

Pathophysiology-based classification of male infertility: evidence from an 800-patient prospective cohort

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Abstract

Context Male factor infertility (MFI) is frequently labeled idiopathic when evaluation relies primarily on semen analysis, potentially overlooking endocrine and pathophysiological mechanisms relevant for targeted management.

Objective The objective of this study was to phenotypically define MFI and develop a pathophysiology-based classification aimed at reducing idiopathic infertility following comprehensive evaluation.

Design and Setting This was a prospective monocentric cohort study conducted at a tertiary referral academic center.

Patients A total of 800 partners of infertile couples were evaluated between October 2024 and January 2026 after exclusion of isolated female factor infertility.

Main Outcome Measures Prevalence of MFI categories, hormonal patterns across phenotypes, and proportion of idiopathic infertility after comprehensive work-up.

Methods All patients underwent standardized clinical evaluation, hormonal assessment (total testosterone, follicle-stimulating hormone [FSH], luteinizing hormone), testicular ultrasound, and complete semen analysis. Microbiological testing, transrectal ultrasound, and genetic analyses were performed according to guidelines.

Results Primary spermatogenic failure was the most prevalent category (56.0%), followed by infection/inflammation (22.4%) and hypogonadotropic hypogonadism (8.5%). Idiopathic infertility was identified in only 5.3% of cases. Distinct endocrine profiles were observed, with high-FSH spermatogenic failure representing the dominant phenotype.

Conclusion Comprehensive phenotyping markedly reduces the proportion of patients classified as having idiopathic infertility and identifies clinically relevant subgroups. This prospective study provides validation of a pathophysiology-based classification and supports a shift toward endocrine-integrated diagnostic strategies in male infertility, with potential implications for targeted management.

Keywords male factor infertility, couple infertility, FSH, semen infections, semen inflammations, idiopathic infertility

Background

Couple infertility is a common clinical condition, being defined as the inability to conceive after at least 12 months of regular and unprotected sexual intercourse (1). Male factor infertility (MFI) is present, alone, or in combination with female factor infertility, in at least half of the cases (2–5).

In clinical practice, the evaluation of MFI is often—inappropriately—limited to semen analysis, and approximately 50% of male

partners of infertile couples with abnormal semen parameters are classified as having “idiopathic male infertility” and are referred directly to assisted reproduction technologies (ARTs) without further evaluation (6, 7).

Indeed, a complete and accurate evaluation of the male partner of an infertile couple is reasonable and recommended; while semen analysis is a pivotal tool, it is not an absolute marker of fertility or infertility (8). Therefore, starting from semen analysis,

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further evaluations are recommended, including an accurate medical history and physical examination, hormonal evaluation, testicular ultrasound, and prostatic ultrasound, semen microbiological evaluation, and genetic analyses when needed (2, 3, 7, 9). This is consistent with the evaluation of the female partner, which requires multiple assessments and a multistep diagnostic approach (10).

These findings highlight that infertility should not be viewed as a condition solely related to the woman or the man, but rather as an issue that involves the couple as a whole (11). Therefore, gynecologists and andrologists should manage the couple jointly to restore natural fertility when possible, ensure access to ARTs, or optimize ART outcomes.

Male factor infertility can arise from diverse etiologies and pathophysiological mechanisms, involving a wide array of risk factors (2, 12, 13). These conditions include genital or seminal tract infections and inflammation, testicular disorders, drug exposure, testicular trauma, cancer and its treatments, adverse lifestyle factors, hormonal disorders, and metabolic alterations. Therefore, the rate of idiopathic or unexplained infertility depends on the number and depth of evaluations performed in both partners of the couple, and this term should be used only following a complete and accurate diagnostic work-up.

The classical distinction of MFI into pretesticular, testicular, and posttesticular causes has limited utility in clinical practice and does not lead to uniformly applicable treatment options. A new diagnostic–therapeutic classification of MFI has been recently proposed (11), involving 6 major categories of MFI. This classification is based on pathophysiological mechanisms and may more easily guide treatment choices, as it is intended for therapeutic management and considers simple clinical aspects. Nevertheless, a comprehensive diagnostic work-up is still necessary to provide an etiologic and pathophysiological explanation of couple infertility.

The aim of the present study is to verify this pathophysiology-based classification, adapt it to a tertiary center, and investigate the integration of clinical hormonal, genetic, imaging, and seminal data in reducing the rate of unexplained infertility. Unlike previous conceptual classifications, our study provides quantitative validation and demonstrates the distribution of pathophysiology-based categories in a large, deeply phenotyped clinical cohort.

Materials and methods

This is a prospective and monocentric study. The study protocol followed the standard clinical approach and the principles outlined in the Declaration of Helsinki. The design of the study was approved by the institutional review board (University of Padova, Italy; n. AOP 3329, September 5, 2024).

We included all male partners of infertile couples referred to our Unit (Unit of Andrology and Reproductive Medicine, University Hospital of Padova, Italy), between October 2024 and January 2026. Patients were consecutively enrolled and evaluated according to a predefined diagnostic protocol. We excluded partners of infertile couples with known female factors of infertility (following a complete gynecological evaluation, as suggested by recent guidelines (10)).

All patients underwent a comprehensive evaluation, including familial and personal histories, physical examination, and semen

analysis according to the WHO manual for semen examination (1). In addition, they underwent fasting hormonal evaluation (total testosterone [TT], follicle-stimulating hormone [FSH], luteinizing hormone [LH]) (14). Testicular ultrasound was used to measure testicular volumes, echogenicity, homogeneity, and vascularization, as well as to assess the characteristics of the epididymis, the proximal tract of the vas deferens, and the presence of varicocele (15, 16). Mean testicular volume was calculated after bilateral assessment.

Semen microbiological evaluation (for general bacteria, atypical bacteria, and human papillomavirus [HPV]) was performed in patients with clinical signs or suspicion of semen infection, reduced sperm parameters (below the 5th percentile), or abnormalities in semen characteristics, including volume (below the 5th percentile or above 95th percentile), pH, viscosity, or liquefaction, as well as leukocytospermia, sperm agglutination or the presence of antisperm antibodies (ASAs), and in those with ultrasound findings suggestive of male genital infection or inflammation (2, 16). Transrectal ultrasonography (TRUS) of the prostate and seminal vesicles was performed to evaluate prostate volume and signs of male accessory gland infection or inflammation (2, 16, 17). Transrectal ultrasonography was indicated for patients presenting with clinical signs or symptoms of semen inflammation, suspected ductal obstruction, or a reduction in sperm parameters (below the 5th percentile). In addition, it was utilized for patients exhibiting alterations in semen volume (below the 5th percentile or above 95th percentile), pH, viscosity, or liquefaction, as well as those with leukocytospermia, sperm agglutination, ASAs, or prior ultrasound findings suggestive of male genital tract infection or inflammation. Both scrotal and transrectal ultrasound examinations were performed by 2 trained and highly experienced operators (P.P. and N.C.) at an Italian Society of Andrology and Sexual Medicine-accredited Center of Excellence in Andrological Ultrasound. All examinations were conducted according to a standardized institutional protocol and predefined diagnostic criteria. In cases of uncertain or equivocal findings, images were jointly reviewed, and a consensus assessment was reached. Karyotyping was performed in subjects with a sperm concentration of <10 million/mL; analysis of microdeletions in the long arm of the Y chromosome (Yq) was performed in subjects with a sperm concentration of <5 million/mL; CFTR gene mutation analysis was performed in subjects with obstructive azoospermia and suspected mild forms of androgen resistance (normal/high TT with normal/high LH); and targeted NGS gene panel—including 31 genes—was performed in subjects with congenital hypogonadotropic hypogonadism (HH), as recommended by the most recent literature on the genetic evaluation of MFI (2, 18, 19). Specifically, targeted NGS panel testing was performed in patients with congenital hypogonadotropic hypogonadism and in patients with hypogonadotropic hypogonadism without identifiable secondary causes. The gene panel comprised 31 genes known to be associated with hypogonadotropic hypogonadism: ANOS1 (KAL1), CCDC141, CHD7, DUSP6, FEZF1, FGF17, FGF8, FGFR1, FLRT3, FSHB, GNRH1, GNRHR, HS6ST1, IL17RD, KISS1, KISS1R, LEP, LEPR, LHB, NDNF, NSMF, PLXNA1, PROK2, PROKR2, SEMA3A, SOX10, SOX2, SPRY4, TAC3, TACR3, and WDR11. Variants were classified according to American College of Medical Genetics and Genomics (ACMG) guidelines.

We then classified the patients to a novel classification of MFI (Table 1).

Table 1 Classification of MFI

Category	Subcategory	Description
I	IA	Isolated infection
	IB	Isolated inflammation
	IC	Combined infection and inflammation
II		Congenital/acquired complete obstruction
III	IIIA	Primary spermatogenic failure with high FSH
	IIIB	Primary spermatogenic failure with normal FSH
IV		Secondary testicular dysfunction
V		Idiopathic semen alterations
VI		Unexplained couple infertility
VII		Clinically significant varicocele
VIII		Immunity-related infertility (sperm autoantibodies)

In detail, we defined the categories as follows:

Category I (to minimize misclassification, strict combined clinical, microbiological, and imaging criteria were required to define infection-/inflammation-related MFI):

Patients with normal hormonal concentrations (LH, 1.5–9.4 IU/L; FSH, 1.5–8.0 IU/L; and TT > 12 nmol) and normal testicular volumes (12–26 mL for the right testis, 11–24 mL for the left testis) (15), but with altered sperm parameters (below the 5th percentile), and meeting at least one of the following A criteria and at least one of the following B criteria:

A Criteria:

1. Anamnesis and/or physical examination findings suggestive of infection/inflammation/obstruction and/or semen findings suggestive of infection/inflammation, including semen volume < 1.4 or > 6.2 mL, semen pH < 7.2 or > 8.5, increased semen viscosity, altered semen liquefaction, leukocytospermia > 10⁶/mL, sperm agglutination, and/or the presence of ASAs as previously stated
2. Epididymal ultrasonographic features suggestive of inflammation/obstruction, defined as the presence of > 2 of the following findings: enlargement of the epididymal head (cranio-caudal diameter > 12 mm), body (> 5 mm), and/or of the tail (> 6 mm) (unilateral or bilateral); multiple microcysts in the epididymal head and/or tail (mono or bilateral); low or high echogenicity (unilateral or bilateral); large unilateral or bilateral hydrocele; enlargement of the superior portion of the epididymal head with a superior-to-inferior diameter of > 1; and unchanged anteroposterior diameter of the epididymal tail after ejaculation (16, 17)

B Criteria:

1. Prostate and seminal vesicle ultrasonographic features suggestive of inflammation/obstruction on TRUS, defined as the presence of > 2 of the following findings. For the prostate: asymmetry of prostate volume, areas of low echogenicity, areas of high echogenicity, dilatation of the periprostatic

venous plexus, single or multiple areas of acinar ectasia, and area(s) of moderately increased vascularity (focal or multiple). For the seminal vesicles: increased anteroposterior diameter (> 16 mm) (unilateral or bilateral), asymmetry > 2.5 mm compared with the contralateral vesicle, reduced anteroposterior diameter (< 6 mm) (unilateral or bilateral), thickened and/or calcified glandular epithelium, polycyclic areas separated by hyperechoic septa in 1 or both vesicles, fundus-to-body ratio > 2.5 or < 1, and an unchanged anteroposterior diameter after ejaculation (16, 17)

2. Presence of infection on semen microbiological evaluation

Moreover, patients were further subcategorized into subcategory IA (semen infection without inflammation), subcategory IB (semen inflammation without infection), and subcategory IC (combined semen infection and inflammation).

Because this classification required the simultaneous assessment of semen infection (in B Criteria) and clinical, seminal, and ultrasonographic evidence of inflammation, this classification minimized the risk of overdiagnosing nonspecific infections.

Category II:

Patients with normal hormonal concentrations (LH, FSH, and TT) and normal testicular volumes who have obstructive azoospermia due to an anatomic alteration at any level of the seminal tract (eg, congenital bilateral absence of vas deferens [CBAVD] or iatrogenic postsurgical bilateral damage) or retrograde ejaculation.

Category III:

Patients with primary spermatogenic alterations and, therefore, a reduced sperm count (subcategory IIIA when the FSH concentration was > 8 IU/L, subcategory IIIB when the FSH concentration was normal 1.5–8.0 IU/L), in the presence of reduced testicular volumes and/or genetic alterations associated with primary testicular diseases—karyotype alterations and Yq microdeletions—with a normal LH–TT axis. This category also includes patients with AR gene mutations.

Category IV:

Secondary testicular dysfunction, characterized by low TT concentration (< 12 nmol/L) with low or inappropriate low LH (< 9.4 IU/L) and FSH (< 8.0 IU/L) concentrations, including both congenital and acquired hypogonadotropic hypogonadism, as well as both organic and functional forms (eg, obesity-related hypogonadism).

Category V:

Idiopathic semen alterations (below the 5th percentile).

Category VI:

Unexplained couple infertility (no male and female factors identified).

Category VII:

Altered sperm parameters (below the 5th percentile) in the presence of an ultrasound-defined high-grade varicocele (> 3rd grade according to the Sarteschi-Liguori and/or European Academy of Andrology (EAA) classification (15)) without any other identifiable cause of MFI.

Category VIII:

Presence of ASAs (according to mixed antiglobulin reaction, above 50%), without any other identifiable cause of MFI.

The classification was defined a priori before data analysis based on the pathophysiology-oriented framework recently proposed by Santi et al (11). Patients were assigned to a single category according to the predominant pathophysiological

mechanism considered most directly responsible for semen impairment. When multiple abnormalities coexisted, a predefined diagnostic hierarchy was applied prioritizing primary spermatogenic failure, obstructive causes, hypogonadotropic hypogonadism, infection/inflammation, varicocele, and immunological factors. Although MFI is frequently multifactorial, mutually exclusive allocation was adopted to allow a pragmatic clinical classification.

Statistical analysis

Statistical analyses were performed using SPSS Statistics (IBM Corp., Armonk, NY, USA). Normality of data distribution was assessed using the Shapiro–Wilk test. Categorical variables were reported as percentages. Continuous variables were expressed as mean $\pm$ SD for statistical analyses. Given the non-normal distribution of several parameters, particularly semen variables, boxplots displaying medians and IQRs were used for graphical representation to better illustrate data distribution. Between-group comparisons were performed using Student's t-test or 1-way ANOVA, as appropriate. Additional exploratory analyses were performed within patients classified as primary spermatogenic failure (categories IIIA and IIIB). Comparisons between genetically defined subgroups were performed using Student's t-test for continuous variables and chi-square test (or Fisher's exact test when appropriate) for categorical variables. Multivariable binary logistic regression models were used to identify independent factors associated with category IIIA and to evaluate predictors of azoospermia and severe sperm impairment within patients in category III. Follicle-stimulating hormone was excluded from models comparing categories IIIA and IIIB to avoid circularity, since it was part of the classification criteria. Results are reported as odds ratios (ORs) with 95% CIs. A 2-sided *P* value of $<.05$ was considered statistically significant.

Results

We enrolled 800 male partners of infertile couples after exclusion of female causes of infertility. Of these, 176 (22.0%) had previously undergone ARTs without any preliminary andrological evaluation other than semen evaluation.

A descriptive analysis of the patient cohort is reported in Table 2.

Table 3 reports the classification of patients according to the new MFI classification.

The most frequently represented category was primary spermatogenic failure (category III, 448/800, 56.0%). Within this group, high FSH (category IIIA) was found in 305/800 patients (38.1%) and normal FSH (category IIIB) in 143/800 patients (17.9%). Approximately one-quarter of the patients (179/800, 22.4%) were classified in the infection-/inflammation-related MFI category according to the predefined diagnostic criteria (category I). Hypogonadotropic hypogonadism was present in 8.5% of the overall sample. Idiopathic or unexplained infertility was present only in 42/800 patients (5.3%), including idiopathic semen alterations (category V) in 15/800 (1.9%) and unexplained couple infertility (category VI) in 27/800 (3.4%). Finally, varicocele as the primary cause of MFI (category VII) was present only in 21/800 patients (2.6%), while antisperm autoimmunity was present in 5/800 (0.6%) patients.

Table 2 Descriptive analysis of the cohort of 800 male partners of infertile couples

Parameter	Normal values	Mean $\pm$ SD
Age of M (years)	n.a.	36.2 $\pm$ 7.7
Age of F (years)	n.a.	34.7 $\pm$ 5.1
Time of infertility (years)	n.a.	2.6 $\pm$ 1.9
BMI (kg/m ²)	19.1 to 24.9	26.3 $\pm$ 5.4
Sperm concentration (million/mL)	>16	11.6 $\pm$ 27.7
Total sperm count (million/ejaculate)	>39	32.4 $\pm$ 80.0
FSH (IU/L)	1.5 to 8.0	11.0 $\pm$ 10.5
LH (IU/L)	1.5 to 9.4	6.8 $\pm$ 4.3
TT (nmol/L)	>12	15.3 $\pm$ 9.1
Right testis volume (mL)	12 to 26	12.0 $\pm$ 5.3
Left testis volume (mL)	11 to 24	11.5 $\pm$ 5.0
Prostate volume (mL)	15 to 35	22.4 $\pm$ 7.2

Abbreviations: BMI, body mass index; F, female partner; FSH, follicle-stimulating hormone; LH, luteinizing hormone; M, male partner; n.a., not available; TT, total testosterone.

The most frequent etiologies were infection/inflammation (22.4%) and genetic causes (15.9%), the latter of which included karyotype alterations (6.9%), Yq microdeletions (5.1%), CFTR gene mutations (2.6%), AR gene mutations (0.6%), and mutations in HH-related genes (0.7%). Other frequent causes were cryptorchidism (11.3%) and functional acquired HH (6.9%), which was mainly represented by overweight/obesity. Overall, idiopathic primary spermatogenic failure was the most frequent diagnosis (23.6%).

No significant differences were observed among the groups regarding the age of the male and female patients at the first evaluation, the duration of infertility, or the body mass index (BMI) of male patients. The BMI of the patients in category IV was not significantly different from the BMI of other categories, probably due to the reduced number of patients in this category, as well as the combined effect of congenital and acquired hypogonadotropic hypogonadism within it.

Patients in category IIIA had, by definition, higher FSH concentrations (21.5 $\pm$ 12.4 IU/L) than patients in all other categories (*P* $<.001$) (Fig. 1A). Patients in category IIIA also exhibited higher LH concentrations (9.5 $\pm$ 5.0 IU/L) than patients in the other remaining categories (Fig. 1B). No differences were observed regarding TT concentrations across the different patient subgroups, with the exception of significantly reduced TT concentrations in category IV, as per definition (Fig. 1C).

Excluding category IV, hypogonadism—defined according to TT concentrations—was present in 43.2% of patients in category IIIA.

Sperm concentration and total sperm count were significantly lower in patients in category IIIA (1.68 $\pm$ 6.84 million/mL and 6.2 $\pm$ 29.1 million/ejaculate, respectively) than in patients in categories I, IIIB, and VI (*P* $<.001$), particularly when compared with patients in category IIIB (12.9 $\pm$ 35.1 million/mL and 25.4 $\pm$ 104.0 million/ejaculate, respectively) (Fig. 2).

Finally, regarding testicular volumes, patients in category IIIA had lower mean testicular volumes than patients in other categories (*P* $<.001$; Fig. 3).

Table 3 Diagnostic classification and putative causes within a cohort of 800 male partners of infertile couples

Category	Subcategory	Associated etiology	N (%)	
I (infection/inflammation)	IA	Isolated infection	35/800 (4.4%)	179/800 (22.4%)
	IB	Isolated inflammation	64/800 (8.0%)	
	IC	Combined infection and inflammation	80/800 (10.0%)	
II (congenital/acquired total obstruction)		CBAVD (with CFTR gene mutations)	21/800 (2.6%)	37/800 (4.6%)
		CBAVD (without CFTR gene mutations)	3/800 (0.4%)	
		Acquired/iatrogenic obstruction	8/800 (1.0%)	
		Sexual dysfunctions, including retrograde ejaculation	5/800 (0.6%)	
III (primary spermatogenic failure)	IIIA		305/800 (38.1%)	448/800 (56.0%)
	IIIB		143/800 (17.9%)	
		Karyotype alterations, including Klinefelter syndrome	55/800 (6.9%)	
		Y chromosome microdeletions	41/800 (5.1%)	
		Androgen receptor mutations	5/800 (0.6%)	
		Postsurgery/infection/trauma	25/800 (3.1%)	
		Previous testicular cancer	21/800 (2.6%)	
		Previous oncological therapy	22/800 (2.8%)	
		Cryptorchidism	90/800 (11.3%)	
		Idiopathic primary spermatogenic failure	189/800 (23.6%)	
		Congenital hypogonadotropic hypogonadism.	6/800 (0.7%)	
		Acquired—organic hypogonadotropic hypogonadism	7/800 (0.9%)	
IV (secondary testicular dysfunction)		Acquired—functional hypogonadotropic hypogonadism	55/800 (6.9%)	68/800 (8.5%)
V (idiopathic semen alterations)				15/800 (1.9%)
VI (idiopathic couple infertility)				27/800 (3.4%)
VII (varicocele)				21/800 (2.6%)
VIII (immunity-related semen conditions)				5/800 (0.6%)

Abbreviation: CBAVD, congenital bilateral absence of vas deferens.

Additional exploratory analyses were performed to further characterize patients with primary spermatogenic failure and to investigate independent predictors of severe spermatogenic impairment.

Compared with those in category IIIB, patients in category IIIA showed significantly higher LH concentrations, lower mean testicular volume, and lower sperm concentration and total sperm count.

Among genetically tested patients with primary spermatogenic failure, genetic abnormalities consistent with primary testicular dysfunction, including karyotype alterations and Y chromosome microdeletions, were significantly enriched in category IIIA compared with category IIIB (32.6% vs 7.8%; OR 5.71, 95% CI 2.18-14.96, $P < .001$). Patients carrying these abnormalities showed higher FSH and LH concentrations, lower TT levels, lower mean testicular volume, and more severe impairment of sperm concentration and total sperm count compared with genetically negative patients.

In a multivariable logistic regression analysis, excluding FSH to avoid circularity, category IIIA remained independently associated with higher LH concentrations (OR 1.42, 95% CI 1.15-1.76, $P = .001$), lower mean testicular volume (OR 0.71, 95% CI

0.61-0.83, $P < .001$), and the presence of genetic abnormalities (OR 12.71, 95% CI 1.49-108.72, $P = .020$).

Within the genetically tested patients in category III (IIIA + IIIB), FSH independently predicted azoospermia (OR 1.06 per IU/L, 95% CI 1.01-1.11, $P = .021$), while genetic abnormalities showed a borderline association (OR 2.63, 95% CI 0.96-7.24, $P = .061$). Severe oligozoospermia/azoospermia could not be reliably modeled when including genetic abnormalities because all patients carrying pathogenic genetic alterations exhibited severe semen impairment, resulting in quasi-complete separation of the outcome; in the model excluding genetic abnormalities but including all patients in category III, FSH independently predicted severe sperm impairment (OR 1.15 per IU/L, 95% CI 1.07-1.24, $P < .001$).

Discussion

Couple infertility is a common clinical condition that affects the overall well-being of a couple, with MFI contributing to at least half of the overall cases, either alone or in combination with a female factor (2, 3). Nevertheless, MFI is often—and unfortunately

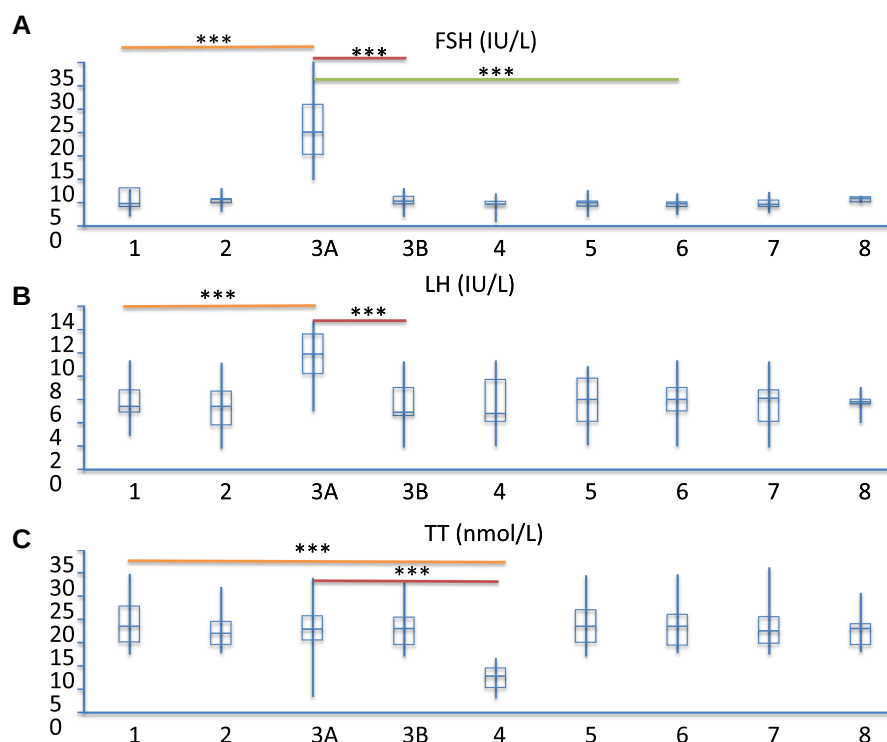


Figure 1 Endocrine phenotype across MFI categories. Boxplots represent FSH, LH, and TT concentrations across MFI categories. Sample sizes were as follows: category I, n = 179; category II, n = 37; category IIIA, n = 305; category IIIB, n = 143; category IV, n = 68; category V, n = 15; category VI, n = 27; category VII, n = 21; and category VIII, n = 5. Patients in category IIIA showed significantly higher FSH concentrations compared with patients in categories I, IIIB, and V–VIII groups (** $P < .001$). LH concentrations showed a moderate increase in category IIIA, whereas TT was reduced only in category IV (by definition). Boxes represent IQR, median lines are shown, and whiskers indicate range.

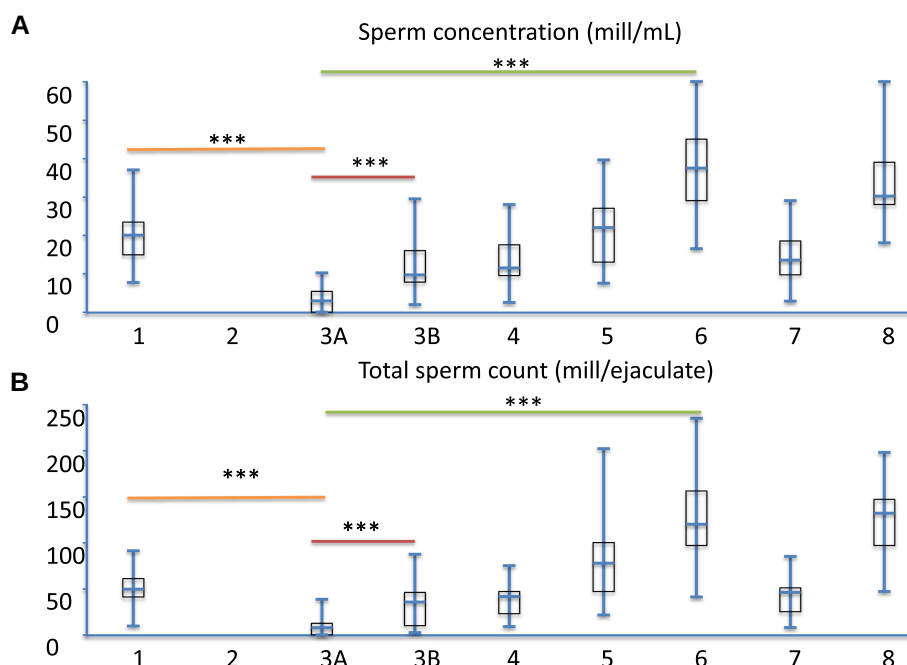


Figure 2 Semen parameters across MFI categories. Boxplots represent (A) sperm concentration and (B) total sperm count across the different categories of MFI. Sample sizes were as follows: category I, n = 179; category II, n = 37; category IIIA, n = 305; category IIIB, n = 143; category IV, n = 68; category V, n = 15; category VI, n = 27; category VII, n = 21; and category VIII, n = 5. Patients in category IIIA showed significantly lower sperm concentration and total sperm count compared with patients in categories I, IIIB, and VI ($P < .001$). These findings confirm the severe impairment of spermatogenic function in the high-FSH phenotype. Boxes represent IQR, horizontal lines indicate medians, and whiskers represent range.

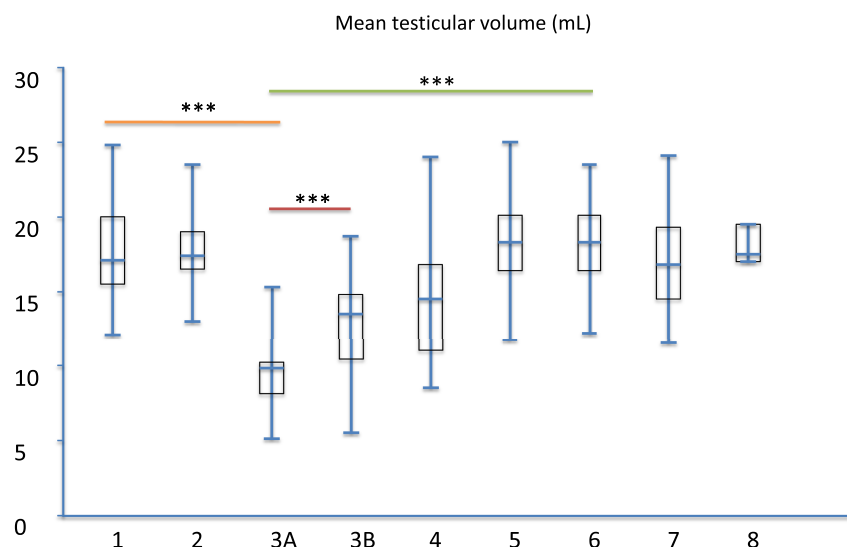


Figure 3 Mean testicular volume across MFI categories. Sample sizes were as follows: category I, $n = 179$; category II, $n = 37$; category IIIA, $n = 305$; category IIIB, $n = 143$; category IV, $n = 68$; category V, $n = 15$; category VI, $n = 27$; category VII, $n = 21$; and category VIII, $n = 5$. The mean testicular volume (calculated as the average of right and left testicular volumes) is significantly reduced in category IIIA compared with other categories ($*** P < .001$), reflecting structural impairment associated with high-FSH spermatogenic failure.

—evaluated solely through semen analysis, and couples with abnormal semen parameters are frequently referred directly for ARTs without further diagnostic evaluation of the male partner (3, 6). In this study, we evaluated a large cohort of male partners of infertile couples. Both partners underwent a complete, parallel, and multistep evaluation according to guidelines, recommendations, and position statements (2, 10). The prospective design of this study strengthens the reliability of phenotype allocation and reduces classification bias. This study moves beyond conceptual classifications by providing a prospective validation of a standardized diagnostic framework.

We observed a 22% prevalence of previous ART cycles initiated without prior andrological evaluation. This is in line with previous studies that have highlighted this trend, thereby underlining that about one-fifth of male partners of infertile couples are referred to ARTs without any pretreatment andrological evaluation (20).

This large tertiary-care cohort demonstrates that male infertility is characterized by distinct endocrine phenotypes, with high-FSH spermatogenic failure representing the dominant biological cluster identified through deep hormonal stratification. Therefore, the primary endocrine finding emerging from this study is that high-FSH spermatogenic failure represents the predominant hormonal phenotype in evaluated infertile men. Indeed, primary spermatogenic failure (both with high and normal FSH) represents the major pathophysiological mechanism of MFI (accounting for 56.0% of patients), followed by male tract infection/inflammation (22.4%). Of note, 15.9% of cases have a genetic origin, with Klinefelter syndrome and Yq microdeletions being the most frequent etiologies. Because our unit is a tertiary referral center (21), the high prevalence of primary spermatogenic failure and genetic abnormalities observed in this cohort may be partially influenced by referral bias. Nevertheless, the substantial proportion of patients carrying genetic abnormalities further underscores the pivotal role of genetic testing in the diagnostic work-up of men with MFI. Beyond establishing an

etiological diagnosis, genetic evaluation may provide prognostic information and guide clinical management and reproductive counseling. Moreover, the contribution of genetic factors to male infertility is likely to increase in the coming years, as advances in next-generation sequencing technologies continue to expand the number of validated infertility-associated genes and improve our understanding of the molecular mechanisms underlying impaired spermatogenesis (2, 18, 19, 22, 23).

Importantly, patients with MFI in category IIIA exhibited not only higher FSH levels but also higher LH concentrations than patients in other categories with normal TT concentrations. This aspect underscores the distinct clinical profile of category III (primary spermatogenic failure) within this new diagnostic-therapeutic classification framework, thereby justifying its characterization as a state of overall subclinical, compensated hypogonadism where the impairment predominantly affects spermatogenic development (2, 24).

The subgroup of patients classified as category IIIB deserves specific consideration. Indeed, additional analyses further supported the biological validity of this distinction. Even after excluding FSH from the multivariable models to avoid circularity, category IIIA remained independently associated with higher LH concentrations, lower testicular volume, and a greater burden of genetic abnormalities. The observation that elevated FSH independently predicted azoospermia and severe sperm impairment supports the biological relevance of the proposed subdivision of primary spermatogenic failure according to gonadotropin profile.

Moreover, karyotype abnormalities and Yq microdeletions were markedly enriched in category IIIA, supporting the concept that this subgroup represents a more severe form of primary testicular dysfunction. Although patients within category IIIB showed FSH concentrations within the reference range, they fulfilled the criteria for primary spermatogenic failure because of impaired sperm production associated with reduced testicular volume and/or the presence of genetic abnormalities known to

affect testicular function. Physiologically, circulating FSH concentrations reflect the complex interaction between hypothalamic–pituitary regulation and Sertoli cell-derived inhibin B secretion and therefore do not invariably mirror the severity of spermatogenic impairment. Accordingly, normal FSH concentrations do not exclude primary testicular dysfunction and may be observed in patients with milder, focal, unilateral, or heterogeneous forms of spermatogenic failure. This subgroup likely represents a biologically heterogeneous population, including subjects with partial Sertoli cell dysfunction, early-stage testicular damage, FSHB and FSHR gene polymorphisms, or genetic conditions associated with impaired spermatogenesis despite preserved endocrine feedback.

It is noteworthy that idiopathic infertility—comprising category V (idiopathic semen alterations) plus category VI (unexplained couple infertility)—was present in only 5.3% of the couples. Importantly, this finding should not be interpreted as a true reduction of idiopathic disease burden. Rather, the comprehensive diagnostic work-up allowed for a more refined pathophysiological categorization of patients who might otherwise have been labeled as idiopathic if evaluated using semen analysis alone.

In everyday clinical practice, a diagnosis of idiopathic infertility is too often assigned without a thorough diagnostic procedure (8). Indeed, our results, which are consistent with previous observations from a multicenter study on infertile couples undergoing a complete multidisciplinary assessment (3), showed that a diagnosis of idiopathic infertility must be given only after all known causes have been properly evaluated and excluded, using a detailed and meticulous diagnostic process and considering the fact that semen evaluation, albeit pivotal, is not sufficient to define the status of fertility/infertility of a man (7, 8, 11). In particular, semen evaluation, albeit useful, has several pitfalls that limit its ability to reliably distinguish between fertile and infertile men. These include substantial day-to-day intraindividual variation, interoperator variability, and the inherent ability of conventional semen parameters to accurately predict pregnancy (25). Therefore, the reported rate of idiopathic infertility depends on the number and the depth of the evaluation performed in both the male and female partners.

Importantly, a substantial proportion of patients in category III present with idiopathic primary spermatogenic failure; however, this must not be misinterpreted as idiopathic infertility, since these patients have a clearly defined pathophysiological condition—primary testicular failure—responsible for infertility, even when the underlying etiology remains unidentified after a comprehensive diagnostic work-up. Therefore, even in the absence of an identifiable etiological factor, a well-defined pathophysiological cause of infertility is still present.

The high prevalence of male tract infection/inflammation observed here is in line with our previous multicentric study (3). Among the various causes of MFI, male tract infections are recognized as a significant contributing factor, and previous studies have reported an infection prevalence of up to 40% in infertile males (26). Despite their potentially detrimental impact on male fertility, these infections often remain underdiagnosed and undertreated, primarily due to their frequently asymptomatic nature. In fact, they can be present in completely asymptomatic males as well as in those experiencing clinical symptoms, such as pain, discomfort, or abnormal semen parameters. A variety of bacterial, viral, and fungal pathogens, including HPV, can infect the male

seminal tract and negatively impact fertility through both direct and indirect mechanisms (27–29). Moreover, according to our predefined diagnostic criteria, approximately one-quarter of the patients were classified within the infection-/inflammation-related category. This category included both microbiologically documented infections and inflammatory conditions supported by combined clinical, seminal, and ultrasound findings. Importantly, microbiologically documented infections and inflammation without microbiological confirmation were analyzed as distinct subcategories (IA, IB and IC), thereby avoiding the assumption that all patients classified in category I had an active infectious process. These findings support the potential value of semen microbiological evaluation and TRUS in selected patients undergoing a comprehensive diagnostic work-up for MFI.

The 8.5% prevalence of HH (category IV) and the high prevalence of low testosterone in category IIIA (43%) underline that testosterone deficiency is a common condition in MFI, as previously reported in other studies (30, 31). Importantly, the vast majority of HH cases were acquired functional forms associated with overweight and obesity (about 7%). Furthermore, a high prevalence of subclinical hypogonadism was found in patients in category IIIA (56.8%).

These data not only highlight a treatable form of MFI (combined gonadotropin therapy) in cases of HH, but they also suggest that a primary intervention on lifestyle factors (diet and physical exercise) should be applied in patients with obesity-associated infertility. Furthermore, patients with subclinical hypogonadism should be regularly followed up to monitor the possible progression to overt hypogonadism (24, 32).

Varicocele, although present in approximately one-third of all male infertile patients (33), is rarely—in everyday clinical practice—considered to be the primary cause of semen alterations; indeed, it is typically found in addition to other clinical conditions that cause or are associated with MFI (ie, semen infections/inflammations or primary testicular disease). It is therefore of pivotal importance to understand when infertility is really caused by varicocele. As previously reported (3), we applied the clinical criteria of a high-grade varicocele (>3rd grade according to the Sarteschi scale) in the absence of any other factor causing or associated with infertility.

The proposed classification of patients with MFI may support a more individualized and pathophysiology-oriented clinical management (11). For instance, patients in category I might be treated with antibiotic/anti-inflammatory therapy (34) and patients in category II with urological surgery and sperm retrieval from the testis/epididymis (11), and patients in category IIIB may represent potential candidates for FSH treatment according to current evidence. Meanwhile, patients in category IV could benefit from gonadotropin therapy combined with lifestyle interventions, when needed (3, 11, 35). Finally, patients with idiopathic semen alterations (category V) could benefit from empirical approaches, such as the use of nutraceuticals or FSH (36).

By moving beyond semen analysis and integrating hormonal, ultrasound, and genetic data, this approach provides a clinically applicable framework that could support a more rational, etiology-driven management of infertile couples. Although reproductive outcomes were not the primary endpoint and the aim of this study was to provide a structured pathophysiological framework for MFI, this study provides the necessary framework for future outcome-driven validation.

The present study was designed as a diagnostic phenotyping study and was not thought to primarily assess reproductive outcomes or treatment response. Therefore, future prospective studies should evaluate whether this classification-based management translates into improvements in semen parameters, natural conception rates, ART outcomes, or live birth rates.

Limitations of our study include its monocentric nature and the lack of data across all patients regarding sex hormone-binding globulin (SHBG) concentrations. Measuring SHBG would have allowed for the calculation of free testosterone concentrations, providing a more precise evaluation of the gonadal axis. This limitation is particularly relevant given the high prevalence of overweight and obesity among infertile men, as alterations in SHBG can substantially influence TT concentrations and potentially confound the classification of functional hypogonadism. In addition, because of the monocentric nature of the study, the relative distribution of categories observed in our cohort should be interpreted in the context of a tertiary referral population and might not fully reflect the general infertile population. Therefore, the relative frequency of categories reported in this study should not be interpreted as prevalence estimates of the general infertile population. The referral structure of our tertiary center likely enriched the cohort for patients with severe spermatogenic impairment, genetic abnormalities, and complex endocrine disorders.

Conclusion

Comprehensive clinical, endocrine, imaging, and genetic evaluations substantially reduce the proportion of patients classified as having idiopathic infertility by enabling a more precise pathophysiological characterization of MFI. The proposed classification identifies clinically distinct subgroups and provides a pragmatic framework for future, etiology-oriented management strategies. Further prospective studies are needed to determine whether this classification-based management translates into better reproductive outcomes, in order to improve, when possible, the overall male fertility status and the results of ARTs. These advancements should remain the primary goals when managing infertile couples, reserving ARTs for cases where natural conception is impossible.

Disclosures

The disclosure for authors G.G., A.G., N.C., A.D.M., A.Ga., L.L.-F.L., M.M., G.M., P.P., M.S.R., A.S., R.S., C.V., L.D.T. and A.F. is as follows: The authors have nothing to disclose.

Data availability

Data are available from the corresponding author upon reasonable request.

References

- World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen*. 6th ed. World Health Organization; 2021.
- Ferlin A, Calogero AE, Krausz C, et al. Management of male factor infertility: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS). *J Endocrinol Invest*. 2022;45(5):1085-1113.
- Grande G, Garolla A, Graziani A, et al. Comprehensive diagnostic and therapeutic approach to male factor infertility aimed at natural fertility: a multicentric retrospective cohort study. *Andrology*. 2025;13(8):2122-2130.
- Garolla A, Pizzol D, Carosso AR, et al. Practical clinical and diagnostic pathway for the investigation of the infertile couple. *Front Endocrinol (Lausanne)*. 2020;11:591837.
- Sharma A, Minhas S, Dhillon WS, Jayasena CN. Male infertility due to testicular disorders. *J Clin Endocrinol Metab*. 2021;106(2):e442-e459.
- Fallara G, Cazzaniga W, Boeri L, et al. Male factor infertility trends throughout the last 10 years: report from a tertiary-referral academic andrology centre. *Andrology*. 2021;9(2):610-617.
- Grande G, Graziani A, Ferlin A. Guideline for unexplained couple infertility: misunderstandings on the approach to the male factor. *Hum Reprod*. 2024;39(4):859-860.
- Björndahl L, Esteves SC, Ferlin A, Jørgensen N, O'Flaherty C. Improving standard practices in studies using results from basic human semen examination. *Andrology*. 2023;11(7):1225-1231.
- Graziani A, Rocca MS, Vinanzi C, et al. Genetic causes of qualitative sperm defects: a narrative review of clinical evidence. *Genes (Basel)*. 2024;15(5):600.
- Romualdi D, Ata B, Bhattacharya S, et al. Evidence-based guideline: unexplained infertility. *Hum Reprod*. 2023;38(10):1881-1890.
- Santi D, Corona G, Salonia A, Ferlin A. Current drawbacks and future perspectives in the diagnosis and treatment of male factor infertility, with a focus on FSH treatment: an expert opinion. *J Endocrinol Invest*. 2025;48(5):1085-1100.
- Ferlin A, Foresta C. Infertility: practical clinical issues for routine investigation of the male partner. *J Clin Med*. 2020;9(6):1644.
- Graziani A, Grande G, Martin M, et al. Chronic prostatitis/chronic pain pelvic syndrome and male infertility. *Life*. 2023;13(8):1700.
- Colonnello E, Graziani A, Rossetti R, et al. The chronobiology of hormone administration: "doctor, what time should I take my medication?" *Endocr Rev*. 2025;46(5):670-689.
- Lotti F, Frizza F, Balercia G, et al. The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: clinical, seminal and biochemical characteristics. *Andrology*. 2020;8(5):1005-1020.
- La Vignera S, Crafa A, Condorelli RA, et al. Ultrasound evaluation of patients with male accessory gland inflammation: a pictorial review. *Andrology*. 2021;9(5):1298-1305.
- Lotti F, Frizza F, Balercia G, et al. The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: prostate-vesicular transrectal ultrasound reference ranges and associations with clinical, seminal and biochemical characteristics. *Andrology*. 2022;10(6):1150-1171.
- Ferlin A, Dipresa S, Delbarba A, et al. Contemporary genetics-based diagnostics of male infertility. *Expert Rev Mol Diagn*. 2019;19(7):623-633.

19. Krausz C, Riera-Escamilla A. Genetics of male infertility. *Nat Rev Urol*. 2018;15(6):369-384.
20. Pozzi E, Boeri L, Candela L, et al. Infertile couples still undergo assisted reproductive treatments without initial andrological evaluation in the real-life setting: a failure to adhere to guidelines? *Andrology*. 2021;9(6):1843-1852.
21. Graziani A, Grande G, Martin M, et al. Fertility preservation in more than 7000 male patients: a single-center, tertiary care registry study over 30 years. *Cancers (Basel)*. 2025;17(4):689.
22. Graziani A, Merico M, Grande G, et al. A cryptozoospermic infertile male with Y chromosome AZFc microdeletion and low FSH levels due to a simultaneous polymorphism in the *FSHB* gene: a case report. *Hum Reprod*. 2024;39(3):504-508.
23. Houston BJ, Riera-Escamilla A, Wyrwoll MJ, et al. A systematic review of the validated monogenic causes of human male infertility: 2020 update and a discussion of emerging gene-disease relationships. *Hum Reprod Update*. 2021;28(1):15-29.
24. Isidori AM, Aversa A, Calogero A, et al. Adult- and late-onset male hypogonadism: the clinical practice guidelines of the Italian Society of Andrology and Sexual Medicine (SIAMS) and the Italian Society of Endocrinology (SIE). *J Endocrinol Invest*. 2022;45(12):2385-2403.
25. Furini C, Costantino F, Granata AR, Spaggiari G, Santi D, Simoni M. Still counting sperm? Why novel, truly informative measurements of testis function in male infertility are urgently needed. *Endocrine*. 2025;90(3):1067-1078.
26. Olana S, Mazzilli R, Santino I, et al. Sperm culture and bacterial susceptibility to antibiotics in a large andrological population: prevalence and impact on seminal parameters. *Int Microbiol*. 2022;26(1):69-79.
27. La Vignera S, Vicari E, Condorelli RA, D'Agata R, Calogero AE. Male accessory gland infection and sperm parameters (review). *Int J Androl*. 2011;34(5 Pt 2):e330-e347.
28. Garolla A, Graziani A, Grande G, Ortolani C, Ferlin A. HPV-related diseases in male patients: an underestimated conundrum. *J Endocrinol Invest*. 2023;47(2):261-274.
29. Grande G, Vincenzoni F, Mancini F, et al. Semen proteomics reveals the impact of *Enterococcus faecalis* on male fertility. *Protein Pept Lett*. 2018;25(5):472-477.
30. Spaggiari G, Costantino F, Dalla Valentina L, et al. Are they functional hypogonadal men? Testosterone serum levels unravel male idiopathic infertility subgroups. *Endocrine*. 2024;84(2):757-767.
31. Ferlin A, Garolla A, Ghezzi M, et al. Sperm count and hypogonadism as markers of general male health. *Eur Urol Focus*. 2021;7(1):205-213.
32. Corona G, Rastrelli G, Dicuio M, et al. Subclinical male hypogonadism. *Minerva Endocrinol (Torino)*. 2021;46(3):252-261.
33. Bertolotto M, Freeman S, Richenberg J, et al. Ultrasound evaluation of varicoceles: systematic literature review and rationale of the ESUR-SPIWG Guidelines and Recommendations. *J Ultrasound*. 2020;23(4):487-507.
34. Milardi D, Luca G, Grande G, et al. Prednisone treatment in infertile patients with oligozoospermia and accessory gland inflammatory alterations. *Andrology*. 2017;5(2):268-273.
35. Grande G, Graziani A, Scafa R, Garolla A, Santi D, Ferlin A. FSH therapy in male factor infertility: evidence and factors which might predict the response. *Life*. 2024;14(8):969.
36. Calogero AE, Aversa A, La Vignera S, Corona G, Ferlin A. The use of nutraceuticals in male sexual and reproductive disturbances: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS). *J Endocrinol Invest*. 2017;40(12):1389-1397.