The dual nature of micronutrients on fertility: too much of a good thing?

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Objective: To study the effects of generally considered safe doses of antioxidant micronutrient supplementation on semen parameters, systemic redox balance, sperm DNA structural integrity, and fertility.
Design: Given ethical limitations in human studies, this dose escalation study examined the effects of common water-soluble antioxidant micronutrients (vitamin C, zinc, folate, and carnitine) on semen parameters, redox status, DNA integrity, and fertility outcomes in healthy male mice over one spermatogenic cycle. The study was partially repeated at the highest carnitine dose for pregnancy outcomes and comparatively assessed in subfertile, oxidatively stressed mice.
Subjects: "Fertile/healthy" (CD1) and "Subfertile/oxidatively stressed" (*gpx5*$^{-/-}$) mice.
Exposure: Water-soluble micronutrients (vitamin C, zinc, folate, and carnitine).
Intervention: N/A
Main Outcome Measures: Sperm parameters included count, motility, viability, and acrosome integrity. Systemic redox status was evaluated in blood, measuring malondialdehyde, thiol levels, and total antioxidant capacity. Sperm DNA parameters were examined for oxidation (8-OHdG staining), fragmentation (TUNEL), and decondensation (toluidine blue). Pregnancy outcomes were also assessed in CD1 mice fed carnitine.
Results: In healthy mice, increasing doses of individual micronutrients had minimal effects on semen parameters. However, high doses of all four micronutrients significantly disrupted the redox balance in blood plasma and compromised sperm DNA integrity in an ingredient-specific manner. Moderate to high doses of carnitine caused severe DNA fragmentation, a finding confirmed in a subsequent experiment using the highest carnitine dose. In this follow-up experiment, male mice supplemented with carnitine and mated with females showed decreased pregnancy rates and fewer total pups born. Conversely, in oxidatively stressed mice, high-dose carnitine had the opposite, beneficial effect of improving sperm DNA integrity.
Conclusions: At high doses, antioxidants can induce reductive stress, damaging vital molecular components of sperm cells such as DNA. Although strong evidence supports the use of preconception antioxidants to boost semen quality, healthcare professionals should assess oxidative stress levels when possible and recommend personalized antioxidant doses to avoid reductive stress and prevent adverse reproductive outcomes. (F S Sci® 2025;■:■–■. ©2025 by American Society for Reproductive Medicine.)

Key Words: Oxidative stress, reductive stress, antioxidant micronutrient supplementation, sperm DNA integrity, male infertility

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F&S SCIENCE CLINICAL QUICK TAKE

What clinical problem is addressed by these studies?

- An increasing number of male partners seeking assisted conception are taking preconception supplements to boost fertility; however, evidence supporting their safety and benefits remains limited.
- Among the hundreds of products on the market, many contain high-dose antioxidant micronutrients, which may disrupt redox balance and potentially do more harm than good.

What are the key findings?

- In healthy mice, high-dose antioxidants, particularly carnitine, disrupted redox homeostasis, induced reductive stress, damaged sperm DNA, and lowered fertility.
- Conversely, in oxidatively stressed mice, high-dose carnitine protected sperm DNA integrity.

How do these findings apply to human fertility or the reproductive process?

- The findings in healthy mice suggest that indiscriminate use of preconception supplements with inappropriate doses of micronutrients may harm sperm DNA and fertility.
- Although micronutrients can boost fertility, when possible, healthcare professionals should consider assessment of oxidative stress levels and recommend appropriate doses to avoid oversupplementation and reductive stress.

Human reproductive health is experiencing a significant and alarming decline, presenting urgent challenges that demand immediate action from both medical and political sectors to avert serious long-term repercussions. Subfertility and infertility now affect approximately one in 4 couples of reproductive age (1), with estimates suggesting 10%–25% of women experience miscarriage (2–4). Concurrently, male reproductive health is deteriorating, with men reportedly losing an average of 700,000 sperm cells per year (5) and a steep decline in motile sperm (6).

The causes of fertility decline are multifactorial and complex, rooted in a combination of modern lifestyle habits, environmental exposures, and poor nutrition (7, 8). Among these, sedentary lifestyle, heat exposure, synthetic chemicals (e.g., pollutants, plasticizers, biocides, pharmaceuticals, and food additives), and radiation from cell phones and laptops appear to be the main culprits (9). Although the specific molecular mechanisms by which these factors impair human fertility remain largely unknown (10), oxidative stress is a consistently observed feature in the pathophysiology of reproductive decline (11).

Oxidative stress is characterized by a surplus of Reactive Metabolic Species (RMS), primarily reactive oxygen and nitrogen species, which can cause extensive damage to molecular structures and disrupt cellular function (12, 13). The situation is exacerbated by the poor nutritional quality of food, because modern agricultural practices and processing methods often result in foods that are dilute in essential antioxidant micronutrients (14–17).

Given the multifactorial etiology of oxidative stress, the resulting pool of cellular RMS is likely to be highly heterogeneous (18). Therefore, it stands to reason to bolster antioxidant defenses using a combination of evidence-based micronutrients formulated according to the principles of medicinal chemistry and formulation science (19, 20). The rationale for such formulations, along with supporting preclinical and/or clinical evidence, should also be published. Unfortunately, most supplement manufacturers do not adhere to these strict principles, resulting in a market flooded with arbitrary preconceptual and prenatal supplements (21, 22). These products often vary widely in composition and frequently contain high doses of antioxidants, with little consideration for the potential risks and safety.

Although regulatory authorities have established maximum recommended doses for many nutrients, some, such as carnitines, still lack defined limits. Moreover, existing safety guidelines are primarily based on short-term side effects and drug interactions rather than on long-term use (23, 24). The prevailing medical consensus often considers water-soluble antioxidants to be "harmless," assuming that any excess is safely excreted by the body. However, these guidelines fail to account for the potential of antioxidants to disrupt cellular redox homeostasis, leading to a state of "reductive stress" (25–27).

The dangers of oversupplementation and the resulting reductive stress impacting male fertility were first proposed over a decade ago (19). Despite this and continued warnings (28–32), the status quo has not only persisted but, in fact, intensified, as evidenced by a significant surge in the number of fertility supplements now available on the market (21, 22). Many of these so-called "fertility supplements" likely lead to excessive dosing, potentially inducing a state of reductive stress and, in effect, compromising fertility rather than mitigating oxidative stress and enhancing the chances of a successful pregnancy. To provide evidence for our hypothesis, we conducted a dose escalation study with four common water-soluble micronutrients (vitamin C, zinc, folate, and carnitine) on semen quality, systemic redox balance, and sperm DNA integrity in healthy mice. Additionally, we investigated fertility outcomes with carnitine and its potential beneficial effects in oxidatively stressed mice.

MATERIALS AND METHODS

Animal models

This study was approved by the Regional Ethics Committee for Animal Experimentation (CEMEA-Auvergne, APAFIS #33605-2021101917156568-v4) in compliance with current French legislation on animal experimentation. All procedures adhered to the Guide for Care and Use of Laboratory Animals, as adopted by the Society for the Study of Reproduction, and

the guidelines of the American Veterinary Medical Association. The *gpx5*$^{-/-}$ mice were derived from the C57BL/6 genetic line as previously described (33), and CD1 mice were provided by Charles River (Ecully, France). Mice were received and acclimated to the facility before being randomly allocated to cages for treatment. All cages were cleaned and refreshed daily by facility technicians to maintain optimal living conditions and kept under a controlled 12-hour light/dark cycle. Dose escalation treatments for each ingredient were administered simultaneously to avoid potential confounding variables. After the exposure period, all mice were anesthetized using isoflurane, and blood plasma samples were collected via cardiac puncture before euthanasia through cervical dislocation for tissue collection. Pregnant female mice were euthanized on day 15 of gestation using CO^2 asphyxiation (Minerve, Esternay, France), following established protocols.

Oversupplementation model

Vitamin C (calcium ascorbate), folate (calcium L-5-methyltetrahydrofolate), zinc (zinc gluconate), and L-carnitine (Blend #1: acetyl-L-carnitine and propionyl-L-carnitine) were dissolved in water and administered to adult male CD-1 mice (8–12 weeks old) in place of their regular drinking water supply for 4 weeks. Each experiment included 3 treatment groups (n = 6) with escalating doses, compared with an unsupplemented, water-only control group (n = 24). The treated/untreated water supplies were refreshed every 3 days over the duration of each experiment. The (n = 6) group sizes are widely recognized to be minimum necessary to balance statistical rigor, biological variability, and ethical responsibility in research involving mice.

Our dose range selection was initially guided by a survey of popular, commercially available male fertility supplements, with doses spanning the lowest to highest amounts of ingredients found in these products. These observations were combined with the United States Food and Drug Administration (US FDA) guidelines, where available, to establish the dose ranges tested in our model. The US FDA Recommended Dietary Allowance values for adults are 90 mg/d for vitamin C, 11 mg/d for zinc, and 400 mcg dietary folate equivalents/d for folate (23, 24). No RDA exists for carnitines. The designated "low, moderate, and high" doses for each ingredient were as follows: vitamin C (30 mg, 300 mg, and 3,000 mg), zinc (5 mg, 15 mg, and 45 mg), folate (0.18 mg, 0.36 mg, and 1.2 mg), and carnitines (150 mg, 750 mg, and 3,500 mg), with appropriate control groups (0 mg for all). These doses were converted to animal equivalents using standard pharmaceutical methodologies (34).

High-dose carnitine exposures were repeated with a new composition (Blend #2: L-carnitine, acetyl-L-carnitine, and propionyl-L-carnitine) to closely resemble the reported top-selling male fertility supplement (35). This was tested in CD-1 (n = 8) and *gpx5*$^{-/-}$ male mice. After the 4-week period, CD-1 males were also mated with two virgin females each, and pregnancy rate, fetal number, and resorption rate were evaluated. Fertility outcomes were not assessed in *gpx5*$^{-/-}$ mice because of previously reported minor differences in pregnancy rates from controls (33).

Sperm preparation

Epididymides were excised and cleaned of surrounding connective tissues and fat. The caput and cauda regions were separated and transferred to a glass dish containing 500 μL of M2 medium (Sigma-Merck, Saint-Quentin-Fallavier, France). Spermatozoa were recovered by gently squeezing the epididymides with forceps, followed by perforation with a 26G needle. Samples were incubated for 10 minutes at 37°C to allow sperm dispersion. After incubation, sperm samples were washed with 500 μL of M2 medium, resulting in a final volume of 1 mL.

General sperm parameter assessments

Sperm count was determined using a Malassez hemocytometer. Motility, viability, and acrosome integrity were measured using the Sperm Class Analyzer (SCA, Microptic, Barcelona, Spain). Sperm motility was assessed by diluting sperm in M2 medium and analyzing at least 500 spermatozoa per mouse on a 20 μm deep slide (IMV, L'Aigle, France). Viability was assessed using the FluoVit kit (Microptic, Barcelona, Spain), which differentiates viable (blue) from dead (red) spermatozoa, analyzing at least 300 spermatozoa per smear. Acrosome integrity was measured using the FluoAcro kit (Microptic, Barcelona, Spain) according to the manufacturer's guidelines, with at least 500 spermatozoa per smear.

Blood plasma redox assessments

Blood samples were collected via cardiac puncture after anesthesia, and plasma was cryopreserved at −20°C. Electrochemical plasma static oxidation/reduction potential (sORP) was measured using the e-BQC lab device (Bioquochem, Llanera, Spain) in 40 μL aliquots and expressed as micro-Coulomb (μC) charge units. Thiol content, an important redox marker, was quantified using the BQC Thiol Quantification Assay Kit (Bioquochem, Llanera, Spain). Results were derived from 20 μL aliquots, with a standard curve adjusted for blank-corrected absorbance values and reported as millimolar (mM) units. Malondialdehyde (MDA) content, a widely recognized lipid peroxidation biomarker, was measured on 100 μL aliquots using the BQC MDA-TBARS Assay Kit (Bioquochem, Llanera, Spain) and reported in micromolar (μM) units.

Sperm nuclear integrity assessments

To assess sperm nuclear oxidation, we utilized a previously reported technique (36) to quantify 8-hydroxy-2-deoxyguanosine (8-oxodG) residues using immunofluorescence with the antibody clone 15A3, diluted 1:100 (ab183393, Abcam). Sperm DNA was counterstained with Hoechst 33342 (0.001 mg/mL) for 5 minutes, spread on a glass slide, and mounted with Mowiol (Euromedex, Souffelweyersheim, France). A minimum of 300 spermatozoa per smear

were counted using the SCA platform (Microptic, Barcelona, Spain).

Sperm DNA fragmentation was assessed using the TUNEL assay, following a modified protocol with the "in situ cell death detection" kit (Roche Molecular Biochemicals, Mannheim, Germany). The spermatozoa were counterstained with Hoechst 33342 (0.001 mg/mL). Positive controls were generated by incubating spermatozoa with 0.02% H_2O_2 for 1 hour at room temperature in the dark. The percentage of spermatozoa with fragmented DNA was determined by flow cytometry using the ATTUNE NxT machine (Life Technologies, CA).

Sperm nuclear condensation was assessed by toluidine blue (TB) staining. Spermatozoa (100,000 per sample) were smeared on a glass slide, stained with 1% TB in McIlvain buffer (pH 3.5) for 17 minutes at room temperature, dehydrated in ethanol, and mounted with Cytoseal 60 medium. At least 300 spermatozoa per smear were counted using the SCA platform (Microptic, Barcelona, Spain).

"In-silico" commercial dietary supplement analyses

In the discussion, we provide an additional in silico analyses of supplement fact labels from commercially available products accessed from the US Office of Dietary Supplements database or previous literature. The data was downloaded in Excel format, sorted, and analyzed to extract relevant nutritional information for comparison and discussion.

Statistics

Outliers were identified using ROUT analysis (GraphPad Prism 9.5.1). The small sample sizes within treatment groups limited the reliability of typical Shapiro-Wilk tests for normality and ANOVA, because the low power could result in misleading *P* values that may reflect insufficient sample size rather than true distributional characteristics. Instead, Kruskal-Wallis tests were employed to identify overall significance among groups. For specific pairwise comparisons, Mann-Whitney *U* tests were subsequently performed, with statistical significance set at $P \leq .05$.

RESULTS

These in vivo results are presented in a logical sequence, starting with traditional clinical observations, such as sperm characteristics (e.g., motility and concentration), followed by oxidative stress biomarkers and, finally, the most clinically significant outcome, damage to sperm DNA integrity and pregnancy outcomes, to highlight the potential risks of these findings when translated to human health.

Antioxidants do not significantly alter sperm characteristics

Classical clinical parameters, including sperm concentration, motility, and acrosome integrity, showed minimal changes across the treatment groups. Notably, the highest dose of carnitine Blend #1 led to an increase in sperm concentration; however, this effect was not observed in follow-up experiments. Folate supplementation significantly reduced sperm viability at moderate to high doses, whereas zinc negatively affected acrosome integrity. Histologic analysis of the testis and epididymis revealed no structural changes, even at the highest doses. Because data related to these findings were not consistently significant across the studied ingredients, they have not been included in the main figures but are available in the supplementary materials for readers who wish to explore them further.

Antioxidants alter the blood redox state

We assessed three critical systemic redox markers in blood samples: MDA, total thiol expression, and sORP. The detrimental impact of high levels of antioxidant supplementation was clearly revealed by the MDA data, which indicated highly significant, dose-dependent increases in lipid aldehyde generation (Fig. 1A, $P \leq .0001$) with vitamin C, zinc, and carnitine. Interestingly, folate caused a sharp initial rise in MDA levels at the lowest dose, but this effect reversed at higher doses in keeping with a dramatic increase in the levels of thiol expression (Fig. 1B, $P \leq .001$). In contrast, with vitamin C, zinc, and carnitine, the levels of thiol expression significantly declined at higher doses in concert with the increase in MDA formation (Fig. 1B, $P < .05$). Overall, there were no consistent changes in sORP with the different antioxidants (Fig. 1C), so the changes observed in lipid peroxidation and thiol status were not simply a function of electron availability in circulation.

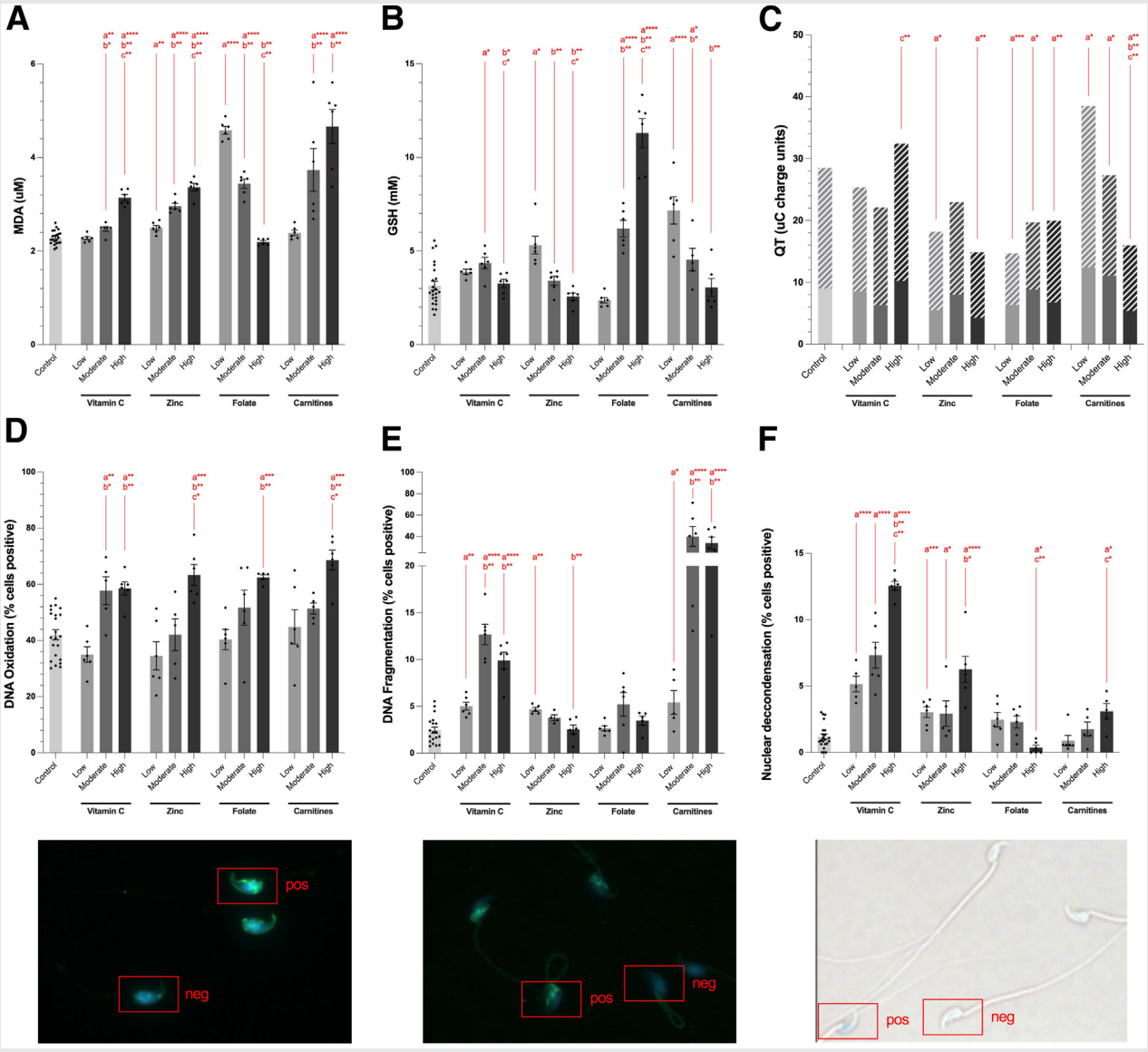
High-dose antioxidants compromise sperm DNA structural integrity

We evaluated key parameters related to sperm DNA structural integrity: oxidation, fragmentation, and nuclear condensation. At the highest doses, all antioxidants exhibited prooxidant behavior, leading to a significant increase in DNA oxidation (Fig. 1D, $P \leq .01$). Only vitamin C and carnitines induced a substantial rise in DNA fragmentation at moderate and high doses (Fig. 1E, $P \leq .0001$). Sperm nuclear decondensation increased significantly in a dose-dependent manner with vitamin C, whereas the effects of zinc and carnitine were less pronounced (Fig. 1F, $P \leq .05$). Interestingly, folate demonstrated an opposing effect, reducing nuclear decondensation at the highest dose (Fig. 1F, $P \leq .05$).

A focus on carnitine redox profile

The unexpected and significant increase in DNA oxidation and fragmentation observed in the initial experiments with carnitine supplementation prompted further studies using an alternative carnitine composition (Blend #2), but only the highest dose was examined. These follow-up experiments largely confirmed the initial findings, showing similar alterations to redox state and DNA structural integrity (Fig. 2A–E, $P \leq .001$). Notably, sperm mitochondrial potential, as assessed by JC-1 flow cytometry, demonstrated a significant increase, providing a potential mechanistic explanation for the observed damaging effects (Supplemental Fig. 2, available online; $P \leq .01$). Additionally, fertility outcomes for carnitine-supplemented males

FIGURE 1



Redox status and DNA integrity biomarkers in healthy mice. The effect of different antioxidants at different doses on biomarkers of oxidative stress in the blood plasma. The data represent mean ± s.e.m. with "a," "b," and "c" denoting statistical difference from the control, low, moderate, and high doses, respectively, and $^{a}P \leq .05$, $^{b}P \leq .01$, $^{c}P \leq .001$, $^{d}P \leq .0001$. Exposition groups (n = 6 mice per treatment, except for Zinc-Moderate, which was n = 5) were assessed against pooled controls (n = 24). The biomarkers assessed include (A) Malondialdehyde, (B) Thiol Quantification, and (C) Electrochemical plasma static oxidation/reduction potential (sORP). Electrochemical sORP measured redox potentials (Q1:lighter shade, Q2:darker shade of stacked column) and expressed the final measurement (QT: full stacked column) as micro-Coulomb charge units. The sperm DNA integrity biomarkers evaluated include (D) sperm DNA oxidation (% of cells positive for 8OHdG) with representative fluorescent microscopy image below, (E) sperm DNA fragmentation (% of cells positive for TUNEL) with representative fluorescent microscopy image below, and (F) sperm nuclear decondensation (% of cells stained via toulidine blue) with representative microscopic image below.

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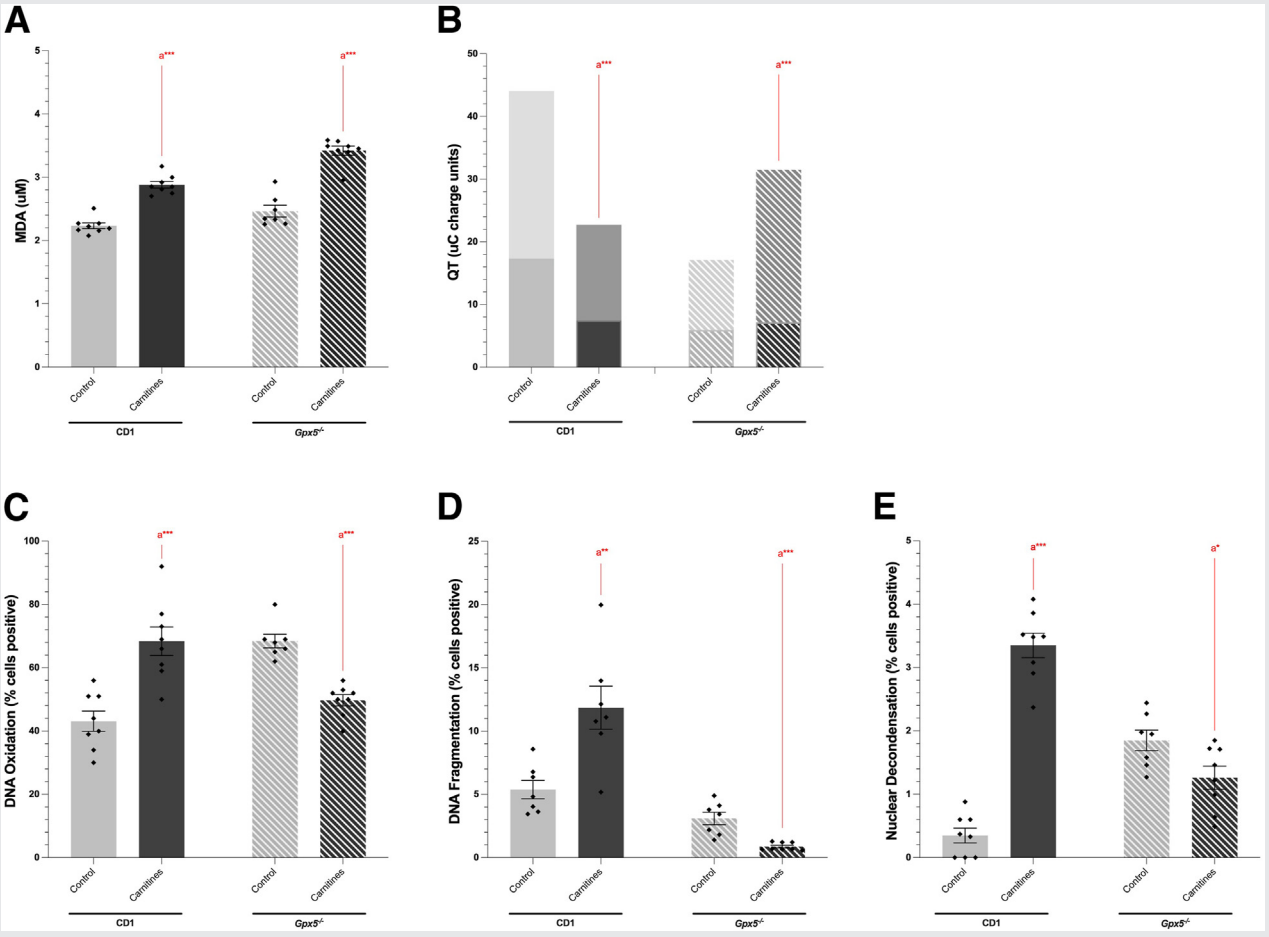
revealed lower mating and pregnancy success rates, although the number of fetuses per pregnancy and the rate of resorptions remained unaffected (Table 1, $P = .07$).

In *gpx5*$^{-/-}$ mice, where oxidative stress is localized to the epididymis, classical sperm parameters remained unaffected (Supplemental Fig. 3, available online). Carnitine supplementation in these mice elevated MDA levels, consistent with findings in CD1 mice but also increased sORP levels (Fig. 2A and B: $P \leq .001$). Interestingly, carnitine supplementation had contrasting effects on DNA integrity in the context of oxidative stress: *gpx5*$^{-/-}$ males showed significant reductions in DNA oxidation, fragmentation, and nuclear decondensation, highlighting a potential protective effect under these conditions (Fig. 2C–E: $P \leq .05$).

FIGURE 2



Redox status and DNA integrity biomarkers in healthy and stressed mice. The effect of high-dose carnitine supplementation on biomarkers of oxidative stress in the blood plasma in healthy (CD1, n = 8) and oxidatively stressed (*gpx5*$^{-/-}$, n = 8) mice. The data represent mean ± s.e.m with 'a' denoting statistical difference from the control dose and $^{a}P \leq .05$, $^{b}P \leq .01$, $^{c}P \leq .001$, $^{d}P \leq .0001$. The biomarkers assessed include (A) Malondialdehyde and (B) Electrochemical plasma static oxidation/reduction potential (sORP). Electrochemical sORP measured redox potentials (Q1:lighter shade, Q2:darker shade of stacked column) and expressed the final measurement (QT: full stacked column) as micro-Coulomb charge units. The sperm DNA integrity biomarkers evaluated include (C) sperm DNA oxidation (% of cells positive for 8OHdG), (D) sperm DNA fragmentation (% of cells positive for TUNEL), and (**e**) sperm nuclear decondensation (% of cells stained via toluidine blue).

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Figure 3 presents a commercial analysis of commonly available dietary supplements, examining the inclusion and doses of ingredients tested in our animal models to assess their relevance to human health. These findings are elaborated on in detail in the discussion section.

DISCUSSION

This preliminary study in mice highlights the complex, dual nature of antioxidants on male reproductive health when administered without prior assessment of redox status. Unlike many synthetic drugs, most natural antioxidant nutrients exhibit similar pharmacologic and biochemical effects across mammalian species, including humans, making animal studies with natural dietary ingredients highly relevant to human health (37).

Although micronutrients such as vitamin C, zinc, folate, and carnitine are widely regarded as safe and beneficial for semen quality, our findings reveal potential risks associated with the high doses of these ingredients commonly found in commercial products. These results support the hypothesis that excessive antioxidant supplementation can disrupt redox homeostasis, inducing reductive stress and leading to adverse reproductive outcomes. However, a potential consideration of this study may be the lack of direct measurement of total daily water intake in mice (typically 2.5–3mL), which may have

TABLE 1

Fertility outcomes in healthy mice.

	Treatment	
	Control	High-dose carnitine
Successful matings, n (%)	7/8 (87.50)	4/8 (50.00)
Pregnant females, n (%)	10/16 (62.50)	5/16 (31.25)[a]
Average fetal # per pregnancy	14.5	14.4
Total fetal number	140	72
Fetal resorptions	0	0

Note: The effect of high-dose carnitine supplementation in healthy CD1 males (n = 8) on fertility outcomes. After the 4-week supplementation period, each male mouse was mated with two (n = 2) virgin, control CD1 female mice.
[a] $P = .07$ via the X^2 test trended toward significance but was not deemed statistically significant.

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introduced slight variability in the doses consumed. Additionally, although no formal power analysis was conducted and the sample sizes were relatively small, strong statistical significance was observed for most measured parameters.

Redox balance and sperm DNA integrity

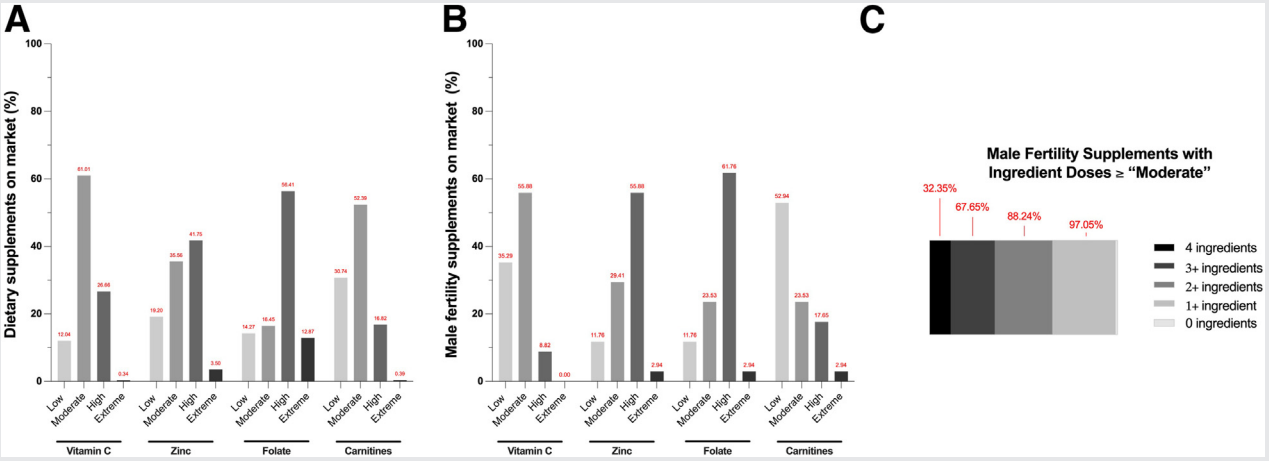
The most striking outcome of our study is the disruption of homeostatic redox state and the corresponding detrimental effects on sperm DNA integrity when antioxidants are given to healthy mice. Both vitamin C and carnitine supplementation exhibited severe damaging effects on sperm DNA integrity. Moderate and high doses induced a significant rise in DNA oxidation, fragmentation, and decondensation, corroborating the notion that at supraphysiological levels, antioxidants can paradoxically act as pro-oxidants or even behave as reducing agents. The pro-oxidant or reducing activity of vitamin C has been previously reported and is likely because of its involvement in the Fenton reaction and redox cycling with metal ions (38). However, the pro-oxidant activity of carnitine has not been reported and may be indirect, likely resulting from increased mitochondrial activity that boosts reactive oxygen species production (39, 40), leaking into the sperm nucleus, and subsequently increasing DNA oxidation and fragmentation.

Therefore, supplementation in nonstressed individuals can result in reduced levels of RMS below physiological norms, leading to a state of reductive stress. Reactive Metabolic Species are not mere metabolic by-products; they are essential signaling molecules crucial for a variety of cellular functions and for maintaining redox homeostasis (13, 41). Reduced levels of RMS can disrupt these vital processes, leading to cellular dysfunction and reproductive pathology. Moreover, the results underscore that antioxidants administered at higher concentrations can turn into pro-oxidants or even reductants depending on the environment (25, 26).

Our findings with vitamin C, and particularly carnitine, are alarming given the widespread use of carnitine doses in excess of 1g contained in many fertility supplements, including in a reported top-selling supplement among men seeking to improve fertility (35). It is also important to note that young, competent oocytes can still repair extensive DNA damage in sperm (42); however, this increases the risk of de novo mutations, potentially predisposing future generations to genetic disorders and disease susceptibility (43).

Interestingly, folate presented a slightly different profile, increasing glutathione (GSH) levels, potentially through the upregulation of glutamate-cysteine ligase (Gclc), an enzyme

FIGURE 3



Commercial supplement analysis for new safety considerations. (A) General dietary supplements analyzed for vitamin C, zinc, folate, or carnitines content and characterized by dose *per* serving into "Low," "Moderate," "High," or "Extreme" ranges as defined by the respective amounts tested in our models. The red number above each bar is the percentage of products that fall within each dosage range *per* ingredient. (B) Male fertility products, specifically, are analyzed in the same manner. (C) Male fertility products were used for the total number of ingredients included in their formulations, which was equal to or greater than the respective "moderate" dose range as defined in our study.

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critical for GSH synthesis (44). It also reduced nuclear decondensation in a dose-dependent manner, likely because of its involvement in the one-carbon cycle, crucial for DNA methylation and nucleotide synthesis (45). However, excessive folate accumulation in mitochondria could lead to dysfunction and elevated reactive oxygen species production (46).

Differential effects in healthy vs. oxidatively stressed mice

In contrast, the effects of antioxidant supplementation in oxidatively stressed *gpx5*$^{-/-}$ mice were more straightforward. A similar composition of carnitine that exacerbated DNA damage in healthy mice appeared to ameliorate DNA damage in *gpx5*$^{-/-}$ mice. This finding supported previous studies suggesting that the redox environment plays a critical role in determining the impact of antioxidant supplementation (20). Under oxidative stress conditions, antioxidants may indeed serve their intended protective function, whereas, in a redox-balanced state, they may tip the scales toward reductive stress with deleterious consequences.

Widespread risk of oversupplementation

Our findings in mice should be highly relevant to human fertility as most natural antioxidants share similar biochemical profiles across mammalian species. The results of this study should raise significant concerns among reproductive health experts, especially considering an estimated 40% of men of reproductive age take general supplements (47), a figure that rises to nearly 70% among men undergoing IVF treatment (48). The US National Institutes of Health Office of Dietary Supplements has registered a staggering 102,530 products for general adult use (49), including 16,805 products with vitamin C, 11,902 with zinc, 10,661 with folate or folic acid, and 2,127 containing carnitines. Alarmingly, $\geq$70% of these products exceed the lowest daily doses recommended by our study (Fig. 3A). These statistics highlight the widespread availability of micronutrients at doses that may pose significant risks to male fertility.

A focus on male fertility products

A review by de Ligny et al. (22) reported that 79% of 34 commercially available male fertility products contained ingredients exceeding the recommended dietary allowances. Our reanalysis of these data revealed even more concerning trends: 97.06% of these products had at least one ingredient above the lowest dose range we tested (Fig. 3B). Furthermore, 88.24% contained at least two ingredients above this range, 67.65% had three or more, and 32.35% included elevated doses of all 4 micronutrients examined in this study (Fig. 3C). These findings underscore a troubling irony: supplements heavily marketed to enhance reproductive health, often without robust clinical evidence (50–52), are more likely to exacerbate fertility issues by overdosing patients with certain ingredients.

Clinical implications

These preliminary invivo findings carry substantial implications for the use of antioxidant supplements in clinical settings. Antioxidants are frequently used to enhance male fertility (35), yet our study highlights the critical importance of considering an individual's redox status before supplementation. Supplementation, especially in individuals without oxidative stress, may not only be ineffective but could also be detrimental, potentially reducing sperm quality and compromising overall fertility, as evidenced here and by previous studies (53–58).

Several clinical trials attempting to improve reproductive outcomes have failed (59, 60), in part because they did not establish oxidative stress as an entry criterion for the enrolled subjects. Without assessing oxidative stress biomarkers, these trials likely included patients who did not require antioxidant intervention, which may have skewed the results and masked potential benefits for those who truly needed supplementation (61). The differential responses between healthy and oxidatively stressed mice observed in our study emphasize the necessity of adopting personalized supplementation strategies rather than a one-size-fits-all approach.

Clinicians should evaluate oxidative stress levels either through validated methods or by assessing the cumulative impact of contributing factors. These factors may include smoking, excessive alcohol consumption, obesity, long-distance cycling and marathons, chronic drug use, and urologic or medical conditions such as varicocele. This approach ensures that antioxidant supplementation is both safe and effective, optimizing reproductive outcomes while mitigating potential risks. By incorporating oxidative stress assessments into clinical decision-making, clinicians can maximize the therapeutic benefits of antioxidants while reducing the possibility of adverse effects from inappropriate dosing.

CONCLUSION

Although these proof-of-concept safety experiments were conducted in mice, the findings are highly likely to be applicable to humans, given the similarity in redox and biochemical profiles of natural antioxidants across most mammalian species (36). The results with carnitine are particularly alarming: although high doses may boost sperm concentration, as reported in several studies, they also lead to significant DNA oxidation and fragmentation. Although confirmation of the results in men is warranted, ethical and safety concerns make such studies extremely difficult and challenging, given the context of fertility and pregnancy outcomes.

Our market analysis shifts the "antioxidant paradox" from an academic discussion to a pressing real-health concern, especially for men of reproductive age. Our findings indicate that men with mild to no nutrient deficiencies or oxidative stress should avoid high-dose antioxidant supplements to circumvent the risk of reductive stress, which could negatively impact semen quality and fertility. This study serves as an important warning to fertility specialists and emphasizes the risks of indiscriminate use of supplements. It highlights the need for patient education on both the potential benefits and risks of unsubstantiated fertility supplements

and stresses the importance of tailored dosing regimens to prevent oversupplementation. Moreover, regulatory bodies such as the FDA and European Food Safety Authority should reevaluate and establish clear safety guidelines for all dietary ingredients on the market, integrating data from redox biomarkers of reproductive health to ensure safe dosing recommendations.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the author(s) used ChatGPT 4o, Version 1.2024.219, to improve the readability of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT Authorship Contribution Statement

Aron Moazamian: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation. **Elisa Hug:** Project administration, Investigation. **Pauline Villeneuve:** Resources, Investigation. **Stéphanie Bravard:** Resources, Investigation. **Richard Geurtsen:** Validation, Resources. **Jorge Hallak:** Writing – review & editing. **Fabrice Saez:** Supervision, Investigation. **Robert John Aitken:** Writing – review & editing. **Parviz Gharagozloo:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Joël R. Drevet:** Writing – review & editing, Supervision, Methodology.

Declaration of Interests

A.M. is an employee of CellOxess Biotechnology, and this study formed the core of his PhD thesis at the Université Clermont Auvergne. E.H. has nothing to disclose. P.V. has nothing to disclose. S.B. has nothing to disclose. R.G. is an employee of CellOxess Biotechnology. J.H. is an honorary scientific adviser to CellOxess Biotechnology. F.S. has nothing to disclose. R.J.A. is an honorary scientific adviser to CellOxess Biotechnology. P.G. is an employee of CellOxess Biotechnology. J.R.D. is an honorary scientific adviser to CellOxess Biotechnology.

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