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REVIEW

Male Infertility

Exploring the potential impact of nutritionally actionable genetic polymorphisms on idiopathic male infertility: a review of current evidence

Sinda Mahbouli¹, Charlotte Dupont^{2,3}, Yaelle Elfassy³, Eric Lameignère¹, Rachel Levy^{2,3}

Infertility affects about 15% of the world's population. In 40%–50% of infertile couples, a male factor underlies the problem, but in about 50% of these cases, the etiology of male infertility remains unexplained. Some clinical data show that lifestyle interventions may contribute to male reproductive health. Cessation of unhealthy habits is suggested for preserving male fertility; there is growing evidence that most preexisting comorbidities, such as obesity and metabolic syndrome, are highly likely to have an impact on male fertility. The analysis of genetic polymorphisms implicated in metabolic activity represents one of the most exciting areas in the study of genetic causes of male infertility. Although these polymorphisms are not directly connected with male infertility, they may have a role in specific conditions associated with it, that is, metabolic disorders and oxidative stress pathway genes that are potentially associated with an increased risk of male infertility due to DNA and cell membrane damage. Some studies have examined the impact of individual genetic differences and gene-diet interactions on male infertility, but their results have not been synthesized. We review the current research to identify genetic variants that could be tested to improve the chances of conceiving spontaneously through personalized diet and/or oral vitamin and mineral supplementation, by examining the science of genetic modifiers of dietary factors that affect nutritional status and male fertility.

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INTRODUCTION

In recent years, several studies have provided evidence that semen quality in humans is decreasing, which may lead to a significant decline in male fertility.¹ Moreover, some studies have investigated the possible association between the infertile male phenotype and specific gene variants.^{2,3} Recent research on impaired sperm quality has demonstrated genetic variation in sperm DNA.⁴ In fact, the growing technology of genetic tools that use genomic information has also provided an avenue for experimental approaches to support the genetic causes of male infertility. Personal genetic testing can provide information that may be used to recommend dietary choices that are more effective at the individual level than the current dietary advice. A recent systematic review found that individuals are more likely to change health behaviors, including their dietary choices, when their genetic information include actionable advice.⁵ A large number of micronutrients are required as cofactors for enzymes, or as part of the structure of proteins involved in DNA synthesis and repair, prevention of oxidative damage to DNA, and maintenance methylation of DNA.⁶ Therefore, genetic variants with poor nutritional and environmental factors may have an impact on fertility, fetal growth, and birth outcomes. To preserve male fertility, the whole person should be

considered, from hormonal balance, nutritional requirements, and optimal gut microbiome to exposure to environmental toxins.

Role of obesity and fatty acids as modulators of sperm function

The prevalence of male obesity in reproductive age has nearly tripled in the last 30 years.⁷ There is increasing awareness that male obesity reduces sperm quality, in particular by altering the physical and molecular structure of germ cells in the testes and mature sperm, and raises the risk of sperm DNA damage linked to excess production of reactive oxygen species (ROS).^{8,9} Recently, growing evidence has linked abdominal obesity, together with insulin resistance and dyslipidemia, to male fertility.¹⁰ Obesity adversely affects male fertility through changes at the hormonal level, as well as by direct changes to sperm function and gamete molecular composition. Adipose tissue depots containing adipocytes and infiltrated immune cells generate inflammatory molecules that may also play an important role in the chronic pro-inflammatory state in the testicular microenvironment and/or excurrent ductal system. Increased adipose tissue is associated with overproduction of adipocytokines, such as leptin, resistin, adiponectin, ghrelin, tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6), which have a negative influence on spermatogenesis.¹¹

¹FabLife, 104 Avenue Albert 1er, Rueil-Malmaison 92500, France; ²Sorbonne Université, Saint Antoine Research Centre, INSERM Genetic and Acquired Lipodystrophies Team, Reproductive Biology and CECOS, AP-HP, Tenon Hospital, Paris F-75020, France; ³Reproductive Biology and CECOS, AP-HP, Tenon Hospital, Paris F-75020, France.

Correspondence: Dr. R. Levy (rachel.levy@aphp.fr)

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Although some data are currently available on dietary modulation of lipid metabolism, little is known about the nutritional regulation of energy metabolism in sperm. In this regard, lipid profile alterations have been correlated with male infertility.¹² There are three types of natural fatty acids, namely, saturated, monounsaturated, and polyunsaturated. Polyunsaturated fatty acids (PUFAs) are essential because they cannot be synthesized by the body. Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and α -linolenic acid are the main omega-3 PUFAs. Linoleic acid, γ -linolenic acid, and arachidonic acid are the main omega-6 PUFAs. The first mechanism by which PUFAs affect spermatogenesis is their incorporation into the spermatozoon cell membrane. PUFAs are structural components of spermatozoon membranes.^{13,14} Conquer *et al.*¹⁵ reported that DHA levels were lower and oleic acid levels were higher in spermatozoa of patients suffering from asthenozoospermia, compared to that of a control group. Another case-control study of idiopathic infertile men and a healthy control group showed that blood and spermatozoa levels of omega-3 were significantly higher in fertile men compared to that of their infertile counterparts. Moreover, the serum omega-6 to omega-3 proportion was considerably lower in fertile individuals.¹⁶ Some studies have reported a detrimental impact of body mass index (BMI) on sperm parameters, notably a decrease in sperm concentration.^{17,18} Consequently, the possibility of improving semen quality through weight reduction has also been considered. Improvement of hormonal status was mainly observed after weight loss;¹⁹ furthermore, a positive impact of weight loss on semen parameters has been observed.²⁰

Association between nutritional factors and the risk of male infertility

Nutritional factors are known to be critical determinants of normal reproductive function.²¹ Indeed, worldwide, environmental conditions have changed dramatically, especially with respect to diet and exercise, and the pronounced changes in these factors suggest that they may be involved in the etiology of declining male fertility and impairment of sperm production.²² In fact, diet and obesity are two important lifestyle factors that can influence spermatogenesis; in terms of both macro- and micro-nutrient intake, they have major effects on normal reproductive function.²³ Within the last few decades, reproductive-age people have started eating more highly refined carbohydrate-rich food, food high in saturated fat and trans fatty acids, and sodium and ultra-processed food, while simultaneously consuming less fresh fruit and vegetables.¹⁶ There is increasing evidence indicating a potential relationship between incorrect nutritional attitudes and lower sperm quality.¹² Essential nutrients, especially vitamins such as folate, are involved in DNA and RNA synthesis, and thus play an important role in spermatogenesis by protecting the sperm's DNA from free radical damage.²⁴ A recent meta-analysis of randomized clinical trials suggests that some dietary supplements could beneficially modulate sperm quality parameters and affect male fertility.²⁵ However, no consensus has been reached on systematic recommendation of oral supplementation.

Impacts of oxidative stress on male reproduction

Evidence has been increasing in recent years for oxidative stress playing a vital role in the pathogenesis of idiopathic male factor infertility. One of the factors that may contribute to the onset of male infertility is the overproduction of ROS.²⁶ Aitken²⁷ (2016) reviewed twenty studies of infertile male patients treated with antioxidants. The review showed a significant decrease in oxidative stress and improved motility in asthenozoospermic patients, but only 50% of the studies reported a pregnancy rate.²⁸ However, there is weak evidence

from a few randomized controlled trials suggesting that antioxidant supplementation in subfertile males may improve live birth rates in couples attending fertility clinics.²⁵ Further studies are required to formulate an optimal dosage and ideal combination of nutrients, both necessary to provide the appropriate response to each patient.

GENETIC VARIANTS ASSOCIATED WITH NUTRITION AND MALE REPRODUCTIVE POTENTIAL

Single-nucleotide polymorphisms (SNPs) represent genetic variation among individuals in a population. These variations in the DNA sequence may significantly affect an individual's response to certain drugs or influence the risk of developing certain diseases. In the field of reproductive medicine, considerable research effort has been devoted to identifying polymorphisms which may influence steroidogenesis and fertility. Genetic risk involved in spermatogenesis is considered one of the main factors in male infertility. In recent years, various studies have reported possible associations between infertile phenotypes and specific genetic variants. The group of variants related to energy metabolism, folate metabolism, and antioxidant defense probably influences male fertility, with the impact of the variants potentially modulated by nutritional interventions. Genetic variation affecting responses to various micro- and macronutrients, as well as bioactives such as folate, and male infertility risk will be reviewed in this paper. Genes were selected based on their potential contribution to infertile male phenotypes and a presumed involvement in various parts of the pathogenic processes of male infertility.

Genetic variants involved in energy balance and lipid metabolism, and their influence on male fertility

The physiological mechanisms that control energy balance are reciprocally linked to those that control reproduction; these mechanisms optimize reproductive success under fluctuating metabolic conditions. Mechanisms regulating energy balance involve complex interactions between genetic, environmental, and behavioral factors. The major driving force behind obesity in modern society is overeating, which is largely coded in genes that are responsible for appetite and satiety regulation. About fifty genes that regulate satiety in humans have been reported; fat mass and obesity-associated gene (*FTO*) and melanocortin 4 receptor (*MCR4*) are the two best-known examples (**Table 1**). *MCR4* codes for a protein that is mainly found in the hypothalamus, an area responsible for controlling appetite and satiety.²⁹ The *FTO* gene has been reported to demethylate mRNA N⁶-methyladenosine (m⁶A) in mammalian cells.³⁰ *FTO*-depleted cells exhibit higher levels of m⁶A than control cells; the demethylase activity of *FTO* protein is required for differentiation of preadipocytes.³¹ Indeed, the discovery of two missense mutations with potentially detrimental effects on the functionality of the methylation protein *FTO*, as well as a genetic variant of the same protein that is associated with altered semen quality, suggests that aberrant demethylation of mRNA is a factor involved in reduced male fertility.³² In addition to low satiety, *FTO* risk variants can also affect food preferences; *FTO* carriers tend to overeat and prefer high-sugar and high-fat foods. For these reasons, it is essential to adapt the dietary intervention in order to modulate the impact of genetic variations, which can also induce alteration in sperm parameters due to disturbance of the energy balance. The crucial role of lipid homeostasis and energy balance in endocrine regulation of spermatogenesis is well known. Thus, genes of the spermatozoon membrane structure represent a logical target for mutational analysis in infertile males. Cholesterol and lipid homeostasis are important for male fecundity.³³ It is reported that 65% of infertile men show hypercholesterolemia

Table 1: Summary of genetic variants involved in energy balance and homeostatic lipid metabolism, and their influence on male fertility

Gene symbol	Gene name	SNPs	Gene consequence	Phenotype impact	Study	Protein function	Dietary recommendation based on
FTO	FTO, alpha-ketoglutarate-dependent dioxygenase	rs9939609	Intron variant	Obesity risk	32,104	RNA demethylase that mediates oxidative demethylation of different RNA species Acts as a regulator of fat mass, adipogenesis, and energy homeostasis	Genetic profile matched to low-fat, low-carbohydrate, Mediterranean or balanced diet, including genetic risks for metabolic health factors (e.g., blood sugar, lipids)
		rs1558902	Intron variant	Type 2 diabetes risk			
		rs7193144	Intron variant	Low fat oxidation rates Higher weight and abdominal circumference			
MC4R	Melanocortin 4 receptor	rs17782313	Intergenic variant	Increased appetite and decreased satiety Overweight Autosomal dominant obesity Higher BMI	29,105	Plays a central role as a leptin-targeted neural circuit in energy homeostasis and somatic growth	
APOE	Apolipoprotein E	rs429358	Missense variant	Hypertriglyceridemia Hyperlipoproteinemia	37	Transports lipoproteins, vitamins, and cholesterol, particularly in the brain	
		rs7412	Missense variant	Cardiovascular risk Atherosclerosis Increased risk factor for male infertility			
TCF7L2	Transcription factor 7-like 2	rs12255372	Intron variant	Increased type 2 diabetes risk	106,107	Implicated in blood glucose homeostasis	
		rs7903146	Intron variant	Reduction of insulin sensitivity Fasting proinsulin			

SNPs: single-nucleotide polymorphisms; BMI: body mass index

and/or triglyceridemia.³⁴ Moreover, Ergün *et al.*³⁵ (2007) reported that increased very-low-density lipoprotein (VLDL) impaired seminal parameters, and that increased triglycerides may have deleterious effects on spermatogenesis. In addition, Schisterman *et al.*³⁶ reported that lipid concentrations may affect semen parameters, specifically sperm head morphology, highlighting the importance of cholesterol and lipid homeostasis for male fecundity. Lipid components of the spermatozoon have an important role in the functional activity of this cell.

Apolipoprotein E (APOE) has a central role in lipid transport by mediating the binding of lipoprotein particles to their receptors.³⁷ APOE is a constituent apoprotein of VLDL, intermediate-density lipoprotein, high-density lipoprotein (HDL), and chylomicron particles.³⁸ Three important polymorphisms have been discovered in the human APOE gene. These co-dominant alleles, designated $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, give rise to functionally distinct proteins, namely, APOE2, APOE3, and APOE4.³⁹ APOE polymorphisms have also been found to affect male fertility and cause marked differences in reproductive efficiency.⁴⁰ Setarehbad *et al.*³⁷ found significant differences in the distribution of APOE allelic combinations between fertile men and infertile men, with a higher percentage of fertile males possessing the $\epsilon 3/\epsilon 3$ genotype. APOE genotypes may be linked to differences in the efficacy of the expressed APOE isoforms in promoting sperm maturation during epididymal transit.³⁷ Diet is the major reason for variation in lipid metabolism in human populations. Several recent studies have demonstrated that a specific dietary intervention may elicit extraordinary effects in certain genetic subgroups of patients.⁴¹ The ability of dietary intervention to improve plasma lipoprotein-lipid profiles varies greatly among individuals with different APOE genotypes. Ordovas and Galluzzi⁴² focused on the APOE genotype and dietary response in a comprehensive review of 27 studies. In general, the $\epsilon 4$ allele appears to be the most responsive to a low-fat and low-cholesterol dietary intervention; however, it may not be the most responsive to changes in other aspects of the diet. For example, subjects carrying the $\epsilon 2$ allele had the greatest change in total and LDL cholesterol in response to wheat- or oat-bran supplementation. Plasma lipid levels in subjects carrying the APOE2 allele show a more favorable response to tea drinking, and possibly to a fruit-and-vegetable

diet. A long-term increase in dietary soluble fiber does not affect fat metabolism after meals in subjects with the APOE4 allele; however, it does enhance fat absorption in subjects with the APOE3/3 phenotype.

Hyperinsulinemia and hyperglycemia are common occurrences in obese individuals, and are constant confounding factors in many rodent studies of male obesity.⁴³ Both hyperinsulinemia and hyperglycemia have been shown to have an inhibitory effect on sperm quantity and quality, and, therefore, may contribute to the reduced fertility seen in obese men.⁴⁴ One of the gene variants that plays an important role in the development of type 2 diabetes mellitus in individuals with metabolic syndrome is transcription factor 7-like 2 (TCF7L2) gene (Table 1). Variants of this gene, such as rs12255372 and rs7903146, have been consistently shown to raise the genetic risk of β -cell dysfunction and development of type 2 diabetes.⁴⁵ Increased ROS and sperm DNA damage are also seen in diabetic patients with commonly altered markers of sperm function. High circulating levels of insulin are suggested as one possible mechanism for the above effects, with increased insulin reducing the production of sex hormone-binding globulin (SHBG).^{44,46} The decreased levels of SHBG to sustain homeostatic levels of testosterone could contribute to the decreased levels of testosterone and reduced sperm counts seen in these patients.

Genetic variants that affect the one-carbon metabolic pathway and their influence on male fertility

Male fertility depends on the normal process of spermatogenesis which is itself a complex process regulated by a number of genes involved in growth, differentiation, apoptosis, and DNA damage. This complex pathway is associated with another important pathway, one-carbon metabolism. Folate and the normal activity of one-carbon metabolic enzymes are central to nucleotide synthesis, methylation, and maintenance of genomic integrity, as well as protection from DNA damage. The key enzymes implicated in these metabolic pathways are methylene-tetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR) (Table 2).⁴⁷ Polymorphisms of important genes in the one-carbon metabolic pathway that affect several physiological processes also have an impact on spermatogenesis, and may directly or indirectly influence the quality of sperm parameters and male infertility.⁴⁸ Homocysteine

is a sulfur-containing amino acid, which is an intermediate product in the metabolism of the amino acid methionine. The homocysteine, folate, and methyl group metabolic pathways are linked processes. The choline, methionine, and folate metabolic pathways interact at the point where homocysteine is converted to methionine (**Figure 1**). Homocysteine can be methylated to form methionine⁴⁹ by two parallel pathways, both of which decrease homocysteine concentrations.⁵⁰ The alternative pathway for methylation of homocysteine to form methionine is catalyzed by betaine-homocysteine S-methyltransferase (BHMT).⁵¹ Betaine, derived from dietary choline by the action of choline dehydrogenase (CHDH), is the methyl group donor in this reaction, and supplemental oral betaine can lower plasma homocysteine concentrations.⁵² Dysfunction of folate metabolism pathways due to insufficient dietary folate intake, vitamin B9 deficiency,⁵³ and genetic variations that impair the activity of enzymes involved in these processes will lead to a reduction in the conversion of homocysteine to methionine.^{54,55} This may lead to hyperhomocysteinemia associated with an increased risk of cardiovascular disease and disturbed DNA synthesis and/or DNA methylation reactions, causing DNA mutations and altered gene expression.

A few studies have assessed an association between male infertility and variants in key genes, *MTR*, *MTRR*, and *MTHFR*, for enzymes involved in methylation and homocysteine metabolism, with mixed

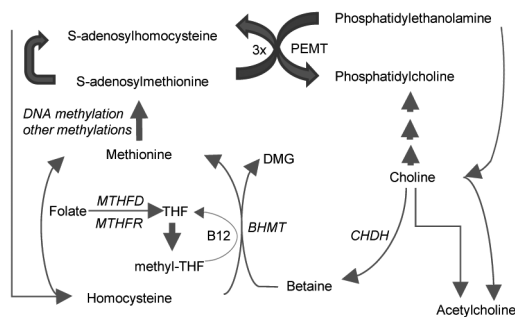


Figure 1: Choline metabolism and its links to methionine and folate metabolism. The pathways described are all present in the liver, with other tissues having one or more of these pathways. B12: cobalamin; BHMT: betaine-homocysteine S-methyltransferase; CHDH: choline dehydrogenase; DMG: dimethylglycine; MTHFD: methylene-tetrahydrofolate dehydrogenase; MTHFR: methylene-tetrahydrofolate reductase; PEMT: phosphatidylethanolamine N-methyltransferase; THF: tetrahydrofolate.

results.^{48,56} MTHFR is one of the main regulatory enzymes involved in folate metabolism, DNA synthesis, and remethylation reactions. Moreover, *MTHFR*, which performs a key function in the metabolism of folate and homocysteine, is potentially one of the candidates for genetic vulnerability to spermatogenic failure. Carriers of the *MTHFR* rs1801133 (C677T) variant have been shown to have decreased activity of MTHFR enzyme – by 35% in the presence of heterozygosis and 70% in homozygosis.⁵⁷ Similarly, the *MTHFR* rs1801131 (A1298C) polymorphism has been shown to be associated with lower enzymatic activity *in vitro*, but to a lesser degree than *MTHFR* rs1801133,⁵⁸ with a resultant increase in homocysteine levels.⁵⁹ Some authors have described a statistically significant correlation between *MTHFR* polymorphisms and human male infertility.^{60–62} Possible negative effects of the *MTHFR* rs1801133 mutation on male fertility may be caused by alteration of the expression of genes involved in spermatogenesis induced by undermethylation,⁶³ or spermatozoa may be damaged by higher production of ROS metabolites, causing DNA damage (**Figure 2**) and reduced sperm counts.^{64,65} Significant experimental data show that the chief enzymes of the folate metabolism cycle are vital to male spermatogenesis.⁶⁰

Genetic variants involved in vitamin D metabolism and their influence on male fertility

The male reproductive tract is one of the sites where vitamin D is metabolized. Vitamin D receptor (VDR) expression in various reproductive tissues, such as the smooth muscles of the epididymis, spermatogonia, Sertoli cells, and spermatozoa (especially the midpiece and nucleus), shows that it plays a crucial role in reproduction, and, therefore, fertility (**Table 3**).⁶⁶ The role of vitamin D in the modulation of testicular functions, including hormone production and spermatogenesis, has been investigated in animals and humans.⁶⁷ Experimental studies support a beneficial effect of vitamin D on male fertility, by modulating hormone production through genomic and nongenomic actions, and, particularly, by improving semen quality, essentially through nongenomic actions.⁶⁸ Indeed, vitamin D seems to contribute to the modulation of the bioavailable rather than total testosterone. Moreover, although an increased prevalence or risk of testosterone deficiency was reported in men with vitamin D deficiency in observational studies, most interventional studies demonstrated a lack of effect of vitamin D supplementation on circulating levels of testosterone. The most consistent effect of vitamin D was reported for

Table 2: Summary of genetic variants involved in folate metabolism, and their influence on male fertility

Gene symbol	Gene name	SNPs	Gene consequence	Phenotype impact	Study	Protein function	Dietary recommendation based on
<i>MTHFR</i>	Methylene-tetrahydrofolate reductase	rs1801133 rs1801131	Missense variant Missense variant	Lower folate status Idiopathic male infertility Homocystinuria Reduced activity of MTHFR	57,108	Converts MeTHF to MTHF	Genetic profile matched to folic acid-fortified foods and vitamin B-supplemented diet, including genetic risks of higher blood homocysteine
<i>MTRR</i>	5-methyl-tetrahydrofolate-homocysteine methyltransferase reductase	rs1801394	Missense variant	Homocystinuria Reduced activity of MTRR enzyme Hyperhomocysteinemia	109	Regenerates functional methionine synthase via reductive methylation	
<i>BHMT</i>	Betaine-homocysteine S-methyltransferase	rs7356530	Intron variant	Increased homocysteine levels in the blood	110	Converts betaine and homocysteine to dimethylglycine and methionine	
<i>CHDH</i>	Choline dehydrogenase	rs12676	Missense variant	Choline deficiency Changes in human sperm cell function	111	Involved in step 1 of the subpathway that synthesizes betaine aldehyde from choline	

SNPs: single-nucleotide polymorphisms; MeTHF: 5,10-methylenetetrahydrofolate; MTHF: 5-methyltetrahydrofolate

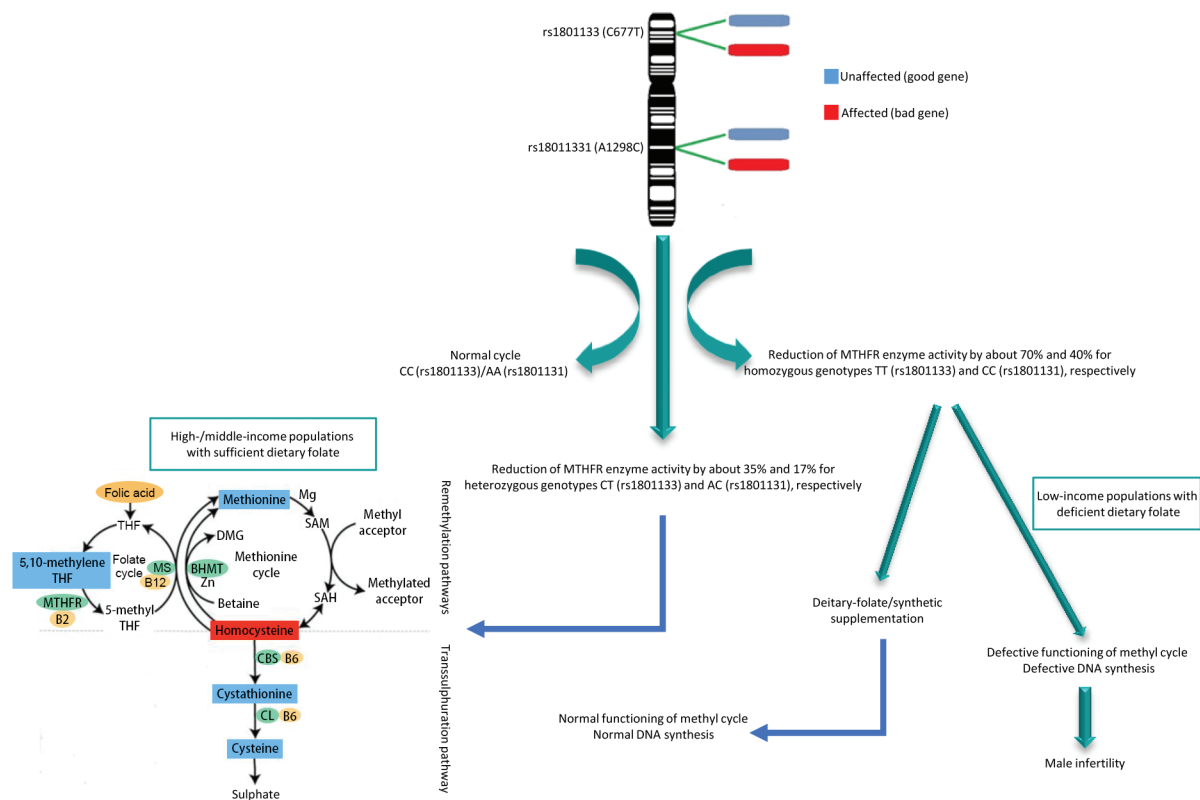


Figure 2: Effects of *MTHFR* polymorphisms on male fertility. The *MTHFR* gene, located on the short arm of chromosome 1 (1p36.3), is composed of 11 exons. It possesses 14 common or rare single-nucleotide polymorphisms that are associated with enzymatic deficiency. Among them, rs1801133 (C677T) and rs1801131 (A1298C) are the most reported to possibly reduce *MTHFR* activity to various degrees. Reduced enzymatic activity due to *MTHFR* polymorphisms is considered a risk factor for many diseases, including infertility. B2: riboflavin; B6: pyridoxine; B12: cobalamin; BHMT: betaine-homocysteine methyltransferase; CBS: cystathionine- β -synthase; CL: cystathionine- γ -lyase; DMG: dimethylglycine; MS: methionine synthase; MTHFR: methylenetetrahydrofolate reductase; SAM: S-adenosyl methionine; SAH: A-adenosyl homocysteine; THF: tetrahydrofolate.

Table 3: Summary of genetic variants involved in vitamin D metabolism, and their influence on male fertility

Gene symbol	Gene name	SNPs	Gene consequence	Phenotype impact	Study	Protein function	Dietary recommendation based on
VDR	Vitamin D receptor	rs2228570	Start-lost	Deficient in vitamin D	112	Plays a key role in the absorption of calcium from the gut, which is required for healthy bone formation, muscle, and heart activity, as well as numerous other cell functions	Genetic predisposition to food and nutrient needs profile and sensitivity to vitamin deficiency
GC	GC Vitamin D binding protein	rs2282679	Intron variant	Associated with reduced levels of vitamin D	113	Responsible for binding with the bioactive form of vitamin D, calcitriol, and shuttling it through the circulatory system into tissue and then presenting it to the VDR to allow its binding	

SNPs: single-nucleotide polymorphisms; VDR: vitamin D receptor

semen quality.⁶⁹ Vitamin D has been shown to increase sperm motility by increasing intracellular calcium concentrations in spermatozoa through the VDR. We conclude that VDR polymorphism may play a major role in male factor infertility, either directly or indirectly, by reducing the effects of vitamin D.⁷⁰ Four SNPs are associated with changes in VDR activity, one of which is the rs2228570 variant (Table 3). Furthermore, one SNP within the *GC* gene is associated with reduced levels of vitamin D. Studies have shown that men with higher dietary and supplemental intake of vitamin D may produce sperm with less DNA damage.⁷¹

Genetic variants that affect antioxidant defense and their influence on male fertility

Extensive research suggests that oxidative stress may be an important cause of male infertility, and that the pathology of infertility in

30%–80% of infertile men may be oxidative damage to spermatozoa.⁷² This is primarily due to DNA and cell membrane damage; however, little is known about the genetic causes underlying suboptimal functioning of the seminal enzymatic antioxidant system. Oxidative stress induces sperm DNA damage,⁷³ a reduction in sperm motility,⁷⁴ a decline in sperm's fertilizing ability,⁷⁵ a reduction in membrane fluidity, apoptosis,^{27,76} and defective sperm membrane integrity by the way of lipid peroxidation.⁷⁷ The presence of ROS in seminal plasma is normally balanced by homeostatic antioxidant systems that facilitate an appropriate level of ROS required for normal physiological processes, such as sperm capacitation, hyperactivation, acrosome reaction, and sperm–oocyte fusion.⁷⁸ Semen is shown to possess large amounts of antioxidants to counterbalance the effects of ROS, thereby protecting mature spermatozoa from oxidative damage. The antioxidants in

semen are present both in spermatozoa and the seminal plasma; however, they are most abundant in the latter because the amount of spermatozoan cytoplasm is small, thereby limiting antioxidant defense within the cells.⁷⁹ Seminal plasma is enriched with both enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST), and nonenzymatic antioxidants, such as glutathione, vitamin A, vitamin C, vitamin E, and coenzyme Q10.⁸⁰ SOD2 is an enzyme that catalyzes the detoxification of superoxide radicals in the mitochondrion. CAT has the capacity to detoxify hydrogen peroxide (H_2O_2) by converting it to water (H_2O) and oxygen (O_2). GPx1 is related to the final electron transporter in mitochondria and neutralizes peroxide radicals into water, whereas GST conjugates toxic electrophiles and other intermediates, with glutathione neutralizing their toxicity. Dietary selenium intake is an important determinant of GPx1 activity, with selenium supplementation leading to an increase in blood GPx1 activity. Large interindividual differences have been observed in the response of GPx1 to selenium supplementation.⁸¹ These variations are mainly due to differences in the baseline selenium status; however, interactions between genetic polymorphisms in the *GPX1* gene and dietary selenium intake may account for some of the interindividual variations.⁸²

Deficiencies in enzymatic or nonenzymatic antioxidant systems in seminal plasma are widely associated with male infertility as the absence of any of these systems leads to the accumulation of excessive levels of ROS, resulting in impairment of both the structural and functional integrity of spermatozoa.⁸³ Although these enzymes appear to be conserved phylogenetically, intraspecific polymorphisms may still cause changes in their activities, and, therefore, they may be useful in understanding the underlying origins of idiopathic infertility. Genetic variation in one or more enzymes involved in redox balance may induce alteration of antioxidant activity in hypofertile men (Table 3). Paraoxonase 1 (*PON1*) is one such gene that can have an impact on male infertility. PON proteins, localized in the seminiferous tubules and spermatozoa, have been implicated in the pathogenesis of male infertility.^{84,85} Impaired oxidative stress regulation in the seminal plasma of patients with abnormal sperm parameters has been proposed to be the result of decreased PON1 activity.⁸⁶ PON possesses antioxidant properties and protects cells against oxidative stress.⁸⁷ Lazaros *et al.*⁸⁸ showed, for the first time, the association between *PON* gene variants and semen concentration and motility. Glutathione S-transferase mu 1 (*GSTM1*) and glutathione S-transferase theta 1 (*GSTT1*) have the highest frequency of polymorphisms among all glutathione S-transferase genes (Table 4). They are characterized by deletion polymorphisms manifested in the absence of enzymatic activity of relevant proteins, and with a frequency of 42%–60% for *GSTM1* and 13%–26% for *GSTT1* in Caucasian populations.^{89–91} Genetically determined imbalance in the detoxification system, due to reduced activity of glutathione system enzymes, can be the cause of various pathological processes, including infertility.⁹²

Nitric oxide, produced by nitric oxide synthase 3 (NOS3), is considered to be an important mediator of oxidative stress in testicular tissue.⁹³ Studies have shown that NOS is involved in sperm motility, capacitation, and acrosome reaction.⁹⁴ Nitric oxide, as one of the most potent free radicals of nitrogen, reacts rapidly with superoxide (O_2^-) to form highly toxic peroxynitrite ($ONOO^-$). Both superoxide and peroxynitrite have the ability to damage DNA directly. Excessive concentrations of nitric oxide in the semen of asthenozoospermic patients have overall negative effects on the kinetic characteristics of spermatozoa and, consequently, reduce sperm motility and sperm DNA

integrity.⁹⁵ Nitric oxide concentration has been found to be significantly higher in the seminal plasma of some infertile males than in that of healthy males.⁹⁶ Associations between *NOS3* gene polymorphisms and male infertility have been reported.⁹⁷ Multivariate logistic regression analyses revealed that carriers of the *NOS3* rs1799983 variant among GT heterozygotes were associated with a marginally significant increase in the risk of male infertility.⁹⁸ In the dominant model, combined rs1799983 genotypes (GT/TT) were associated with a significant 34% increase in the risk of male infertility. Buldreghini *et al.*⁹⁹ (2010) showed that the T allele of *NOS3* rs1799983 polymorphism (Table 4) contributed to poor sperm motility. Common genetic variants affecting uptake, distribution, transport, or metabolism of dietary antioxidants have been linked to variation in antioxidant serum levels and response to supplementation.^{100,101} Elucidating the relationship between common genetic variants and antioxidant status may have important health implications through identification of individuals and subgroups that benefit the most from dietary intervention or supplementation with antioxidants.

CONCLUSIONS

This paper provides an overview of the current science linking genetic variants to nutritional or supplemental needs with a focus on direct and indirect factors that influence male infertility. This concept is nutrigenomic which assesses interaction between nutrients and gene expression. It is a preliminary review of the potential impacts of various genetic polymorphisms, associated with efficiency of energy expenditure, antioxidant defense, and energetic metabolism, that influence male fertility. It is conceivable that the effects of some genes on fertility phenotypes may be nutrient sensitive. Indeed, diet composition may modulate gene expression through complex transcriptional mechanisms as well as more downstream processes involving the gene products.

Following the present review, a clinical trial is being set up to study the effect of a personalized lifestyle and nutrition program based on a specific set of genetic variants that we identify in this paper. The study was approved by the French ethics committee and registered in ClinicalTrials.gov with reference number NCT03475199.

With the advent of personalized medicine, identification of polymorphisms related to the reproductive function in men and elucidation of their functional importance remain an important area of research. Indeed, epigenetic modifications play a potential role in spermatogenesis via regulation of molecular pathways to maintain testicular homeostasis. The best-known epigenetic process is DNA methylation. A recent genome-wide study has shown that aberrant DNA methylation is imprinted, and developmental genes may have a role in male infertility.¹⁰² In addition to genetic factors, environmental genotoxins, endocrine disruptors, and micronutrient deficiency play an important role in the increasing rates of human infertility. These factors may have deleterious effects on human reproductive health through numerous mechanisms and may also explain some cases of idiopathic infertility in men, especially when the male factor in infertility problems of couples has been neglected for a long time. Emerging observations support the conclusion that parental influences begin before conception and compel us to further explore preconception pathways by which parents contribute more than genetic material to offspring.¹⁰³ There is now clear evidence that beyond genetic alterations alone, there is an epigenetic transmission from the father to his offspring. In fact, paternal smoking, age, and occupational chemical exposure are well known to be linked to increased risk of cancer and neurological disorders in children. It is less appreciated that the father's body mass has a greater

Table 4: Summary of genetic variants involved in redox balance, and their influence on male fertility

Gene symbol	Gene name	SNPs	Gene consequence	Phenotype impact	Study	Protein function	Dietary recommendation based on
<i>SOD2</i>	Superoxide dismutase 2	rs4880	Missense variant	Idiopathic male infertility Low pregnancy rates in IVF Sperm concentration Sperm motility DNA fragmentation	114	Plays an important role in protecting spermatozoa from oxidative damage	Genetic profile and sensitivity to antioxidative stress system matched to antioxidant supplementation, including genetic risks for oxidative stress.
<i>CAT</i>	Catalase	rs1001179	Regulatory region variant	Lower susceptibility to male infertility Idiopathic male infertility Higher distribution of fat	115	Plays a defensive role against oxidative stress. Detoxifies both intracellular and extracellular H ₂ O ₂ to water and oxygen	
<i>PON1</i>	Paraoxonase 1	rs662	Missense variant	Coronary artery disease Decreased sperm motility Decreased semen quality Decrease in antioxidant PON1 activity Increase in lipid peroxidation Higher oxidative stress levels	116	Removes harmful oxidized lipids Protects against the development of atherosclerosis	
<i>GPX1</i>	Glutathione peroxidase 1	rs1050450	Regulatory region variant	Increased risk of damage caused by oxidative stress Risk of male infertility	117	Catalyzes the reduction of organic hydroperoxides and H ₂ O ₂ by glutathione, thereby protecting cells against oxidative damage	
<i>GSTT1</i>	Glutathione S-transferase theta 1	Null alleles	Null deletion	Decreased detoxification Susceptibility factors of male infertility Susceptibility to spermatogenesis	118	Catalyzes the conjugation of glutathione to a wide range of potential toxins as the first step in detoxification	
<i>GSTM1</i>	Glutathione S-transferase mu 1	Null alleles	Null deletion	Idiopathic male infertility Oligozoospermia Varicocele	119	Catalyzes the reaction of glutathione, a reaction that is sometimes a first step in a detoxification process leading to mercapturic acid formation	
<i>GSTP1</i>	Glutathione S-transferase pi 1	rs1695	Missense variant	Development of male factor infertility High oxidative stress	92	Neutralizes xenobiotic reactive oxygen species on other molecules	
<i>NOS3</i>	Nitric oxide synthase 3	rs1799983	Missense variant	Increased risk of male infertility Higher levels of sperm DNA fragmentation Oxidative stress Sperm DNA damage Poor sperm motility	93	Plays a key role in regulation of nitric oxide	

SNPs: single-nucleotide polymorphisms; IVF, *in vitro* fertilization; H₂O₂: hydrogen peroxide; PON1: paraoxonase 1

impact than the mother's on prepubertal child's body fat and metabolic measures. In addition to sperm DNA damage, in some instances, there is accumulating evidence for pathways of parental transgenerational epigenetic effects, attributable to sperm and seminal fluid, that transmit the effects of environmental exposure to the next generation.

Ultimately, once pathways are defined and prioritized according to their importance for health outcomes, it will be possible to define how prospective parents can alter their lifestyles and food choices and adopt interventions to protect children from adverse outcomes. However, further research is required to fully clarify the potential effect of specific SNPs involved in metabolism on male infertility.

AUTHOR CONTRIBUTIONS

SM reviewed the literature, collected data, and wrote the manuscript. CD, YE, EL, and RL collaborated in writing, revising, and editing the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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