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Seminal Plasma Neutral alpha Glucosidase as a Predictive Biomarker of Epididymal Dysfunction and Association with Body Mass Index in Male Partners of Infertile Couples

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ABSTRACT:

Abnormal body mass index (BMI) is linked to metabolic disturbances that may affect spermatogenesis. Alpha-neutral glucosidase (NAG) is a biochemical marker of sperm maturation and epididymal function. Abnormal BMI has not been associated with seminal plasma NAG among Nigerian men. This study investigates the relationship between seminal plasma NAG activity and BMI among male partners of infertile couples. This study involved 200 infertile men (test group) and 100 age-matched fertile men (control group). Semen analysis was performed according to the World Health Organization guidelines. BMI was calculated using standard protocols, and seminal plasma NAG was determined by enzyme-linked immunosorbent assay. Data were analyzed using appropriate statistical tools, and significance was set at $p<0.05$. The test group had a significantly higher BMI than controls ($p<0.05$). Sperm count, motility, normal morphology, and NAG activity were significantly lower in the test group ($p<0.001$) than in the control group. NAG activity decreased progressively across BMI categories, with the highest activity in normal-weight men and the lowest in obese subjects ($p<0.001$). Significant negative correlations were observed between BMI categories and sperm count ($r=–0.425$), motility ($r=–0.401$), and NAG activity ($r=–0.451$). The area under the curve for NAG (0.744, $p<0.001$) was significant with sperm count, but motility (0.503) and morphology (0.537) were insignificant ($p>0.05$). Increased BMI is associated with reduced NAG activity and impaired sperm quality, suggesting that obesity negatively affects epididymal function and male fertility. Weight reduction programs may be integrated into fertility treatment for overweight and obese men.

Keywords: Male, Semen, Glucosidases, Biomarkers, Humans

INTRODUCTION

Male infertility is a significant contributor to couples' difficulty conceiving and may arise due to several etiological conditions. Accurate identification of the underlying cause is important because treatment and prognosis differ substantially. Routine semen analysis provides essential information about sperm concentration, motility, morphology, and semen volume, but these parameters do not always identify the anatomical or functional source of the problem [1,2].

The epididymis plays a central role in sperm maturation, transport, and acquisition of progressive motility. Neutral α-glucosidase (NAG) is an enzyme secreted predominantly by the epididymis and measured in seminal plasma. Because its concentration reflects epididymal secretory activity, low seminal plasma neutral α-glucosidase may indicate impaired epididymal function or obstruction of the male reproductive tract [3]. It may therefore complement routine semen analysis, particularly when sperm concentration is low or absent.

Despite its potential, the clinical usefulness of seminal plasma NAG is not fully established in all populations and laboratory settings [4]. Results may vary by assay method, reference limits, sample collection and handling, and the characteristics of the men studied. In addition, enzyme levels need to be interpreted alongside clinical findings and other semen parameters. Further

research is needed to determine how accurately the marker distinguishes epididymal dysfunction or obstructive infertility from other causes of male infertility.

A recent report highlighted that excess body weight is linked to a higher incidence of abnormal semen parameters in a group of infertile men. Individuals who were overweight or obese experienced a greater degree of combined and severe semen abnormalities compared to those with a normal BMI. It underscores the significance of BMI as a critical clinical factor in assessing male infertility and suggest that maintaining a healthy weight might be an essential part of fertility-focused management [5]. Several studies have assessed the effects of BMI on semen quality, sperm DNA integrity, and reproductive hormones [6-8]. However, there is a lack of research investigating whether BMI influences epididymal secretory function, as indicated by α-NAG activity among Nigerian male partners of infertile couples. A decrease in its activity could suggest a link between obesity and impaired epididymal function, further contributing to male infertility. Understanding this relationship is crucial for developing targeted interventions for infertile males with metabolic dysfunctions [9].

Therefore, the problem addressed by this study is the lack of sufficient local evidence on whether seminal plasma NAG can reliably help predict epididymal dysfunction and its association with sperm quality parameters as causes of infertility among men. The study will assess its levels and examine their association with body mass index and semen-analysis findings to determine whether the test may provide useful additional information in the diagnostic evaluation of male infertility.

This study investigates seminal plasma NAG and sperm quality parameters among male partners of infertile couplesen with infertility, focusing on their relationship with BMI in male partners of infertile couples. Evaluating the marker against relevant clinical and laboratory findings may clarify its role as an adjunct to routine diagnostic assessment.

MATERIALS AND METHODS

Study Design

This is a cross-sectional study involving 200 male partners of male partners of infertile couples (mean age 36.5±2.8 years) presenting at the fertility clinic with an established diagnosis of infertility after review by a clinician. After physical examination by the attending Physician, the anthropometric measurements were made in the clinic; the subjects were referred to the Chemical Pathology Laboratory for investigation. One hundred (100) males (mean age 34.2 ±2.2 years) with proven fertility recruited from among staff and Students of the University served as controls. Specimens were collected between November 2023 and December 2024.

Study Area

The study was conducted at the Human Reproduction and Research Program Unit, University of Benin Teaching Hospital, Benin City, Nigeria. Participants were selected on the basis of detailed history, clinical examination, and laboratory investigations.

Inclusion and Exclusion Criteria

After physical and clinical examinations, individuals with known pathological or congenital conditions such as severe hypertension, diabetes mellitus, sexually transmitted diseases, testicular varicocele, and genital warts were excluded from the study. In addition, individuals who were currently on antioxidant food supplements, smoked cigarettes, and consumed alcohol excessively were also excluded due to their high seminal reactive oxygen species levels and possibly low antioxidant activity, which could have led to decreased motility and abnormal sperm morphology. Participants with no history of infertility and normal semen analysis, with at least 50% motility and >30% normal sperm morphology and a count of $\geq 15 \times 10^6$ cells/mL were enrolled as controls.

Ethical Consideration

Ethical approval was sought and obtained from the Health Research Ethics Committee of the Edo State Ministry of Health, Benin City, Nigeria (Reference number: HA/737/23/C/10270196; Date of issuance: 27th October, 2023). Participants were informed in the appropriate language about the nature of the research, purpose, procedures, potential risks, and their right to withdraw at any time without consequence before consent was obtained. The study was conducted in accordance with ethical guidelines as set out in the Declaration of Helsinki [10]. All data collected were kept confidential, and participants remained anonymous in the reporting of results.

Consent to Participate

Informed consent was obtained from all participants before their enrolment in the study. Participation was voluntary, and participants were informed of their right to withdraw from the study at any time without penalty.

Sample Size

The sample size for this study was determined using the sample size determination formula for health studies [11]. As previously reported, 18.5% prevalence of male factor infertility was used to calculate the sample size [12].

$$N = \frac{z^2 p (1-p)}{d^2}$$

Where

N = required sample size

Z = confidence level interval 95% (standard value of 1.96)

P = 18.5 %; Prevalence of overweight and obesity in the Southwestern states of Nigeria among the target population from the previous study [12].

D = margin of error at 5% (standard value of 0.05)

$$N = \frac{1.96^2 * 0.185 (1 - 0.85)}{0.05^2} = 232$$

N = 232

A total of 232 male partners of infertile couples evaluated for infertility were initially enrolled in the study. Twenty-two (22) were excluded using the exclusion criteria, while 10 participants did not complete the investigation. Therefore, 200 participants who completed the investigation are presented in this study. While 100 male subjects with proven fertility were enrolled as controls.

Anthropometric Measurements:

Anthropometric measurements were performed to determine the body mass index (BMI) of each participant. BMI is a widely used method to assess an individual's nutritional status and obesity, which could influence fertility. The height and weight of each participant were measured using calibrated, standardized equipment. Height was measured in centimeters (cm) using a stadiometer,

with the participant standing upright, wearing light clothing, barefoot, and with their head positioned in the Frankfort horizontal plane. The participant's weight was recorded in kilograms (kg) using a calibrated digital weighing scale. BMI was then calculated using the following formula: weight (kg) divided by height squared (m^2). Based on BMI values, participants were classified according to the World Health Organization's BMI categories:

- Underweight: BMI < 18.5
- Normal weight: BMI 18.5 - 24.9
- Overweight: BMI 25 - 29.9
- Obese: BMI ≥ 30

Semen Collection:

To minimize variations in sperm parameters, each participant was instructed to abstain from sexual activity for 48 to 72 hours before sample collection. This period was considered essential to allow for sperm maturation and to standardize the quality of the sample. The semen samples were collected by masturbation into sterile containers. Immediately after collection, the semen samples were carefully transported to the laboratory to prevent deterioration and ensure accuracy during the subsequent analysis.

Semen Analysis:

Semen samples were analyzed in accordance with World Health Organization (WHO) guidelines and reference values [13] for semen analysis within 60 minutes after ejaculation. Macroscopic examination was performed to determine pH, volume, colour of semen, liquefaction, and consistency. Microscopic examination was also performed to determine sperm count, sperm morphology, and sperm motility.

Evaluation of *Neutral* alpha *glucosidase* (NAG)

The supernatant of the centrifuged semen, seminal plasma, was used for the assay. NAG was measured using a commercially available ELISA kit (*Biorex Diagnostics* (Antrim, Northern Ireland, United Kingdom), following the manufacturer's instructions.

Statistical Analysis

Data were analyzed using SPSS software (version 25.0). Results were expressed as mean ± standard deviation (SD). Comparisons between groups were performed using t-tests or analysis of variance (ANOVA), and statistical significance set at $p < 0.05$.

RESULTS

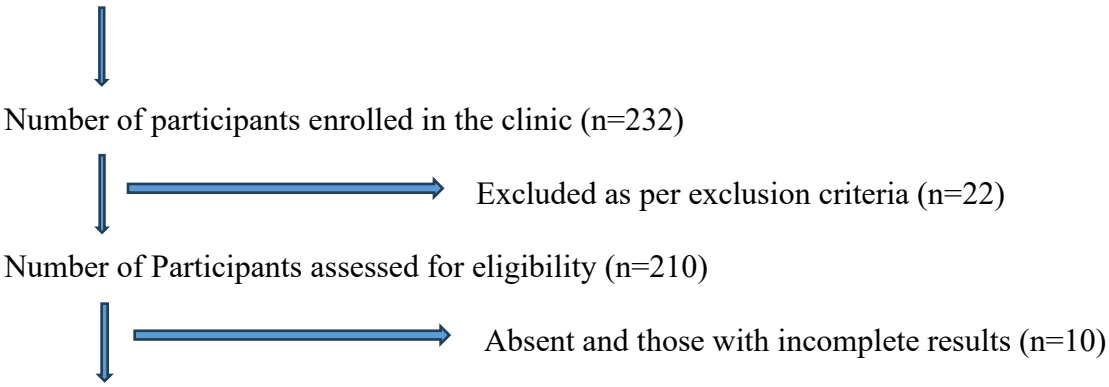

Figure 1: Flowchart of participants enrolled for the study according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines

At the beginning of the study, 232 male partners of infertile couples were identified in the clinics. After the eligibility assessment, only 210 participants were selected, while 22 men were excluded as per the exclusion criteria. However, at the end of the study, 200 participants completed the

investigations. Therefore, the results of 200 male partners of infertile couples who completed data collection are presented, as shown in Figure 1.

This study involved 200 male partners of infertile couples presenting at the fertility clinic with an established diagnosis of infertility after review by a clinician, and 100 males with proven fertility served as controls.

The socio-demographic variables of the two hundred (200) subjects in the study revealed that most of the study participants were married and were civil servants. The sperm quality indices indicate that most of the control subjects had a significantly higher proportion of normospermia (98%), while the test group showed a wider distribution of abnormalities, with 33.5% normozoospermia. Abnormal sperm quality included mild oligospermia (16.5%), severe oligospermia (5%), azoospermia (10%), teratozoospermia (15%), asthenoteratozoospermia (13.5%), and oligoasthenoteratozoospermia (6.5%), indicating compromised fertility status. Based on BMI classification, the control group predominantly had normal weight (88%), while the test group displayed a notable proportion of overweight (31.5%) and obesity (33.5%), with normal weight (33.5%), and underweight (1.5%), suggesting a potential link between increased BMI and impaired sperm quality (Table 1).

Table 1: Frequency of participants' demographics and measured parameters

Variables	**Test [Frequency (%)]**	**Control [Frequency (%)]**
Age (Years)	36.5± 0.8(100)	34.2±0.2(100)
Marital status		
Married	197 (98.5)	96 (96)
Unmarried	03 (1.5)	4 (4)
Total	200 (100)	100 (100)
Occupation		

Civil Servant	193 (96.5)	92 (92)
Artisan	07 (3.5)	8(8)
Total	200 (100)	100 (100)
Sperm Morphology		
Normospermia	67 (33.5)	98 (98)
Mild Oligospermia	33(16.5)	2(2)
Severe Oligospermia	10 (5.0)	0(0)
Azospermia	20 (10.0)	0(0)
Tetratozospermia	30(15.0)	0(0)
Asthenotetrazospermia	27 (13.5)	0(0)
Oligoasthenotetrazospermia	13 (6.5)	0(0)
BMI		
Underweight (<18.5)	03 (1.5)	0 (0)
Normal (18.5-24.9)	67 (33.5)	88 (88)
Overweight (25.0-29.9)	63 (31.5)	12 (12)
Obese (>30.0)	67 (33.5)	0 (0)
Total	200 (100)	100 (100)

Table 2 presents the comparison of mean values of measured variables between male partners of infertile couples and control subjects. The test group was older (36.5 ± 0.8 years) compared to the control group (34.2 ± 0.2 years), with no significant difference ($p > 0.05$). Semen volume was also significantly lower in the test group than in controls, indicating reduced ejaculate volume among infertile men. BMI was notably higher in the test group compared to the control group, showing a significant association between increased body weight and infertility ($p < 0.05$). Furthermore, sperm motility and viability were markedly lower in the test group, while sperm count, percentage of normal forms, and neutral alpha-glucosidase (NAG) activity were all significantly lower ($p < 0.001$). Conversely, the percentage of abnormal sperm forms was significantly higher among

the test subjects compared to controls. These findings collectively suggest a strong link between impaired sperm parameters, elevated BMI, and reduced epididymal function (as indicated by lower NAG activity) in infertile men.

Table 2: Mean values of measured variables between Male partners of infertile couples and control subjects

Variable	Test (n=200)	Control (n=100)	t	p-value
Age (Yrs)	36.5±0.8	34.2±0.2	5.635	**P<0.001**
Semen volume (mL)	2.06±0.06	3.56±0.78	3.771	**P<0.001**
BMI (kg/m^2)	28.70±1.55	22.50±0.28	2.031	**P<0.05**
Sperm Motility (%)	36.10±3.02	63.95±1.35	-8.392	**P<0.001**
Viability (%)	32.6±2.2	30.0 ±1.7		**P<0.05**
Sperm count (X10^6/mL)	29.10±1.71	58.38±1.71	-12.653	**P<0.001**
Normal Form(%)	**5.7**±0.50	**16.0**±1.2	7.234	**P<0.001**
Abnormal form(%)	**83.5**±1.2	**50.6** ±3.60	13.63	**P<0.001**
Neutral alpha glucosidase (NAG) activity (µU/mL)	15.21 ±0.64	26.30± 0.69	14.7	**P<0.001**

Table 3 shows the comparison of sperm quality parameters across the different BMI categories among male partners of infertile couples investigated. The results show a significant increase in age with increasing BMI, with obese participants being the oldest and underweight participants the youngest ($p < 0.001$). Sperm count and motility significantly declined with increasing BMI, with obese men showing the lowest sperm count and motility compared to normal-weight individuals, who recorded the highest values ($p < 0.001$). Viability followed a similar trend, decreasing as BMI increased. Notably, NAG activity was highest in normal -weight individuals and lowest in the obese participants ($p < 0.001$), suggesting compromised epididymal function with increasing adiposity. Although semen volume did not differ significantly across BMI groups ($p > 0.05$), the percentage of abnormal sperm forms was significantly higher in overweight and obese individuals. These findings strongly indicate that elevated BMI is associated with deteriorating sperm quality and reduced epididymal enzyme activity in infertile men.

Table 3: Comparison of sperm Quality parameters based on BMI categories of men investigated for infertility using Analysis of Variance (ANOVA)

Variable	Underweight (<18.5) (n=3)	Healthy weight (18.5-24.9) (n=67)	Overweight (25.0-29.9) (n=63)	Obese (>30.0) (n=67)	F	P-value
Age (Yrs)	31.00±0.00	33.27±0.70	34.91±0.74	37.71±0.69	2.054	P>0.05
Semen Volume (mL)	2.50±0.00	2.99±0.10	1.98±0.13	2.06±0.12	0.250	0.861
Sperm count ($x10^6$/mL)	28.00±0.00	34.21±2.30	22.54±3.05[a]	18.44±2.54[b]	19.746	P<0.001
Motility (%)	38.00±0.00	37.36±2.10	34.14±2.23[a]	29.85±2.22[b]	32.245	P<0.001
Viability (%)	51.4±2.30	60.75±3.04	45.50±3.26	34.45±2.02[b]	32.120	P<0.001
Abnormal form(%)	52.4 ±2.41	50.6±2.10	68.3±2.11	68.2±3.10	28.921	P<0.001
NAG(μU/mL)	22.30± 0.61	26.30± 0.69	17.02 ±0.94[b]	8.21 ±0.64[b]	12.8	P<0.001

a= p<0.05; b=p<0.001; NAG=neutral-α-glucosidase

Table 4 shows the correlation between Body Mass Index (BMI) and various sperm quality parameters among infertile men. A non-significant positive correlation was observed between BMI and semen volume ($r^2 = 0.152$, $p > 0.05$), suggesting that, BMI had minimal effect on semen volume. However, statistically significant negative correlations were observed between BMI and sperm count ($r^2 = -0.425$, $p < 0.001$), motility ($r^2 = -0.401$, $p < 0.002$), and neutral alpha-glucosidase (NAG) activity ($r^2 = -0.451$, $p < 0.001$), indicating that increased BMI was strongly associated with reduced sperm quantity, reduced motility, and impaired epididymal function. In contrast, a significant positive correlation was observed between BMI and abnormal sperm forms ($r^2 = 0.428$, $p < 0.001$), suggesting a link between higher BMI and poorer sperm morphology. Viability also showed a significant positive correlation with BMI ($r^2 = 0.398$, $p < 0.005$), which may reflect complex biological interactions. Overall, the findings highlight that increased BMI adversely affects several critical markers of sperm quality and reproductive function.

Table 4: Correlation between Sperm Quality Parameters, Neutral α- glucosidase activity, and Body Mass Index among male partners of infertile couples

Correlation	**R2**	**P-Value**
Body Mass Index/Semen Volume	0.152	0.20
Body Mass Index/Sperm Count	-0.425	0.001
Body Mass Index/Motility	-0.401	0.002
Body Mass Index/Viability	0.398	0.005
Body Mass Index/Abnormal Form	0.428	0.001
Body Mass Index/ Neutral Alpha glucosidase activity	-0.451	0.001

Table 5 indicates the mean NAG activity among male partners of infertile couples according to sperm quality compared to control subjects. The results indicate that the NAG activity among the azoospermic and oligozoospermic subjects is significantly lower ($p < 0.001$) than that of the normospermic and control subjects. NAG activity among normospermic subjects was not significantly different ($p > 0.05$) from that of the control subjects.

The mean NAG activity among subjects with asthenozoospermia was significantly lower ($p < 0.05$) than that of the control subjects, while the subjects with normospermia showed no significant difference.

The mean NAG activity among subjects with teratozoospermia was significantly lower ($p < 0.05$) than that of the control subjects, but no significant difference ($p>0.05$) was observed when the NAG activity of subjects with Normal Sperm Morphology was compared with the control.

Table 5: Level of *neutral alpha-glucosidase* (*NAG*) activity based on semen quality among study participants

Parameters	**N**	***NAG* activity (Mean ± SEM) (µU/mL)**	**F-value**	**P-value**
Sperm motility (%)				
≥ 40	70	30.33±0.79*	15.87	**0.001**
≤ 40	82	26.30± 0.69*		**0.001**
Control	100	33.45± 1.00		
Sperm count (10^6 cells/ mL)				

Normospermia (>15 x10^6/mL)	82	32.79 ±1.00	49.52	0.001
Oligospermia (<15x10^6/mL)	70	25.75 ±0.40*		0.001
Azoospermia	48	22.68 ±0.54*		0.001
Control (>15 x10^6/mL)	100	33.45 ±1.00		
Morphology (%)				
>4	**86**	30.49 ±0.74	21.72	0.001
<4	**66**	24.91 ±0.48*		0.001
Control	**100**	33.44 ±1.00		0.001

*; n = Number of subject; *=P<0.05 is considered statistically significant; NAG;Neutral alphaglucosidase*

Table 6 shows the performance of NAG activity in predicting Sperm count, sperm motility, and sperm morphology among study participants. It was observed that NAG *activity* shows an acceptable measure of separability with an AUC of 0.744, p<0.001; the ROC curve showed a sensitivity of (80.5%) and a specificity of (61.6%) at a cut-off value (27.9µU/mL), a model of fair performance in predicting sperm count.

However, the model shows a relatively poor measure of separability with an AUC of 0.507 p>0.05) for sperm motility and morphology and AUC of 0.537, indicating a poor diagnostic tool in predicting total sperm motility and morphology.

Table 6: Performance of *Neutral Alpha-Glucosidase Activity* in Predicting Sperm Count, Total Sperm Motility, and Sperm Morphology

Variables	Area	P-value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
Sperm count (x10^6/mL)	.744	.001	.667	.822
Total Sperm Motility (%)	.503	.956	.410	.598
Sperm Morphology (%)	.537	.431	.445	.629

DISCUSSION

One of the critical determinants of male factor infertility is the sperm quality parameters and biochemical markers such as NAG, an indicator of accessory gland function.. BMI, though a crude measure, is frequently used to assess adiposity and its metabolic effects. Several studies have highlighted that increased BMI is associated with hormonal imbalance, oxidative stress, testicular dysfunction, and poor semen quality [14,15]. However, the relationship between BMI and epididymal function, particularly as it relates to α-NAG activity in seminal plasma, remains under-explored. This study sought to elucidate the relationship between BMI and seminal α-NAG activity among male partners of infertile couples, using men of proven fertility as controls.

A high proportion of both test and control groups were married, indicating shared reproductive motivations, while the predominance of civil servants suggests a similar socioeconomic and lifestyle background, particularly sedentary habits known to influence body weight and reproductive health [16]. Notably, the test group exhibited a broader range of abnormal sperm quality parameters such as oligospermia, azoospermia, and teratozoospermia compared to the control group, which predominantly had normospermia (98%), aligning with prior findings linking semen abnormalities to infertility [17,18]. BMI classifications further emphasized this distinction, with the test group showing a higher proportion of overweight and obesity, while the control group remained largely within the normal range. This supports evidence that increased BMI adversely affects sperm quality and seminal fluid enzymatic activity, including NAG, thereby contributing to compromised fertility [19,20].

Notably, increased BMI was significantly associated with a decline in sperm count, motility, and morphology, confirming earlier reports that overweight and obese men had lower semen quality compared to men with normal[21,22].

A key highlight of this study was the significant reduction in seminal plasma α-NAG activity among infertile men compared to controls, indicating impaired epididymal function. Furthermore, a strong negative correlation was observed between BMI and α-NAG activity, suggesting that increasing adiposity adversely affects epididymal secretion and function. This relationship aligns with a previous study [19], which reported lower α-NAG activity in obese men with abnormal sperm parameters, emphasizing the impact of metabolic disturbances on the epididymis.

The epididymis plays a central role in the post-testicular maturation of spermatozoa, and α-NAG, secreted by epididymal principal cells, is crucial for sperm motility and fertilizing ability [23]. A decline in NAG activity, therefore, reflects a disruption in this essential maturation process. The

significant reduction in this enzyme in overweight and obese participants reinforces the detrimental impact of adiposity on male reproductive tract function, extending beyond spermatogenesis.

When stratified by BMI categories, infertile men in the obese group exhibited the lowest sperm count, motility, and α-NAG activity, with statistically significant differences observed across groups ($p < 0.001$). Conversely, participants in the normal BMI range had significantly better outcomes across all sperm parameters and the highest α-NAG activity. These findings highlight a clear relationship between sperm quality, epididymal function, and BMI.

Interestingly, semen volume was not significantly affected by BMI, which is consistent with prior studies that found semen volume is more influenced by accessory glands such as the seminal vesicles and prostate rather than by body fat [24].

While viability showed a mild positive correlation with BMI, the biological plausibility of this finding remains unclear and may be attributed to compensatory mechanisms or variability in viability assays.

The strong association between increased BMI and reduced seminal α-NAG activity observed in this study highlights the need for weight management as a modifiable intervention in male infertility care. Given that NAG is a reliable marker of epididymal function, its consistent reduction in overweight and obese individuals provides a tangible target for assessing treatment efficacy and monitoring metabolic improvement.

Furthermore, incorporating biochemical assessments such as NAG activity into routine infertility evaluation could offer deeper insights into the pathophysiology of idiopathic infertility. Lifestyle interventions aimed at weight reduction may improve epididymal function [25].

Lower seminal NAG activity among obese men with asthenozoospermia was also observed, which aligns with previous studies [26]. However, the present study is distinctive in its combined analysis of BMI categories, semen quality parameters, and NAG activity, providing a more holistic perspective.

The findings in this study implied that seminal plasma NAG activity has differential diagnostic performance across the assessed semen parameters, with better discrimination for sperm count than for sperm motility or morphology. The area under the ROC curve (AUC) for sperm count indicates a fair to acceptable discriminatory ability, suggesting that NAG activity may help distinguish participants with and without the defined sperm-count abnormality. At the selected cut-off value of 27.9 μU/mL, the sensitivity was 80.5%, while specificity was 61.6%. These results suggest that the selected threshold may correctly identify a substantial proportion of participants with the target sperm-count abnormality, although its ability to correctly exclude those without the abnormality is more limited. Thus, NAG activity may have potential as an adjunctive biomarker for identifying men who require further assessment of sperm production and reproductive function. However, the moderate specificity indicates that false-positive classifications may occur, limiting the use of NAG activity as a standalone diagnostic test.

The relatively stronger performance of NAG activity for sperm count may be biologically plausible because NAG is an established marker of epididymal secretory function. The epididymis provides the microenvironment required for sperm maturation, including the acquisition of progressive motility and other functional characteristics. Impaired epididymal function may therefore be associated with changes in sperm quality and, in some circumstances, sperm numbers. Nevertheless, sperm count is influenced by several additional factors, including testicular spermatogenesis, hormonal regulation, reproductive tract obstruction and ejaculate characteristics.

Consequently, the observed association does not establish that NAG directly measures sperm production or that reduced NAG activity causes a low sperm count.

In contrast, the AUC of 0.507 for sperm motility indicates virtually no discriminatory ability beyond chance, while the AUC of 0.537 for sperm morphology indicates poor discrimination. These findings suggest that NAG activity alone may not adequately distinguish participants with normal and abnormal sperm motility or morphology. Although epididymal secretions contribute to sperm maturation, motility and morphology are influenced by multiple testicular, epididymal, metabolic, oxidative and environmental factors. Morphological abnormalities, in particular, may originate during spermatogenesis and cannot necessarily be identified through a marker of epididymal secretory activity. Similarly, sperm motility may be affected by oxidative stress, mitochondrial function, infection and other factors that are not directly captured by NAG measurement.

Overall, the findings support the potential role of seminal plasma NAG activity as an adjunctive marker for assessing sperm-count abnormalities, rather than as a universal predictor of semen quality. Its clinical usefulness would require validation against an appropriate reference standard, assessment of confidence intervals and consideration of other relevant clinical and laboratory variables. Because sperm count, motility and morphology are distinct reproductive outcomes, NAG activity should not replace conventional semen analysis.

Conclusion

This study has demonstrated a significant negative relationship between BMI and seminal plasma NAG activity among male partners of infertile couples. Elevated BMI was associated with reduced NAG activity, indicating compromised epididymal function, as well as poorer semen parameters

including lower sperm count, motility, and abnormal morphology. These findings suggest that excess body weight may impair male fertility not only through testicular dysfunction but also by affecting post-testicular sperm maturation processes within the epididymis. Therefore, maintaining a healthy BMI may play an important role in preserving male reproductive health, and NAG may serve as a useful biochemical marker in the evaluation of epididymal function among infertile men.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to applicable ethical requirements and data confidentiality.

Consent to publish: Not Applicable

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AUTHORS CONTRIBUTIONS:

Conceptualization, MAE; Data Curation, MAE, JBA, JTO, OJA; Methodology, MAE, IA, GEO,KI; Project Administration, MAE, KI, OJA Resources, MAE, JBA, JTO, OJA, IA, GEO, KI, Software, JBA,JTO, OJA, IA, GEO; Supervision, MAE,KI; Writing—Original Draft, JBA, JTO, OJA, IA, GEO; Writing—Review and Editing, MAE. All authors have read and agreed to the published version of the manuscript.

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