DOI: 10.69946/ojms/2026.04.01.03, pp(22-26)

Association of Serum and Seminal Afamin and Vitamin E with Hormonal Profiles Across Different Semen Phenotypes in Infertile Men

Almuhanad Jamal Mawlood[1], Amal Abdulwahid Mohammed[2], Ula Mohamed Al kwaz[3]

[1,2,3]High Institute for Infertility Diagnosis and Assisted Reproductive Technologies Al-Nahrain University, Baghdad, 10006, Iraq

Corresponding Author email: muhanadjamal44@gmail.com

Abstract

Background: Male infertility is a complex disorder affected by hormonal imbalances, poor quality of semen, and oxidative stress. The defense mechanisms against oxidative damage to spermatozoa require antioxidant defense systems. Afamin is a transport protein of vitamin E, a lipid soluble antioxidant that helps in maintaining the stability of sperm membrane and cell integrity. Nonetheless, the differences in the levels of afamin and vitamin E in various semen phenotypes and reproductive hormones are not well defined.

Objective: Analyzing the levels of association between afamin and vitamin E concentrations in the serum and seminal plasma and the hormonal profile in infertile males.

Method: The study was conducted as a cross-sectional study involving ninety infertile men. Semen analysis was performed. Both serum and seminal afamin and vitamin E were measured. Hormonal profile was also evaluated in serum.

Result: Significant differences between groups were found in seven parameters: FSH (p =0.034), LH (p =0.011), Testosterone (p =0.049), afamin serum (p =0.006), vit-E serum (p =0.002), afamin semen (p =0.001), and E semen (p =0.001). There were strong positive and negative correlations between LH and seminal afamin, LH with seminal vitamin E in asthenozoospermic men (r=0.6032; *p*-value=0.001; r=-0.6108; *p*-value=0.012), respectively.

Conclusion: Results indicate that localized seminal biochemical markers have a pivotal diagnostic value of male factor infertility that cannot be fully reflected by serum analysis. The increased levels of gonadotropins in oligozoospermic phenotypes also indicate the existence of compensatory mechanisms of the HPG axis to the disrupted spermatogenesis.

Keywords: Afamin, vitamin E, male infertility, FSH, LH.

1. Introduction

Infertility is an increasing international health issue with about 15 percent of couples of reproductive age being affected across the world [1]. Recent epidemiological statistics show that male factors are a cause of almost half of them, and the deterioration of sperm quality in different geographical locations has increased over the past ten years [2]. Although the latest technologies have been

DOI: 10.69946/ojms/2026.04.01.03, pp(22-26)

developed in the field of diagnosis, the primary examination of male fertility continues to depend on the traditional semen analysis. Yet, the conventional parameters can hardly reflect the multifaceted molecular and hormonal landscape of the male reproductive tract, which is why idiopathic infertility cases are very frequent [3].

The hypothalamic-pituitary-gonadal (HPG) axis controls most of the processes involved in the regulation of spermatogenesis. The FSH and LH are synergistic hormones that keep the testicles functional; FSH stimulates Sertoli cells to multiply and helps the Leydig cells to manufacture testosterone that is critical in the process of completing spermatogenesis [4], [5]. Any imbalance in this fine feedback mechanism may be reflected as clinical phenotypes Oligozoospermia (OL), Asthenozoospermia (AS), or the more extreme Oligoasthenoteratozoospermia (OAT).

Oxidative stress is a critical determinant in the pathogenesis of male infertility due to its development as a result of the lack of balance between the reactive oxygen species (ROS) and the antioxidant defenses of the body [6]. Vitamin E, a potent fat-soluble antioxidant, is one of the essential factors in the defense of sperm cell membranes against lipid peroxidation [7]. Vitamin E preserves the structure and motility of the spermatozoa by neutralizing the free radicals. Recent meta-analyses have established that supplementation of Vitamin E or sufficient seminal levels are strongly linked with better sperm concentration and DNA integrity [8], [9].

Vitamin E is transported by specialized proteins, the most common of which is Afamin, the bioavailability of Vitamin E in the reproductive system is alpha-tocopherol [10]. Afamin is a vitamin E-binding glycoprotein, which has recently become an important indicator of the oxidative-antioxidant balance of the male reproductive system [11]. Current studies have shifted to localized biochemical indices in serum and seminal plasma [12].

Although the roles of reproductive hormones and antioxidant defense systems in male fertility are established, there is limited comparative analysis of systemic (serum) and localized (seminal) levels of afamin and vitamin E in various pathological sperm phenotypes. Hence, the purpose of the present study was to compare the concentrations of FSH, LH, testosterone, prolactin, afamin, and vitamin E in serum and seminal plasma in five groups, including asthenoteratozoospermia (ASTR), asthenozoospermia (AS), oligoasthenoteratozoospermia (OAT), oligozoospermia (OL), and normozoospermic controls (N). The study also aimed at assessing possible variations in endocrine-antioxidant relationships between these different semen phenotypes.

2. Materials and Methods

A. Patients and Study Details

The research was applied as cross-sectional and comparative study. The case group comprises of infertile men with abnormal semen parameters (asthenozoospermia (AS), astheno-teratozoospermia (AST), oligozoospermia (OL), oligo-astheno-teratozoospermia (OAT) and infertile men with normozoospermia (NO) considered as internal control groups. This study involved an experimental part, which was carried out between October 2025 and January 2026 at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies. Informed consent was written by all the patients after a clear explanation of the research objectives and the purpose of the questionnaire. Afterwards, blood and semen samples were obtained to be analyzed further at Al-Nahrain University in Baghdad, Iraq. Afamin and vitamin E were evaluated in blood and semen with hormones utilizing

DOI: 10.69946/ojms/2026.04.01.03, pp(22-26)

immunoassay Florence (Cobas E411, Roche/Germany). ELISA kit, (ELK Biotechnology, China; Afamin: Cat. No. ELK3008, Vitamin E: Cat. No. ELK7865). Adapted to the strict requirements of its manufacturer instructions, satisfactory to achieve the right results. To identify hormones, the serum was preserved at -4 °C and had previously thawed, and then whirled to be homogenized.

B. Inclusion criteria

- Infertile males with abnormal semen analysis.
- Male ages 20-40.

C. Exclusion criteria

- Azoospermia.
- Hormonal administration.
- Chronic disease.

D. Study Ethical Code 0702-MM-2025A69-C2

E. Sample Size

The last study group was identified due to the particular time constraints of the study period and the strict inclusion criteria stated in the research protocol. Though the predetermined sample size was 90-106 specimens, 16 samples were later omitted in the final analysis because of poor quality of the analysis or unexpected technical difficulties. This was a very strict process of selection that guaranteed the integrity and reliability of the resulting data.

F. Semen analysis

All samples of semen were examined based on the standardized protocols in the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen, 6th edition (2021).

G. Statistical analysis

IBM SPSS Statistics 26.0 was used to analyze the data. Since the variables were non-parametrically distributed, Kruskal-Wallis H test was applied to identify whether statistically significant differences were present between the five study groups (ASTR, AS, NO, OAT, and OL). Post-hoc pairwise comparisons were made on all the variables where the Kruskal-Wallis test showed a p value $p < .05$. In order to keep the threshold strict and reduce the probability of Type I errors (false positives) of multiple comparisons, the Bonferroni correction was used to correct the significance levels. All findings are listed in terms of the H statistic (Chi-square), degrees of freedom (df), and adjusted p-values (p). The predetermined level of statistical significance was alpha = 0.05. The degree of association between continuous variables was calculated by Pearson's correlation coefficient (r), and the results were considered statistically significant when the p-value was equal to or less than 0.05.

3. Result

A. General Comparison of Hormonal and Biochemical Markers

The Kruskal-Wallis H test was used to determine the difference in hormonal and biochemical parameters among the five study groups: Asthenoteratozoospermia (ASTR), Asthenozoospermia (AS), Normozoospermia (N), Oligoasthenoteratozoospermia (OAT), and Oligozoospermia (OL).

DOI: 10.69946/ojms/2026.04.01.03, pp(22-26)

The analysis of the data revealed that seven of the eight variables being tested had significant differences between the groups of data ($p < 0.05$). It is important to note that the only variable that failed to provide significant variation was Prolactin which had $H = 0.505$ and $p = 0.973$. The most significant statistical variations were seen in the seminal markers, namely Afamin (semen) ($H = 19.444$, $p = 0.001$) and Vitamin E (semen) ($H = 18.225$, $p = 0.001$), illustrated in Table 1.

B. Hormonal Profiles (FSH, LH, and Testosterone)

There were significant differences in FSH ($p = 0.034$), LH ($p = 0.011$), and Testosterone ($p = 0.049$). Pairwise Bonferroni-corrected post-hoc comparisons found the specific differences between the groups: FSH: There was a significant difference between the ASTR and OL groups (Adjusted $p = 0.037$). LH: A substantial difference was found between Normozoospermic (N) control and OL (Adjusted $p = 0.026$). Testosterone: The global Kruskal-Wallis test was significant ($p = 0.049$), but, when compared individually after Bonferroni correction, the individual pairwise comparisons were not significant, suggesting a small, diffuse difference between the cohorts presented in Table 1.

C. Serum and Seminal Concentrations of Afamin and Vitamin E

The research identified very significant differences in both serum and seminal Afamin and Vitamin E levels. Afamin: * ASTR versus AS, AS versus N, there were significant differences in serum ($p = 0.008$ and $p = 0.014$). The N group of semen was significantly different compared to the OAT group ($p = 0.002$) and the OL group ($p = 0.050$). Likewise, there was a large variance in ASTR and OAT ($p = 0.022$).

Vitamin E: The level of serum Vitamin E was significantly different between the ASTR group and the N group ($p = 0.005$) and the OAT group ($p = 0.039$). Seminal Vitamin E recorded the largest range of variation with a significant difference between AS and OL ($p = 0.008$), AS and N ($p = 0.037$), and ASTR and N ($p = 0.047$), as illustrated in Table 2.

D. The correlation between hormonal profiles with antioxidant biomarker among the studied groups

There were strong positive and negative correlations between LH and seminal afamin, LH with seminal vitamin E in asthenozoospermic men (r=0.6032; p-value=0.001; r=-0.6108; p-value=0.012), respectively. While in normozoospermic men showed a strong positive relationship between LH and serum afamin, LH and seminal vitamin E (r=0.7775; p-value=0.001; r=0.8073; p-value=0.001), respectively. Likewise, there were positive correlations found in Testosterone with seminal vitamin E and prolactin with serum afamin (r=0.5018; p-value=0.0173; r=0.5445; p-value=0.0131) respectively. In contrast, prolactin revealed a strong negative correlation with seminal afamin (r=-0.8196; p-value=0.01). On the other hand, the OAT group presented a strong negative relationship concern FSH vs serum afamin and prolactin vs seminal afamin (r=-0.6724; p-value=0.0332; r=-0.8787; p-value=0.008). While in the OAT group showed strong positive correlation in FSH vs seminal afamin, LH vs seminal vitamin E, and testosterone vs seminal afamin (r=0.7256; p-value=0.0175; r=0.846; p-value=0.002; r=0.8127; p-value=0.0043) respectively. Oligozoospermic men exhibited a negative association between testosterone and seminal afamin (r=-0.5241; p-value=0.0372), as illustrated in Table 3.

DOI: 10.69946/ojms/2026.04.01.03, pp(22-26)

The overall correlation between hormones and antioxidant biomarkers revealed a positive significant association related to LH and seminal afamin (r=0.234; p-value=0.039) and a negative relationship concerning testosterone vs serum vitamin E (r=-0.227; p-value=0.037), and prolactin vs seminal afamin (r=-0.369; p-value=0.001) denoted in Table 4.

Table 1: Kruskal-Wallis H Test Summary enrolled in the present study

Parameters	Chi-Square (H)	df	p-value	Significance
FSH	10.432	4	0.034	*
LH	13.067	4	0.011	*
Testosterone	9.526	4	0.049	*
Prolactin	0.505	4	0.973	ns
Afamin (Serum)	14.606	4	0.006	* *
Vitamin E (Serum)	16.602	4	0.002	* *
Afamin (Semen)	19.444	4	0.001	* * *
Vitamin E (Semen)	18.225	4	0.001	* * *

Note: H = Kruskal-Wallis test statistic.
df = degrees of freedom. Statistical significance was determined at the alpha = 0.05 level.
*p < 0.05 (Significant).
** p < 0.01 (Highly Significant).
*** p < 0.001 (Very Highly Significant).
ns = not significant (p > 0.05).

Table 2: Significant Post-Hoc Pairwise Results (Bonferroni test)

Dependent Variable	Significant Pairwise Comparison	Adjusted p-value
FSH	ASTR vs OL	0.037
LH	N vs OL	0.026
Afamin (Serum)	ASTR vs AS	0.008
	AS vs N	0.014
Vitamin E (Serum)	ASTR vs N	0.005
	ASTR vs OAT	0.039
Afamin (Semen)	N vs OAT	0.002
	ASTR vs OAT	0.022
	N vs OL	0.050
Vitamin E (Semen)	AS vs OL	0.008
	AS vs N	0.037
	ASTR vs N	0.047

Pairwise comparisons were performed. All p-values presented in this table have been adjusted using the Bonferroni correction to account for multiple comparisons and control the family-wise error rate.

DOI: 10.69946/ojms/2026.04.01.03, pp(22-26)

Table 3: Significant Correlations within Specific Groups

Asthenozoospermia

Hormones	Markers	r	*p*-value	Level of significance
LH	Afamin semen	0.603	0.001	**
	Vit E semen	-0.610	0.012	**

Normozoospermia

Hormones	Markers	r	*p*-value	Level of significance
LH	Afamin serum	0.7775	0.001	***
	Vit E semen	0.807	0.001	***
Testosterone	Vit E semen	0.501	0.0173	**
prolactin	Afamin serum	0.544	0.0131	**
	Afamin semen	-0.819	0.01	***

Oligo-Astheno-Teratozoospermia (OAT)

Hormones	Markers	r	*p*-value	Level of significance
FSH	Afamin serum	-0.672	0.033	**
	Afamin semen	0.725	0.017	***
LH	Vit E semen	0.846	0.002	***
Testosterone	Afamin semen	0.812	0.004	***
prolactin	Afamin semen	-0.878	0.008	***

Oligozoospermia

Hormones	Markers	r	*p*-value	Level of significance
Testosterone	Afamin semen	-0.5241	0.0372	**

r: Pearson's correlation coefficient; NS: Not significant ($p > 0.05$).
* indicate week correlation, ** moderate correlation, *** strong correlation.
(-) Minus values indicate negative correlation, whereas positive values indicate positive correlation.
Bold values indicate that a statistically significant difference exists between the specific pairs ($p<0.05$).

Table 4: Pearson's correlation coefficient between hormones and biochemical markers

Hormones	Marker	R	*p*-value
FSH	Afamin serum	-0.1181	0.303
FSH	Afamin semen	-0.147	0.166
FSH	Vit E serum	0.071	0.517
FSH	Vit E semen	-0.036	0.742
LH	Afamin serum	0.234	0.039
LH	Afamin semen	-0.118	0.268
LH	Vit E serum	-0.125	0.256
LH	Vit E semen	0.154	0.157
Testosterone	Afamin serum	-0.155	0.175
Testosterone	Afamin semen	-0.179	0.091
Testosterone	Vit E serum	-0.227	0.037
Testosterone	Vit E semen	-0.027	0.803
Prolactin	Afamin serum	0.198	0.08
Prolactin	Afamin semen	-0.369	0.001
Prolactin	Vit E serum	-0.144	0.191
Prolactin	Vit E semen	-0.014	0.896

r: Pearson's correlation coefficient; NS: Not significant ($p > 0.05$)

Bold values indicate that a statistically significant difference exists between the specific pairs ($p<0.05$).

DOI: 10.69946/ojms/2026.04.01.03, pp(22-26)

4. Discussion

The current research examined the biochemical differences among different semen phenotypes in relation to hormones and antioxidant-related parameters and found that there were substantial differences in reproductive hormones and oxidative stress - related biomarkers in infertile men. With non-parametric analysis, we can prove that the changes in serum and seminal afamin and vitamin E are phenotype-dependent and closely associated with semen quality.

A. Hormonal Profile Differences across Semen Phenotypes

There were significant subgroup differences in FSH, LH, and testosterone, but no differences in prolactin were found in all groups. Several high levels of FSH, especially in asthenoteratozoospermic and oligozoospermic men, are probably evidence of poor spermatogenesis and dysfunction of the Sertoli cell, which has also been reported by other authors as an indicator of the damage of seminiferous tubules [13], [14]. The large disparity in LH between normozoospermic and oligozoospermic groups could reflect Leydig cell compensation or a change in hypothalamic - pituitary - gonadal axis regulation as has been reported in men with low sperm production [15]. Though the level of testosterone was found to have a significant global difference, the lack of significant pair-wise differences indicates that there was subtle, diffuse hormonal changes and not overt androgen deficiency.

B. Afamin and Vitamin E Variations in Serum and Seminal Plasma

The strongest differences in this analysis were found on seminal afamin and seminal vitamin E which had the highest Kruskal - Wallis H values. The significance of the local seminal antioxidant environment has been highlighted at the expense of systemic circulation. Afamin as a particular vitamin E transporting molecule in extravascular fluids has been suggested to control vitamin E bioavailability in the male reproductive tract [12].

Important pairwise differences between serum afamin of asthenoteratozoospermic, asthenozoospermic, and normozoospermic men indicate that the systemic antioxidant transportation mechanisms could be sensitive to the defects of sperm motility and morphology. On the same note, low levels of seminal afamin in OAT and oligozoospermic relative to normozoospermic controls confirm the earlier results that a defective spermatogenesis is linked to low seminal antioxidant activity [16].

There was a significant difference in vitamin E levels especially in seminal plasma among different semen phenotypes. The reduced seminal vitamin E of asthenozoospermic and oligozoospermic men is in line with the literature that has reported the depletion of lipid-soluble antioxidants in the face of high oxidative stress [17], [18]. These results confirm the idea of the antioxidant redistribution, as the more oxidative burden one has the more he consumes vitamin E locally or transports it insufficiently to semen plasma.

C. Association of oxidative biomarkers afamin and vitamin E with hormonal profile

The present study is demonstrate the phenotype – specific communication between the fertility hormones and antioxidant biomarkers, especially afamin and vitamin E, through highlighting of the complicated relationship between endocrine regulating and oxidative balance in the male fertility.

DOI: 10.69946/ojms/2026.04.01.03, pp(22-26)

The findings indicated that this interaction was preserved under physiological condition but it becomes dysregulated in pathological semen characteristics.

In normozoospermia there were strong positive correlations observed between LH and serum afamin, LH seminal vitamin E and testosterone with seminal vitamin E. which indicate preserved endocrine – antioxidant coordination in normal reproductive physiology. The adequate of LH stimulation supports Leydig cell function as well as testosterone production, which may promote antioxidant defense system within the reproductive tract. Oxidative stress is a major cause of impaired male reproductive health due to its interference with the hypothalamic-pituitary-gonadal (HPG) axis. This interference can be either direct or indirect by interacted with the hormonal crosstalk between different endocrine pathways, which ultimately inhibits the vital processes of sperm development and maturation [19]. This support the results observed in the positive relationship between testosterone and seminal vitamin E in the present study. Furthermore, the positive association between LH and afamin may reveal the hormonally regulated of antioxidant transport due to afamin considered as a vitamin E binding glycoprotein involved in the systemic antioxidant distribution.

In contrast, asthenozoospermic men exhibited a distinct pattern. Which characterized by the positive correlation between LH and seminal afamin, also a negative correlation between LH and seminal vitamin E. Asthenozoospermia is widely linked with increased of the oxidative stress and lipid peroxidation, which primarily affects sperm motility [20]. Vitamin E is a lipid soluble antioxidant agent, which was consumed during membrane protection against the damage caused by ROS [7]. Therefore, the negative correlation observed in LH and seminal vitamin E may reflect the increased of antioxidant utilization under the oxidative conditions. Meanwhile, the afamin elevated in relation with LH may represent the compensatory response in aimed to maintaining the antioxidant transport capacity. Recent studies have indicated that afamin is a dynamic oxidative stress marker rather than a passive carrier protein [21], which was supported the interpretation that its increase can be indicative of adaptive redox control.

In the OAT patients, the elevation of FSH is generally indicative of spermatogenesis impairment. The relationship between FSH and seminal afamin was negative, which indicated that the systemic depletion of antioxidants as a result of chronic testicular dysfunction. In oligozoospermia, the negative association between the testosterone and seminal afamin may suggest altered of the androgen with antioxidant interaction in the quantitative of sperm deficiency.

Overall, this result was supported the concept that afamin and vitamin E participate in the oxidative-endocrine network of the male reproductive system. The significant correlation between LH and seminal afamin in asthenozoospermia was represented a notable findings and may indicate that compensatory endocrine – antioxidant axis. Further study with the larger cohorts sample are required to confirm these relationship clarify the underline mechanisms.

D. Clinical and Biological Implications

Collectively, these findings suggest that antioxidant imbalance in male infertility is systemic and local with seminal plasma changes being more sensitive markers of semen impairment. The high discriminatory ability of seminal afamin and vitamin E indicates that these two can be used as complementary biomarkers to measure the oxidative stress - related sperm dysfunction. Their

DOI: 10.69946/ojms/2026.04.01.03, pp(22-26)

phenotype specific variation also substantiates the clinical significance of antioxidant assessment of semen and not serum measurements.

E. Study Limitations

Although this study has its strengths, it is constrained by the cross-sectional design that does not allow making causal inferences. Also, the markers of oxidative stress were not directly measured, but calculated indirectly because of the levels of antioxidants. Longitudinal studies to include direct oxidative stress indices and assisted reproductive outcomes are recommended in the future to further elucidate the mechanistic and clinical role of the afamin - vitamin E role in male infertility.

5. Conclusion

This results shows that there are major differences in hormonal and antioxidant profiles of various semen phenotypes. The strongest differences were observed with seminal afamin and vitamin E, which supports the significance of the local seminal antioxidant environment of male infertility. Although FSH, LH and testosterone differed across groups, prolactin did not. These results indicate that afamin and vitamin E could be involved in the regulation of the oxidative balance and spermatogenic activity. Seminal afamin and vitamin E may be used to complement biomarkers of oxidative stress - related sperm dysfunction, and should be further investigated.

Acknowledgment

We would like to be grateful the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University.

Funding

This project received no funding.

References

[1] Agarwal, A., Baskaran, S., Parekh, N., Cho, C.-L., Henkel, R., Vij, S., Arafa, M., Panner Selvam, M. K., & Shah, R. (2021). Male infertility. *The Lancet, 397*(10271), 319–333. https://doi.org/10.1016/S0140-6736(20)32667-2

[2] Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Jolles, M., Pinotti, R., & Swan, S. H. (2022). Temporal trends in sperm count: A systematic review and meta-regression analysis of samples collected globally in the 20th and 21st centuries. *Human Reproduction Update, 29*(2), 157–176. https://doi.org/10.1093/humupd/dmac035

[3] Leslie, S. W., Siref, L. E., & Khan, M. A. (2023). Male infertility. *StatPearls Publishing*. https://www.ncbi.nlm.nih.gov/books/NBK562258/

[4] Li, L., Lin, W., Wang, Z., Huang, R., Xia, H., Li, Z., Deng, J., Ye, T., Huang, Y., & Yang, Y. (2024). Hormone regulation in testicular development and function. *International Journal of Molecular Sciences, 25*(11), Article 5805. https://doi.org/10.3390/ijms25115805

[5] Singla, B. (2025). Role of follicle-stimulating hormone (FSH) and testosterone in abnormal sperm parameters: A review article. *Medical Research Archives*, 13(11). https://doi.org/10.18103/mra.v13i11.7075

[6] Rashki Ghaleno, L., Alizadeh, A., Drevet, J. R., Shahverdi, A., & Valojerdi, M. R. (2021). Oxidation of sperm DNA and male infertility. *Antioxidants, 10*(1), Article 97. https://doi.org/10.3390/antiox10010097

[7] Ahmed, A. E., Alshehri, A., Al-Kahtani, M. A., Eldin, S., Alshehri, M. A., Shati, A. A., Alfaifi, M. Y., Al-Doais, A. A., Taha, R., Morsy, K., & El-Mansi, A. A. (2020). Vitamin E and selenium administration synergistically mitigates ivermectin and doramectin-induced testicular dysfunction in male Wistar albino

rats. *Biomedicine & Pharmacotherapy, 124*, Article 109841. https://doi.org/10.1016/j.biopha.2020.109841

[8] Agarwal, A., Majzoub, A., Baskaran, S., Panner Selvam, M. K., Cho, C. L., Henkel, R., Finelli, R., Leisegang, K., Sengupta, P., Barbarosie, C., Parekh, N., Alves, M. G., Ko, E., Arafa, M., Tadros, N., Ramasamy, R., Kavoussi, P., Ambar, R., Kuchakulla, M., . . . Shah, R. (2020). Sperm DNA fragmentation: A new guideline for clinicians. *The World Journal of Men's Health, 38*(4), 412–471. https://doi.org/10.5534/wjmh.200128

[9] de Ligny, W., Smits, R. M., Mackenzie-Proctor, R., Jordan, V., Fleischer, K., de Bruin, J. P., & Showell, M. G. (2022). Antioxidants for male subfertility. *Cochrane Database of Systematic Reviews, 2022*(5), Article CD007411. https://doi.org/10.1002/14651858.cd007411.pub5

[10] Ma, Y. L., Li, Z. W., Wang, Z., & Yang, A. J. (2024). Afamin ameliorates testosterone propionate (TP)-induced oxidative stress and mitochondrial damage in human ovarian granulosa tumor cells (KGN) by upregulating the expression of SIRT1. *Molecular Biology, 58*(6), 1250–1267. https://doi.org/10.1134/s0026893324060074

[11] Liu, J., Huang, X., Cao, X., Feng, X., & Wang, X. (2020). Serum biomarker analysis in patients with premature ovarian insufficiency. *Cytokine, 126*, Article 154876 https://doi.org/10.1016/j.cyto.2019.154876

[12] Nuñez-Calonge, R., Cortes, S., Caballero Peregrín, P., Gutierrez Gonzalez, L. M., & Kireev, R. (2021). Seminal plasma and serum afamin levels are associated with infertility in men with oligoasthenoteratozoospermia. *Reproductive Sciences, 28*(6), 1665–1673.

[13] https://doi.org/10.1007/s43032-020-00436-8

[14] Eisenberg, M. L., Esteves, S. C., Lamb, D. J., Hotaling, J. M., Giwercman, A., Hwang, K., Cheng, C. Y., Gazzano, G., & Ebner, T. (2023). Male infertility. *Nature Reviews Disease Primers, 9*(1), Article 49. https://doi.org/10.1038/s41572-023-00459-w

[15] Esteves, S. C., Zini, A., Coward, R. M., Evenson, D. P., Gosálvez, J., Lewis, S. E. M., Agarwal, A., Humaidan, P., & Alviggi, C. (2020). Sperm DNA fragmentation testing: Summary evidence and clinical practice recommendations. *Andrologia, 53*(2), Article e13874. https://doi.org/10.1111/and.13874

[16] Moretti, E., & Signorini, C. (2024). Antioxidants in male and animal reproduction: Applications and critical issues. *Antioxidants, 13*(11), Article 1283. https://doi.org/10.3390/antiox13111283

[17] Agarwal, A., Rana, M., Qiu, E., AlBunni, H., Bui, A. D., & Henkel, R. (2018). Role of oxidative stress, infection and inflammation in male infertility. *Andrologia, 50*(11), Article e13126. https://doi.org/10.1111/and.13126

[18] Moretti, E., Signorini, C., Corsaro, R., Giamalidi, M., & Collodel, G. (2023). Human sperm as an in vitro model to assess the efficacy of antioxidant supplements during sperm handling: A narrative review. *Antioxidants, 12*(5), Article 1098. https://doi.org/10.3390/antiox12051098

[19] Manocha, A., Kankra, M., Singla, P., Sharma, A., Ahirwar, A. K., & Bhargava, S. (2018). Clinical significance of reproductive hormones. *Current Medicine Research and Practice, 8*(3), 100–108. https://doi.org/10.1016/j.cmrp.2018.05.006

[20] Lahimer, M., Mustapha, H., Bach, V., Khorsi-Cauet, H., Benkhalifa, M., Ajina, M., & Ali, H. B. (2023). Oxidative stress in male infertility and therapeutic approach: A mini-review. *Asian Pacific Journal of Reproduction, 12*(6), 249-255. DOI: https://doi.org/10.4103/2305-0500.390299

[21] Mo, L., Wu, H., Zhang, M., Zhang, P., Peng, W., He, Y., & Gao, F. (2025). The mechanism of oxidative stress in asthenozoospermia and antioxidant strategies: A review. *Frontiers in Endocrinology, 16*, Article 1670762.

[22] Zhang, Q., Zheng, X., Zhang, X., & Zheng, L. (2024). Protective effect of afamin protein against oxidative stress related injury in human ovarian granulosa cells. *Journal of Ovarian Research*, *17*(1), Article 189. https://doi.org/10.1186/s13048-024-01511-3 .