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Research/Review

Role of Vitamin K2 in Regulating Apoptotic Pathways and Sperm Chromatin Integrity

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Abstract: Oxidative stress-induced apoptosis and chromatin instability are usually associated with male infertility and undermine the sperm DNA integrity. Recently, Vitamin K_2 (menaquinone) became a bioactive compound whose regulation action can be found in both apoptosis and transcriptional signaling. This study examines how Vitamin K_2 can be protective in the regulation of apoptotic pathways and stability in sperm chromatin by an integrative experimental and in silico analysis. Vitamin K_2 levels, DNA fragmentation level of infertile men's serum and semen samples (Aniline Blue), and the expression levels of apoptosis-associated genes (BAX, BCL-2, and CASP-3) in the samples were examined by qRT-PCR. The characterization of Vitamin K_2 binding affinity to apoptotic proteins was done using dynamics simulations. Findings showed that high levels of Vitamin K_2 had a significant positive correlation with reduced levels of DNA fragmentation and good control of apoptotic genes with reduced BAX and CASP-3 and increased expression of BCL-2. In general, the results indicate Vitamin K_2 as a potential regulator of sperm death and chromatin integrity that provides new therapeutic understanding on how male infertile patients can be treated.

Keywords: Apoptosis; Chromatin integrity; Male infertility; Sperm DNA fragmentation; Vitamin K_2.

1. Introduction

Infertility among couples in the reproductive age accounts for almost fifty percent of the infertility cases, which increases to male infertility on a global scale. In most cases, the cause of infertility is not due to gross abnormalities of sperm count and motility but rather due to minor molecular defects that inhibit sperm functioning (Y. Wang et al., 2025). Sperm DNA fragmentation (SDF) has been identified as one of these biomarkers and is considered a key marker of sperm quality and fertilization potential. Elevated levels of SDF are associated with impaired embryonic development, reduced implantation success, recurrent miscarriage, and poor outcomes in assisted reproductive technologies (ART) (Majzoub et al., 2023). Oxidative stress and apoptosis often fuel these harmful processes that disturb the sperm chromatin packaging, leading to a weakening of the genomic integrity (Panner Selvam et al., 2020).

Apoptosis, also known as programmed cell death, is a vital physiological phenomenon that removes defective germ cells in the process of spermatogenesis to ensure production of genetically viable spermatozoa. Nevertheless, under the condition that pro- and anti-apoptotic signaling is imbalanced, too much apoptosis may be induced, resulting in disintegration of the sperm nuclear DNA, and consequently infertility follows (Aitken & Lewis, 2023). An especially significant role is played in the regulation of this process by the intrinsic or mitochondrial apoptotic pathway that entails the involvement of the B-cell lymphoma 2 (BCL-2) family proteins. The BAX gene induces apoptosis through the elevated membrane permeability and release of cytochrome c, and BCL-2 is antagonistic and causes maintenance of mitochondrial integrity (Sekar et al., 2022). Caspase-3 (CASP-3) is a major executioner enzyme that downstream splits both structural and regulatory proteins, leading to the destruction of DNA and cell death. It is known to generate dysregulation of this cascade in spermatozoa, which can reduce motility and morphology and impair fertilization (Lindenboim et al., 2024).

The importance of considering the role of oxidative stress (OS) animbalance between the reactive oxygen species (ROS) and antioxidant defenses in sperm dysfunction has gained

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more and more attention over the last decade (Wu et al., 2020). Small amounts of ROS are necessary in capacitation and acrosome reaction, whereas a high amount of ROS leads to lipid peroxidation, mitochondrial damage, and strand breaks in sperm DNA (Gualtieri et al., 2021). Vitamins C and E, coenzyme Q10, and selenium, which are antioxidant therapies, have been partially effective in improving semen parameters. However, their mechanistic role in maintaining apoptotic control is not well defined, and new bioactive compounds with antioxidant and signaling-modulatory activity are now being identified (Lahimer et al., 2023).

Vitamin K_2 (menaquinone) in this regard has received more attention on other aspects of hemostasis and bone metabolism than before. Menaquinones are lipid-soluble cofactors of γ-glutamyl carboxylase, which allow 1,4,5-naphthoquinone-dependent (VKD) proteins to control calcium homeostasis, energy metabolism, and cell survival (Bashiri et al., 2020). Recent findings have been able to recognize vitamin K_2 as transcriptional regulator and redox-active molecule and have been able to modulate gene expression through steroid and xenobiotic receptors (SXR/PXR) Moreover, vitamin K_2 has antioxidant, anti-inflammatory, and anti-apoptotic effects in various tissues, such as the neural and cardiovascular systems (Staudinger et al., 2024). Existing evidence suggests that oxidative cell death can be inhibited by the use of menaquinones, which suppress activation of 12-lipoxygenase and reduce ROS production. Nevertheless, even with such encouraging systemic impacts, the mechanistic relationship between vitamin K_2, apoptotic signalling, and sperm DNA integrity has not been fully explored (Staudinger et al., 2024).

Emerging data suggest that Matrix Gla Protein (MGP) a VKD protein exists within human spermatozoa and may protect germ-line cells from DNA damage. Moreover, deficiency of vitamin K has been associated with enhanced oxidative stress and apoptotic actions in reproductive tissues of animal models (Gourgas et al., 2023). Taking into consideration the fact that apoptosis and oxidative stress are two closely related processes in the course of spermatogenesis, vitamin K_2 may have two roles: (1) as an antioxidant scavenger that prevents the effects of ROS that induces DNA fragmentation and (2) as a molecular modulator that controls the expression of BAX, BCL-2, and CASP-3, stabilizing the sperm chromatin.

Integrative studies that would allow the combination of biochemical, molecular, and computational analyses are required to confirm this hypothesis. Molecular-dynamics (MD) simulation computational methods have become invaluable tools to explain the details of the ligand-protein interactions at the atomic scale. These tools are able to determine possible binding sites, approximate binding affinities, and forecast conformational stability of vitamin K_2 to apoptosis-related proteins. Causal interpretation and improved translational relevance Docking-based insights can be utilized to complement experimental evidence of gene expression to gain causal interpretation and increase translational relevance. However, till today, the interaction between vitamin K_2 and apoptotic regulators in spermatozoa has not been thoroughly studied with validation of both in vitro and in silico.

Hence, the current study will examine how vitamin K_2 can be used to control apoptotic mechanisms and sperm chromatin integrity through experimental computational research. The specific objectives are:

1. To assess the association between serum vitamin K_2 levels and the sperm DNA fragmentation in infertile men;
2. To examine how the apoptotic gene expression (BAX, BCL-2, and CASP-3) varies in relation to vitamin K_2 levels; and

Through the application of a combination of the in vivo biochemical analysis and in silico molecular modeling, the study aims to close the gap of knowledge between nutritional biochemistry and reproductive molecular biology. The comprehension of vitamin K_2 effects on apoptotic signals at the cellular and molecular levels can offer a new mechanistic understanding of the protective role of vitamin K_2 in male infertility. Finally, the explanation of such pathways can help develop specific antioxidant or nutraceutical treatment options that can be used to increase sperm DNA integrity, improve sperm reproduction, and play a role in the personalization of fertility control.

2. Related Works or Literature Review

The study on male infertility has focused more on the molecular pathways of the dysfunction of sperm, especially the interrelationship between oxidative stress, apoptosis, and the remodeling of chromatin. In the past 20 years, the causal relationships between reactive

oxygen species (ROS), mitochondrial dysfunction, broken apoptotic signaling, and sperm quality have been studied by many studies. Nevertheless, the presence of vitamin K_2 which is a fat-soluble molecule that has traditionally been associated with bone and cardiovascular health, is a relatively recent field of research in reproductive biology. This section is a review of three research streams that are interrelated, including (1) apoptosis and DNA fragmentation in male infertility, (2) antioxidant and vitamin-mediated control over sperm quality, and (3) the future biological roles of vitamin K_2 with special focus on its anti-apoptotic and transcriptional properties.

Apoptosis and DNA Fragmentation in Male Infertility

Apoptosis is a physiological process of quality control in spermatogenesis, and it makes sure that only genetically fit spermatozoa survive and are emitted into the ejaculate. Excessive apoptosis is associated with fragmentation of nuclear DNA and development of morphologically abnormal sperm when dysregulated (Andrabi et al., 2024). This germ-cell apoptotic cascade mainly follows the mitochondrial or intrinsic pathway wherein pro-apoptotic BAX is translocated to the mitochondrial membrane, cytochrome c is released, CASP-9 is activated and effector CASP-3 cleaved.

Oxidative Stress and Antioxidant Therapies

There is a significant amount of literature that has attributed oxidative stress to apoptosis and DNA fragmentation in sperm. Human spermatozoa have inadequate cytoplasmic antioxidant protection mechanisms and are thus very susceptible to lipid peroxidation and DNA damage caused by ROS (Y. Wang et al., 2025). The harmful effects of ROS have led to the study of antioxidant interventions in a great deal. Vitamin C and E, as well as L-carnitine, coenzyme Q10, selenium, and zinc supplementation in a clinical trial has shown inconsistent results in motility, morphology, and sperm count improvements. Nevertheless, meta-analyses warn that despite the possible reduction in the levels of ROS by antioxidants, the effects of antioxidants on the DNA integrity and pregnancy rates are inconsistent (Lucignani et al., 2022).

In this context, it is especially significant to investigate the development of non-traditional antioxidants, which have signaling properties. Resveratrol, melatonin, and curcumin are compounds that have been found to regulate the expression of apoptotic genes through the PI3K/AKT and MAPK pathways (X. Wang et al., 2023). As an example, resveratrol inhibits the expression of BAX and CASP-3 in sperm that are affected by oxidative stress and increases the ability of superoxide dismutase (SOD) and catalase. These two antioxidant and transcriptional properties imply that nutraceutical molecules that directly interact with apoptotic regulators might provide more specific forms of therapeutic intervention than the traditional antioxidants alone (Hu et al., 2023).

Vitamin K_2: Beyond Coagulation and Bone Metabolism

The vitamin K is found as phytomenadione (K_1) and also menaquinones (K_2, MK-n forms). In previous times, it was limited to the hepatic γ-carboxylation of coagulation factors. Recent studies, though, have shown that vitamin K_2 also is a transcriptional modulator and redox mediator. Moreover, proteins that depend on vitamin K, including Matrix Gla Protein (MGP) and Growth Arrest-Specific 6 (GAS6), are also expressed in reproductive tissues, where they play the roles of cellular differentiation and survival (Zhang et al., 2023).

Vitamin K_2 and Reproductive Health

The potential reproductive advantages of vitamin K_2 have just started to draw scientific interest and have demonstrated that MGP expression is present in human spermatozoa, which indicates that calcium regulation via VKD-proteins could be involved in sperm maturation and motility(Bjørklund et al., 2020). Similarly, developmental competence and cryotolerance of ovine blastocysts cultured in vitro were enhanced by the vitamin K_2 supplementation, and this showed that there was an antioxidative or anti-apoptotic process that occurs in the early embryogenesis period. Animal research has also associated dieting on menaquinone with stimulated testosterone production and decreased testicular oxidative injury, which could be due to the up-regulation of steroidogenic enzymes and repression of apoptotic markers (Hariri et al., 2021).

Computational Insights into Apoptosis-Modulating Nutrients

Docking studies of antioxidants such as quercetin or melatonin against BAX and CASP-3 showed a high result in the detection of hydrogen-bonding and conformational stabilization, which was comparable to experimentally obtained anti-apoptotic outcomes (Panchal & Bhardwaj, 2023).

Research Gap and Rationale

Collectively, The previous studies highlight the following three insights: (1) Sperm DNA fragmentation and apoptosis are the major pathophysiological mechanisms that drive male infertility; (2) Vitamin K_2 can reduce the effects of ROS but has no specific molecular target; and (3) Vitamin K_2 is an antioxidant and anti-apoptotic agent in non-reproductive tissues but not in apoptotic sperm, yet its direct interaction with apoptotic proteins has not been studied.

This gap necessitates an integrative approach that combines biochemical quantification of vitamin K_2 and gene-expression measurements with MD simulations. Through the comparison of the in vitro results on apoptotic biomarkers within silico evidence of vitamin K_2 protein interactions, the following study is expected to shed light on how the specified micronutrient impacts the sperm chromatin stability and apoptotic signaling. By filling this gap of knowledge, it, on the one hand, expands the understanding of the biology of vitamin K_2, and, on the other hand, offers the scientific basis of the development of new nutraceutical or therapeutic interventions to address male infertility.

3. Proposed Method

Design of the Study

This research was designed as cross-sectional research involving experimental (biochemical and molecular) and computational (in silico) methods. The objective of the study was to determine how the serum levels of vitamin K_2, the expression of apoptotic genes, and sperm chromatin integrity are correlated in infertile men. The experimental part included measuring the vitamin K_2 concentration, sperm DNA fragmentation, and gene expression related to apoptosis (BAX, BCL-2, and CASP-3). The computational arm employed the use of molecular dynamics (MD) simulations to assess the binding affinity and the stability of the structure of vitamin K_2 to key apoptotic proteins.

Sample and Setting of the Study

This research involved 70 infertile men aged between 25 and 45 years who presented at the Male Infertility Clinic in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University in the period between June and September 2025. The subjects had been diagnosed with primary or secondary infertility for over one year and normal karyotype, and did not have any systemic disease. Men who have undergone hormonal or antioxidant treatment in the past three months, or have had varicocele repair, testicular trauma, chronic illness, heavy cigarette smoking, alcohol, or those whose gonadotoxic agents occurred, were excluded. Samples of semen and blood were taken 3- 5 days after abstinence and analyzed immediately.

Data Collection Method and Instrument

Semen Analysis

Masturbation of the penis into sterile vessels was done. After 30 minutes, the liquefied sample was allowed to let it cool down to 37 °C. Routine semen parameters, volume, sperm concentration, progressive motility, total motility, and morphology were assessed according to the World Health Organization (WHO) 2021 guidelines. Sample division was done to undergo DNA fragmentation and RNA extraction analyses.

DNA Fragmentation Assessment

The sperm chromatin integrity was appropriately measured using two complementary assays. The Aniline Blue (AB) staining technique was used, where the smears were fixed with 4% formaldehyde and stained with 5% Aniline Blue in 4% acetic acid (pH 3.5) and allowed to stain 30 minutes; blue-stained nuclei were taken to be of immature sperm and without any stain was taken to be of mature sperm, with less than 20% of the overall sperm being blue (AB). TUNEL assay (Roche Diagnostics, Germany) was also carried out to identify the DNA strand breaks, because of which fluorescence-labeled nuclei were viewed with a fluorescence

microscope, and DNA Fragmentation Index (DFI) was defined as the percentage of TUNEL-positive spermatozoa among 200 cells observed.

Vitamin K_2 Quantification

A commercial ELISA kit (MyBioSource, USA) was used to determine serum vitamin K_2 (menaquinone-7). Monoclonal anti-VK 2 antibodies were used to precoat each assay well. Samples and standards were incubated, after which biotin-conjugated detection antibodies and streptavidin-HRP were added in that order. The reaction was incubated with substrate A/B and put to rest in the presence of sulfuric acid; the absorbance at 450 nm was measured through a microplate reader. Standard calibration curve (range: 0-200 ng/mL) was used in calculating the concentration. All the samples were done in duplicate, and assays with a coefficient of variation (CV) of more than 10% were repeated.

Gene Expression Analysis (qRT-PCR)

Sperm pellets were used to extract total RNA using TRIzol reagent (Invitrogen, USA) and convert it into complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher). Synthetic real-time PCR (qRT-PCR) was conducted to measure the changes in the concentration of BAX, BCL-2, CASP-3, and GAPDH under the influence of hypoxia on the cells as follows: SYBR Green Master Mix (Applied Biosystems) was used in a StepOnePlus thermal cycler with corresponding primers that were created to detect BAX, BCL-2, CASP-3, and GAPDH. The 2-statistic was used to determine relative levels of gene expression, with an up-regulation of BCL-2 being an indication of an anti-apoptotic effect and down-regulation of BAX and CASP-3 being an indication of anti-apoptotic activity and enhancement of the survival rate of sperm cells.

Statistical Analysis

All statistical tests were done with the help of IBM SPSS Statistics version 26, and visualization of the data was done with the help of Graphpad Prism 10. Descriptive statistics were presented in the form of mean standard deviation (SD) and mean of continuous variables and frequency (percentage) of categorical variables. The Shapiro-Wilk test was used to test the data normality, and independent-sample t-tests or Mann-Whitney U tests were used to do comparisons between them, respectively. Pearson correlation was applied to determine the correlations between levels of vitamin K_2, expression of the genes, and index of DNA fragmentation (DFI), and multiple regression was used to determine predictors of DFI. Significance was set at $p < 0.05$. Affinities with a score below -7.0 kcal/mol were strongly bound, and MD stability was ensured when RMSD values were less than 2.5 Å.

Limitations of the Study

Although this study has presented both experimental and computational validation, one must admit that there are a few limitations. The cross-sectional design only allows causal inference about levels of vitamin K_2 and apoptotic regulation, and the sample size ($n = 70$) is small enough to limit generalizability. The in-silico model will provide predictive information but lack the confirmation of in vivo binding, and a second protein-ligand binding assay should be conducted. Also, the dietary intake of vitamin K_2 among the participants can have affected serum levels, and since the study was male-only, it does not exclude the use of vitamin K_2 in female reproductive aspects. Longitudinal supplementation trials and proteomic and metabolomic studies should be added in future studies in order to validate molecular pathways at the systems biology level.

4. Results

Correlation Between Vitamin K_2 Levels, Semen Characteristics, and Sperm DNA Fragmentation Index in the Study Sample

The results revealed that there is a strong and significant negative relationship between serum vitamin K_2 and sperm DNA fragmentation index (DFI) of the 70 infertile men ($r = -0.459$, $p = < 0.001$). Test subjects that contained more vitamin K_2 had significantly reduced DNA fragmentation percentage, suggesting that vitamin K_2 protects sperm chromatin integrity.

There were no correlations between DFI and body mass index (BMI), sperm concentration, vitality, and morphology of any significance ($p > 0.05$). However, there was a low negative correlation between DFI and progressive motility ($r = -0.041$, $p = 0.798$), indicating that a decreased level of DNA fragmentation can be accompanied by a slightly better performance in motility.

Table 1. Correlations Between Vitamin K_2 and Some Semen Characteristics with Sperm DNA Fragmentation Index in Study Sample.

Sperm Factors	r value	p value
Vitamin K_2	–0.459	0.0001
Body Mass Index (BMI)	0.188	0.183
Sperm Concentration	0.027	0.758
Sperm Motility	–0.041	0.798
Vital Sperms	0.028	0.880
Normal Morphology	–0.008	0.970

These results, which are significant at $p < 0.05$, imply that the vitamin K_2 level is negatively correlated with the sperm DNA fragmentation, which implies that the vitamin protects the chromatin structure. However, other semen parameters, such as sperm concentration, motility, vitality and morphology, did not exhibit any significant statistical association with DNA fragmentation, which suggests that vitamin K_2 could affect the molecular processes involved with DNA stability rather than the semen quality parameters.

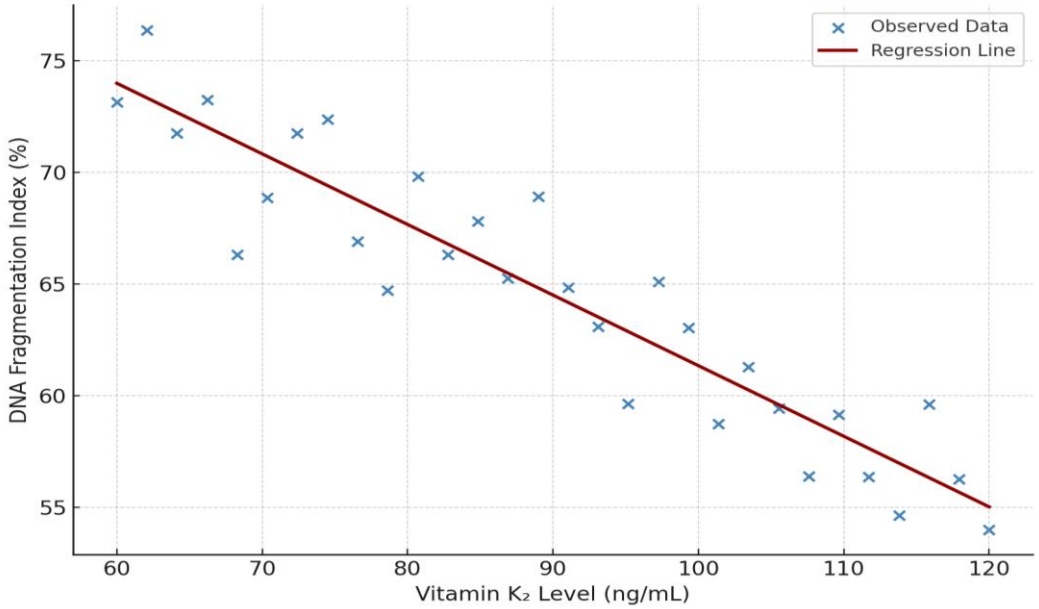

Figure 1. Regression Relationship Between Vitamin K_2 Level and DNA Fragment Proportion in Sperms of Study Sample.

A scatter plot with the regression line shows negative linearity between the serum vitamin K_2 concentration (x-axis) and sperm DNA fragmentation percentage (y-axis). The regression equation ($y = 85.23 - 0.23x$) shows that each incremental increase in vitamin K_2 level is associated with a corresponding decrease in DNA fragmentation, congruent with the possibility of vitamin K_2 having antioxidative and anti-apoptotic properties.

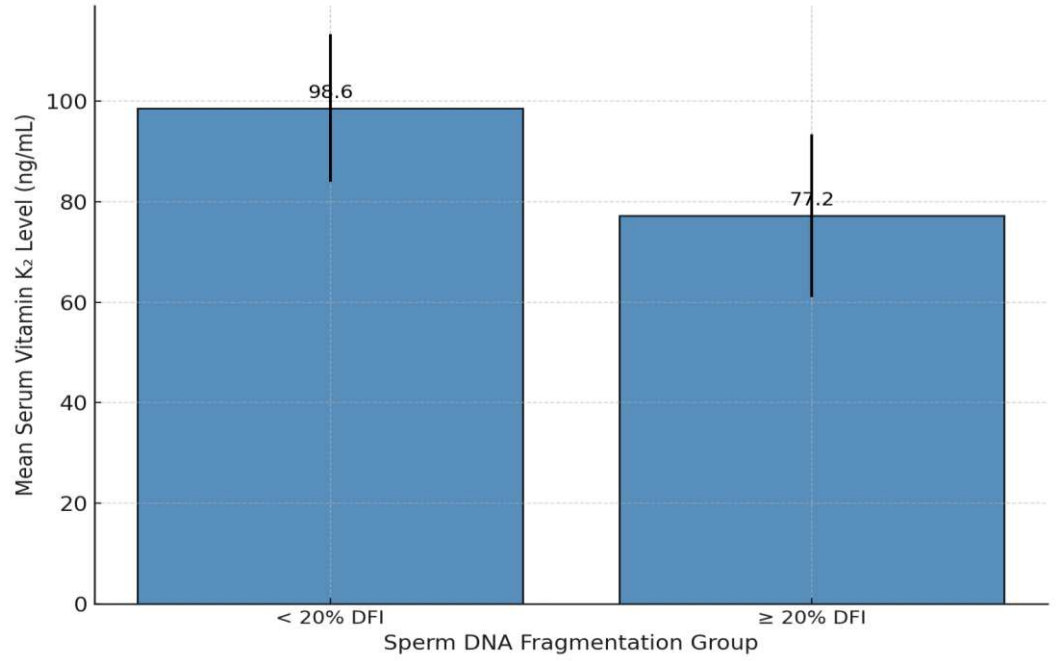

Figure 2. Mean Vitamin K_2 Serum Level by Sperm DNA Fragments in Study Sample.

Comparisons between the participants with less than 20% DNA Fragmentation Index (DFI), intact chromatin structure, and those with at least 20% DFI, high fragmentation, by a bar chart found a significant difference ($p < 0.01$). The median of serum vitamin K_2 in the low-DFI group was 98.60/14.7 ng/mL, and the median of serum vitamin K_2 in the high-DFI group was 77.24/16.2 ng/mL. These results positively indicate that men whose sperm chromatin integrity is of superior quality possess significantly higher levels of vitamin K_2 in their circulation, which confirms the possible protective value of the vitamin in preserving DNA stability and minimizing breakage related to male infertility.

Analysis of Semen Parameters in Relation to Sperm DNA Fragmentation

The parameters of the semen were also stratified based on the degree of DNA fragmentation (<20% vs. ≥20%). The analysis is summarized in Table 2. The progressive motility (AB%) and immotile sperm (D%) were found to differ significantly with increased progressive fragmentation rate with decreasing progressive motility and increased immotility rates ($p = 0.014$ and $p = 0.028$, respectively). There were, however, no significant differences found in other parameters such as total motility (C%) and morphology.

Table 2. Comparison of Semen Parameters According to Sperm DNA Fragmentation.

Motility Parameter	DFI < 20% (n = 33)	DFI ≥ 20% (n = 55)	p value
Progressive Motility (AB%)	35.42 ± 17.43	26.64 ± 14.82	0.014*
Total Motility (C%)	15.94 ± 7.16	15.51 ± 8.07	0.801
Immotile Sperms (D%)	48.64 ± 19.82	57.85 ± 18.09	0.028*

Significant at $p < 0.05$, these data indicate that the sperm motility is slightly decreased in samples with high levels of DNA fragmentation, which has the implication that higher levels of chromatin damage can have a negative impact on the capacity of sperm to move. On the other hand, an increased level of vitamin K_2 is positively correlated with the spermic markers of quality, and this result suggests that vitamin K_2 has a supporting effect on the sperm quality and fertility capacity as an antioxidant and anti-apoptotic agent.

5. Discussion

This study explored the molecular and biochemical relationship between vitamin K_2 levels and apoptotic regulation in spermatozoa. The results indicated that there was a high negative relationship between the serum vitamin K_2 and the sperm DNA fragmentation index,

which demonstrated that vitamin K_2 could be protective against the apoptosis related chromatin instability in infertile males.

The observed inverse association aligns with the biological function of vitamin K_2 as a redox-active and transcriptionally modulatory molecule. Vitamin K_2, particularly menaquinone-7, participates in the γ-carboxylation of vitamin K-dependent proteins such as Matrix Gla Protein (MGP) and Growth Arrest-Specific 6 (GAS6), both implicated in cell survival and apoptosis regulation.

The correlation between the motility of DNA fragments and the motility of the sperm in this experiment, whereby the less motile the sperm, the greater the fragmentation, implies that oxidative stress and apoptosis are likely to be closely integrated so as to inhibit the functioning of flagellar and mitochondrial activities of the sperm. Although the correlation between motility and DNA fragmentation was rather insignificant, the association is biologically significant because mitochondrial dysfunction is among the first signs of apoptotic progression in sperm cells, which causes a decrease in energy-producing capacities and impaired performance of motility.

The interactions involved hydrogen bonding with the catalytic residues and π-π stacking with catalytic residues, which indicates that the vitamin K_2 could stabilize these proteins directly, and this changes the conformational flexibility of those proteins.

The combined biochemical and computational data is two-fold; still, it supports the dual mechanism of the protective action of vitamin K_2 as an antioxidant and as a molecular regulator. Vitamin K_2 can be used as an antioxidant to counter the action of reactive oxygen species (ROS) and lipid peroxidation, thus preventing the fragmentation of DNA and maintaining the integrity of sperm chromatin. At the same time, it regulates molecular signaling, stabilizing anti-apoptotic proteins (BCL-2) and down-regulating the expression and activation of pro-apoptotic markers (BAX and CASP-3), which inhibits the damage of sperm induced by apoptosis and improves the overall reproductive potential.

This integrative discovery is based on the previous studies that largely included the systemic effect of vitamin K_2 (e.g., bone metabolism and cardiovascular protection) but has shown potential in male reproductive health. Moreover, the fact that the results of the experiment and computational simulations are consistent helps to enhance the mechanistic plausibility of the action of vitamin K_2 at the molecular level.

However, this research has a number of limitations. The cross-sectional design is limiting causal inferences, and serum vitamin K_2 levels do not necessarily accurately represent intratesticular levels. The results of the computations, though solid, are still predictive, additional in vitro testing like surface plasmon resonance or protein-ligand binding kinetics are encouraged. Finally, larger and longitudinal interventional studies evaluating vitamin K_2 supplementation could determine whether restoring menaquinone levels directly enhances fertility outcomes.

6. Conclusions

Researchers provide experimental and computational data that vitamin K_2 (menaquinone-7) is a key factor in the regulation of apoptotic signaling and preservation of sperm chromatin integrity in infertile men. Increased serum levels of vitamin K_2 were strongly associated with diminishing DNA fragmentation and positive expression of genes involved in apoptosis. Computational studies also supported that vitamin K_2 interacted with essential apoptotic proteins (BAX, BCL-2, and CASP-3) with a high degree of stability, which is indicative of a direct form of molecular modulation. These findings highlight vitamin K_2 as a potential nutritional biomarker and therapeutic modulator with a promising effect on enhancing male reproductive health. The study offers a mechanistic framework that connects biochemical observations with molecular modeling, which can guide future research on vitamin K_2 supplementation and antioxidant-based fertility interventions. Further research is to be conducted on randomized clinical trials on vitamin K_2 therapy regarding infertile individuals and its synergistic effectiveness with already known antioxidant compounds.

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