Article

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Discriminative Performance of Semen, Hormonal, and Clinical Parameters for Male Fertility Based on WHO 2021 Reference Values

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Background: The diagnosis of male infertility often relies solely on semen analysis, despite its well-recognized limitations. The clinical utility of the updated World Health Organization (WHO) 2021 reference standards, especially when integrated with other clinical markers, requires validation. This study aimed to evaluate the discriminative performance of semen parameters, testicular volume, and hormonal profiles in differentiating fertile from infertile men using WHO 2021 criteria.

Methods: This retrospective study included 417 men (315 infertile, 102 fertile). All parameters were re-evaluated according to WHO 2021 standards. Receiver operating characteristic (ROC) curve analysis and multivariable logistic regression were performed to identify independent predictors and construct an integrated predictive model.

Results: Fertile men had significantly better semen parameters and larger testicular volume, while infertile men showed higher follicle-stimulating hormone (FSH) and prolactin, and lower testosterone ($p < 0.05$). Sperm concentration (area under the curve [AUC] = 0.984) and morphology (AUC = 0.913) showed the strongest discrimination. Multivariable analysis identified sperm concentration, morphology, mean testicular volume, and FSH as independent predictors of fertility status. The combined model demonstrated excellent performance (AUC = 0.994; 95% confidence interval [CI]: 0.988–0.999), confirmed by Bootstrap internal validation (optimism-corrected AUC = 0.992). Sensitivity analyses showed high discriminative power across all phenotypes, including normozoospermic infertility (AUC = 0.921).

Conclusion: Sperm concentration and morphology remain the most robust indicators of male fertility within the WHO 2021 framework. The integration of clinical, hormonal, and semen parameters improved predictive accuracy and supports a multidimensional approach to the evaluation of male infertility, while confirming the applicability of WHO 2021 reference standards in this population.

Keywords: male infertility; semen analysis; WHO 2021; hormone profile; testicular volume

Introduction

Couples are generally diagnosed with infertility when pregnancy is not achieved after 12 months of regular, unprotected intercourse, a condition estimated to impact 15% of the global population. In nearly 50% of these instances, male-related factors either contribute to or are the primary cause of the reproductive challenge [1]. Effective clinical management and accurate diagnosis of male infertility rely heavily on a systematic evaluation process that encompasses comprehensive patient history, clinical examination, and standardized semen analysis.

The primary tool for assessing male reproductive potential remains semen analysis [2,3]. This diagnostic approach has undergone several revisions by the World Health Organization (WHO), most notably moving from the long-standing 5th Edition (2010) to the updated 6th Edition (2021), which introduced recalibrated reference intervals [3,4]. Because semen quality is subject to environmental, genetic, and lifestyle influences, reference values can vary considerably across different populations. Consequently, the use of population-specific reference data is essential for precise clinical interpretation [5]. For instance, Tang *et al.* [6] highlighted that fertile men in China exhibit semen characteristics distinct from Western cohorts, underscoring the demand for region-specific reference standards.

Understanding male fertility requires a multidimensional look at testicular function, where testicular volume and the hypothalamic-pituitary-gonadal axis serve as vital indicators. While testicular volume often correlates with the capacity for sperm production [7], elevated follicle-stimulating hormone (FSH) concentrations typically indicate damage to the germinal epithelium [8]. Additionally, subnormal testosterone levels may impair both reproductive function and spermatogenesis [9]. Therefore, integrating hormonal profiles with seminal data is a prerequisite for a thorough diagnostic workup.

This study sought to identify which clinical and seminal parameters most effectively distinguish fertile from infertile individuals. By analyzing semen characteristics, testicular measurements, and endocrine profiles, we aimed to evaluate the applicability of WHO 2021 reference benchmarks in our local population.

Methods

Study Framework and Participant Selection

This retrospective clinical study analyzed medical records from a urology outpatient population treated between January 2016 and December 2020. Our reporting follows the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist (**Supplementary File 1**). The study protocol received approval from the local institutional ethics committee of Cukurova University (Meeting 57, Decision 39) and adhered to the Declaration of Helsinki. Given the anonymized nature of the retrospective data, the requirement for individual informed consent was waived by the institutional review board.

The study cohort comprised 417 individuals divided into two primary groups. The infertile group (n = 315) included men unable to achieve conception after one year of regular, unprotected intercourse. The fertile group (n = 102) consisted of men who had successfully fathered a child naturally within the preceding 12 months without requiring fertility interventions. Exclusion criteria were strictly applied to minimize confounding, and excluded men with a history of orchiectomy, genetic syndromes, prior varicocele repair, oncological treatments (chemotherapy or radiotherapy), testicular malignancy, acute genital infections, or incomplete clinical or laboratory records.

Clinical Assessment and Laboratory Procedures

Clinical data, including demographics and medical history, were systematically documented. Physical examinations focused on varicocele detection and the measurement of testicular volume via a Prader orchidometer (Huaiyin Medical Instruments Co., Ltd., Huai'an, China). Semen samples were collected following a 3-to-7-day period of sexual abstinence and processed after complete liquefaction according to the standardized protocols of our center.

Semen volume was measured using a graduated collection. Sperm concentration was measured using a Neubauer hemocytometer (Marienfeld Superior, Lauda-Königshofen, Germany) and expressed as the number of spermatozoa per milliliter (10^6/mL). The total sperm count (10^6 per ejaculate) was subsequently derived by multiplying the sperm concentration by the total semen volume, as per the WHO 2021 manual guidelines. Sperm motility was assessed by light microscopy and classified as progressive (grades A+B) or non-progressive according to standard criteria. Sperm morphology was assessed using DiffQuick staining (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) based on strict Kruger criteria [10]. Semen parameters, including sperm concentration, motility, morphology, and volume, were retrieved from medical records. For the purposes of the present study, the recorded semen parameters were re-evaluated and interpreted in accordance with the reference values established in the WHO 2021 laboratory manual for the examination and processing of human semen.

Endocrine profiles were assessed from fasting venous blood samples collected between 08:00 and 10:00 AM. Chemiluminescence immunoassay was utilized to measure serum concentrations of FSH, luteinizing hormone (LH), prolactin, total testosterone, and estradiol using the UniCel DxI 800 Access Immunoassay System (Beckman Coulter Inc., Brea, CA, USA) [11].

Sample Size and Power Analysis

Sample size estimation was based on the expected difference in sperm morphology between fertile and infertile men, as reported by Guzick *et al.* [12] in a large multicenter study. Using the observed effect size from prior literature and our pilot data, a minimum of 200 participants (100 per group) was required to achieve 95% power at a two-sided α of 0.05. The final sample size of 417 men (315 infertile and 102 fertile) exceeded the minimum required number based on the a priori power analysis, providing adequate statistical precision for the planned analyses. All analyses were performed using G*Power software (version 3.1.9.7; Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany).

Statistical Analysis

Statistical processing was conducted using SPSS (version 26.0; IBM Corp., Armonk, NY, USA) and R software (version 4.3.0; R Foundation for Statistical Computing, Vienna, Austria) with the 'rms' package (version 6.7-0). Data distribution and homogeneity of variance were assessed using the Shapiro-Wilk and Levene tests, respectively. As continuous variables did not follow a normal distribution variables ($p < 0.05$), data are presented as median (interquartile range [IQR]) and compared via the Mann-Whitney U test.

Receiver operating characteristic (ROC) curve analysis was used to evaluate the discriminative ability of clinical, semen, and hormonal parameters. The area under the curve (AUC) with 95% confidence intervals (CIs) was calculated, and optimal cut-off values were determined using the Youden index. Univariate logistic regression analysis was first performed to assess the association between each variable and fertility status. Odds ratios (ORs) with 95% CIs were calculated for all candidate predictors, including semen parameters (sperm concentration, morphology, progressive motility), mean testicular volume, and hormonal parameters (FSH, LH, testosterone, prolactin). Variables

Table 1. Comparison of semen analysis parameters between groups.

Semen Parameters	Fertile group (n = 102)	Infertile group (n = 315)	U value	*p* value
Semen volume (mL), Median (IQR)	2.0 (2.0–3.0)	2.0 (1.0–2.0)	21,067.0	<0.001
Sperm concentration ($\times 10^6$/mL), Median (IQR)	33.0 (23.8–50.0)	8.0 (5.0–12.0)	25,611.5	<0.001
Total sperm count ($\times 10^6$/ejaculate), Median (IQR)	75.0 (50.8–120.0)	16.0 (10.0–23.5)	25,804.0	<0.001
Progressive motility (A+B) (%), Median (IQR)	70.0 (60.0–70.0)	60.0 (50.0–65.0)	18,806.0	<0.001
Morphology (Kruger) (%), Median (IQR)	5.0 (4.0–7.0)	2.0 (1.0–2.0)	21,975.5	<0.001

IQR, interquartile range.

Table 2. Comparison of serum hormone levels between groups.

Hormonal parameters	Fertile group (n = 102)	Infertile group (n = 315)	U value	*p* value
FSH (mU/mL), Median (IQR)	4.9 (3.1–6.1)	8.2 (3.1–14.5)	10,185.0	<0.001
LH (mU/mL), Median (IQR)	4.6 (3.2–6.3)	5.2 (2.5–7.9)	14,855.0	0.021
Prolactin (ng/mL), Median (IQR)	6.2 (4.2–8.2)	7.4 (5.0–9.8)	12,242.0	0.001
Total testosterone (ng/mL), Median (IQR)	3.9 (2.8–4.6)	3.3 (2.4–3.9)	20,140.0	<0.001
Estradiol (pg/mL), Median (IQR)	25.0 (17.7–36.8)	33.8 (20.9–40.5)	13,623.5	0.251

FSH, follicle-stimulating hormone; LH, luteinizing hormone.

achieving statistical significance ($p < 0.05$) were then considered for inclusion in the multivariable model. Sensitivity analyses were performed to assess the robustness of the multivariable model across clinically relevant infertility phenotypes defined according to WHO 2021 criteria, including azoospermia, oligo-teratozoospermia, oligozoospermia, teratozoospermia, and normozoospermia (unexplained infertility). For each subgroup, the discriminative performance of the multivariable model was evaluated by calculating the AUC with 95% CI, using a randomly selected subset of 30 fertile men as the reference group. Multicollinearity was assessed using the variance inflation factor (VIF).

To prevent model overfitting, internal validation was executed through bootstrap resampling with 1000 iterations, yielding optimism-corrected AUC values and calibration curves. Subgroup analyses were subsequently conducted according to WHO 2021 sperm parameter classifications (e.g., azoospermia, oligozoospermia).

All tests were two-tailed, and statistical significance was set at $p < 0.05$.

Results

The mean age of the 417 participants was 32.11 ± 5.96 years. No statistically significant difference in age was observed between the fertile (32.60 ± 5.12 years) and infertile (31.95 ± 6.21 years) groups ($p = 0.265$).

Physical Examination Findings

Mean bilateral testicular volume was significantly greater in fertile men than in infertile men (**Supplementary Table 1**).

Semen Parameters

Fertile men demonstrated significantly more favorable values across all principal semen parameters compared with infertile men (Table 1).

Hormonal Profile

Infertile men had markedly higher median FSH levels than fertile men [8.2 (11.7) vs 4.9 (3.2) mIU/mL; $p < 0.001$]. Similarly, median prolactin levels were significantly elevated in the infertile group. Median LH levels were also significantly higher in infertile men compared with fertile men [5.2 (5.5) vs 4.6 (3.3) mIU/mL; $p = 0.021$]. In contrast, median total testosterone levels were higher in the fertile group [3.9 (1.6) ng/mL] compared to the infertile group [3.3 (1.6) ng/mL, $p < 0.001$]. No statistically significant between-group difference was observed for estradiol levels ($p = 0.251$) (Table 2).

ROC Analysis and Discriminative Performance of Parameters

ROC curve analysis demonstrated that semen parameters were the strongest discriminators of fertility status. Sperm concentration (AUC = 0.984) and sperm morphology (AUC = 0.913) showed excellent

Among hormonal parameters, FSH exhibited limited but significant discriminative ability (AUC = 0.683). Testosterone (AUC = 0.627) and prolactin (AUC = 0.619) showed statistically significant but clinically modest discriminative performance, primarily owing to low specificity. LH did not show significant discriminative ability (AUC = 0.462, $p = 0.253$).

Detailed AUC values, optimal cut-off points, sensitivity, specificity, and predictive values are presented in Table 3. The comparative ROC curves, illustrating the superior discriminative ability of sperm concentration and morphology over hormonal markers, are presented in Fig. 1.

Table 3. Diagnostic performance of clinical, semen and hormonal parameters in discriminating between fertile and infertile men.

Parameters	AUC (95% CI)	Cut-off value	Sensitivity (95% CI)	Specificity (95% CI)	PPV/NPV (%)	*p* value
Sperm concentration	0.984 (0.976–0.995)	14 (million/mL)	90.16 (86.3–93.2)	98.04 (93.1–99.8)	99.3/76.3	<0.001
Morphology (Kruger)	0.913 (0.851–0.930)	3 (%)	89.81 (85.9–92.9)	85.29 (76.9–91.5)	94.9/73.1	<0.001
Progressive motility (A+B)	0.769 (0.662–0.783)	60 (%)	74.60 (69.4–79.3)	67.65 (57.7–76.6)	87.7/46.3	<0.001
Mean testicular volume	0.812 (0.749–0.848)	15 (mL)	79.68 (74.8–84.0)	64.71 (54.6–73.9)	87.5/50.8	<0.001
FSH (mIU/mL)	0.683 (0.604–0.715)	4.73	66.67 (59.3–73.4)	58.82 (48.6–68.5)	74.4/49.6	<0.001
LH (mIU/mL)	0.462 (0.418–0.597)	Not applicable	-	-	-	0.253
Testosterone (ng/mL)	0.627 (0.609–0.689)	2.75	79.4 (71.6–87.2)	36.5 (31.2–41.8)	28.8/84.6	<0.001
Prolactin (ng/mL)	0.619 (0.545–0.680)	9.54	86.3 (79.6–93.0)	27.9 (23.0–32.8)	27.9/86.3	<0.001

AUC, Area Under Curve; CI, Confidence Interval; PPV, Positive Predictive Value; NPV, Negative Predictive Value.

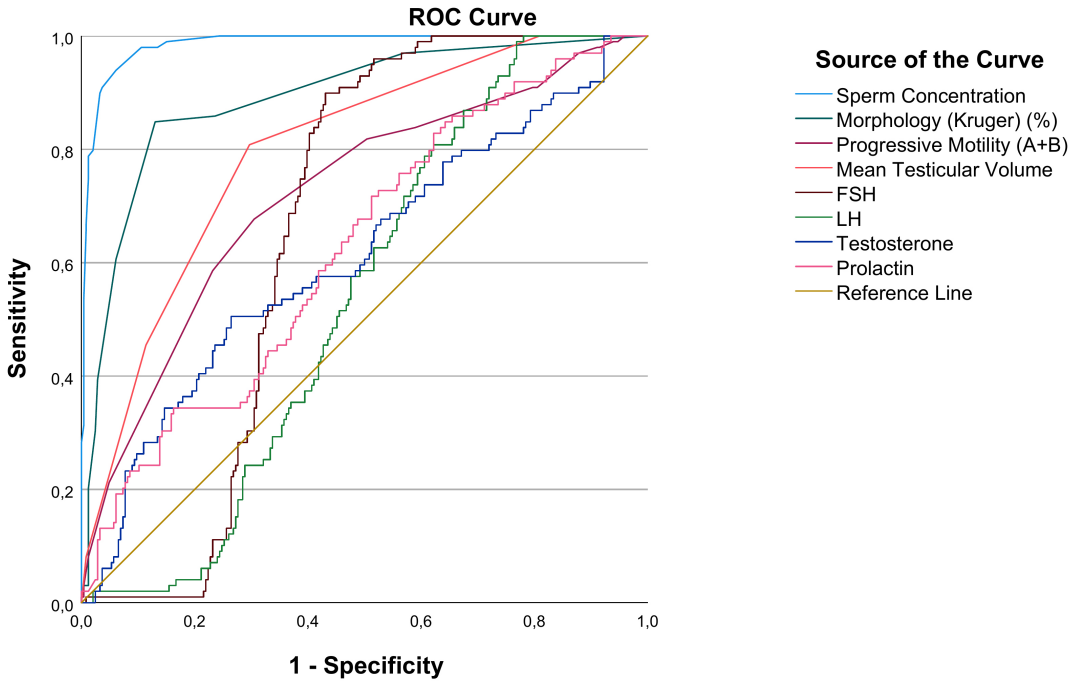

Fig. 1. ROC curves for clinical, semen, and hormonal parameters in predicting male fertility. ROC, receiver operating characteristic.

Univariate and Multivariable Logistic Regression Analysis

In univariate logistic regression analysis, sperm concentration, morphology, progressive motility, mean testicular volume, and serum levels of FSH, testosterone, and prolactin were all significantly associated with fertility status (all $p < 0.05$; Table 4). However, LH levels ($p = 0.058$) and age ($p = 0.350$) did not reach statistical significance. To preserve model parsimony and avoid multicollinearity, testosterone and prolactin were excluded from the final multivariable model despite their univariate significance, given their strong biological correlation with the hypothalamic-pituitary-gonadal axis and testicular volume. In the multivariable logistic regression analysis, sperm concentration, sperm morphology, mean testicular volume, and serum FSH level remained independent predictors of fertility. Higher sperm concentration, normal sperm morphology, and greater testicular volume were each associated with increased odds of fertility, whereas elevated FSH was associated with reduced fertility. Progressive motility did not retain independent predictive significance after adjustment for other covariates in the multivariable model (Table 4).

The combined multivariable model demonstrated excellent discriminative performance, with an AUC of 0.994 (95% CI: 0.988–0.999), outperforming all individual semen and hormonal parameters (Fig. 2). To validate the robustness of the multivariable model, internal validation was performed using Bootstrap resampling with 1000 iterations. The original AUC was 0.993, and the optimism-corrected AUC was found to be 0.992. The calibration

Table 4. Univariate and multivariable logistic regression analysis for independent predictors of fertility.

Variable	Univariate analysis OR (95% CI)	*p* value	Multivariable analysis OR (95% CI)	*p* value
Sperm concentration	1.355 (1.261–1.456)	<0.001	1.43 (1.26–1.63)	<0.001
Morphology (Kruger, %)	2.096 (1.770–2.488)	<0.001	1.47 (1.17–1.84)	0.001
Mean testicular volume	1.585 (1.418–1.773)	<0.001	1.52 (1.10–2.09)	0.011
FSH (mIU/mL)	0.864 (0.821–0.908)	<0.001	0.73 (0.57–0.93)	0.010
Progressive motility	1.059 (1.037–1.082)	<0.001	0.99 (0.95–1.03)	0.577
Testosterone (ng/mL)	1.441 (1.172–1.773)	<0.001	—*	—*
Prolactin (ng/mL)	0.862 (0.799–0.930)	<0.001	—*	—*
LH (mIU/mL)	0.937 (0.877–1.002)	0.058	—	—
Age (years)	1.017 (0.981–1.056)	0.350	—	—

Model performance: Nagelkerke $R^2 = 0.908$, Hosmer–Lemeshow $p = 0.326$.
*Variables excluded from the multivariable model due to multicollinearity or to maintain model parsimony.

curve demonstrated excellent agreement between the predicted and observed probabilities of fertility (Mean Absolute Error = 0.033), indicating minimal overfitting (Fig. 3).

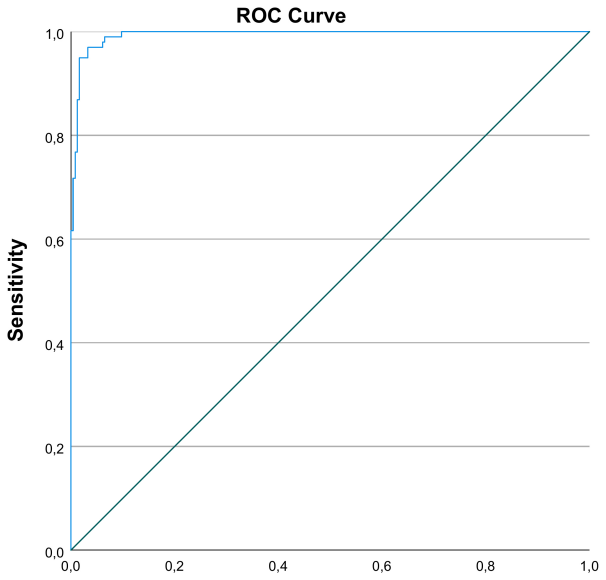

Fig. 2. ROC curve of the multivariable predictive model. The model combines mean testicular volume, sperm concentration, progressive motility (A+B), morphology, and serum FSH levels to predict fertility status. ROC, receiver operating characteristic.

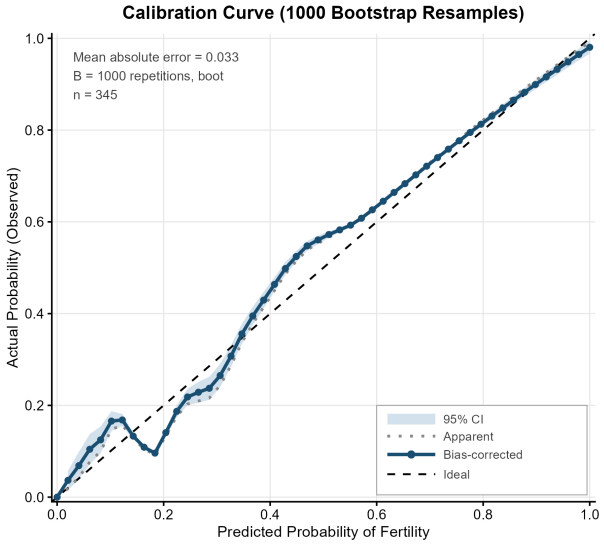

Fig. 3. Calibration plot of the multivariable model using 1000 bootstrap resamples (n = 345). The x-axis shows the predicted probability of fertility, while the y-axis shows the actual observations. The proximity of the bias-corrected line (solid) to the 45-degree ideal line (dashed) indicates excellent model calibration. A mean absolute error of 0.033 confirms high predictive accuracy and minimal overfitting.

The distribution and baseline clinical characteristics of the infertile subgroups are presented in Table 5. Sensitivity analyses revealed that the model maintained excellent discriminative performance across all phenotypes. The multivariable model demonstrated high diagnostic accuracy with AUC values of 0.998 (95% CI: 0.992–1.000) for oligo-teratozoospermia, 0.994 (95% CI: 0.985–1.000) for oligozoospermia, and 0.982 (95% CI: 0.955–1.000) for teratozoospermia. Notably, even in the normozoospermic (unexplained) infertility subgroup, the model retained a strong discriminative ability with an AUC of 0.921 (95% CI: 0.845–0.996) (Table 6).

Discussion

Male infertility is a multifaceted condition in which reproductive success depends on the interplay between endocrine regulation, sperm functional integrity, and quantitative output. This study provides a detailed assessment of the discriminative ability of testicular volume, hormonal profiles, and seminal parameters differentiate

Table 5. Baseline clinical characteristics of the infertile population subgroups (WHO 2021 criteria).

Subgroup category	n	Age (Years), Median (IQR)	FSH (mIU/mL), Median (IQR)	Mean testicular volume (mL), Median (IQR)
Azoospermia	59	31.0 (27.0–36.5)	12.5 (8.2–18.4)	7.5 (5.0–11.5)
Oligo-teratozoospermia	183	31.0 (28.0–34.0)	8.3 (2.8–14.4)	15.0 (15.0–17.5)
Oligozoospermia	31	32.0 (28.0–37.5)	8.1 (1.5–11.8)	15.0 (15.0–16.3)
Teratozoospermia	10	29.5 (28.2–31.5)	7.1 (3.4–15.9)	15.0 (15.0–15.0)
Normozoospermia (Unexplained)	32	33.5 (29.0–37.0)	10.7 (4.7–15.3)	15.0 (14.0–15.0)

Table 6. Sensitivity analysis: predictive performance of the multivariable model across different infertility phenotypes.

Infertility phenotype (Cases)	n (Cases)	Control group (n)	AUC (95% CI)
Overall population (Excl. Azoospermia)	256	30	0.992 (0.988–0.999)
Oligo-teratozoospermia	183	30	0.998 (0.992–1.000)
Oligozoospermia	31	30	0.994 (0.985–1.000)
Teratozoospermia	10	30	0.982 (0.955–1.000)
Normozoospermia (Unexplained)	32	30	0.921 (0.845–0.996)

fertile individuals from infertile ones under the WHO 2021 framework. Our results indicate that traditional semen parameters—specifically morphology and sperm concentration—continue to be the most robust clinical markers for fertility. These findings emphasize the enduring importance of standardized semen evaluation, consistent with the evidence-based revisions incorporated in the 6th Edition of the WHO manual [13].

Notably, the optimal concentration threshold identified in our study (14 million/mL) was remarkably consistent with the WHO 2021 lower limit (16 million/mL), validating the clinical relevance of these international standards for our specific population. A similar concordance was noted for morphology (3% vs the WHO's 4%). These results are supported by large-scale evidence suggesting that morphology and concentration are the most reliable predictors of a couple's ability to conceive naturally [14]. Although the clinical significance of strict morphology criteria remains a matter of debate, our findings highlight its high discriminatory power, likely reflecting its role as a proxy for genomic integrity and overall spermatogenic health.

Although progressive motility showed moderate diagnostic accuracy (AUC = 0.769)—which matches reports linking low motility to mitochondrial issues and oxidative stress [15]—it did not surface as an independent predictor in our multivariable analysis. This finding is likely attributable to the strong correlation between the effect of motility and sperm concentration and morphology. Beyond conventional semen analysis, structural assessment of the testes directly reflects the underlying parenchymal integrity and the size of the germ cell population [16]. In contrast, mean testicular volume not only provided strong discrimination (AUC = 0.812) but also remained an independent predictor, reinforcing its established connection to Sertoli cell mass and overall sperm production capacity. Our findings suggest that whereas semen parameters offer a functional snapshot of an ejaculate, testicular volume serves as a more stable structural surrogate for the quantitative potential of the germinal epithelium.

Hormonal markers, although statistically significant, offered comparatively modest clinical utility as standalone discriminators. FSH showed limited standalone accuracy (AUC = 0.683) but remained a valuable independent predictor, acting as a biochemical indicator of germinal epithelium status [17]. Conversely, despite reasonable sensitivity, prolactin and testosterone demonstrated low positive predictive values and specificity, restricting their use as primary diagnostic tools. These results suggest that endocrine parameters should serve to contextualize semen findings rather than replace them as primary diagnostic markers, consistent with current clinical practice guidelines [18].

A key strength of our study lies in the integrative multivariable approach. The combined model incorporating sperm concentration, morphology, mean testicular volume, and FSH demonstrated excellent discriminative performance (initial AUC = 0.993). To address potential concerns regarding model overfitting and optimistic bias, we performed internal validation using 1000 bootstrap resamples. The resulting optimism-corrected AUC was found to be 0.992, with a mean absolute error of 0.033 in the calibration curve analysis. These findings underscore the additive value of integrating structural, functional, and endocrine parameters, confirming that the near-perfect discriminative performance is robust and is maintained after rigorous internal validation. Although future studies involving external validation in independent cohorts remain essential to confirm generalizability, our results demonstrate that this integrated model provides a highly reliable predictive framework for male fertility evaluation within the WHO 2021 context.

Recent literature has increasingly focused on sperm DNA fragmentation (SDF) and oxidative stress as potential diagnostic enhancements [19,20]. Although these molecular assays can provide additional mechanistic insights in

complex or unexplained cases, our data demonstrate that conventional parameters, when carefully interpreted, already possess substantial predictive power. Accordingly, emerging biomarkers should be regarded as complementary tools in borderline or unexplained cases, rather than replacements for classical semen analysis.

Several limitations merit consideration. First, the retrospective single-center design may limit the generalizability of the findings to broader populations with different environmental or genetic backgrounds. Furthermore, semen parameters were evaluated from a single semen analysis per participant; however, current clinical guidelines recommend repeated assessments given the known intra-individual variability, which may not have been fully captured in the present study. Second, while we utilized bootstrap resampling to mitigate bias, the lack of external validation in an independent cohort means the model's robustness across broader populations still needs to be confirmed. Third, advanced molecular biomarkers—including sperm DNA fragmentation (SDF), oxidative stress markers, and seminal microbiome profiling—were not assessed, precluding any mechanistic interpretation of the findings. Fourth, potential confounding variables such as detailed lifestyle factors, occupational exposures, and environmental influences were not quantitatively incorporated into the regression models. Finally, the cross-sectional design precludes causal inference.

Despite these limitations, this study provides robust, population-specific evidence confirming the applicability of WHO 2021 reference values and demonstrates that a multidimensional integrative approach substantially enhances diagnostic discrimination. Our findings reaffirm that sperm concentration and morphology remain the most powerful clinical indicators of male fertility status, and that their integration with structural and endocrine parameters may further optimize individualized diagnostic strategies.

Conclusion

Sperm concentration and morphology were the most powerful discriminatory parameters for distinguishing fertile from infertile men, confirming their central role in male fertility assessment under the WHO 2021 criteria. Mean testicular volume and serum FSH provided complementary structural and endocrine information, whereas the remaining hormonal markers demonstrated limited standalone diagnostic value. An integrated evaluation combining seminal, clinical, and hormonal parameters substantially improved discriminative performance, supporting a multidimensional approach to the assessment of male infertility. These findings validate the applicability of WHO 2021 reference values in our population and highlight the added clinical value of a comprehensive, multidimensional interpretation in reproductive medicine.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

BK: Conceptualization, methodology, and formal analysis. IHS: Data curation and investigation. FO: Analysis and interpretation of data, and writing—original draft preparation. All authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

This study was approved by the local institutional ethics committee of Cukurova University (Meeting No. 57, Decision No. 39). The study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Due to the retrospective nature of the study and the use of anonymized clinical data, the requirement for written informed consent was waived by the ethics committee.

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of Generative AI and AI-Assisted Technologies in Manuscript Preparation

During the preparation of this manuscript, AI-assisted language tools (Claude, Anthropic) were used exclusively for language editing and text paraphrasing. All scientific content, data analysis, and conclusions are solely the work of the authors. The authors take full responsibility for the integrity and accuracy of the reported research.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202638209.146.

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