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# Protective Effects of Vitamins D and E on Sperm Quality and Quantity in Vodka - Exposed Male Wistar Rats ( *Rattus norvegicus* )

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## ABSTRACT
Infertility is a significant global health problem, with male factors responsible for nearly half of all cases. Alcohol exposure, particularly vodka, generates oxidative stress that disrupts sperm production and function. This study examined the protective effects of vitamin D, vitamin E, and their combination in male Wistar rats exposed to vodka. Twenty five rats were divided into five groups: control, vodka - only, vodka with vitamin D, vodka with vitamin E and vodka with combined vitamins D and E. Rats that received vodka showed a sharp decline in sperm quality, with concentration reduced to about forty two million per milliliter, motility decreased to around ten percent, and normal morphology present in only about one quarter of cells. Supplementation with vitamin D or vitamin E improved these parameters, while the combined treatment produced the greatest recovery, restoring concentration to more than sixty million per milliliter, motility to nearly one half, and normal morphology to almost two thirds. These findings suggest that the combination of vitamins D and E provides stronger protection against alcohol induced reproductive toxicity than either vitamin alone.

***Keywords:*** *Vitamin D, Vitamin E, Oxidative Stress, Alcohol Exposure, Male Fertility, Sperm Quality*

## INTRODUCTION
Infertility contributes substantially to global reproductive health challenges, with an estimated 15 % of couples affected worldwide (Skakkebæk et al., 2022), with male factors contributing to nearly half of all cases (Agarwal et al., 2021; Esteves et al., 2021). Alcohol consumption is a well established factor in male reproductive dysfunction, particularly distilled beverages such as vodka, which induce oxidative stress, impair Leydig cell steroidogenesis, and reduce testosterone levels (Cui et al.,

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2020; Huang et al., 2020; La Vignera et al., 2020). Elevated reactive oxygen species ( ROS ) trigger lipid peroxidation, DNA fragmentation, mitochondrial dysfunction in spermatozoa, and apoptosis of germ cells (Drobnis & Nangia, 2021; Panner Selvam & Agarwal, 2019; Zhu et al., 2021).

Recent studies emphasize the role of antioxidants in mitigating oxidative damage. Vitamin D supports spermatogenesis through steroidogenic regulation and calcium signaling (Dissanayake et al., 2021; Jensen et al., 2020), while vitamin E protects sperm membranes by preventing lipid peroxidation and improves motility and morphology in both clinical and animal models (Alahmar, 2022; Kim et al., 2022). Several studies demonstrate that supplementation with either vitamin D or vitamin E ameliorates oxidative stress in male reproductive tissues (Cannarella et al., 2022, 2023; Sharma et al., 2020). However, limited research has explored the combined role of vitamins D and E in alcohol induced reproductive impairment, creating a distinct knowledge gap.

This study aimed to determine the protective effects of vitamins D and E on sperm concentration, motility, and morphology in vodka exposed male Wistar rats.

## METHODS

This experimental study employed a post-test only control group design and was conducted at the Pharmacology Laboratory, Faculty of Medicine, University of North Sumatera, April - May 2025. Ethical approval was obtained from the Ethics Committee of Universitas Prima Indonesia ( Approval No : 097/KEPK/UNPRI/III/2025 ).

Twenty five male Wistar rats ( 3 - 4 months, 150 - 200 g ) were housed under standard laboratory conditions. Rats were divided into five groups ( n = 5 ) : **K1 ( Negative Control )** distilled water 2 mL/day orally for 10 days**. K2 ( Positive Control )** received vodka at a dose of 2.7 mL/day orally for 10 days. **P1** received vodka for the first five days, followed by vitamin D (0.2 µg/day) for five subsequent days. **P2** received vodka for five days, then vitamin E (1.44 mg/days) for the following five days. **P3** received vodka for five days, followed by a combination of vitamin D and E at the same doses for five days.

On day 11, rats were anesthetized, and cauda epididymis samples were collected. Sperm concentration was measured using a hemocytometer. Motility was evaluated microscopically ( ≥ 200 cells/ sample ), and morphology was assessed on Giemsa stained slides ( ≥ 100 cells/ sample ) (World Health Organization (WHO), 2021).

Statistical analyses were performed using one way analysis of variance ( ANOVA ) followed by the Games-Howell post hoc test. A significance level of $p < 0.05$ was considered statistically meaningful (Sharma et al., 2020).

## RESULTS

Vodka administration markedly reduced sperm quality compared with the control group. Concentration decreased by more than third, motility declined to nearly one quarter of the control value, and normal morphology fell below half. These differences were statistically significant by ANOVA ( $p < 0.05$ ).

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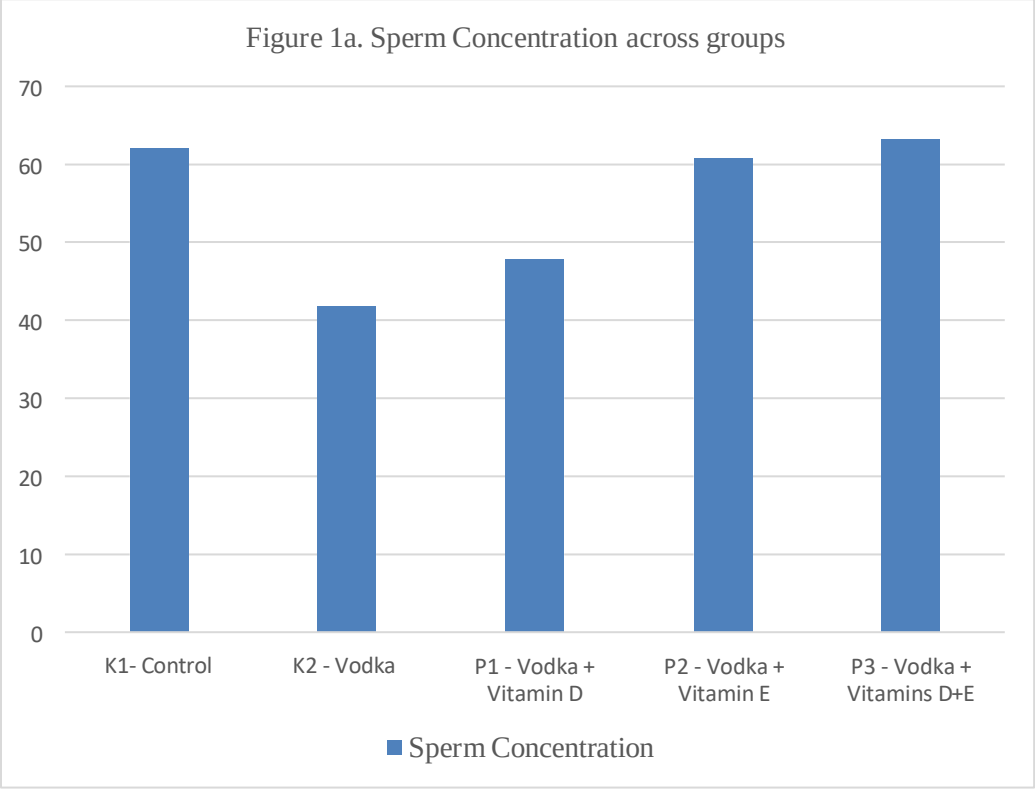
Vitamin D supplementation increased concentration and motility, while vitamin E supplementation enhanced motility and morphology. Post hoc analysis confirmed significant differences between each treatment and the vodka group ( $p < 0.05$ ).

The combined vitamin D and E treatment produced the strongest recovery, restoring concentration close to control values, motility to almost one half of the control, and morphology to more than two thirds. This effect was significantly superior to either vitamin alone (Cannarella et al., 2023; Dai et al., 2023)

**Table 1. Mean Sperm Concentration, Motility, and Morphology Across Groups**

| Group | Consentration ( x $10^6$ mL ) | Motility ( % ) | Normal Morphology ( % ) |
|---|---|---|---|
| K1 ( Negative Control ) | 62.00 ± 11.51 | 44.80 ± 3.96 | 55.20 ± 3.96 |
| K2 ( Vodka Only ) | 41.80 ± 4.92 | 10.00 ± 3.81 | 24.20 ± 5.12 |
| P1 (Vodka + Vitamin D ) | 47.80 ± 1.92 | 40.40 ± 2.07 | 35.00 ± 2.23 |
| P2 (Vodka + Vitamin E ) | 60.80 ± 4.45 | 39.00 ± 4.00 | 57.60 ± 2.70 |
| P3 (Vodka +Vitamins D+E) | 63.16 ± 1.92 | 44.20 ± 1.92 | 63.60 ± 1.14 |



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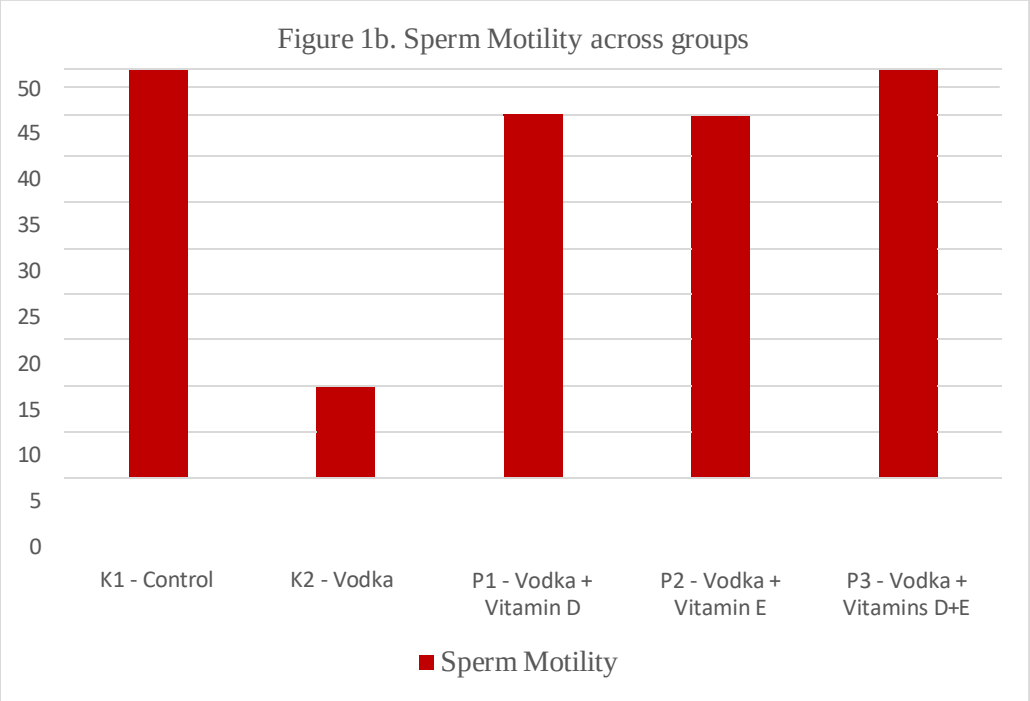


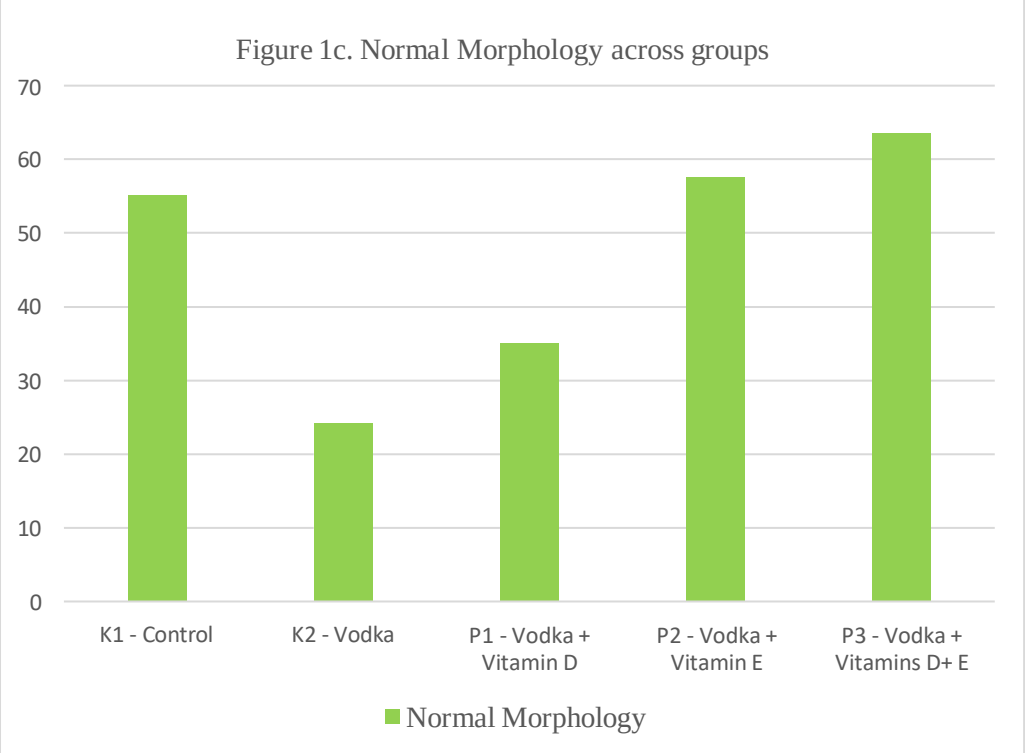


**Figure 1. Mean Sperm Parameters**

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To further confirm group differences, Welch's ANOVA and Games-Howell post hoc analyses were conducted. ANOVA revealed significant differences among groups for all sperm parameters ( $p < 0.05$ ). Post hoc testing indicated that the vodka only group ( K2 ) was significantly different from all other groups. Additionally, the combined vitamin D+E group ( P3 ) showed significantly higher values compared with either vitamin D ( P1 ) or vitamin E ( P2 ) alone ( $p < 0.05$ ).

**Table 2. Results of Welch's ANOVA and Games-Howell Post Hoc Test for Sperm Parameters**

| Parameters | Welch's ANOVA ( p-value ) | Significant Comparisons | Post Hoc ( Games-Howell ) |
|---|---|---|---|
| Concentration | < 0.001 | K2 vs all ; P3 vs P1, P2 | Significant differences confirmed |
| Motility | < 0.001 | K2 vs all ; P3 vs P1, P2 | Significant difference confirmed |
| Normal Morphology | < 0.001 | K2 vs all ; P3 vs P1, P2 | Significant difference confirmed |

## DISCUSSION

This study confirmed that ethanol induced oxidative stress significantly compromises sperm quality, consistent with earlier findings reporting that alcohol exposure leads to impaired spermatogenesis and reduced male fertility (La Vignera et al., 2020; Skakkebæk et al., 2022). These results demonstrated that supplementation with either vitamin D or vitamin E improved sperm parameters, while the most pronounced effect was observed in the combined treatment group, suggesting a synergistic interaction between these antioxidants, consistent with previous evidence that vitamin supplementation supports male fertility (Cannarella et al., 2022).

At the molecular level, ethanol exposure enhances the generation of reactive oxygen species ( ROS ), which induces lipid peroxidation, mitochondrial dysfunction, DNA fragmentation, and ultimately apoptosis in germ cells (Alahmar & Sengupta, 2021; Cui et al., 2020; Kumar & Singh, 2020). These processes destabilize sperm membranes, reduce motility, and impair morphology. Vitamin D contributes to reproductive protection by modulating calcium homeostasis and promoting testosterone synthesis through luteinizing hormone regulation (Dissanayake et al., 2021). In parallel, vitamin E, a potent lipid soluble antioxidant, interrupts ROS driven chain reactions and prevents peroxidative damage to the sperm plasma membrane, thereby preserving motility and morphology (Kim et al., 2022). When administered together, these antioxidants complement each other by restoring mitochondrial function, reducing apoptotic signaling, and improving the structural and functional integrity of spermatozoa (Dai et al.,2023).

Beyond these established mechanisms, additional biomolecular pathways may contribute to the observed protective effects. Vitamin D has been shown to regulate the expression of genes involved in spermatogenesis, such as those controlling calcium channels in the epididymis,

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which facilitate sperm maturation. Furthermore, vitamin E stabilizes mitochondrial membranes and supports ATP production, which is essential for flagellar movement and progressive motility. Together, these compounds also enhance the activity of endogenous antioxidant enzymes, such as superoxide dismutase ( SOD ), catalase, and glutathione peroxidase ( GPx ), thereby strengthening the cellular defense against oxidative insults. By attenuating oxidative stress, both vitamins protect sperm DNA integrity, reduce apoptosis via inhibition of caspase pathways, and maintain the structural integrity of acrosomes, which is crucial for fertilization.

These findings are consistent with prior evidence that antioxidant supplementation mitigates ethanol induced reproductive toxicity. For instance, Cannarella et al., (2023) reported that antioxidant therapy improved sperm motility and morphology in clinical studies, though effects varied by compound. Similarly, Dai et al., (2023) demonstrated that combined antioxidants, such as vitamin C and E, act synergistically to restore sperm mitochondrial function and reduce apoptosis in animal models. In line with these studies, the current results confirm that vitamins D and E together exert greater protection than either alone, supporting the hypothesis of a complementary mechanism. While individual antioxidants target specific pathways, their combination provides broader protection against oxidative stress, membrane damage, and DNA fragmentation. These comparative data strengthen the translational relevance of these findings and highlight combined antioxidant strategies as promising interventions for alcohol related male infertility. Despite the promising outcomes, this study is limited by its relatively small sample size and the use of single dose regimens. Further investigations incorporating oxidative stress biomarkers such as malondialdehyde ( MDA ), SOD, and GPx, together with hormonal profiling and chronic ethanol exposure models, are warranted to provide stronger translational relevance and a deeper mechanistic understanding. In addition to the sperm quality parameters analyzed in the present study, future investigations are encouraged to evaluate biomarkers that directly reflect oxidative stress and antioxidant defense systems. Measurements of malondialdehyde ( MDA ) as an index of lipid peroxidation, together with enzymatic antioxidants such as superoxide dismutase ( SOD ) and glutathione peroxidase ( GPx ), would provide quantitative evidence of the redox balance in testicular tissue following vodka exposure and vitamin supplementation. These data would strengthen the mechanistic interpretation that vitamins D and E act through modulation of oxidative pathways. Moreover, hormonal profiling including serum testosterone, luteinizing hormone ( LH ), and follicle stimulating hormone ( FSH ) should be incorporated in future studies to clarify the endocrine regulation underlying the observed improvements in sperm concentration, motility, and morphology. Correlating these hormonal and biochemical parameters with sperm indices would offer a more integrated understanding of how antioxidant therapy restores reproductive function after vodka induced toxicity.

This study demonstrates that antioxidant therapy targeting ethanol induced oxidative stress can restore sperm quality through multiple biomolecular mechanisms. The combination of vitamin D and E not only reduces ROS mediated lipid peroxidation and DNA fragmentation but also preserves mitochondrial integrity, supports steroidogenesis, and promotes sperm maturation. These findings underline the translational potential of combined antioxidant strategies as adjunctive therapies for male infertility associated with lifestyle related oxidative stress.

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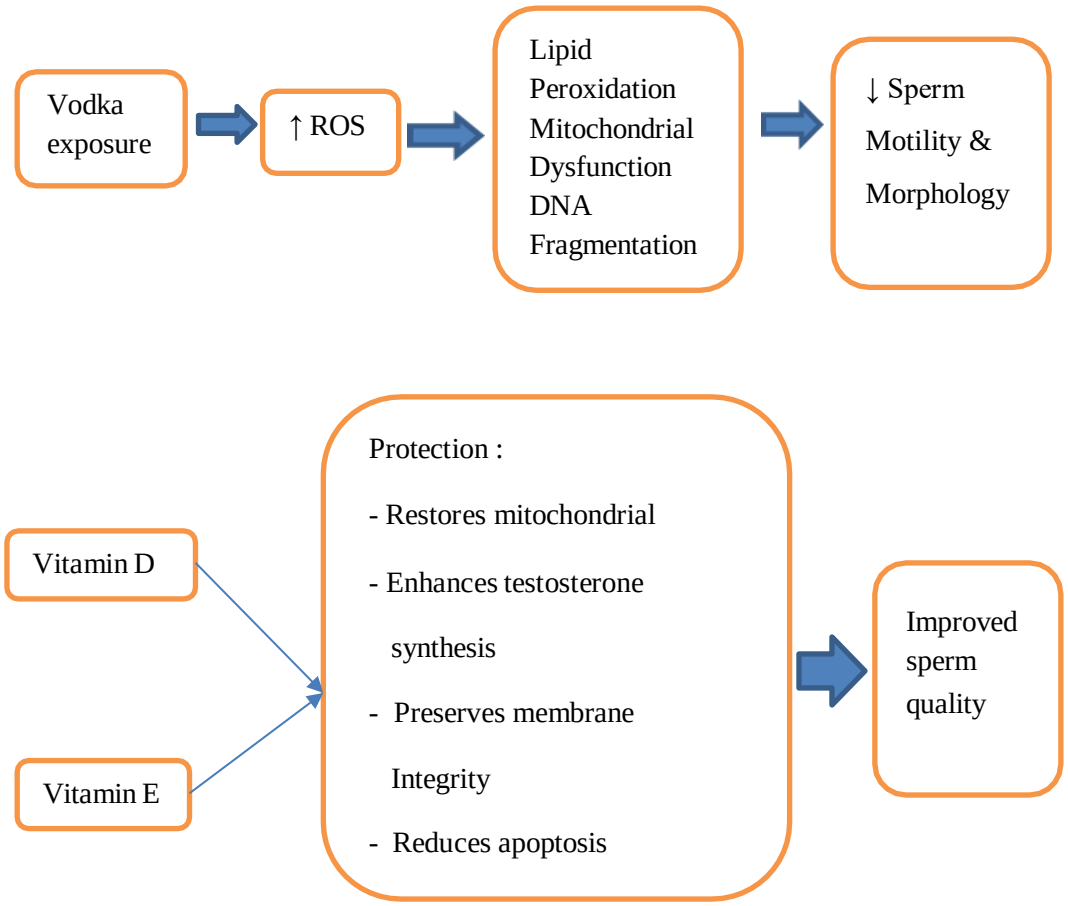
**Biomolecular Mechanisms of Vitamin D and E Protection on Ethanol induced Sperm Damage :**




Future studies should explore molecular mechanisms in greater depth, including vitamin D regulation of steroidogenic enzymes and vitamin E protection of mithochondrial DNA integrity in spermatozoa (Skakkebæk et al., 2022; Tuttelmann et al., 2021).

## CONCLUSION

Vodka exposure significantly impairs sperm quality in male Wistar rats. Supplementation with vitamin D or vitamin E improves sperm concentration, motility, and morphology, while combined supplementation offers the strongest protective effect. Future studies are recommended to include biochemical and hormonal assessments such as MDA, SOD, GPx, testosterone, LH and FSH to confirm the antioxidant and endocrine mechanisms through which vitamins D and E exert their protective effects. The integration of these biomarkers will enhance translational relevance and provide stronger molecular evidence for antioxidant based interventions in alcohol related male infertility. Co-supplementation with vitamin D and E may serve as a promising approach to mitigate alcohol induced reproductive toxicity

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