heme to non-heme iron, which is absorbed less efficiently than heme iron. Both low iron status and iron overload are conditions that can adversely impact the reproductive system. Monitoring dietary and genetic factors can help to achieve iron homeostasis.

Calcium

Calcium is important for a variety of functions in the body including promoting bone health, heart function, blood clotting and muscular contractions (170). It also plays a role in reproductive health due to its effects on vitamin D homeostasis, inflammation, and facilitating fertilization (171). Circulating calcium levels and, therefore, calcium available to reproductive tissues can be determined in part by individual genetic differences. The *GC* gene encodes vitamin D-binding protein, which helps regulate vitamin D absorption and transport. This affects circulating calcium levels since vitamin D is necessary for its metabolism and homeostasis. Individuals with the G allele of *GC* rs7041 and C allele of *GC* rs4588, which affects approximately 60% of the population (172,173), are at a higher risk for low circulating vitamin D, which is associated with lower circulating calcium, when calcium intake is low (39).

In men, calcium is known to regulate sperm motility (174)

and is responsible for triggering the acrosome reaction, which allows for effective fertilization (175,176). Epididymal and prostate fluid contains 2–3 times the amount of calcium found in serum (177), suggesting its essence in sperm development and function. Though the mechanism through which calcium regulates fertility in men is not fully known, it has been demonstrated that vitamin D deficient mice that become hypocalcemic can restore fertile capabilities with calcium supplements only (171,178). This may be due to the positive effect of calcium on sperm maturation (40), motility (41), morphology (42) and overall function (43,44).

Other food bioactive ingredients and male fertility

Gluten

Celiac disease (CD) is an autoimmune-mediated enteropathy of the small intestine characterized by intolerance to dietary gluten in individuals with susceptible genotypes, human leukocyte antigens (HLA)-DQ2 and -DQ8, which are necessary but not sufficient for onset of disease (62). Ninety-nine percent of people with CD possess the DQ2 and DQ8 risk variants, which can be determined by 6 SNPs in the HLA gene region, compared with 30% of the general population (179,180). Two immune pathways are involved in CD; one through deamidation of glutamine residues in gluten peptides by human transglutaminase 2 and generation of autoantibodies, and the other by activation of the innate immune system, leading to atrophy of intestinal villi (181). A viral infection model suggests that reovirus infections may trigger loss of oral tolerance of gluten (182). In addition, the microbiome plays pathogenic and protective roles through interactions that may modulate autoimmune risk in individuals with HLA-DQ2 (183).

CD is associated with extra-intestinal symptoms, such as infertility and decreased bone density (63,184,185). Before treatment with a strict gluten-free diet, men may experience impaired pituitary regulation of gonadal endocrine function (186). The inflammatory response to gluten consumption in individuals with gluten intolerance creates an adverse environment for reproductive tissue maintenance and function (187). Available evidence does not show an increased risk of subfertility in men with CD, although auto-antibodies can be found in seminal fluid of men with unrecognized disease (187,188).

Anti-sperm antibodies associated with the autoimmune response that CD incurs when gluten is consumed, as well as compromised nutritional status due to the disease, may be

related to the pathogenesis of reduced sperm morphology and motility (64). The gut is a site of conversion of testosterone to dihydrotestosterone and influences hormone metabolism (189). In untreated celiac disease, low levels of testosterone and subsequent hormone imbalances can cause hypogonadism (63) and hypothalamic pituitary resistance, oligospermia, and azoospermia, disrupting reproductive function (64,190). However, with the removal of gluten from the diet, semen parameters can increase and fertility can be restored (191). Further consequences of untreated CD in men include micronutrient deficiencies. These can include folate, vitamin A, vitamin E, zinc, and selenium, all of which play an important role in maintaining reproductive health and protecting fertile tissues (192). Further research on this topic is warranted as more fortified, functional gluten-free foods come to the marketplace and these may reduce the risk of micronutrient deficiencies associated with a gluten-free diet (193,194).

Caffeine

Studies linking caffeine consumption to various health outcomes remain inconsistent. This may be due, in part, to genetic differences in caffeine metabolism. The cytochrome P450 1A2 (*CYP1A2*) gene codes for the CYP1A2 enzyme, which is responsible for 95% of caffeine metabolism within the body (65,195). Individuals with the CA or CC genotypes of rs762551 make up approximately 60% of the population (196), and are known to metabolize caffeine slower than those who have the AA genotype (65). Slower clearance of caffeine from the bloodstream in combination with high consumption is associated with increased risk of a number of adverse health outcomes, including heart attack (65).

In men, caffeine crosses the blood-testes barrier, and can be harbored in the gonadal tissues and excreted into the semen (197,198). There is evidence to suggest that caffeine consumption is associated with increased incidence of aneuploidy, and other DNA damage in sperm cells (199). Though some studies have not found a positive or negative effect of this phenomenon on fertility (67,200–203), others have reported dose-dependent effects of caffeine on sperm motility, number and morphology, such that consuming 1–2 cups of coffee per day had a positive effect on semen parameters, whereas consumption of zero or more than 2 cups per day was associated with diminished sperm motility and count, as well as poor morphology (66). However, higher intake of caffeine (>175 mg/day) has

been positively associated with semen volume and circulating testosterone in men (67). There may be an inverse relationship between higher caffeine intake in men and lower fecundity (204,205), but additional studies that include analysis of participant *CYP1A2* genotype are needed.

Macronutrients and male fertility

Fat

Dietary fat has several important functions within the body. Its physiological roles include acting as an energy source, insulating organs and playing a crucial part in the creation of hormones, cell membranes and tissue membranes (206). However, diets high in fat can increase serum and semen triglyceride levels, which may increase oxidative stress in reproductive tissues (207), and have been linked to a higher risk of obesity (208,209). Genetics play a role in dietary preference for fat. The cluster of differentiation 36 (*CD36*) gene impacts the transport of fat in the blood throughout the body, and overall perception of dietary fat. As such, “super tasters,” or carriers of the GA or GG variants, can detect dietary fat at a heightened level in comparison to a “typical taster,” or an individual with the AA variant of *CD36* (210). It has been suggested that “super tasters” of fat may consume less dietary fat than “typical tasters” (211).

Transcription factor-7 like 2 (*TCFL2*) genotype controls the impact of dietary fat on body composition and several metabolic factors. Individuals who carry the TT genotype for rs7903146 comprise approximately 10% of the population. Those with the TT variant who consume a high proportion of dietary fat may be more likely to be overweight and experience insulin resistance compared to those with the CC or TC genotypes (45,46). Both of these outcomes can have negative implications on fertility due to hormonal imbalances, oxidative stress causing sperm damage, and increased testicular temperature (208,209,212,213).

In men, total dietary fat intake has been negatively associated with sperm count and concentration (47). Diets higher in fat can lead to hormonal disruption and a lack of testicular energy supply, compromising germ cells and mitochondria (214). Observed effects include disrupted sperm membranes, impaired motility and function, and decreased sperm quality (48). However, the most important factor when analyzing the relationship between dietary fat and fertility in men is the type of fat, as the

ratio of unsaturated to saturated fat can influence semen quality (47,48).

Omega-3 fat

Polyunsaturated omega-3 fat is a component of a healthy diet, found in fatty fish, nuts, seeds, and oils, and can help to maintain healthy levels of circulating triglycerides. The protein nitric oxide synthase (NOS) regulates the interaction between dietary fat and plasma triglycerides and is encoded by the nitric oxide synthase-3 gene (*NOS3*). Variation in this gene changes how the NOS protein is expressed, and therefore different genotypes can affect circulating triglyceride levels in response to plasma omega-3 levels. In one study, individuals with the GT or TT genotype of *NOS3* had higher circulating triglycerides when plasma omega-3 fats were low, compared to those with the GG genotype (49). Circulating triglycerides have been shown to be positively correlated with sperm concentration, as well as having a concentration-dependent effect on sperm morphology (215).

Omega-3 and omega-6 fatty acids are metabolized into long-chain polyunsaturated fatty acids (LC-PUFAs) that modulate blood pressure, blood clotting, and inflammation through the formation of eicosanoids (216). The production of LC-PUFAs is regulated by the fatty acid desaturase enzymes encoded by the *FADS* gene cluster. In particular, polymorphisms of the *FADS1* and *FADS2* genes are known to affect the rate of LC-PUFA synthesis (50). Polymorphisms in these genes influence the circulating levels of various metabolic forms of n-3 and n-6 fatty acids including eicosanoid precursors (217). Multiple studies have found that carriers of the C allele in the *FADS1* gene (rs174547) had reduced endogenous production of LC-PUFAs (51,52,218). SNPs in the *FADS2* gene (rs2727270, rs498793) have also been correlated with altered PUFA metabolism (51,52). The effects of these *FADS* polymorphisms on circulating lipid profiles and levels of eicosanoid precursors may affect male reproductive health through altering inflammatory responses and sperm membrane characteristics.

Omega-3 fats have been suggested to be the most important component in sperm membranes because of their contribution to sperm motility and membrane fluidity, as well as fertile potential of sperm (53,54). Polyunsaturated fats in the sperm membrane are targets of lipid peroxidation, creating oxidative stress in the semen (219). Therefore,

antioxidants such as vitamins E and C are necessary to protect unsaturated fats composing sperm and oocyte membranes, maintaining the integrity and function of these structures (59,220). Lower ratios of omega-6 to omega-3, and saturated to unsaturated fatty acids have been associated with better semen parameters, specifically sperm count, motility and morphology in oligoasthenoteratozoospermic men (221,222).

In a 3-month trial of omega-3 fatty acid-based supplements from fish and algal oils for 10 men with asthenozoospermia, reduced concentrations of omega-3 fatty acids in seminal plasma and sperm fatty acid profile were seen following supplementation, but no changes in seminal parameters were observed (223). In contrast, a randomized, double blind study of men undergoing evaluation for infertility who were given 1,500 mg per day of docosahexaenoic acid (DHA, a type of omega-3 fatty acid)-enriched oil over a 10-week period resulted in improvement in DHA and omega-3 fatty acid content in seminal plasma, and a reduction in the percentage of spermatozoa with DNA damage (224). Overall, the majority of studies suggest that increased intakes of omega-3 fatty acids are correlated with improved semen quality parameters (225). However, future research should focus on examining how *NOS3* and *FADS1/2* genotypes directly modify these associations.

Saturated fat

Saturated fat has long been regarded as a marker of poor diet quality, due to its suggested link to diseases such as obesity (226). Apoprotein A-II (*APOA2*), a major protein of HDL, reduces the reverse efflux of cholesterol transport and its antioxidant function and is regulated by the *APOA2* gene (55). Variation in the *APOA2* (rs5082) gene is known to affect the way saturated fat intake impacts body mass index (BMI), such that carriers of the CC genotype had a stronger correlation between saturated fat intake and BMI than those with the TC or TT genotypes (55). Individuals with a higher BMI score and the CC genotype have been observed to have higher saturated fat intakes than lower-weight carriers of the T allele of the *APOA2* gene (55), which can impact circulating fatty acids (226) and the composition and quality of reproductive tissues in men (47,227). Further, higher BMI is a risk factor for infertility (209,212). Therefore, carriers of the *APOA2* CC genotype may benefit from a limited intake of saturated fats.

Dietary saturated fat has been shown to negatively

affect sperm count and concentration (47,228,229), as well as poor sperm motility (229,230) and morphology (231,232). Saturated fat content in sperm membranes has been shown to be higher in infertile men, specifically with asthenozoospermia and oligozoospermia when compared to normozoospermic men (56,57). Sperm membrane composition is integral to fluidity and function, as well as sperm motility, viability and susceptibility to lipid peroxidation (233-235), which are compromised in infertile men (56,236).

Sugar

The 2015–2020 Dietary Guidelines for Americans recommend an intake of no more than 10% of calories from added sugars (237). The glucose transporter type-2 (*GLUT2*) protein influences glucose levels in the body and variation in the *GLUT2* (rs5400) gene dictates its production. Carriers of the T allele have been shown to have a lower sensitivity and thus higher preference for glucose when compared to carriers of the CC genotype (58). Sugar intake can be an important factor contributing to daily caloric intake, and in excess can drive the development of chronic diseases such as obesity and type 2 diabetes (238), which can have negative implications on fertility (239-242). The existing literature focuses on one concentrated source of added sugar: sugar-sweetened beverages (SSBs).

Higher sugar-sweetened beverage consumption has been associated with lower sperm motility and sperm count, after adjustment for caffeine and BMI (243). Male mice fed an SSB analog had reduced fertility rates by 25% in comparison to controls not provided an SSB analog (244). In a large cohort of Asian men, higher intake of sweet snacks and SSBs was associated with lower sperm count (245). Additionally, a prospective cohort study of 2,554 Danish men found that self-reported consumption of SSBs negatively correlated with sperm count, concentration, and morphology. However, the study's results may have also been influenced by poorer lifestyle factors among the high SSB-consuming individuals, including higher self-reported consumption of red meats and alcohol (59). Other health effects of excess sugar consumption in men include insulin resistance, oxidative stress, and an altered hormone profile, all of which are linked to poor semen parameters and infertility (213,246). However, some studies have found no association between SSB intake and semen parameters (67,247).

Fiber

Whole grain wheat is a rich source of methionine, betaine, choline and folate, all of which are involved in the methylation of DNA. Whole grains also provide dietary fiber, which can mitigate rapid increases in blood glucose following ingestion of carbohydrates. Diets rich in fiber are known to be inversely associated with insulin resistance and incidence of type 2 diabetes (248,249). The transcription factor-7 like-2 (TCF7L2) protein plays a role in the development of type 2 diabetes by impacting insulin secretion and hepatic glucose production, and is encoded by the *TCF7L2* gene (60). Possession of the T allele of the *TCF7L2* (rs12255372) gene is associated with greater expression of the TCF7L2 protein, impaired insulin sensitivity, and thus a higher risk of developing type 2 diabetes, which can have detrimental effects on male fertility (250). As an endocrine disorder, type 2 diabetes can alter a man's hormone profile such that spermatogenesis, steroidogenesis, sperm maturation, and ejaculation are negatively impacted (61). Glucose transporter function and therefore the bioavailability of glucose are integral to processes such as sperm production, maturation, and fertilization (236,251). The presence of type 2 diabetes may compromise sperm motility, DNA integrity and seminal composition (251,252), negatively impacting overall fertility (241). Sperm DNA damage may be attributable to diabetes-induced oxidative stress and lipid peroxidation (253), which can result in poor implantation, embryonic development, and low clinical pregnancy rate (252).

Research has shown that a diet rich in whole grains promotes better sperm morphology and motility (26,254). High fiber diets can play an important role in supporting greater fecal microbiome diversity, but little evidence exists on the direct influence of diet on the reproductive microbiome. It is known that host genetics and diet influence the development of the microbiome (255,256). Also, obesity, insulin resistance, inflammation and dyslipidemia occur less frequently in individuals with greater microbiome diversity (257). There is a need for research exploring the interaction between diet, reproductive health and the microbiome (258).

Conclusion and future directions

Overall, evidence to date suggests that nutritional status plays an important role in male fertility, and common genetic variations influence nutrient metabolism and

response to dietary intake. The interaction between nutrition, male fertility and genetic variation remains little explored, but there is evidence that these three factors are interrelated and should be examined together in scientific literature and clinical practice to increase our understanding of reproductive function and enhance fertility outcomes. Men experiencing infertility may seek support from registered dietitians to ensure dietary intakes meet requirements for essential nutrients. Future research may provide clarity by focusing on tailoring dietary recommendations to individuals based on genetic susceptibilities to deficiencies and toxicities.

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