Prevalence and clinical implications of biochemical hypogonadism in patients with nonobstructive azoospermia undergoing infertility evaluation

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Objective: To investigate the prevalence and clinical implications of biochemical hypogonadism in infertile men with nonobstructive azoospermia (NOA).
Design: Cohort study.
Setting: University-affiliated tertiary center for male reproductive health.
Patients: 767 consecutive normogonadotropic or hypergonadotropic patients with NOA undergoing infertility evaluation from 2014 to 2021.
Intervention: Patients aged 23–55 years underwent comprehensive clinical, hormonal, genetic, semen analysis, and histopathology evaluations and were classified on the basis of predefined baseline follicle-stimulating hormone (12 IU/L) and total testosterone (350 ng/dL) serum levels cutpoints into four groups: hypergonadotropic hypogonadal, hypergonadotropic eugonadal, normogonadotropic hypogonadal, and normogonadotropic eugonadal. All patients were naïve regarding previous sperm retrieval (SR) or hormonal therapy use.
Main Outcome Measures: The period prevalence of biochemical hypogonadism, defined as testosterone levels of <350 ng/dL, and the distribution of patients per group were computed. The associations between hypogonadism, clinical factors, and SR success were evaluated using multivariable logistic regression analyses. Adjusted relative risks (aRRs) and 95% confidence intervals (CIs) were estimated to assess the association between SR and patient classification.
Results: The overall period prevalence of biochemical hypogonadism was 80.8% (95% CI 77.9%–83.4%). The prevalence of patients by group was hypergonadotropic hypogonadal (42.4%, 38.9%–45.9%), normogonadotropic hypogonadal (38.5%; 35.1%–41.9%), hypergonadotropic eugonadal (8.3%; 6.6%–10.5%), and normogonadotropic eugonadal (10.8%; 8.8%–13.2%). Reduced testicular volume and lower estradiol levels were associated with an increased likelihood of hypogonadism. Paternal age was also an independent predictor, with higher age linked to an increased likelihood of hypogonadism. Hypogonadism was less likely in patients with germ cell maturation arrest and more likely in those with Sertoli cell-only. Patients with hypergonadotropic hypogonadism had lower SR success than normogonadotropic eugonadal counterparts (aRR 0.611; 95% CI 0.398–0.855). In the subset of hypogonadal men, hypergonadotropic patients had lower SR success than normogonadotropic participants (aRR 0.632; 0.469–0.811).
Conclusion: The prevalence of biochemical hypogonadism among men with NOA is substantial. Hypogonadism is associated with testicular volume, estradiol levels, age, and histopathology patterns. This condition impacts SR success and emphasizes the need for improved care for men with NOA. (Fertil Steril Rep® 2024;5:14–22. ©2023 by American Society for Reproductive Medicine.)
Key Words: Male infertility, nonbstructive azoospermia, hypogonadism, testosterone, prevalence

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Nonobstructive azoospermia (NOA) is characterized by the absence of spermatozoa in semen due to spermatogenic failure (1), affecting 5%–15% of men undergoing infertility evaluation and accounting for approximately 60% of all azoospermia cases (2). Nonobstructive azoospermia can result from various factors leading to severe testicular deficiencies, like genetic abnormalities, congenital diseases, gonadotoxin exposure, orchitis, and testicular trauma, but many cases remain idiopathic (3). Despite its prevalence, few studies have investigated the hormonal profile of men with NOA seeking fertility (4, 5).

Men with NOA may exhibit biochemical hypogonadism (4, 6), indicated by low circulating levels of total testosterone (T) (7), suggesting concurrent Leydig cell insufficiency. Because the testis produces >95% of total T concentrations, its circulating levels are used to estimate intratesticular testosterone (ITT) production. In nonobese men, there is a fair correlation ($r = 0.82$) between circulating T and ITT levels (8), even though ITT concentrations are much higher (9, 10).

Intratesticular T levels play a vital role in spermatogenesis, especially during the final stages, acting through androgen receptors in Sertoli cells, which produce T-dependent paracrine stimuli for germ cell development (11, 12). In men with NOA exhibiting biochemical hypogonadism, there may be reduced ITT concentrations (8), potentially impairing spermatogenesis. Studies in rodents have shown that reductions in ITT levels are associated with disrupted spermatogenesis (13), and in humans, serum T levels appear to have a positive relationship with sperm retrieval (SR) rates (14, 15). However, the exact prevalence of hypogonadism in men with NOA is not well documented, and data on the clinical factors associated with this condition are lacking.

This study aims to determine the prevalence of biochemical hypogonadism in men with NOA and explore its relationship with clinical factors and SR outcomes. Our goal is to provide valuable insights that can improve clinical practice and research, ultimately leading to enhanced diagnosis, counseling, and the potential for improved quality of life (QoL) and fertility outcomes for NOA patients.

MATERIALS AND METHODS

Study design

We conducted a retrospective analysis using data from 767 consecutive patients with NOA with either normogonadotropic or hypergonadotropic profiles seeking paternity. The study was conducted at a university-affiliated tertiary center for male reproductive health between January 2014 and September 2021. The research adhered to the Guidelines for Accurate and Transparent Health Estimates Reporting and Strengthening the Reporting of Observational Studies in Epidemiology guidelines (16, 17).

Ethical approval

Approval (CAAE #23217119.9.0000.5404) was obtained from the Ethics Committee at the Faculty of Medical Sciences, State University of Campinas (Campinas, São Paulo, Brazil).

Study population

All patients underwent a comprehensive diagnostic evaluation for NOA, including clinical, hormonal, and genetic assessments (i.e., karyotype and Y-chromosome microdeletion screening) as well as semen analysis, as described previously (3). At least one additional semen analysis was performed to confirm the initial diagnosis of azoospermia. Testicular volume was estimated using the Prader orchidometer. Histopathological data were obtained from patients who underwent SR, all of whom were naïve regarding previous SR attempts or hormonal therapy use. Patients with obstructive azoospermia, hypogonadotropic hypogonadism, concomitant thyroid diseases, or with a history of current or past use of medication affecting total T concentrations (e.g., T replacement therapy, selective estrogen-receptor modulators, aromatase inhibitors, gonadotropins, and antiepileptic drugs) were excluded. Patients with a history of previous SR were also excluded.

Assessment of reproductive hormones

Morning venous samples collected between 8:00 AM and 10:00 AM were used to determine levels of T, follicle-stimulating hormone (FSH), luteinizing hormone (LH), sex steroid-binding hormone (SHBG), and estradiol (E) levels using an electrochemiluminescence immunoassay (Cobas e, Roche Diagnostics, Mannheim, Germany). Reference values, lower detection limit, and intra- and interassay variabilities for the analytes were as follows, respectively: T (240.0–816.0 ng/dL, 2.5 ng/dL, $\leq$3.5%, and $\leq$9.3%), FSH (1.5–12.0 UI/L, 0.1 IU/L, $\leq$2.8%, and $\leq$4.5%), LH (1.7–9.0 UI/L; 0.1 IU/L, $\leq$2.0%, and $\leq$2.0%), SHBG (18.0–54.0 nmol/L; 0.35 nmol/L, $\leq$5.6%, and $\leq$6.0%), E (11.3–43.2 pg/mL, 5 pg/mL, $\leq$6.7%, and $\leq$10.6%), and calculated free-T (4.7–25.0 ng/dL). Free-T level was calculated on the basis of SHBG and albumin levels (18).

Data input and extraction

Patient data were systematically collected and extracted using clinical management software (Clinisys, Brazil). Initial work-up data included age, body mass index, smoking status, infertility duration, NOA etiology, testicular volume, reproductive hormone levels (FSH, LH, E, T, T-to-E ratio, SHBG, and calculated free-T), and presence of clinical varicocele (yes or no). Nonobstructive azoospermia etiology categories included cryptorchidism, genetic, postgonadotoxin therapy, postinfection, posttrauma, and idiopathic. Testicular histopathology data were obtained from a biopsy taken during SR. Data validation was performed for implausible values due to data entry errors or missing values, and incongruencies were resolved with the investigators. Individual-level clinical data with nonresolved implausible values and/or missing values were excluded. After data processing, patients were categorized into distinct groups on the basis of their hormonal profiles.

Hypogonadism definition and patient classification

Biochemical hypogonadism was defined as T levels of <350 ng/dL, confirmed on a second analysis at least 1 week apart. The 350 ng/dL threshold level was adopted on the basis of professional society guidelines (e.g., European Academy of Andrology, European Association of Urology, and International Society for the Study of the Aging Male) (19–22). An upper limit threshold level of 12 IU/L for FSH was used to classify patients as having elevated (hypergonadotropic) circulating FSH concentrations, as reported commonly (23).

On the basis of these criteria, patients were grouped as follows: hypergonadotropic hypogonadal (elevated FSH and low T levels; group 1), hypergonadotropic eugonadal (elevated FSH and normal T levels; group 2), normogonadotropic hypogonadal (normal FSH and low T levels; group 3), and normogonadotropic eugonadal (normal FSH and normal T levels; group 4).

Sperm Retrieval

Sperm retrievals were performed using microdissection testicular sperm extraction (micro-TESE) by a senior reproductive urologist (S.C.E.), as described previously (24). Sperm retrieval success was defined as the presence of any number of viable spermatozoa during extractions. Only patients who underwent SR without current or past use of hormonal therapy were included in this analysis.

Testicular histopathology

Histologic assessment of testicular biopsies taken during micro-TESE was performed on Bouin-fixed specimens, followed by hematoxylin-eosin staining (25). Specimens were classified on the basis of the predominant histologic pattern as follows: normal spermatogenesis, hypospermatogenesis, spermatogenic maturation arrest, germ cell aplasia (Sertoli cell-only [SCO] syndrome), and tubular sclerosis (26).

Main outcome measures

The primary outcomes were the period prevalence of biochemical hypogonadism in the entire cohort and the period prevalence within each patient group. The secondary outcomes were the relationship between clinical factors and biochemical hypogonadism and the relationship between SR rates and patient groups, adjusted for relevant confounders such as age, body mass index, smoking status, infertility duration, NOA etiology (i.e., idiopathic, cryptorchidism, genetic, postinfection, postgonadotoxic therapy, and posttrauma), testicular volume, and the presence of clinical varicocele. The period prevalence was defined as the proportion of patients with biochemical hypogonadism at the initial evaluation. The SR rate was the proportion of patients with SR success after micro-TESE. Reproductive outcomes were beyond this study's aim.

Statistical analysis

Period prevalence and 95% confidence intervals (CIs) were computed using the Bonferroni-adjusted method of Goodman (27). Categorical data were presented as the number of cases and percentages, whereas continuous data were reported as median and interquartile range. Pearson χ^2 test and Wilcoxon tests were used to analyze categorical and continuous data, respectively, with statistical significance set at $P<.05$.

Multivariable nominal logistic regression analysis was performed on the entire dataset to explore the relationship between clinical factors and biochemical hypogonadism. Significant P values were adjusted to ensure a false discovery rate of 5% or lower. Additionally, nominal logistic regression analysis, adjusted for patient characteristics, was conducted to assess the likelihood of SR success according to patient groups. This analysis was on the basis of the subset of patients who had undergone SR. Odds ratios obtained from logistic regression analyses were used to calculate relative risks along with their corresponding 95% CIs, using the method described by Zhang and Yu (28). The analyses were performed using JMP PRO 13 and SAS 9.3 (SAS Institute, Cary, NC).

RESULTS

Participants

A total of 1,003 patients with NOA were evaluated initially, and 767 met the inclusion and exclusion criteria, representing 76.5% of all patients with NOA evaluated at the institution during the study period. The cohort comprised 8.9% of the total male patients (767 out of 8,568) and 49.7% of azoospermic individuals (767 out of 1,542) attending the Clinic.

Table 1 shows the baseline characteristics of the patient cohort. Patients with hypogonadism (groups 1 and 3 combined) exhibited significantly lower testicular volume ($P<.0001$), higher baseline serum levels of FSH ($P=.0009$) and LH ($P=.01$), and lower baseline serum levels of E ($P=.02$), T ($P<.0001$), free-T ($P<.0001$), and T-to-E ratio ($P<.0001$) than their eugonadal counterparts (groups 2 and 4 combined). Testicular histopathology results also differed significantly between hypogonadal and eugonadal patients ($P=.0004$), with hypogonadal patients more frequently showing SCO or hypospermatogenesis and eugonadal patients having a higher proportion of biopsies exhibiting spermatogenic maturation arrest.

Main outcome measures

The overall prevalence of biochemical hypogonadism was 80.8% (Table 2). The prevalence among patient groups was as follows: group 1 (hypergonadotropic hypogonadal), 42.4%; group 2 (hypergonadotropic eugonadal), 8.3%; group 3 (normogonadotropic hypogonadal), 38.5%; and group 4 (normogonadotropic eugonadal), 10.8%. A detailed distribution of reproductive hormone levels is provided in Supplemental Figure 1 (available online).

Figure 1 illustrates the clustering of patients by groups on the basis of predefined baseline T and FSH levels cutpoints. Among patients with hypogonadism (groups 1 and 3 combined), 52.4% (325 out of 620) were hypergonadotropic (FSH > 12 IU/L), whereas 47.6% (295 out of 620) were normogonadotropic (within-range FSH levels) ($P=.23$). Among patients with eugonadism (T ≥350 ng/dL; groups 2 and 4

TABLE 1

Baseline characteristics of the 767 patients with nonobstructive azoospermia, overall, and stratified by the presence or absence of biochemical hypogonadism.

Parameter	All patients (n = 767)	Biochemical hypogonadism (n = 620)	Biochemical eugonadism (n = 147)	*P* value
Age (y)	35 (32; 39)	35 (32; 39)	35 (31; 39)	.15[a]
Body mass index (kg/m^2)	27.1 (24.5; 30.9)	27.4 (24.8; 31.0)	26.4 (24.1; 31.2)	.33[a]
Infertility duration (mo)	57.6 (38.4; 96.0)	57.6 (38.4; 96.0)	62.4 (38.4; 98.8)	.12[a]
Smoker	61 (9.9)	62 (10.0)	18 (12.2)	.42[b]
Etiology				.17[b]
Idiopathic	532 (69.4)	432 (69.7)	100 (68.0)	
Cryptorchidism	115 (15.0)	98 (15.8)	17 (11.6)	
Genetic	43 (5.6)	35 (5.7)	8 (5.4)	
Postinfection	34 (4.4)	23 (3.7)	11 (7.5)	
Postgonadotoxic therapy	39 (5.1)	28 (4.5)	11 (7.5)	
Posttrauma	4 (0.5)	4 (0.6)	0 (0.0)	
Testicular volume (mL)				
Total (right + left)	20.0 (16.0; 27.0)	20.0 (16.0; 27.0)	24.0 (20.0; 31.0)	**<.0001**[a]
Baseline hormonal levels				
FSH (mIU/mL)	12.1 (8.6; 17.6)	12.3 (8.9; 18.2)	11.0 (7.5; 16.0)	**.0009**[a]
LH (mIU/mL)	6.0 (4.7; 8.8)	6.2 (5.0; 8.9)	5.4 (4.4; 8.2)	**.01**[a]
Estradiol (pg/mL)	26.0 (21.0; 31.0)	26.0 (20.1; 31.0)	28.0 (22.0; 35.0)	**.002**[a]
Total testosterone (ng/dL)	290.0 (245.0; 333.0)	276.0 (233.0; 304.0)	395.0 (367.0; 408.0)	**<.0001**[a]
Testosterone-to-estradiol ratio	10.7 (8.4; 14.5)	10.1 (8.0; 13.2)	14.2 (11.2; 17.1)	**<.0001**[a]
Free testosterone (ng/dL)	7.0 (6.0; 8.0)	6.9 (6.0; 7.4)	8.0 (7.0; 9.0)	**<.0001**[a]
SHBG (nmol/L)	29.0 (24.5; 38.6)	28.6 (22.8; 39.1)	32.4 (27.2; 38.5)	.34[a]
Varicocele	203 (32.9)	196 (31.6)	51 (34.7)	.39[b]
Testicular Histopathology[c]				**.0004**[b]
Sertoli cell-only	232 (56.5)	195 (60.0)	37 (43.0)	
Maturation arrest	118 (28.7)	78 (24.0)	40 (46.5)	
Hypospermatogenesis	61 (14.8)	52 (16.0)	9 (10.5)	

Note: Values are given as medians (quartiles) or n (%). Bolded *P*-values are statistically significant.
FSH = follicle-stimulating hormone; LH = luteinizing hormone; SHBG = sex hormone binding globulin.
Biochemical hypogonadism defined as serum testosterone levels of <350 ng/dL.
[a] Wilcoxon test.
[b] Pearson χ^2 test.
[c] On the basis of data from 411 patients, all were naïve regarding previous sperm retrieval attempts or hormonal therapy use.

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combined), 43.5% (64 out of 147) were hypergonadotropic, and 56.5% (83 out of 147) were normogonadotropic (*P*=.12).

Data on SR were available for 411 patients, all of whom were naïve regarding previous SR attempts or hormonal therapy use. The overall SR rate in this cohort was 51.6% (212 out of 411). The SR rates among hypogonadal (groups 1 and 3 combined) and eugonadal (groups 2 and 4 combined) patients were 49.8% (162 out of 325) and 58.1% (50 out of 86), respectively. Among patient groups, SR rates were 38.6% (63 out of 163; hypergonadotropic hypogonadal; group 1), 48.3% (14 out of 29; hypergonadotropic eugonadal; group 2), 61.1% (99 out of 162; normogonadotropic hypogonadal; group 3), and 63.1% (36 out of 57; normogonadotropic eugonadal; group 4) (*P*=.0002). In the overall assessment (Supplemental Table 1), we found no clinically meaningful differences between the groups that underwent SR and those that did not, although certain variables did exhibit statistically significant differences.

Logistic regression analyses

In the multivariable logistic regression analysis investigating the relationship between clinical factors and biochemical hypogonadism (Supplemental Table 2), testicular volume (*P*<.0001) and E levels (*P*=.0002) emerged as significant independent predictors. Reduced testicular volume and lower E levels were associated with an increased likelihood of hypogonadism. Higher paternal age (*P*=.004) was also linked to an increased likelihood of hypogonadism. Furthermore, testicular histopathology emerged as a significant predictor (*P*=.003); patients with germ cell maturation arrest were less likely to have hypogonadism, although those with SCO or hypospermatogenesis were more likely to experience it. These associations retained their significance even after adjusting the *P* values to ensure a 5% false discovery rate (Supplemental Table 2).

In the multivariable logistic regression analysis assessing factors influencing SR success, hypergonadotropic hypogonadism (*P*=.002) and normogonadotropic eugonadism (*P*=.03) were significant predictors. Testicular volume was also a significant predictor (*P*=.01), with increased testicular volume indicating a higher likelihood of SR success. Adjusted relative risks (aRRs) for SR success among patient groups are reported in Table 3, showing that patients with hypergonadotropic hypogonadism (group 1) had a lower probability of successful SR compared with their normogonadotropic eugonadal counterparts (group 4) (*P*=.001). In a subset analysis

TABLE 2

Periodic prevalence of biochemical hypogonadism in the total population and patient groups according to predefined follicle-stimulating hormone (FSH) and testosterone (T) threshold levels.

Groups	No. of patients	Prevalence (%)	95% Confidence interval
All patients			
Biochemical hypogonadism	620/767	80.8	77.9–83.4
Patient groups[a]			
Hypergonadotropic hypogonadal	325/767	42.4	38.9–45.9
Normogonadotropic hypogonadal	295/767	38.5	35.1–41.9
Hypergonadotropic eugonadal	64/767	8.3	6.6–10.5
Normogonadotropic eugonadal	83/767	10.8	8.8–13.2

[a] Hypergonadotropic hypogonadal (elevated FSH and low T), hypergonadotropic eugonadal (elevated FSH and normal T), normogonadotropic hypogonadal (normal FSH and low T), and normogonadotropic eugonadal (normal FSH and normal T).

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focusing on hypogonadal men (groups 1 and 3 combined), hypergonadotropic patients (group 1) demonstrated a significantly lower probability of SR success than their normogonadotropic counterparts (group 3) ($P<.0001$).

DISCUSSION

Main findings

Our study highlights a high prevalence of biochemical hypogonadism among patients with NOA undergoing infertility evaluation, with an 80.8% period prevalence rate. Within our patient cohort, reduced testicular volume, lower E levels, and higher paternal age increased the likelihood of hypogonadism. Additionally, our investigation into testicular histopathology patterns showed that patients with hypogonadism exhibited SCO or hypospermatogenesis more commonly, whereas patients with eugonadism had a higher proportion of cases with germ cell maturation arrest. We introduced a novel classification on the basis of predefined T and FSH threshold levels, categorizing patients into four groups. Hypergonadotropic hypogonadal individuals had a notably higher prevalence within this classification. Importantly, we observed differences in the SR success rates among these groups, with patients with hypergonadotropic hypogonadism exhibiting a significant reduction in SR success compared with their normogonadotropic eugonadism counterparts. These findings suggest that FSH and T levels are pivotal in determining SR success rates in patients with NOA.

Interpretation of findings

Our findings confirm the common occurrence of hypogonadism in patients with NOA undergoing infertility evaluation. The narrow CIs for period prevalence support the certainty of our estimates. However, caution is needed when generalizing our results, as factors such as patient characteristics, clinical practices, and diagnosis criteria can influence the prevalence rates.

The relatively higher prevalence observed in our study, approximately 80%, than in previous reports, approximately 50% (4, 6), may be attributed to different T threshold levels used to define T deficiency. The 300 ng/dL T threshold level, used in previous studies, was derived from research on aging men (29) and has been considered too low for identifying hypogonadism in young men (30). By contrast, we adopted the 350 ng/dL threshold level, endorsed by most professional societies. If we had used the 300 ng/dL threshold level, 56.3% (432/767; Supplemental Fig. 1) of our patient population would have been classified as having biochemical hypogonadism, thus similar to previously reported.

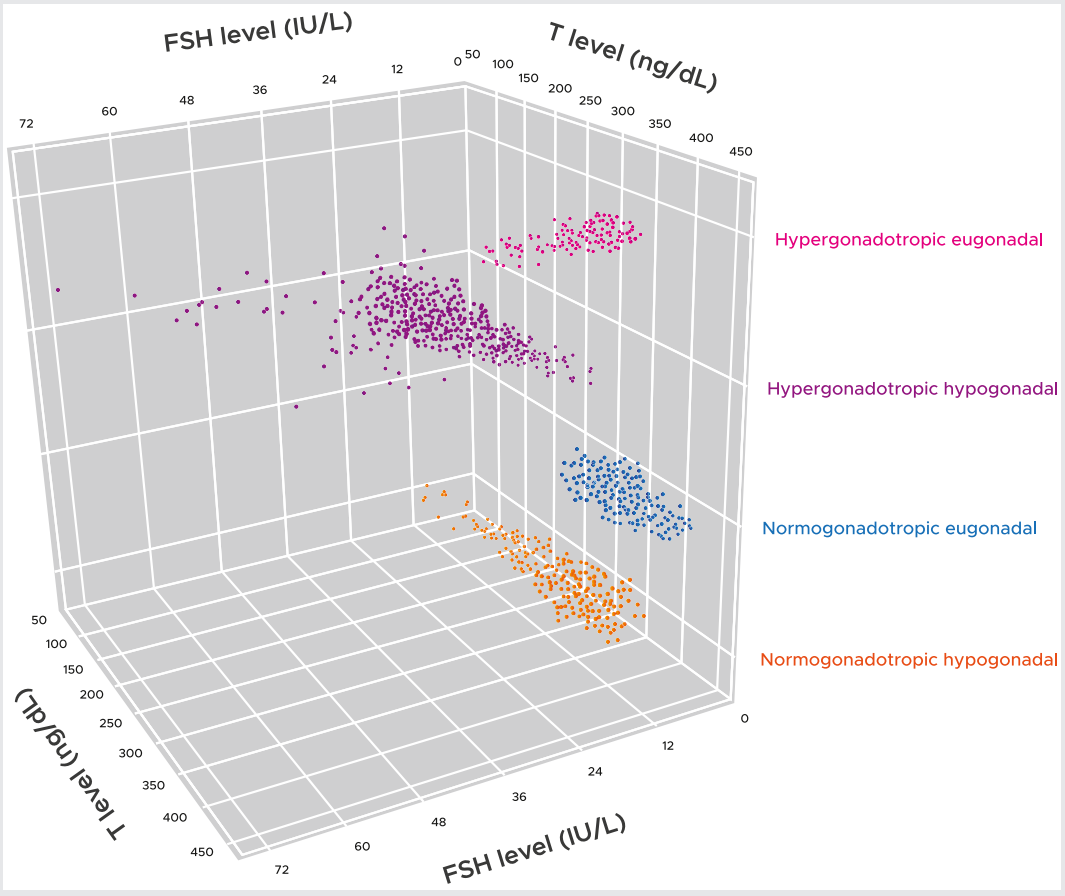
The 350 ng/dL threshold level is likely more appropriate for the younger infertile male population because it is based on a study of 456 healthy, nonobese men aged 19–40 years (Framingham Heart Study Generation 3) (31). In this study, the median T level was 698.7 ng/dL (296.5 ng/dL), and T levels below the 2.5th percentile (348.3 ng/dL) of the reference sample were considered low. Yet, there is no consensus on the ideal T deficiency threshold levels for men of reproductive age.

Age, E levels, testicular volume, and histopathology patterns emerged as significant factors influencing hypogonadism. Advanced paternal age has been associated with an increased risk of hypogonadism because of the age-related gradual decline in T production (32, 33). Aging may also affect SHBG levels, influencing circulating free-T levels (34). Notably, our study did not establish a specific cutoff to define paternal age. Instead, we treated patient age as a continuous variable in our multivariable model. Our findings indicate that aging may magnify the already reduced global testicular function in men with NOA. Additionally, our study reveals a negative relationship between hypogonadism and serum E concentration in men with NOA, possibly attributed to reduced peripheral aromatization of T in fatty tissues among patients with hypogonadism (35).

We also found an association between testicular volume and hypogonadism, which is expected given the relationship between testicular size, spermatogenesis, and androgen production (36, 37). Although most of the testicular parenchyma is implicated in spermatogenesis, varying degrees of Leydig cell insufficiency can coexist in the testes of men with NOA, thus reducing T production (36). Hypogonadic NOA men tend to exhibit more pronounced testicular deficiency, as evidenced by the increased frequency of biopsies showing SCO and hypospermatogenesis. In contrast, germ cell maturation arrest was more common in patients with eugonadism, indicating that their infertility is related primarily to defects in spermatogenesis (38, 39).

Our patient classification on the basis of FSH and T threshold levels provides nuanced insights into the hormonal profile of men with NOA, with potential clinical implications for counseling and management. Patients with hypergonadotropic hypogonadism exhibited significantly lower SR success rates than their normogonadotropic eugonadal counterparts, emphasizing the clinical relevance of this classification. Additionally, within the hypogonadism subset, patients

FIGURE 1



Three-dimensional scatterplots show the patient distribution (n = 767) into groups on the basis of FSH (12 IU/L) and T (350 ng/dL) threshold levels. Hypergonadotropic hypogonadal (FSH > 12 IU/L and T < 350 ng/dL), hypergonadotropic eugonadal (FSH > 12 IU/L and T ≥ 350 ng/dL), normogonadotropic hypogonadal (FSH ≤ 12 IU/L and T < 350 ng/dL), and normogonadotropic eugonadal (FSH ≤ 12 IU/L and T ≥ 350 ng/dL). FSH = follicle-stimulating hormone; T = testosterone.

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with elevated FSH levels achieved lower SR rates than those with normal FSH levels. These findings highlight the importance of FSH and T levels as markers of testicular reserve in patients with NOA, reflecting spermatogenic and androgenic functions and their impact on SR success.

Clinical implications

Our study offers valuable insights into the prevalence of biochemical hypogonadism in men with NOA, aiding in identifying at-risk patients and developing prevention and treatment strategies. Moreover, we also identified potential causal associations between patient characteristics and hypogonadism, which can inform infertility research, clinical practices, and public health policies.

The high prevalence of biochemical hypogonadism in patients with NOA underscores the importance of endocrine evaluation. Hypogonadism is linked to reduced QoL, increased morbidity, and decreased life expectancy (32, 40, 41). Although interventions to mitigate this burden are still under investigation, treating male hypogonadism with gonadotropins has shown promise for improving QoL (42). Our data suggest that early diagnosis and treatment may partially alleviate the burden of hypogonadism in this population.

Our findings reveal variations in SR success between patients with hypogonadism and eugonadism with normal or elevated FSH levels. This has implications for counseling patients about testicular sperm extraction outcomes. A 2021 review demonstrated that patients with normal T levels achieved higher SR success rates than those with subnormal T levels (2,029 patients; OR 1.63, 95% CI 1.08–2.45, $P=.02$) (43). However, the evidence is not unequivocal because others have reported no significant difference in SR success among patients with eugonadism and hypogonadism (6).

Human spermatogenesis is physiologically regulated by the combined and synergic action of FSH- and LH-dependent ITT, and both are needed to obtain qualitatively and quantitatively adequate spermatogenesis (44). Although normal ITT levels are considered crucial for spermatogenesis, the fact that men with maturation arrest

TABLE 3

SRR and aRR according to patient groups.

Patient groups	SRR	aRR	95% CI	*P* value
All patients (n = 767)				
Normogonadotropic eugonadism (group 4; n = 57)[a]	63.1	1	-	-
Hypergonadotropic hypogonadism (group 1; n = 163)	38.6	0.611	0.398–0.855	**.001**
Hypergonadotropic eugonadism (group 2; n = 29)	48.3	0.764	0.433–1.105	.186
Normogonadotropic hypogonadism (group 3; n = 162)	61.1	0.967	0.723–1.181	.784
Hypogonadal cohort (n = 620)				
Normogonadotropic (group 3; n = 162)[a]	61.1	1	-	-
Hypergonadotropic (group 1; n = 163)	38.6	0.632	0.469–0.811	**<.0001**

Note: Patient groups: hypergonadotropic hypogonadal (elevated FSH and low T), hypergonadotropic eugonadal (elevated FSH and normal T), normogonadotropic hypogonadal (normal FSH and low T), and normogonadotropic eugonadal (normal FSH and normal T). Bolded *P*-values are statistically significant.
Hypergonadotropic = FSH levels > 12 IU/L; normogonadotropic = within-range FSH levels; BMI = body mass index; CI = confidence interval; FSH = follicle-stimulating hormone; NOA = nonobstructive azoospermia; T = testosterone.
[a] Reference category; confounders included in the analysis: age, body mass index, smoking status, testicular volume, nonobstructive azoospermia etiology (i.e., idiopathic, cryptorchidism, genetic, postinfection, postgonadotoxic therapy, and posttrauma), and the presence of clinical varicocele.

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experience disrupted spermatogenesis despite having normal serum T levels implies a high degree of complexity in this process (45–47). Further research is needed to unravel the complexities of spermatogenesis and the role of hormonal balance, genetics, and the testicular microenvironment.

Our findings of differential SR rates among patient groups suggest a promising avenue for further investigation—the potential benefits of enhancing ITT levels using hormonal therapy before SR in suitable subgroups. Previous literature has hinted at the utility of hormonal treatment in improving SR rates among patients with NOA. In a 2022 review encompassing evidence from 28 studies, treated patients exhibited a higher overall SR success rate (39.4%; N = 1,403) compared with their untreated counterparts (33.8%; N = 1,564), with an OR of 1.29 (95% CI 1.11–1.50; *P*=.0007) favoring hormonal therapy (44). Another 2022 meta-analysis involving 22 studies and 1,706 patients indicated that SR rates were notably higher in individuals pretreated with hormonal therapy than those without, with an OR of 1.96 (95% CI 1.08–3.56, *P*=.03) (23). However, in the latter study, a subgroup analysis on the basis of baseline FSH levels showed that the improvement was statistically significant only in normogonadotropic men; however, results stratified using T levels were not reported (23). These findings highlight the potential benefits of enhancing ITT levels using hormonal therapy before SR in selected patients. Although the evidence for such interventions is still evolving, our classification system may guide future research in exploring the clinical utility of hormonal optimization to increase SR success in men with NOA.

Strengths and limitations

Our study includes a large sample size, consecutive patients with complete data records, and an in-depth analysis of the hormonal profiles of men with NOA. It provides valuable insights into the prevalence of biochemical hypogonadism and its associated factors in a well-characterized NOA patient cohort. Our data shed light on SR outcomes in patients with NOA and hypogonadism, an area with limited information, and underscore the impact of patient classification on SR success.

However, our study has limitations. First, it is retrospective and single-centered, limiting its generalizability. Testosterone measurements relied on electrochemiluminescence immunoassays, which may have limitations in accuracy and precision (48). Pregnancy data and information on other potential causes of hypogonadism and their symptoms were not available. Our dataset also lacked inhibin B and antimüllerian hormone values, which may be relevant in predicting SR success in patients with NOA (49, 50). Future research should address these limitations and explore interventions to improve SR rates in patients with NOA.

CONCLUSIONS

Our study reveals a substantial prevalence of biochemical hypogonadism, affecting approximately 80% of men with NOA undergoing infertility evaluation. This condition is intricately linked to specific factors such as testicular volume, E levels, patient age, and testicular histopathology patterns. Our patient classification on the basis of well-defined FSH and T threshold levels underscores significant differences in SR rates. Importantly, our findings indicate hypogonadism is associated with reduced SR success rates, particularly when accompanied by elevated FSH levels. Concerted efforts are required to address the impact of biochemical hypogonadism on patients with NOA. These efforts should focus on improving diagnosis accuracy, providing comprehensive counseling, and developing tailored treatment strategies to enhance the overall management of patients with NOA and potentially improve their reproductive outcomes.

CRediT Authorship Contribution Statement

Arnold P.P. Achermann: Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Sandro C. Esteves:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Interests

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