

Addressing Complete Separation in Male Infertility Risk Factor Modeling: A Firth Logistic Regression of BMI and Vitamin E

Noor Kamil Hussein Al-Mayahy *, Mohammed Talib Al-Tameemi

Department of Medical Biotechnology, College of Biotechnology, Al-Qasim Green University, 51013 Al-Qasim, Babylon, Iraq.

* Corresponding author: noorkamil@biotech.uoqasim.edu.iq

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Abstract

Background: About 7% of men are affected by male infertility, up to 50% of cases remaining idiopathic. Proposed risk factors such as elevated BMI and reduced vitamin E show inconsistent evidence. Standard logistic regression often fails against Quasi-complete separation, a common statistical issue in case-control studies, and no data exist from Iraqi populations. **Objective:** To assess the independent associations of BMI and serum vitamin E with idiopathic male infertility using Firth's penalised logistic regression to overcome separation. **Materials and Methods:** This case control study included 160 men (80 infertile men, 80 controls) from Najaf, Iraq. BMI was recorded, and serum vitamin E was measured by a validated colorimetric assay. Firth's logistic regression was applied. **Results:** Infertile men have significantly increased BMI compared to control (median 28.6 vs. 25.9 kg/m²; $p < 0.001$), and decreased levels of vitamin E (median 11.4 vs. 12.5 µg/mL; $p < 0.001$). Each 1-kg/m² increase in BMI increased odds for infertility OR=2.53; 95% CI, 1.89–3.61; $p < 0.001$, and each 1-µg/mL increase of vitamin E decreased odds by 61% (OR=0.39; 95% CI, 0.24–0.60; $p < 0.001$). The model demonstrated to have an excellent discrimination (AUC=0.912; 95% CI, 0.869-0.955) and good calibration (Hosmer-Lemeshow $p = 0.586$). **Conclusions:** Higher BMI and low vitamin E are independently associated with male infertility. Firth's regression effectively resolved quasi complete separation. BMI may be considered a useful screening tool in routine andrological assessment, while vitamin E's therapeutic role requires further study.

Keywords: Male infertility, Body mass index, α -tocopherol, Vitamin E, Firth logistic regression; Quasi-complete separation; ROC curve

Introduction

Male infertility is an important global health problem, accounting for almost half of all cases of couple infertility and affecting an estimated 7% of men, with up to 50% of male factor cases remaining idiopathic despite extensive clinical workups.[1] This persistent diagnostic gap points to an urgent clinical need to identify modifiable risk factors to inform prevention and targeted interventions. Among these, elevated body mass index (BMI) and low serum vitamin E (α -tocopherol) have become particularly promising candidates due to their strong biological plausibility and global epidemiological signifi-

cance. [2–4] Obesity negatively influences spermatogenesis by disrupting the hypothalamic pituitary gonadal axis, inducing scrotal hyperthermia, promoting systemic inflammation and altering sperm proteomic profiles. [4,5] In contrast, vitamin E is the most important chain-breaking antioxidant that is lipid soluble and plays an important role in protecting sperm membranes from peroxidative injury [6,7] Current systematic reviews have shown a significant link between increased BMI and declining sperm quality and hormonal status, [3] while lifestyle modifications have shown modest improvements in sperm motility and morphology

but inconsistent effects on conception outcomes. [8] Similarly, meta-analyses of antioxidant supplementation including selenium and vitamin E show biochemical improvements in sperm parameters, but inconclusive evidence for clinical endpoints such as pregnancy or live birth. [9,10] Despite strong mechanistic underpinnings, the clinical evidence base is highly inconsistent. Observational studies frequently demonstrate significant protective or detrimental associations; however, large-scale interventional trials, including the landmark MOXI randomized controlled trial, have largely failed to demonstrate meaningful benefits of antioxidant supplementation on conventional semen parameters. [11] Likewise, meta-analyses examining lifestyle-induced weight reduction have often shown that BMI decreases do not reliably translate into improved spermatogenesis, suggesting that these relationships are non-linear, confounded by population heterogeneity, or masked by methodological limitations. [4,8] Critically, a major yet frequently overlooked statistical issue in case-control studies is quasi-complete separation, a phenomenon occurring when predictors perfectly classify the outcome, causing standard logistic regression to yield infinite, unstable, and non-interpretable estimates. Firth's penalised maximum likelihood approach offers a robust correction, providing finite and asymptotically unbiased estimates, yet its application in andrological research is virtually non-existent [12,13]. Furthermore, a pronounced geographical gap persists, with the overwhelming majority of evidence derived from Western populations and data from Iraqi cohorts remain relatively scarce, where distinct dietary habits and environmental exposures may modulate both adiposity and antioxidant status. Nevertheless, a limited number of previous local studies have explored these variables, highlighting the need for more rigorously designed

investigations in this population. To bridge these multifaceted gaps, we conducted a rigorously controlled case-control study evaluating the independent associations of BMI and serum vitamin E with idiopathic male infertility in a well-characterized Iraqi population. By employing Firth's penalised logistic regression to overcome statistical separation, we aimed to derive stable, clinically interpretable odds ratios and provide methodologically robust evidence to enhance andrological risk stratification and guide future interventional strategies in this understudied population.

Materials and Methods

Study Design and Population

A total of 160 men were enrolled and allocated into two equal groups: 80 patients with idiopathic male infertility (case group) and 80 fertile men (control group) This case-control study was conducted at the Fertility and Infertility Prevention Center, Al-Sadr Medical City, Najaf, Iraq, between November 2025 and February 2026. The study protocol was approved by the Institutional Ethics Committee of AL-Qasim Green University (approval no. Qgec/21/2025). All procedures were performed in accordance with the principles of the Declaration of Helsinki, and written informed consent was obtained from each participant before enrolment.

Participant Selection and Inclusion/Exclusion Criteria

Infertile group: Men were diagnosed with idiopathic infertility after failing to achieve pregnancy after 12 months of regular unprotected intercourse, combined with normal physical examination, endocrine profiles, and standard semen analyses that did not identify a clear cause. Oligozoospermia, asthenozoospermia, and teratozoospermia were defined

according to the criteria of the WHO Laboratory Manual for the Examination and Processing of Human Semen (6th edition, 2021).[1]

Control group: Healthy, fertile men with proven paternity within the last 1-2 years were included. Exclusion criteria for all participants: Azoospermia or obstructive infertility; clinical varicocele or genital tract infection; history of hormonal therapy, chemotherapy, or radiotherapy; known genetic or endocrine disorders; major systemic diseases (diabetes, cardiovascular disease, chronic renal failure); use of antioxidant supplements or specific medications within the previous 3–6 months; any history of smoking (cigarettes, e-cigarettes, waterpipes), alcohol consumption, or occupational toxin exposure. All participants were non-smokers, had no history of radiation or urogenital surgery, and were fasting when blood was drawn

Anthropometric Measurement

BMI was calculated using the standard weight in kilograms divided by height in meters squared formula. Weight was measured to the nearest 0.1 kg using a calibrated digital scale, and height to the nearest 0.1 cm using a wall mounted stadiometer, with participants in light clothing and barefoot.

Blood Collection and Serum Preparation

On the same day as the semen analysis, we drew approximately 5 mL of venous blood from the antecubital vein into plain serum gel tubes. The blood was left to clot at room temperature for 30 minutes, then centrifuged at 3000×g for 10 minutes. We aliquoted the separated serum into sterile tubes and stored them at -80 °C until biochemical analysis. All assays were run in duplicate.

Serum Vitamin E Measurement

Serum vitamin E (α -tocopherol) concentration was quantified using a commercially available colorimetric assay kit (Elabscience® Vitamin E (VE) Colorimetric Assay Kit, Cat. No. E-BC-K033-S). The assay principle is based on the reduction from ferric (Fe^{3+}) to ferrous (Fe^{2+}) by vitamin E in the presence of ferroin, followed by reaction with phenanthroline to form a pink complex measured spectrophotometrically at 532 nm. The detection range of the kit is 0.09–40 $\mu\text{g/mL}$, and the analytical sensitivity is 0.09 $\mu\text{g/mL}$. The intra-assay and inter-assay coefficients of variation were <5.2% according to the manufacturer's instructions. All measurements were performed according to the protocol provided by the manufacturer.

Statistical Analysis

Data were analysed using SPSS (version 27.0) and JASP (version 0.19.1). Normality of continuous variables was assessed using the Shapiro–Wilk test. Baseline characteristics between groups were compared using the Mann–Whitney U test because most variables showed non-normal distribution. Data are presented as Median (IQR). To examine the independent associations of BMI and serum vitamin E with male infertility, we initially attempted standard binary logistic regression including age, BMI, and vitamin E. However, the model suffered from quasi-complete separation, causing non-convergence of the maximum likelihood algorithm. To overcome this issue, we applied Firth's penalised maximum likelihood logistic regression (Firth logistic regression), which introduces a Jeffreys prior penalty term to the likelihood function, guaranteeing finite and stable parameter estimates even in the presence of separation. [12,13] Age was excluded from the final model because it was not significantly different between groups and its inclusion

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contributed to convergence failure. The final model included BMI and serum vitamin E as independent variables, with infertility status (0 = control, 1 = patient) as the dependent variable. Results are reported as regression coefficients (B), odds ratios (OR) with 95% confidence intervals (CI), and p-values. Model performance was assessed using the Cox & Snell R^2 , Nagelkerke R^2 , Hosmer–Lemeshow goodness of-fit test, overall classification accuracy, and the area under the receiver operating characteristic (ROC) curve (AUC). An AUC > 0.90 was considered indicative of excellent discriminative performance [14]; however, it is important to acknowledge that the presence of quasi-complete separation in the initial unpenalized model may partially inflate the discriminative estimates. Therefore, while the AUC of 0.912 is promising, this value should be interpreted with caution and validated in future external cohorts. All statistical tests were two-sided, and a p-value < 0.05 was considered statistically significant.

Results:

Participant characteristics

A total of 160 men (80 controls and 80 patients with infertility) were included in the analysis. Table 1 summarizes the baseline characteristics of the two groups. The groups were comparable with respect to age, with no statistically significant difference (Mann-Whitney $U = 3179.5$, $p = 0.944$). In contrast, patients had a significantly higher body mass index (BMI) than controls (median 28.6 [IQR: 27.3–29.9] vs. 25.9 [IQR: 24.3–27.3] kg/m^2 ; Mann-Whitney $U = 852.0$, $p < 0.001$). Likewise, serum vitamin E levels were significantly lower in the patient group (median 11.4 [IQR: 10.4–12.2] vs. 12.5 [IQR: 11.4–13.7] $\mu\text{g/mL}$; Mann-Whitney $U = 1593.0$, $p < 0.001$).

Table 1. Demographic and clinical characteristics of the study participants.

Variable	Control (n = 80)	Patient (n = 80)	p-value*
Age (years)	34.0 (29.3 – 38.8)	34.0 (30.0 – 39.0)	0.944
BMI (kg/m^2)	25.9 (24.3 – 27.3)	28.6 (27.3 – 29.9)	< 0.001
Vitamin E ($\mu\text{g/mL}$)	12.5 (11.4 – 13.7)	11.4 (10.4 – 12.2)	< 0.001

* p-values were calculated using the Mann-Whitney U test. Data are presented as median (interquartile range, IQR), BMI: body mass index.

Logistic regression analysis

Because the initial logistic regression model that included age, BMI and vitamin E suffered from quasi-complete separation (the model failed to converge), we applied Firth's penalised maximum likelihood logistic regression a method specifically designed to provide stable estimates in the presence of separation. Age was not retained in the final model because it was not significantly different between groups and its inclusion prevented convergence. The final model therefore included only BMI and vitamin E as independent predictors of infertility status (0 = control, 1 = patient). The results of the Firth logistic regression are presented in Table 2. BMI showed a strong positive association with infertility: each one-unit increase in BMI (kg/m^2) was associated with a 2.53 fold higher odds of being in the patient group (OR = 2.53; 95% CI, 1.89–3.61; $p < 0.001$). Conversely, vitamin E exhibited a protective effect. For every 1 $\mu\text{g/mL}$ increment in serum vitamin E, the odds of infertility decreased by approximately 61% (OR = 0.39; 95% CI, 0.24–0.60; $p < 0.001$). The model explained a substantial proportion of the variance: Nagelkerke $R^2 = 0.630$ and Cox & Snell $R^2 = 0.472$. The Hosmer-Lemeshow goodness of fit test showed no evidence that the fit was poor ($\chi^2 = 6.553$, $\text{df} = 8$, $p = 0.586$). Overall classification accuracy was 81.9%.

Table 2. Result of Firth logistic regression model for infertility (Control=Base group).

Variable	B	SE	OR	95% CI for OR	p-value
BMI	0.927	0.161	2.53	(1.89 – 3.61)	< 0.001
Vitamin E	−0.935	0.232	0.39	(0.24 – 0.60)	< 0.001
Constant	−14.06	4.77	–	–	0.002

B: regression coefficient; SE: standard error; OR: odds ratio; CI: confidence interval.

Model discrimination – ROC analysis

We constructed a receiver operating characteristic (ROC) curve using the predicted probabilities derived from the logistic model. To evaluate the ability of the combined BMI vitamin E model to discriminate between infertile patients and controls. The area under the ROC curve (AUC) was 0.912 (95% CI, 0.869–0.955; $p < 0.001$) see Figure 1. This AUC value indicates excellent discriminative performance: the model correctly distinguishes infertile from fertile men in more than 91% of random pairs.

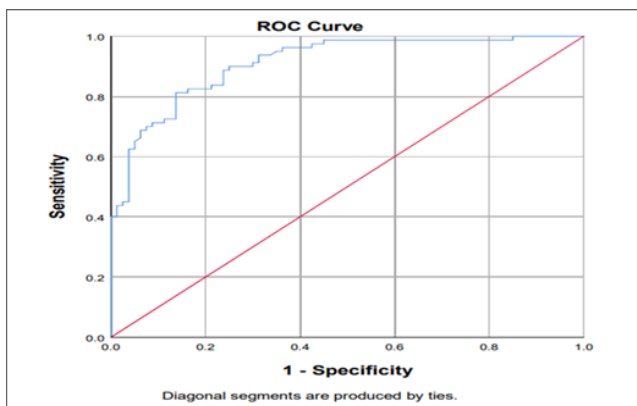


Figure 1: Receiver operating characteristic (ROC) curve of the logistic regression model including both BMI and vitamin E for predicting infertility (Male fertility status: 0 = Control, 1 = Patient). The area under the curve (AUC) is 0.912 (95% CI: 0.869–0.955, $p < 0.001$), indicating excellent discriminative ability. AUC: area under the curve; CI: confidence interval.

Discussion

This case-control study reports an independent association between body mass index (BMI) and serum vitamin E (α -tocopherol) in idiopathic male infertility among a defined Iraqi population.

One methodologically unique aspect of this study was the fact that conventional binary logistic regression encountered quasi-complete separation and therefore, Firth's penalized maximum likelihood was used to derive stable, interpretable estimates. The final model achieved a Nagelkerke R^2 of 0.630, an overall classification accuracy of 81.9%, and an area under the receiver operating characteristic curve (AUC) of 0.912 which all indicated excellent discriminative performance collectively. We do discuss our more substantive findings; a higher BMI and lower serum vitamin E levels in infertile men versus controls, relative to published literature, both supporting and contradicting evidence for these conditions.

Methodological Considerations: Addressing Quasi-Complete Separation in the Context of Clinical Risk Factor Modeling

Before interpreting the independent associations of body mass index (BMI) and serum vitamin E (α -tocopherol) with idiopathic male infertility, it is essential to address a statistical issue that arose during the initial model fitting process. When we attempted to fit a standard binary logistic regression model that included age, BMI, and vitamin E as independent predictors of infertility status, the model failed to converge and produced unstable, non-interpretable estimates. This failure was attributable to a phenomenon known as quasi-complete separation, which occurs when a linear combination of predictors perfectly, or nearly perfectly, classifies the binary outcome variable. In such circumstances, the standard maximum-likelihood estimation algorithm attempts to estimate regression coefficients that tend toward infinity and the model therefore fails to produce finite, reliable parameter estimates. This issue is particularly common in clinical datasets with small to moderate sample sizes and highly discriminating

predictors, as was the case in the present study, where the strong between-group differences in both BMI and serum vitamin E individually and jointly created the conditions for quasi-complete separation [12,13]. To overcome this methodological challenge, we applied Firth's penalized maximum likelihood logistic regression, a bias-reduction method first proposed by Firth [12] and subsequently advocated and refined by Heinze and Schemper [13] for use in logistic regression models affected by separation. The method introduces a Jeffreys prior as a penalty term to the log-likelihood function, which effectively shrinks the parameter estimates toward zero and guarantees finite, asymptotically unbiased coefficient estimates even under conditions of complete or quasi-complete separation. This approach yields considerably less biased estimates than conventional maximum-likelihood estimation in small to moderate sample sizes and provides more reliable profile penalized likelihood confidence intervals compared with standard Wald-based intervals [13]. The practical application of this penalized approach in clinical and preclinical settings has been further illustrated in recent methodological tutorials, which provide step-by-step guidance on how to implement Firth's logistic regression to obtain stable estimates when separation occurs [15]. Importantly, this methodological choice was not merely a technical adjustment; it directly enabled us to derive stable and clinically interpretable odds ratios for BMI and vitamin E—the central aim of our study. Without the application of this penalized approach, the strength and direction of the associations we observed could not have been reliably estimated, and the clinical conclusions drawn from the model would have been invalid. The appropriateness and success of this methodological approach in the present study are supported by several model perform-

ance indicators. The Hosmer Lemeshow goodness of fit test yielded a χ^2 value of 6.553 with 8 degrees of freedom and a p-value of 0.586, indicating that the predicted probabilities derived from the Firth-corrected model were well calibrated to the observed outcomes across all decile groups. There was therefore no evidence of poor model fit. Furthermore, the area under the receiver operating characteristic (ROC) curve was 0.912 (95% confidence interval: 0.869–0.955), exceeding the conventional threshold of 0.90, which is widely considered indicative of excellent discriminative performance [14]. These findings confirm that the Firth-corrected model provides valid, stable, and clinically useful inferences, while also demonstrating that the two-predictor model (BMI and vitamin E) possesses substantial practical utility in distinguishing between infertile and fertile men. It is also important to justify the exclusion of age from the final regression model. Age was initially included as a potential confounder; however, the two study groups were closely matched with respect to age, with no statistically significant difference observed between infertile patients and fertile controls (Mann-Whitney U = 3179.5, p = 0.944). The inclusion of age in the initial standard logistic regression model contributed to the convergence failure, and given the absence of a meaningful age difference between groups, retaining age in the final model would have introduced unnecessary complexity without improving model fit or predictive performance. The decision to exclude age from the final model is therefore consistent with the principle of parsimony in clinical prediction modeling and is supported by the findings of similar case-control studies that have also reported homogeneous age distributions between infertile and fertile male cohorts. This design strength enhances the interpretability of the regression coefficients for BMI and vitamin E

and strengthens the internal validity of the study. In summary, the transparent reporting and appropriate resolution of quasi-complete separation through the application of Firth's penalized logistic regression represent a key methodological contribution of the present study. This approach not only resolved a critical statistical issue but also enabled us to produce stable, unbiased, and clinically interpretable estimates of the associations between BMI, vitamin E, and idiopathic male infertility, thereby directly serving the primary aim of the investigation.

BMI and Male Infertility: Supporting and Contradictory Evidence

In this study, the infertile patient group had a statistically significantly higher mean BMI ($28.60 \pm 1.66 \text{ kg/m}^2$) than the fertile controls ($25.93 \pm 1.62 \text{ kg/m}^2$; $p < 0.001$). The Firth logistic regression model showed that for every unit increase in BMI there was a 2.53 fold increased odds of being in the infertile group (OR = 2.53; 95% CI, 1.89-3.61), a strong and clinically relevant association. These results are consistent with a large and growing body of literature implicating excess adiposity as a modifiable determinant of male reproductive dysfunction. A large meta-analysis of 14 studies comprising 8,443 participants confirmed that increased BMI is significantly associated with impaired sperm quality in multiple parameters including normal morphology, total and progressive motility, sperm concentration, volume, and total sperm count, as well as alterations in sex hormone profiles including total testosterone, FSH, and LH.[3] A seminal population-based study by Jensen et al. [2] Demonstrated that overweight men (BMI 25-29.9) had 20% greater odds of infertility (OR = 1.20; 95% CI, 1.04-1.38), and obese men (BMI 30-34.9) had 36% greater odds (OR = 1.36; 95%

CI, 1.13-1.63) compared to men with low-normal BMI, after adjusting for multiple confounders. These findings are generally in concordance with the considerably stronger association observed in the current study, although the magnitude of the odds ratio reported here (OR = 2.53) is larger, a difference that may partly reflect the more homogeneous, strictly controlled case definition used in the present study (idiopathic infertility with detailed exclusion of confounding pathologies) and the narrower BMI range across groups. Obesity does not affect male fertility through a single pathway; rather, it disrupts multiple biological systems at once, and these systems interact with each other in ways that collectively impair reproductive function. The Service et al.[4] systematic review in Fertility and Sterility identified three principal pathways: (1) hypothalamic pituitary gonadal (HPG) axis disruption via leptin resistance and hyperleptinemia, which suppresses Leydig cell testosterone synthesis; (2) increased peripheral aromatisation of androgens to oestrogens within excess adipose tissue, leading to secondary hypogonadism; and (3) localised scrotal heat stress from increased adipose padding, which impairs thermoregulation of the testicular microenvironment. Pini et al [17], in a proteomics-based study, further identified that sperm from obese men exhibited alterations in proteins involved in oxidative stress, inflammation, DNA damage repair, and translation regulation, underlining the broad molecular impact of adiposity on spermatogenesis. More recently, a multi-decade retrospective study by Santi et al. [18] demonstrated that obesity had a detrimental impact on the temporal decline of sperm quality in normospermic men, suggesting that adiposity-related impairment is both cross-sectional and longitudinal in nature. Notwithstanding the above, the evidence relating BMI to semen

parameters is not universally consistent. Li et al. [16] performed a systematic review and meta-analysis and found heterogeneity among the studies, with some studies failing to report significant associations between BMI and the traditional semen parameters in multivariate models. Wang et al. [19] performed a retrospective study and found only weak correlations between body mass index (BMI) and progressive motility ($r = 0.076$, $p < 0.05$), and between waist-to-hip ratio and semen parameters, pointing to a potential non-linear association of adiposity with fertility whereby associations with body fat distribution may be greater than total adiposity as measured objectively by BMI alone. A systematic review and meta-analysis by Peel et al. [8] investigated the impact of obesity interventions on male fertility and reported a mean difference (MD) of 1.29 million/mL (95% CI: -1.03 to 3.61; $p = 0.26$) in sperm concentration between intervention and control groups, suggesting that short-term weight loss does not consistently translate into improved spermatogenesis. A critical methodological consideration in reconciling these discrepancies relates to study population heterogeneity. Studies that include men with identified etiologies of infertility (varicocele, hormonal disorders, genetic anomalies) alongside idiopathic cases may dilute the predictive signal of BMI, whereas the strict exclusion criteria applied in the present study which removed all men with known comorbidities, substance use, radiation exposure, and medication use may have amplified the BMI infertility association by reducing residual confounding. The exclusive focus on idiopathic infertility, in which the proximal cause is unidentified and systemic metabolic disturbances are therefore more likely to be operative, is consistent with the observed effect size.

Serum Vitamin E and Male Infertility

In our study, infertile men had significantly lower mean serum vitamin E concentrations ($11.35 \pm 0.96 \mu\text{g/mL}$) than fertile controls ($12.49 \pm 1.22 \mu\text{g/mL}$; Mann Whitney $U = 1593.0$, $p < 0.001$). In the Firth logistic regression model, a $1 \mu\text{g/mL}$ increase in serum vitamin E corresponded to an odds ratio of 0.39 (95% CI: 0.24–0.60), indicating a strong inverse statistical association with infertility status. This association should be interpreted as correlational rather than causal, given the cross-sectional design of the study. These results support the known role of α -tocopherol as the main lipid-soluble, chain-breaking antioxidant in biological membranes and as an important factor to keep sperm membrane integrity. The theoretical basis for vitamin E's protective role in male reproduction is well-characterised. Spermatozoa are uniquely vulnerable to reactive oxygen species (ROS)-mediated peroxidative damage owing to the exceptionally high polyunsaturated fatty acid (PUFA) content of their plasma membranes, which are required for the membrane fluidity essential to capacitation and the acrosome reaction. Kaltsas [7] reviewed evidence that elevated ROS in seminal plasma impairs sperm motility, morphology, and DNA integrity by initiating lipid peroxidation cascades, and that α -tocopherol acts by interrupting these cascades at the membrane level. Additionally, Dutta et al. [6], review published in Antioxidants, which supports the positive correlation of higher seminal vitamin E levels with improved sperm motility and that supplementation with α -tocopherol results in a decrease in ROS-mediated sperm DNA fragmentation. The in vitro effect of vitamin E on asthenoteratozoospermic men was quantified by Ghafarizadeh et al. [20] who reported significant improvements in sperm motility and viability, in agreement with an antioxidant mechanism of action. These

mechanistic and observational findings resonate with the classic work of Omu et al. [21], who, using high-performance liquid chromatography, documented that men with normal sperm parameters had significantly higher serum and seminal plasma α -tocopherol levels than men with oligozoospermia and azoospermia, suggesting a dose response relationship between antioxidant status and spermatogenic function. The cross-sectional comparison between the infertile and control groups in the present study essentially reproduces this pattern within a contemporary Iraqi cohort, strengthening the generalisability of the antioxidant-deficiency hypothesis across geographically and ethnically distinct populations. Compelling corroboration also comes from the domain of antioxidant supplementation research. A systematic review by Majzoub and Agarwal[22] reported that α -tocopherol supplementation significantly improved sperm count and reduced sperm DNA damage when administered as part of multi-antioxidant protocols. More recently, a randomised controlled trial analysed by Nguyen et al. [23] demonstrated that a combined micronutrient formulation with 60 mg vitamin E used in infertile men with high baseline DFI values significantly reduced sperm DNA fragmentation index, providing interventional evidence that correcting antioxidant deficiency can translate into measurable improvements in sperm chromatin integrity. A comprehensive narrative review by Çayan and colleagues [24], which synthesized evidence from multiple meta-analyses on antioxidant therapy, reported that vitamin E monotherapy was associated with pregnancy rates 8.17 times higher than placebo, representing the highest subgroup effect among the antioxidant categories evaluated. However, the clinical evidence base also contains notable contradictions that merit critical consideration. Steiner et al.[11], in the Males, Antioxidants, and

Infertility (MOXI) randomised placebo-controlled trial a rigorously designed multi-centre study found no statistically significant differences in sperm concentration, motility, morphology, or DNA quality between infertile men receiving a combined antioxidant supplement (including vitamins C, E, and D, selenium, L-carnitine, zinc, folic acid, and lycopene) and those receiving placebo. Similarly, some other randomised controlled trials have also reported no significant differences in ongoing pregnancy rates between antioxidant and placebo groups, with some even showing numerically lower rates in the antioxidant arm. These null findings indicate that the relationship between antioxidant status and fertility outcome is more complex than a simple deficiency model would suggest.

Several explanations may reconcile the apparent discrepancy between the observational associations reported in the present study and the null findings from some interventional trials. First, it is possible that antioxidant supplementation in men who are not antioxidant-deficient confers no benefit, since the biological effect of vitamin E is most relevant when it corrects a physiologically meaningful deficiency. As explicitly noted by the controversial review of vitamin E efficacy by Greco et al.[25] and affirmed by Saleh et al.[26], antioxidant therapy may be effective only in subpopulations where oxidative stress is a primary or contributing infertility factor a condition more likely to be met in idiopathic cases with demonstrable antioxidant depletion, such as those enrolled in the present study. Second, the MOXI trial and similar interventional studies typically enrolled heterogeneous infertile populations without pre-stratification by baseline antioxidant status, potentially diluting a treatment effect that may be present only in the deficient subgroup. Third, as cautioned by Sengupta et al. [27], supraphysiol-

ological antioxidant supplementation may paradoxically interfere with redox signalling pathways necessary for sperm capacitation and the acrosome reaction, blunting the net benefit. These nuances together strengthen the argument that lower serum vitamin E concentration is a true risk marker for male infertility in the present cohort, while acknowledging that its clinical utility as a therapeutic target needs further stratified inquiry.

Interplay between BMI, Vitamin E and Oxidative Stress

The concurrent identification of elevated BMI and depleted serum vitamin E as independent predictors of male infertility in the present study highlights an important pathophysiological convergence: obesity itself is a pro-oxidative state that may deplete systemic antioxidant reserves, thus creating a compounding risk profile. This hypothesis is supported by several lines of evidence. Recent observational evidence has demonstrated that infertile men with higher BMI have significantly lower total antioxidant capacity (TAC) scores compared to both fertile controls and infertile men with normal BMI, suggesting that adiposity and antioxidant deficiency are co-occurring and potentially mutually reinforcing. Hussain et al. [28] further reviewed evidence that adipocyte-derived pro-inflammatory cytokines notably TNF- α and IL-6 elevate seminal and systemic ROS, thereby increasing the demand for antioxidant neutralisation and potentially depleting circulating α -tocopherol. Furthermore, the observation in the present study that BMI and serum vitamin E contributed independently to the regression model and that their combined AUC (0.912) substantially exceeded what would be expected from either predictor in isolation is consistent with the hypothesis that these variables capture complementary but distinct dimensions of the

reproductive risk phenotype: systemic metabolic dysregulation (BMI) and oxidative defence capacity (vitamin E). This multivariate discriminative capacity reinforces the clinical utility of assessing both measures in the andrological evaluation of idiopathic infertility.

Comparison between the Infertile and Fertile Groups

In direct group comparisons, the infertile cohort in the present study presented with a median BMI of 28.6 (IQR: 27.3–29.9) kg/m², which falls within the overweight category by WHO criteria, whereas the fertile control group had a median BMI of 25.9 (IQR: 24.3–27.3) kg/m², within the healthy weight range. This 2.67 kg/m² inter-group difference, while modest in absolute terms, was highly statistically significant ($p < 0.001$) and, when modelled continuously, generated a markedly elevated odds ratio. The mean serum vitamin E concentration in the infertile group was 11.35 ± 0.96 μ g/mL versus 12.49 ± 1.22 μ g/mL in the fertile group a difference of 1.14 μ g/mL that, in the context of the Firth model, translated to a 61% reduction in the odds of infertility per unit increase. Both comparisons remained statistically significant after the application of the Mann Whitney U test, appropriate given the non-normal distribution of variables as confirmed by the Shapiro Wilk test, demonstrating that the observed differences were robust to the violation of parametric assumptions. Importantly, the two groups were well matched for age (34.94 ± 5.80 years vs. 34.85 ± 5.67 years; $p = 0.944$), eliminating age as a potential confounder in the observed associations. Age related decline in antioxidant capacity and increasing adiposity with advancing age are well recognised phenomena; by ensuring age comparability between groups, the present study strengthens the argument that the observed BMI and vitamin E differences reflect true

fertility-associated variations rather than artifacts of differential age distribution. This design strength supports the interpretability of the regression coefficients and enhances the internal validity of the study.

Strengths, Limitations, and Future Directions

This study has some methodological strengths. Strengths of this study include the strict case definition of idiopathic infertility, comprehensive exclusion criteria, the use of validated commercial assay kits for vitamin E quantification, Firth's bias-reduced logistic regression was used in analysis in the context of model separation, and an appropriate case-control design (1:1 control to case ratio). The final model (AUC = 0.912) was found to be very discriminative, showing that both predictors together provide a clinically relevant screening signal in this population. However, a number of limitations must be recognised. **First**, the cross-sectional case-control study design precludes causal inference; either vitamin E deficiency precedes and contributes to spermatogenic failure, or whether the infertile state itself promotes antioxidant consumption cannot be determined without prospective longitudinal data. **Second**, the investigation was performed at a single tertiary fertility centre based in Najaf, Iraq and the external generalisability of the results to other populations with different dietary patterns, environmental exposures, and genetic backgrounds may be restricted. **Third**, because serum α -tocopherol was determined rather than seminal plasma vitamin E concentrations that may more closely represent the in vivo antioxidant microenvironment of spermatozoa [6], which may help to explain some of the variability in associations found in investigations of vitamin E status. Going forward, seminal antioxidant profiling should be combined with systemic measurements to provide a more

complete description of the redox phenotype of the infertile male. **Fourth**, restricting smoking, alcohol use, and antioxidant supplement use while enhancing internal validity limits generalizability of the sample to a more general clinical population in which these exposures are common. **Fifth**, the information on sperm DNA fragmentation index, oxidative stress biomarkers (malondialdehyde or 8-hydroxy-2'-deoxyguanosine) or hormonal profiles were not included in the study, whereby the observed associations could have been analysed in a more mechanistic perspective. Indeed, prospective studies investigating whether the weight loss interventions that are effective in overweight and obese infertile men translate into significant improvements in serum vitamin E and fertility would give the interventional data necessary to move the observational data to the threshold of clinical practice. Further studies with stratified analyses considering the effect of the degree of adiposity (as a continuous variable) on the association between serum vitamin E and the risk of infertility would provide additional insight into the interaction between these 2 risk factors. These associations require validation in randomised controlled trials of vitamin E supplementation in selected idiopathic infertile men with proven α -tocopherol deficiency rather than unselected infertile populations.

Conclusion

This case-control study provides methodologically robust evidence for independent and significant associations between raised BMI + vitamin E deficiency and idiopathic male infertility in an Iraqi population. Firth's penalised logistic regression was used to appropriately deal with the quasi-complete separation present in the first model, providing estimates that were stable, unbiased, statistically valid and (in contrast to other techniques)

clinically interpretable. The combined BMI-vitamin E model performed very well (AUC = 0.912), in keeping with the international literature that has typically shown excess adiposity and antioxidant deficiency, as distinct but modifiable risk factors in the infertile male. Although conflicting results exist, especially from interventional trials of antioxidant supplementation, these discrepancies are mostly reconcilable with careful attention to the patients described, baseline antioxidant status and important distinctions between observational biomarker associations and drug-like effects. Clinicians assessing men with idiopathic infertility should consider systematic screening of BMI and serum antioxidants in the andrological workup, while specific lifestyle and nutritional interventions require future clinical trials to investigate as potential therapies.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

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