

## Assessment of Serum Anti-Müllerian Hormone (AMH) as an Independent Marker for Oligozoospermia and Non-Obstructive Azoospermia in Infertile Nigerian Men

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Anti-Müllerian hormone (AMH) is a Sertoli cell-derived glycoprotein that mediates regression of Müllerian duct in male embryos. The present study aims to evaluate the diagnostic efficacy of serum AMH in the detection of oligozoospermia and non-obstructive azoospermia (NOA) in a homogenous population of Nigerian men. This case-controlled prospective study was conducted on eighty male subjects (aged 18-45 years), at the Jos University Teaching Hospital, Nigeria. Subjects were classified as control (n=30), oligozoospermic (n=27) and non-obstructive azoospermia (NOA; n=23) (World Health Organization, 2010). Serum concentrations of various hormones were measured. Statistical analyses were performed using MedCalc. (v.19.5.1, Ostend, Belgium). Serum AMH levels did not differ significantly among the study groups ( $P > 0.05$ ). Serum levels of testosterone were significantly lower, while serum FSH levels were significantly higher in the infertile groups than the control ( $P < 0.000001$ ). Serum LH levels were significantly higher in the NOA men ( $P < 0.000001$ ), while oligozoospermic men showed no significant difference, compared to control. Receiver operating characteristics (ROC) curve analysis depicted the same cut-off value ( $= 1.7$  ng/ml) of serum AMH for oligozoospermia and NOA with low sensitivity and moderate specificity. The findings suggest that serum AMH is not a potent stand-alone marker of NOA or oligozoospermia among Nigerian men.

**Keywords:** Anti-Müllerian Hormone; Azoospermia; Oligozoospermia; Sertoli Cells; Spermatogenesis; Testosterone.

Anti-Müllerian Hormone (AMH) is a Sertoli cell-derived dimeric glycoprotein that acts a pivotal role in the development of male reproductive tract<sup>1</sup>. It induces regression of the Müllerian ducts during male fetal development<sup>2</sup>. AMH is the earliest Sertoli cell-specific protein which is produced by the testes throughout life. It begins to be produced by the testicles as early as the ninth week of pregnancy and continues to be secreted at a high level until puberty<sup>3,4</sup>. The concentration of AMH in the blood drops substantially during puberty and remains at extremely low levels throughout adulthood<sup>5</sup>. AMH has also been found to regulate the proliferation of Leydig cells as well as their steroidogenic function<sup>6</sup>. It inhibits production of pre-pubertal progenitor Leydig cells and prevent regeneration of Leydig cells after chemical ablation<sup>6,7</sup>.

In the recent decades, AMH has gained much attention in male fertility research. There are conflicting reports on whether seminal or serum AMH can serve as a potential marker for disrupted spermatogenesis in infertile male<sup>8,9</sup>. Oligozoospermia and azoospermia are common male reproductive diseases with varied serum AMH levels<sup>10</sup>. It has been reported that infertile men with oligozoospermia have lower serum AMH concentrations than control men. Moreover, the same study has suggested that serum AMH is a superior marker for male factor infertility to seminal AMH<sup>11</sup>. Another study revealed no significant difference in blood AMH between fertile men and men with low sperm counts, but discovered a link between serum AMH, sperm counts, follicle stimulating hormone (FSH), and free testosterone.<sup>12</sup> These studies had small sample sizes and varied underlying reasons of reproductive issues. Another study, a retrospective analysis of 199 well-characterized men with normal or reduced sperm concentration, found that serum AMH levels correlated negatively with FSH and positively with testicular volume and sperm concentration in men with maldescended testes<sup>13</sup>.

Considering the suggested merit of serum AMH in detection of male fertility impairment, on the other hand, the lack of consensus whether AMH can serve as an independent serum marker of male infertility, the present study objects to examine the diagnostic efficacy of serum AMH in detection

of oligozoospermia and NOA in a homogenous population of Nigerian men.

## MATERIALS AND METHODS

### Study setting and Patients

This prospective, analytical study was conducted in the Departments of Chemical Pathology and Obstetrics and Gynecology of Jos University Teaching Hospital (JUTH), Jos, Plateau State, North Central Nigeria. The duration of the study was 15 months (April 2016 to April 2017). Ethical clearance was obtained from research and ethical committee of Jos University Teaching Hospital, Jos Plateau State, Nigeria (JUTH/DCS/ADM/127/XIX/6332) following the World Medical Association's Helsinki Declaration on Human Subject Research. A total of 50 diagnosed infertile male patients, aged between 18 and 45 years (having a fertile female partner) of which 27 were with oligozoospermia and 23 with non-obstructive azoospermia (NOA) attended the Infertility Unit of Department of Obstetrics and Gynecology, JUTH and 30 age-matched fertile control men participated in this prospective case-controlled study. All participants provided written informed consent. Any etiologies connected to physiological abnormalities, e.g. varicocele, as established by physical examination according to the Dublin grading system<sup>14</sup>, (b) males with chronic conditions (diabetes, hypertension, etc. ), (c) men taking antioxidants, anabolic steroids, hormones, or reproductive treatments, and (d) men with autoimmune diseases. The study also excluded data on female infertility. The body mass index (BMI) was estimated by the formula:  $BMI = \text{weight (kg)} / (\text{Height in m})^2$ <sup>15</sup>.

### Semen analysis

Patients included in this study had seminal fluid analysed according to the World Health Organization (WHO) Manual (fifth edition)<sup>16</sup>. Masturbation was used to obtain sperm specimens after a period of 3 - 5 days of sexual abstinence, and following liquefaction, a sperm concentration assay was done to evaluate the sperm concentration in the specimen. Normal values for sperm concentration were  $\geq 15 \times 10^6/\text{ml}$ , patients who had less than  $15 \times 10^6/\text{ml}$  were considered oligozoospermic and patients with no spermatozoa are found in the

sediment of a centrifuged sample were considered as azoospermic patients.

**Serum hormone assays**

Five milliliters of peripheral venous blood were collected from each participant. Blood samples were collected in plain tubes, allowed to clot and then centrifuged at 1500g for five minutes. The serum was separated then frozen at -20oC until the time of analysis. The hormonal analyses were done by Enzyme Linked Immunosorbent Assay (ELISA) for follicle stimulating hormone (FSH) (Monobind Inc., Lake Forest, California, USA), total testosterone (Monobind, Lake Forest, California, USA), luteinizing hormone (LH) (Monobind, Lake Forest, California, USA), and AMH (Monobind, Lake Forest, California, USA).

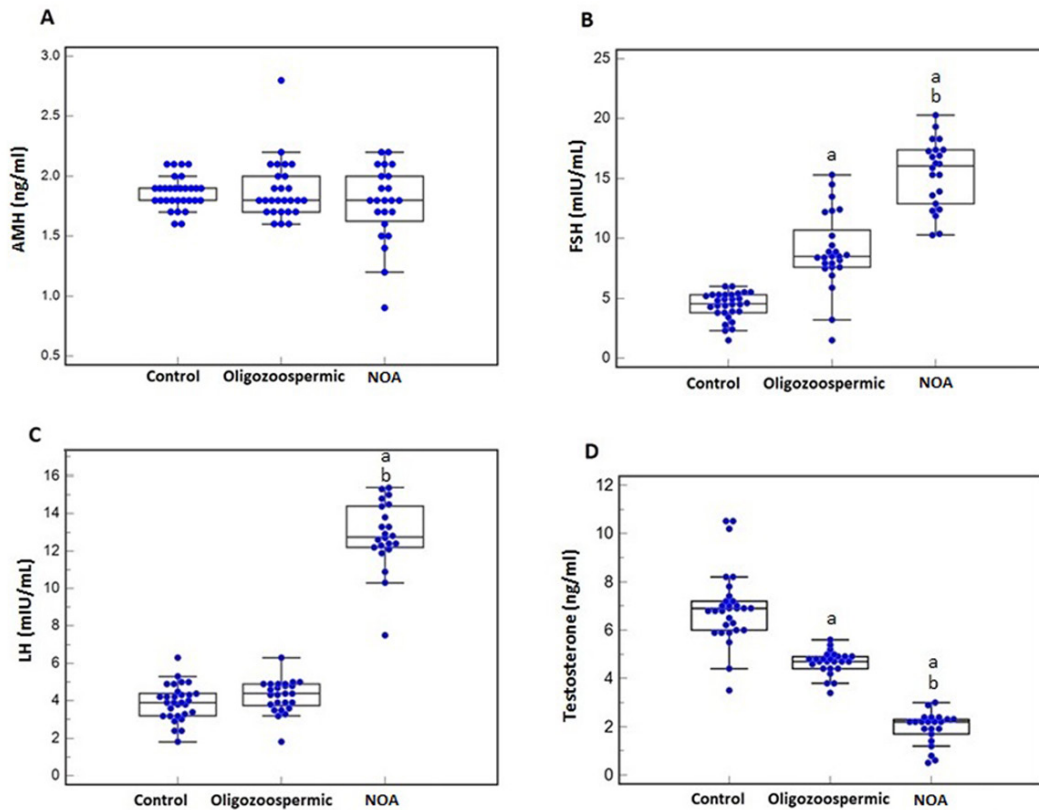
**Statistical analyses**

Data were analysed with MedCalc Statistical software (v.19.5.1, Ostend, Belgium). The Kolmogorov-Smirnov normality test was used to check the distribution of the samples. Comparison

of the seminal and hormonal parameters among control, oligozoospermic and NOA groups, was by the non-parametric Kruskal-Wallis test. If significant differences were found, Dunn’s multiple comparison post-hoc test was used. Spearman rank correlation was used to detect the association of variable parameters in oligozoospermic and NOA patients. Receiver operator characteristic (ROC) analysis was with AMH as continuous variables and sperm concentration as the categorical variables to obtain and compare the area under the curves (AUCs), sensitivities, specificities, Youden’s indices and cut-off values. For all comparisons, P-Values <0.05 were considered statistically significant.

**RESULTS**

The present study included a total of 80 subjects, out of which 33.75% (27 subjects) were recorded with oligozoospermia, 28.75%



**Fig. 1.** Serum AMH (A), FSH (B), LH (C), testosterone (D) levels of control subjects and patients with oligozoospermia and NOA. Superscripts indicate significance levels at P<0.05

(23 subjects) with NOA, whereas and 37.5% (30 subjects) were healthy fertile men. Table 1 presents the age, BMI and seminal parameters of the respondents. Figure 1 shows the serum hormonal levels in control, oligozoospermic and NOA groups. The three groups had similar serum AMH levels ( $P>0.05$ ). Serum FSH levels were significantly higher in the infertile groups than the control ( $P<0.000001$ ). Serum LH levels were significantly higher in the NOA men ( $P<0.000001$ ), while oligozoospermic men showed no significant difference, as compared to control.

The findings of this investigation revealed that there was no statistically significant relationship between blood AMH concentration and serum

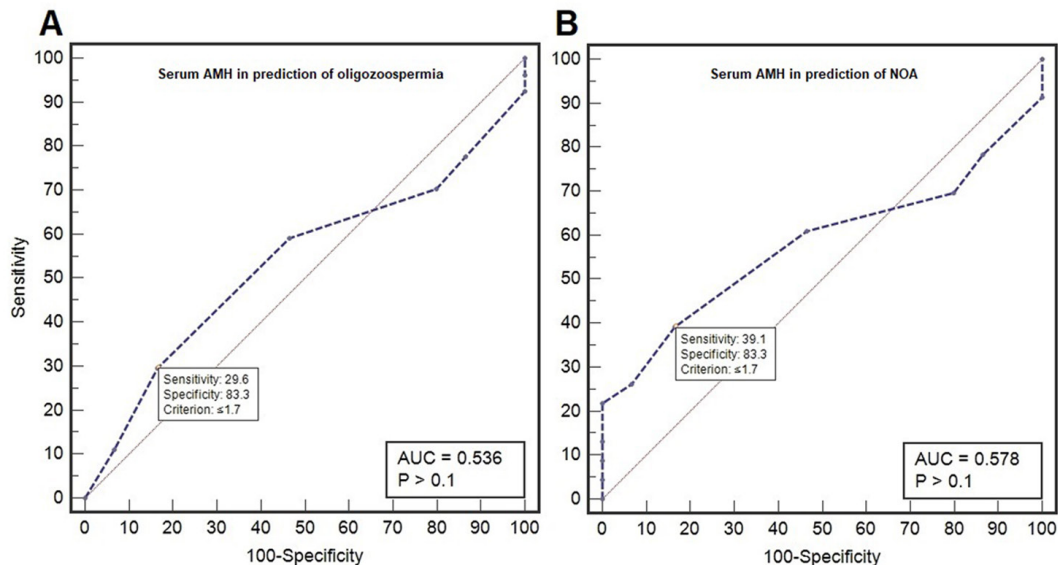
levels of FSH, LH, and testosterone in the control, oligozoospermia, and NOA groups. In addition, there was no statistically significant relationship between serum AMH and the semen parameters in either in the control or infertile groups (Table 2).

The comparison of ROC curves of AMH for prediction of oligozoospermia and NOA are presented in Figure 2. The ROC curve analysis for AMH in predicting oligozoospermia showed AUCs (CI 95%) of 0.536 (0.399 to 0.670) with the cutoff value of  $d^*1.7$  ng/ml, sensitivity of 29.60 and specificity of 83.3. The AUCs (CI 95%) for NOA were 0.578 (0.435 to 0.713) with the cutoff value of  $d^*1.7$  ng/ml, sensitivity of 39.13 and specificity of 83.33 (Figure 2).

**Table 1.** Age, BMI and semen parameters of healthy and infertile subjects.

	Age	BMI (kg/m <sup>2</sup> )	Semen Volume (ml)	Sperm Concentration (10 <sup>6</sup> /ml)	Sperm Motility (%)	Sperm Morphology (%)
Control	31.40±6.891	25.22±3.825	4.527±0.613	52.67±5.768	63.10±6.840	36.73±3.373
Oligozoospermia	31.26±6.377	25.72±4.517	3.722±0.612	5.626±2.616 <sup>a</sup>	33.37±6.362 <sup>a</sup>	26.93±4.094 <sup>a</sup>
NOA	30.57±7.668	26.10±4.259	-	-	-	-

Data are expressed as Mean±SD, <sup>a</sup>vs Control,  $P<0.05$



**Fig. 2.** (A) ROC curves of AMH in the prediction of oligozoospermia (n=27) and (B) azoospermia (n=23). Both ROC curves showed same cut-off values of AMH ( $d^*1.7$  ng/ml) for oligozoospermia and NOA, with AUC values of 0.536 (0.399 to 0.670) and 0.578 (0.435 to 0.713), respectively. P-values of both oligozoospermia (0.643) and NOA (0.364) are statistically non-significant

**Table 2.** Correlation of serum AMH with semen parameters and serum hormonal levels in control, oligozoospermic and NOA men

	Semen Volume <sup>2</sup>	Sperm Concentration <sup>3</sup>	Sperm morphology <sup>4</sup>	Sperm motility <sup>4</sup>	Testosterone <sup>1</sup>	LH <sup>1</sup>	FSH <sup>1</sup>
Control AMH <sup>1</sup>	-0.221 (0.24)	-0.354 (0.055)	-0.227 (0.22)	0.218 (0.24)	-0.019 (0.91)	0.111 (0.56)	0.316 (0.08)
Oligozoospermia AMH <sup>1</sup>	-0.078 (0.69)	-0.146 (0.46)	-0.247 (0.21)	-0.249 (0.21)	0.134 (0.50)	0.046 (0.82)	0.103 (0.60)
NOA AMH <sup>1</sup>	-	-	-	-	-0.110 (0.61)	0.019 (0.93)	-0.05 (0.82)

Data are expressed as r (p), <sup>1</sup>ng/ml, <sup>2</sup>ml, <sup>3</sup>10<sup>6</sup>/ml, <sup>4</sup>%

## DISCUSSION

In a time when there is a declining trend in male fertility across the globe<sup>17</sup> with most of the cases being idiopathic, it is essential to bring forth some simple clinically reliable biomarkers for early detection of male infertility. Lately, there have been contradictory reports on the diagnostic value of serum or seminal AMH as simple markers of impaired spermatogenesis and semen parameters in men with NOA and oligozoospermia<sup>11-13,18-20</sup>. Africa is not an exception among the continents with reduction in male fertility over the last decades<sup>21</sup>, but there is a lack of studies to suggest investigating the efficacy of serum AMH in detection of fertility complications in African men. This study has included a homogeneous cohort of idiopathic infertile Nigerian men with either oligozoospermia or NOA; and investigated whether serum AMH can serve as an independent serum marker for these conditions.

Several studies have been reported the possibilities of serum AMH as a predictor of spermatogenesis in men<sup>18,22</sup>. The present study observed that the serum levels of AMH did not differ significantly among the study groups that included NOA, oligozoospermic and fertile men. Although it is known that Sertoli cells secrete AMH into the seminiferous tubules, the rationale of studying the serum AMH in the present study was that seminal AMH levels may be influenced by the activities of seminal proteases. This also may explain why there is undetectable seminal AMH concentrations even in some fertile donors<sup>22</sup>. Moreover, immature Sertoli cells cannot produce AMH into seminiferous tubules via the apical layer; instead, secretion occurs via the basal layer

into the interstitium. In infertile males, substantial spermatogenic impairment in human seminiferous tubules is related with a prepubertal Sertoli cell population<sup>23</sup>, so more likely to be released into the circulation and detectable in serum. There are studies that revealed that serum AMH were significantly lower in infertile or subfertile men as compared to fertile control<sup>11,24,25</sup>. Thus, circulating AMH content was assumed to be a superior marker of Sertoli cell maturity and spermatogenesis. However, in our study, it may be due to the small sample size that no significant differences in the serum levels of AMH could be detected among the study groups. Alike the present observation, there are several studies that reported no significant changes in the serum AMH levels in infertile/subfertile and fertile men<sup>12,13,26,27</sup>. A largescale study in the Nigerian population should be carried out to validate our observation. Moreover, testicular biopsies in infertile male patients can also further confirm whether AMH could be specific in detecting the type of infertility in men. Another reason for persistent serum AMH levels in all the study groups may be that in most of the NOA or oligozoospermic patients, the functions Sertoli cells and interstitial cells are not completely lost<sup>28</sup>.

The reproductive hormones, namely the FSH, LH and testosterone are the key endocrine regulators of male reproductive functions, while there are several other hormones that can crosstalk with these prime hormones<sup>29</sup>. The present investigation found that both infertile groups had considerably greater FSH serum levels than earlier studies on diverse study populations<sup>30,31</sup> and LH level was significantly higher in the NOA group<sup>30,31</sup>, as compared to the control, while there was significant reduction in the serum levels of

testosterone in the infertile men as compared to the fertile control<sup>12</sup>. Though AMH levels are unrelated to gonadotropin regulation, they have been linked to sperm count and FSH levels<sup>12</sup>. However, in this study, we found no significant correlation of serum AMH with the reproductive hormones (Table 2). Moreover, it is known that testicular AMH production is regulated by androgens, but serum levels of testosterone do not inevitably reflect the concentration of intratesticular androgen and Sertoli cells are regulated by autocrine actions of local androgen rather than by the serum testosterone<sup>33</sup>. Thus, the non-significant correlation of AMH with serum testosterone observed in our study (Table 2) may be relevant in justifying that association between these two hormones are not very essential for prediction of testicular functions.

There is no confirmatory evidence on the diagnostic value of serum AMH and so far, the limited evidence negates the potential of either seminal or serum AMH as a stand-alone marker for prediction of NOA or oligozoospermia<sup>34,35</sup>. Studies by Aksglaede *et al.* (2018) had also suggested that serum AMH is not a predictor of impaired semen quality in infertile men<sup>36</sup>. Moreover, there are studies that suggest that serum AMH may not serve as predictor of sperm recovery in azoospermic men<sup>26,37</sup>. In the present study, we used ROC curves to indicate that AMH is not a good predictor of the incidence of NOA or oligozoospermia among the study patients. We used continuous variables such as AMH and categorical factors such as sperm concentration to illustrate this. The findings revealed that cut-off values of  $d^*1.7ng/ml$  of AMH were effective in predicting both NOA and oligozoospermia, with poor sensitivity and intermediate specificity in both cases in the study.

### CONCLUSION

The present study is the first ever evaluation of the predictive efficacy of serum AMH for infertile men in Nigeria, presented with NOA and oligozoospermia. The results showed that serum AMH may not serve as a single predictor of NOA or oligozoospermia. This is a notable scientific revelation that directs future studies in unveiling other endogenous factors, which together with serum AMH may emerge as predictors of the male infertility subtypes. The study also aims

to encourage further research considering the seminal plasma concentration of AMH including larger sample size. It is thereby suggested that precise prediction of infertility or subfertility in men is warranted by using a multivariate model including all the male reproductive hormones that demonstrate the complex regulation of testicular microenvironment.

### Author Contributions

OBO, AIG, and JOT conceptualized the study; OBO, AIG, JOT, and NSE did bench work; OBO, AIG, JOT, IOC, NSE, OMU, STT, SD, PS did literature search and initial manuscript writing; OBO, AIG, JOT, OMU, SD, PS, SRC did final editing and approved for final publication.

### Ethical Approval

Ethical clearance was obtained from research and ethical committee of Jos University Teaching Hospital, Jos Plateau State, Nigeria (JUTH/DCS/ADM/127/XIX/6332)

### Conflict of Interest

There are no conflict of interest

### Funding Sources

There is no funding sources.

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