

# The Role of Serum and Seminal Fluid Anti-Müllerian Hormone (AMH) in Differentiating Subtypes of Male Infertility

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## Abstract

**Background:** Infertility is the inability to conceive after one year of unprotected intercourse, with male factors accounting for about 50% of cases. Anti-Müllerian hormone (AMH), a glycoprotein belonging to the Transforming growth factor beta family, is secreted from Sertoli cells and plays a key role in male sexual differentiation.

**Objectives:** To examine the potential role of Anti-Müllerian Hormone in differentiating the subtypes of male infertility.

**Cases and Methods:** This case-control study was done at the Infertility Center of Al-Batool Teaching Hospital in Diyala Governorate, Iraq, by the Department of Biochemistry / College of Medicine / University of Baghdad between April 2024 and January 2025. It included 111 males, aged 20-55 years. These subjects were subdivided into four groups based on seminal fluid analysis: The Normozoospermic group (30 males), who served as the control group; the Asthenozoospermia group (29 males); the oligozoospermia group (25 males); and the idiopathic (unexplained) infertility group (27 males). Investigations included serum and seminal fluid measurements of anti-müllerian hormone using enzyme-linked immunosorbent assays.

**Results:** The mean  $\pm$ SD values of anti-müllerian hormone concentrations were significantly higher in the serum compared with the results of seminal plasma in all groups except the oligozoospermia group, where the AMH was higher than in the serum. The receiver operating characteristics (ROC) analysis revealed that Serum AMH exhibited fair to good diagnostic performance with cutoff value between (2.5-3.2 ng/ml), while seminal fluid AMH displayed lower diagnostic accuracy in distinguishing subtypes of male infertility, the cutoff value ranging from (1.75-3.83 ng/ml).

**Conclusion:** Serum AMH levels exhibit fair to good diagnostic performance in differentiating subtypes of male infertility. Seminal fluid AMH displayed lower diagnostic accuracy across all groups, with elevated levels in oligozoospermic subjects. These findings suggest that serum AMH may be a valuable biomarker in evaluating male infertility subtypes.

**Keywords:** Anti-müllerian hormone; Asthenozoospermia; Oligozoospermia; Plasma Seminal fluid; Unexplained infertility.

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## Introduction:

Infertility can be defined as couple's failure to conceive after one year of unprotected intercourse, with male factors contributing to approximately 50% of reported cases (1). There are multiple causes for male infertility, which can be broadly classified due to their general underlying etiology. These include:

- Endocrine disorders (usually due to hypogonadism) estimated at 2% - 5% of cases,
  - Sperm transport disorders (such as vasectomy) at 5%,
  - Primary testicular defects (which involve abnormal sperm parameters without any identifiable cause) at 65% - 80%
  - Idiopathic (where an infertile male has normal sperm and semen parameters) at 10% - 20%.
- Other causes include:
- Acquired urogenital abnormalities (e.g., bilateral orchiectomy),

- Congenital urogenital abnormalities (e.g., undescended testes),
  - Environmental toxins (pesticides, smoking, excess alcohol, etc),
  - Genetic causes (primary ciliary dyskinesia, Klinefelter syndrome, and Y chromosome microdeletion),
  - Malignancies (pituitary macroadenomas, testicular tumors, and adrenal tumors leading to an excess of androgens),
  - Medications or drugs that can cause inhibition of GnRH (exogenous testosterone or androgenic steroids supplementation, chronic glucocorticoid therapy),
  - Sexual dysfunction (premature ejaculation and erectile dysfunction), and
  - Urogenital tract infections (Gonococci, chlamydia, syphilis, tuberculosis, prostatitis) (2,3).
- Male infertility can be manifested through various semen phenotypes, including oligozoospermia

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(reduced spermatozoa count), azoospermia (complete absence of spermatozoa in the ejaculate), necrozoospermia (presence of nonviable sperm) (4), and qualitative defects in sperm cells, such as abnormal morphology (teratozoospermia), decreased motility (asthenozoospermia), or a combination of qualitative and quantitative defects (oligoasthenoteratozoospermia) (5,6). The Anti-Müllerian hormone (AMH) is a homodimer glycoprotein with a molecular weight of 140 kDa connected via disulfide bonds, and it belongs to the transforming growth factor-beta (TGF- $\beta$ ) superfamily (7,8). It binds to its exclusive AMH type II receptor (AMH-R2); AMH is the earliest hormone released by immature Sertoli cells at about eight weeks of gestation and is responsible for the regression of the Müllerian ducts in the male fetus during sexual differentiation (9,10). It acts as an independent biomarker for Sertoli cell state and spermatogenic capacity, serving as a predictor of successful sperm retrieval (SSR) in microdissection testicular sperm extraction (11).

This study aimed to compare the AMH levels in serum and seminal plasma among groups of men with normozoospermic, oligozoospermia, asthenozoospermia, and idiopathic or unexplained infertility to discover its ability to differentiate these subtypes and study the positional correlations with sperms parameters and fertility hormones profile.

### Cases and methods

This case-control study was done at the Infertility Center of Al-Batool Teaching Hospital in Diyala Governorate, Iraq, by the Department of Biochemistry/ College of Medicine/ University of Baghdad, Baghdad, Iraq, during the period from April 2024 to January 2025. The ethical and scientific review board Committee of the Department of Biochemistry, College of Medicine, University of Baghdad gave their stamp of approval to this study. Additionally, the scientific research committee of Diyala Health Directorate, Diyala, Iraq provided their ethical clearance. Subjects verbally agreed before participating in this study. One hundred eleven males, aged (20-55 years) were involved in this study. All participants were diagnosed by a consultant with infertility. These participants were divided into four groups according to seminal fluid analysis (WHO 1999 criteria) (12): The sample size was determined using Fleiss' formula, which related to the calculation of sample size for comparing more than two groups in case-control studies (13).

The study groups were the following: Group 1 (G1) with 30 normozoospermic males as controls, Group 2 (G2) with 29 infertile asthenozoospermic males, Group 3 (G3) with 25 males with oligozoospermia, and Group 4 (G4) with 27 males with idiopathic (unexplained) infertility.

The exclusion criteria included those patients who had diabetes mellitus, Hashimoto's thyroiditis, any other autoimmune diseases, varicocele, undescended testis, and testicular torsion, hypogonadotropic hypogonadism, and those who took any medication

that may have an effect on the male reproduction system based on history, physical examination, and laboratory results.

Seminal fluid analysis was done by Olympus microscope (Japan) to evaluate the seminal fluid parameters, including:

1) determining sperm concentration by calculating the mean number of spermatozoa multiplied by million ( $10^6$ ) in ten random microscopic fields, and this is a measurement of how many sperms are found in each milliliter of seminal fluid,

2) identifying the sperm motility percent and grade of activity which was calculated by applying the following equation:  $\text{Motility} = 100 \times \text{No. of motile sperm in several of fields} / \text{total number of sperm in number of fields}$ . The quantitative parameter of sperm movement represents sperm motility expressed as a percentage. The quality of sperm movement is assessed through sperm progression and expressed on a subjective typical scale like the Macleod Scale of (0-4), which explains the type of movements exhibited by most sperms: Immotile sperm = 0, motile sperm with no progressive forward movement = 1, slow forward progressive movement = 2, moderate forward progressive movement = 3, and rapid, regular forward progression = 4. It is classified into four grades as the following:

Grade A- Rapid linear progressive movement  $25\mu\text{m}/\text{sec}$  at  $37^\circ\text{C}$ , Grade B- Slow or sluggish linear or no linear movement below  $25\mu\text{m}/\text{sec}$  at  $37^\circ\text{C}$ , Grade C- Non-progressive below  $5\mu\text{m}/\text{sec}$  at  $37^\circ\text{C}$ , and Grade D- Immotile, and

3) Sperm Abnormal Morphology Percent.

The normozoospermia group (control group) (G1) were participants who had a total (Grade A + Grade B) motility of more than 50% and total sperm count above  $20 (10^6/\text{ml})$ , with normal morphology (%)  $\geq 14$ . The asthenozoospermic participants (G2) included those with reduced sperm motility (Grade A + Grade B) motility, less than 50% or less than 25%, with rapid and linear progression. In contrast, other parameters, such as sperm count and morphology, still fall within the normal range.

The oligozoospermic patients (G3) are those who had a total sperm count of less than  $20 (10^6/\text{ml})$ , motility and morphology within the normal range of normozoospermic subjects.

The idiopathic infertility participants (G4) were those with normal criteria parameters of seminal fluid analysis, but with no clear cause for infertility.

Blood and seminal fluid samples were taken from each patient and control participant. Five milliliters (ml) of blood were aspirated from the peripheral vein of each patient and normozoospermic subjects (control group) and were allowed to clot for 15 minutes, then centrifuged for 10 minutes at 2500 rounds per minute (rpm). Laboratory testing included serum measurements of anti-müllerian hormone (AMH), Luteinizing Hormone (LH), and Follicular Stimulating Hormone (FSH), which was performed by Enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle (quantify antigens

captured between two layers of antibodies) and free testosterone (FT) done by Competitive-ELISA with the principle that the limited the number of antigen binding sites, forcing a target analyte and a labeled analog to compete for antibody binding according to the manufacturer (Elabscience Company -Houston, Texas, USA).

Two to three milliliters of seminal fluid samples were collected from each participant after the abstinence period (3-5 days). The seminal fluid sample was transported immediately into an incubator for 15-30 minutes to get liquefaction. Then, it was centrifuged for 10 minutes at 2500 rounds per minute (rpm). To get supernatant plasma, which was used for measurements of anti-müllerian hormone (AMH) by using ELISA based on the sandwich principle according to the manufacturer (Elabscience Company -Houston, Texas, USA). Each subject's serum and seminal plasma were divided into two samples and then transported to a 1.5 milliliter Eppendorf tube for freezing at -80 °C until the time of the studied parameter measurements.

### Statistical Analysis

Statistical analysis was done using Microsoft Excel for organized data and Statistical Package for Social Sciences (SPSS) version 26.0. the date was described

using percentages, means and standard deviation (SD). The ANOVA test was employed to compare more than two separate groups to assess the differences between means of numerical data. The correlation between the numerical data was evaluated using the Pearson correlation regression test. A P value of < 0.05 was considered significant. Receiver operating characteristic (ROC) and its area under the curve (AUC) were performed to determine the parameter that is preferred in differentiation subtypes of male infertility.

### Results

Table 1 presents the mean±SD values of demographic data including age, body mass index (BMI) and waist circumference (WC), and the percentage of smokers of the four studied groups. The demographic analysis revealed relative homogeneity across groups in terms of age and anthropometric measures. The mean age values ranged from 29.8 - 31.5 years, the mean BMI values ranged from 26.7 - 27.8 kg/m<sup>2</sup>, and the mean WC values ranged from 85.4 - 89.6 cm, with no statistically significant differences (P-values > 0.05). The percentage of smokers in the asthenozoospermia group (48.3%) was significantly higher than those in other groups (*p-value*= 0.024).

**Table Error! No text of specified style in document.: Demographic and Anthropometrical Characteristics by Fertility Status**

Characteristic	Normozoosp. (n=30)	Asthenozoosp. (n=29)	Oligozoosp. (n=25)	Idiopathic (n=27)	p-value
Age (years) *	29.8 ± 4.71	31.5 ± 7.23	30.2 ± 3.90	30.1 ± 4.52	0.521 <sup>NS</sup>
BMI (kg/m <sup>2</sup> ) *	26.7 ± 4.52	27.3 ± 5.16	27.8 ± 4.81	26.9 ± 3.23	0.483 <sup>NS</sup>
Waist Circumference (cm) *	85.4 ± 11.24	89.2 ± 10.83	89.6 ± 8.44	86.8 ± 4.61	0.312 <sup>NS</sup>
Smokers (%)	8 (26.7%)	14 (48.3%)	9 (36.0%)	7 (25.9%)	0.024 <sup>†</sup>
Non-smokers (%)	22 (73.3%)	15 (51.7%)	16 (64.0%)	20 (74.1%)	

\*Values presented as mean ± standard deviation

†Chi-square test p-values

NS: Non-significant differences

Table 2 presents the mean ± SD values of the seminal fluid analysis for the four studied groups. Normozoospermic and idiopathic groups displayed significantly higher volumes (2.7 ± 0.8 and 2.8 ± 0.5 mL), while the mean of total sperm counts of asthenozoospermic and oligozoospermic subjects was within the normal sperm counts (2.1 ± 0.7 and 2.0 ± 0.8 mL) but was significantly lower than that of normozoospermic and idiopathic participants with (*p-value* <0.001), suggesting potential physiological discrepancies in semen production. A highly significant disparity (*p-value* <0.001) existed, with

normozoospermia showing the highest count (71.8 ± 11.2 M/mL), followed by Idiopathic (45.2 ± 18.7M/mL) and asthenozoospermia, 45.2 (35.4 ± 12.3M/mL). At the same time, oligozoospermia had markedly lower counts (9.3 ± 4.2M/mL). The mean of sperm motility in grade A (*p-value* <0.001) and B (*p-value* <0.001) were significantly lower, in contrast, grade D (*p-value* <0.0001) was significantly higher; Grade C motility showed no statistical variation in asthenozoospermia group compared with other groups.

**Table 1: Semen Parameters among fertility groups**

Parameter		Normozoosp. (n=30)	Asthenozoos.p.(n=29)	Oligozoosp. (n=25)	Idiopathic (n=27)	p-value
Volume (mL)		2.7 ± 0.84 <sup>a</sup>	2.1 ± 0.73 <sup>b</sup>	2.0 ± 0.82 <sup>b</sup>	2.8 ± 0.52 <sup>a</sup>	<0.001
Total Sperm Count (M/mL)		71.8 ± 11.23 <sup>a</sup>	35.4 ± 12.33 <sup>b</sup>	9.3 ± 4.24 <sup>c</sup>	45.2 ± 18.71 <sup>b</sup>	<0.001
Motility (%)	- Grade A	22.3 ± 6.22 <sup>a</sup>	5.4 ± 5.84 <sup>b</sup>	20.2 ± 6.11 <sup>a</sup>	21.8 ± 6.43 <sup>a</sup>	<0.001
	- Grade B	33.5 ± 3.82 <sup>a</sup>	15.8 ± 6.71 <sup>b</sup>	31.2 ± 6.93 <sup>a</sup>	31.0 ± 5.82 <sup>a</sup>	<0.001
	- Grade C	10.2 ± 1.41	10.8 ± 2.32	11.8 ± 2.93	11.5 ± 2.73	0.087
	- Grade D	34.0 ± 8.24 <sup>a</sup>	68.0 ± 11.21 <sup>b</sup>	36.8 ± 4.23 <sup>a</sup>	35.7 ± 4.80 <sup>a</sup>	<0.001

p-values were calculated using one-way ANOVA with Tukey's post-hoc test. Different superscript letters (a, b, c) indicate significant differences between groups in post-hoc analysis.

Table 3 provides the mean $\pm$ SD values of serum free testosterone (FT), LH, and FSH levels of the studied groups. All hormonal parameters demonstrated statistically significant differences among the groups ( $p < 0.001$ ). Serum FT levels exhibited a clear gradient across fertility status groups. Normozoospermic subjects maintained the highest testosterone levels ( $384.2 \pm 121.3$  pg/mL), followed by a significant decrease in asthenozoospermic individuals ( $208.7 \pm 116.8$  pg/mL), and reaching the lowest concentrations in oligozoospermic patients ( $112.4 \pm 65.7$  pg/mL). Idiopathic cases demonstrated intermediate levels ( $264.8 \pm 123.4$  pg/mL),

statistically similar to asthenozoospermic values. Gonadotropin hormones (LH and FSH) showed inverse relationships with fertility status. LH levels were lowest in normozoospermia ( $2.8 \pm 1.1$  mIU/mL), increasing significantly in asthenozoospermia ( $4.2 \pm 3.1$  mIU/mL), and peaking in oligozoospermia ( $7.8 \pm 6.7$  mIU/mL). Similarly, FSH showed elevated levels in both asthenozoospermia ( $18.4 \pm 4.2$  mIU/mL) and oligozoospermia ( $22.7 \pm 14.8$  mIU/mL) compared to normozoospermia ( $5.8 \pm 2.2$  mIU/mL), with idiopathic cases showing intermediate elevation ( $10.3 \pm 4.6$  mIU/mL).

**Table 2: Free testosterone, follicle-stimulating hormone, and luteinizing hormone among fertility groups**

Parameter	Normozoosp. (n=30)	Asthenozoosp. (n=29)	Oligozoosp. (n=25)	Idiopathic (n=27)	p-value
Serum Free Testosterone (pg/mL)	$384.2 \pm 121.30^a$	$208.7 \pm 116.82^b$	$112.4 \pm 65.73^c$	$264.8 \pm 123.43^b$	<0.001
Serum LH (mIU/mL)	$2.8 \pm 1.11^a$	$4.2 \pm 3.14^b$	$7.8 \pm 6.70^c$	$4.1 \pm 2.40^b$	<0.001
Serum FSH (mIU/mL)	$5.8 \pm 2.24^a$	$18.4 \pm 4.23^b$	$22.7 \pm 14.83^b$	$10.3 \pm 4.61^c$	<0.001

p-values were calculated using one-way ANOVA with Tukey's post-hoc test. Different superscript letters (a, b, c) indicate significant differences between groups in post-hoc analysis.

Table 4 depicts the mean $\pm$ SD values of serum and plasma seminal fluid of anti-müllerian hormone (AMH) levels of the four studied groups. The analysis of serum AMH levels revealed distinct patterns across the study groups. Normozoospermic men exhibited the highest mean serum AMH concentration ( $3.84 \pm 1.46$  ng/mL), followed by those with idiopathic infertility ( $3.32 \pm 0.98$  ng/mL). Subjects with asthenozoospermia showed intermediate levels ( $2.30 \pm 0.82$  ng/mL), while oligozoospermic men demonstrated the lowest serum AMH concentrations ( $1.92 \pm 0.71$  ng/mL). The range of serum AMH values was notably wider in the normozoospermic group ( $1.16$ - $6.70$  ng/mL) compared to other groups. The one-way ANOVA revealed statistically significant differences in serum AMH levels across the study groups; the analysis yielded a significant ( $p$ -value < 0.001), indicating substantial variation between groups.

In contrast to serum levels, seminal fluid AMH concentrations displayed a different pattern. Oligozoospermic subjects showed markedly elevated SF AMH levels ( $3.78 \pm 1.52$  ng/mL), which were significantly higher than all other groups.

Normozoospermic and asthenozoospermic men exhibited comparable SF AMH concentrations ( $2.54 \pm 1.03$  ng/mL and  $2.63 \pm 1.12$  ng/mL, respectively), while the idiopathic infertility group demonstrated the lowest mean values ( $2.21 \pm 0.64$  ng/mL). SF AMH concentrations showed significant between-group differences ( $p$ -value < 0.001). Oligozoospermic subjects showed significantly higher levels compared to both normozoospermic (mean difference = 1.24 ng/mL,  $p < 0.001$ ) and idiopathic groups (mean difference = 1.57 ng/mL,  $p < 0.001$ ). The differences between normozoospermic and asthenozoospermic groups did not reach statistical significance ( $p > 0.05$ ). A notable inverse relationship was observed between serum and SF AMH levels, particularly in the oligozoospermic group. While these subjects demonstrated the lowest serum AMH concentrations, they exhibited the highest SF AMH levels among all groups. This inverse pattern was less pronounced in other study groups, suggesting a potential compensatory mechanism specific to oligozoospermia.

**Table 4: Serum and seminal fluid of anti-müllerian hormone (AMH) among fertility groups**

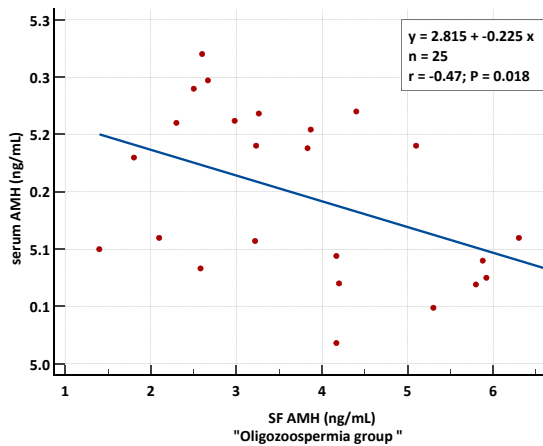
Parameter	Normozoosp. (n=30)	Asthenozoosp. (n=29)	Oligozoosp. (n=25)	Idiopathic (n=27)	p-value
Serum AMH (ng/mL)	$3.9 \pm 1.43^a$	$2.2 \pm 0.92^b$	$1.9 \pm 0.80^b$	$3.3 \pm 1.01^a$	<0.001
Seminal fluid AMH (ng/mL)	$2.54 \pm 1.03^a$	$2.63 \pm 1.12^a$	$3.78 \pm 1.52^b$	$2.21 \pm 0.64^a$	<0.001

p-values were calculated using one-way ANOVA with Tukey's post-hoc test. Different superscript letters (a, b, c) indicate significant differences between groups in post-hoc analysis.

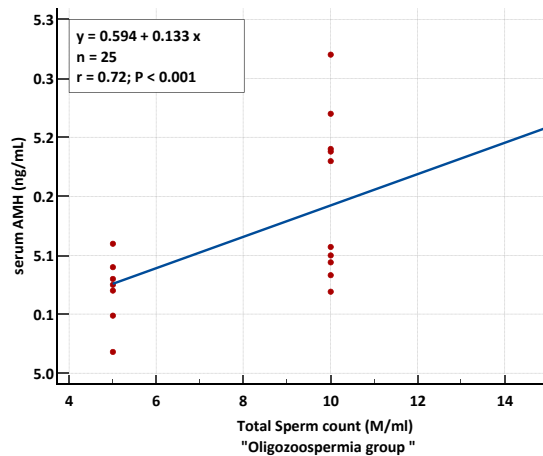
There was a significant negative correlation between serum and seminal plasma AMH in oligozoospermia ( $r = -0.47$ ,  $p = 0.018$ , Figure 1), and a significant positive correlation between serum AMH and total sperm count in oligozoospermia ( $r = 0.72$ ,  $p < 0.001$ ,

Figure 2). Also, there were significant positive correlations between serum AMH and serum FSH in the asthenozoospermia group ( $r = 0.37$ ,  $p = 0.05$ , Figure 3).

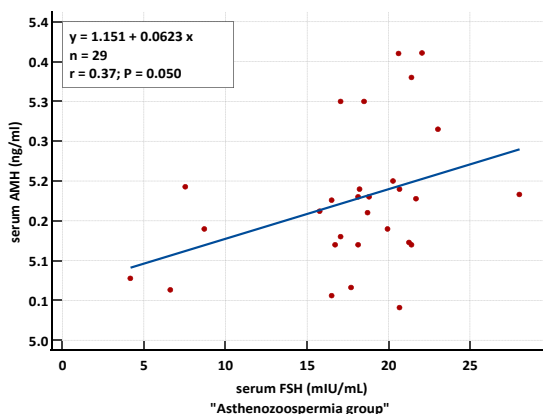




**Figure 1: Scatter plot presenting the correlation between serum and seminal plasma AMH in oligozoospermia**



**Figure 2: Scatter plot displaying the correlation between serum AMH, and total sperm count in oligozoospermia**



**Figure 3: Scatter plot showing the correlation between serum AMH and serum FSH in asthenozoospermia**

The outcomes of receiver operating characteristic (ROC) and area under the curve (AUC) analysis in differentiation between normozoospermia and asthenozoospermia groups revealed that serum AMH shows fair diagnostic performance with AUC of

0.811 (95% CI: 0.688 to 0.901). Using a cutoff value of  $\leq 2.5$  ng/mL, it achieved 80% sensitivity and 75.9% specificity. SF AMH demonstrates relatively poor diagnostic performance (AUC: 0.519) (95% CI: 0.385 to 0.651). Using a cutoff value of  $\leq 2.4$  ng/mL, with low sensitivity=62.1% and specificity=50%. The outcomes of receiver operating characteristic (ROC) and (AUC) analysis in differentiation between normozoospermia and oligozoospermia groups demonstrated that serum AMH showed good diagnostic performance with an AUC of 0.857 (95% CI: 0.736 to 0.937). Using a cutoff value of  $\leq 2.5$  ng/mL, it achieved 70% sensitivity and 96% specificity, resulting in a strong positive likelihood ratio of 17.5. SF AMH demonstrates more modest diagnostic capability, (AUC: 0.763) (95% CI: 0.629 to 0.867). Using a cutoff value of  $\leq 3.83$  ng/mL, it achieved 52% sensitivity and 93.3% specificity. Also, the results (ROC) and (AUC) analysis in differentiation between normozoospermia and idiopathic groups showed that serum AMH demonstrates limited diagnostic value (AUC: 0.576) (95% CI: 0.438 to 0.706). Using a cutoff value of  $\leq 3.2$  ng/mL, it achieved 40.7% sensitivity and 80% specificity. SF AMH shows relatively poor diagnostic performance with AUC values close to 0.572, (95% CI: 0.429 to 0.714). Using a cutoff value of  $\leq 1.75$  ng/mL, with low sensitivity 33.3% and specificity 86.7%, indicating limited discriminatory ability between these two groups.

## Discussion

The results of B.M.I in this study aligns with WHO's classification, placing most participants in the overweight category with no statistically difference among studied groups. The current study shows a higher percentage of smokers in the asthenozoospermia group than the other groups, with the lowest being in the idiopathic group suggesting a potential association between smoking and asthenozoospermia, which agrees with the findings of Ramlau-Hansen et al, who illustrated that smokers exhibited an inverse dose-response relationship with the percentage of motile sperm (14). In males, it has been believed that cigarette smoking adversely impacts every system integral to the reproductive process. Spermatozoa for smokers exhibit diminished fertilization potential, and embryos demonstrate reduced rates of implantation (15). The results of the current study about seminal fluid parameters elucidated that asthenozoospermic and oligozoospermic subjects had lower ejaculate volumes than those with normozoospermic and idiopathic groups, even though they were within the normal limits of the WHO criteria 1999 (12). This agrees with Banihani and Shefa'M, who found that low seminal fluid volume was associated with increased sperm dysfunction, particularly in oligozoospermic and asthenozoospermic patients. The authors linked lower seminal fluid volume to reduced seminal vesicle function, which plays a key role in sperm motility and viability (16). Melnyk et al.

noted a non-significant difference in seminal fluid volume between idiopathic sub-fertile group and normozoospermic males (17). However, this is in disagreement with Ahmad and Al-Murshidi, who reported that there was a non-significant difference in seminal fluid volume among normozoospermic and asthenozoospermic men when controlling for confounding factors like dehydration, sexual abstinence, and ejaculation frequency (18). The finding of the current study about sperm motility corresponds with Bonanno et al., who found a significant decrease in sperm motility in asthenozoospermic patients (19).

The present study also observed that total sperm counts were significantly lower in oligozoospermic subjects compared to the remaining groups. These findings are in agreement with Al-Nedaw and Hassan (20) and Ahmad and Al-Murshidi, who reported that this could be associated with conditions like mutations in chromosomes, gene regulation, infections, endocrine dysfunction, the environment, and the immune system (18). The finding of the present study reported that serum FT was significantly lower; in contrast, serum LH and FSH were significantly higher in the oligozoospermic followed by the asthenozoospermic group, which is consistent with Jaber et al., who found that the decline in testosterone levels could be attributed to the psychological state of infertile individuals, as elevated cortisol levels lead to a reduction in testosterone levels (21). Castellini et al. suggested that lifestyle factors such as smoking, consumption of unhealthy food, inadequate sleep, micronutrient deficiencies (specifically zinc, magnesium, and vitamin D), and the use of plastic materials can contribute to this decrease (22). These factors disrupt the balance of reproductive hormones, particularly testosterone. As a result, it has an impact on male fertility (23).

The present study showed that serum AMH Levels were highest in normozoospermic men, intermediate levels in idiopathic infertility, lower in asthenozoospermic, and lowest in oligozoospermic patients. The levels of AMH in the seminal plasma were as follows: Oligozoospermic men had the highest AMH levels. normozoospermic and asthenozoospermic subjects had comparable levels. The idiopathic infertility group had the lowest AMH levels. In general, we observed that the results of AMH in serum were higher than those in seminal fluid except the finding of AMH in oligozoospermic patients; which was attributed to the instability of hormones of testicular origin compared to blood hormones, as 90% of semen formation depends on glands such as the seminal vesicle and the prostate (21). The results of our study agree with Turhan et al., who reported no correlation between semen and blood AMH levels, noting significant variability in AMH levels of seminal plasma among the studied individuals. They proposed that seminal plasma AMH could serve as a marker for sperm production, although its predictive value is constrained (24). Our results also agree with Jaber et al., who observed that

high levels of AMH in the blood that are compared to seminal plasma may suggest that Sertoli cells are either immature or defective (21). Xiao et al. noticed that AMH is a marker that exhibits the function of Sertoli cells in the male reproductive accessories. Therefore, higher concentrations of seminal AMH may indicate that the Sertoli cells are still in an immature state and their function may be declining (25). The results of the present research disagree with Kang-sheng et al., who reported that the concentrations of AMH in plasma seminal fluid are higher than in the blood (26). There was a significant negative correlation between serum and seminal plasma AMH in oligozoospermic subjects. This inverse relationship may be reflect an increase in seminal AMH production by immature or damaged Sertoli cells (27). Also, there was a significant positive correlation between serum AMH, and total sperm count in oligozoospermic patients. These findings agree with the results observed by Benderradji et al. (28), which can be explained by the significant positive correlation observed between serum AMH and serum FSH in asthenozoospermia group. FSH has been identified as a regulator of Sertoli cell quantity during testicular development (29). Thereby, transcriptionally stimulates AMH via a nonclassical cyclic adenosine monophosphate (cAMP) route by binding to the AMH promoter and enhancing AMH synthesis (30).

### Conclusion

Serum AMH levels exhibit fair to good diagnostic performance in differentiating subtypes of male infertility. Seminal fluid AMH displayed lower diagnostic accuracy across all groups, with elevated levels in oligozoospermic subjects. These findings suggest that serum AMH may serve as a valuable biomarker in the evaluation of male infertility subtypes.

### Authors' declaration

We confirm that all the figures and tables in the manuscript belong to the current study. Authors sign on ethical consideration's approval-ethical clearance: The project was approved by the local ethical committee in the place where the research was conducted, or samples collected and treated according to the code number (444) (27/ 1/ 2025).

**Conflict of interest:** None

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### Authors' Contributions

Study conception & design: (Basil Oied Mohammed Saleh, Osamah Ali Layih). Literature search: (Osamah Ali Layih, Basil Oied Mohammed Saleh). Data acquisition: (Osamah Ali Layih, Basil Oied Mohammed Saleh). Data analysis & interpretation: (Basil Oied Mohammed Saleh, Osamah Ali Layih). Manuscript preparation: (Osamah Ali Layih, Basil Oied Mohammed Saleh). Manuscript editing & review: (Basil Oied Mohammed Saleh, Osamah Ali Layih).

## References

- Ghewade P, Vagha S, Ghewade B, Gadkari P. Role of Dietary Antioxidant Supplements in Male Infertility: A Review. *Cureus*. 2024;16(6). <https://doi.org/10.7759/cureus.61951>
- Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, et al. Male infertility. *The Lancet*. 2021;397(10271):319-33. [https://doi.org/10.1016/S0140-6736\(20\)32667-2](https://doi.org/10.1016/S0140-6736(20)32667-2)
- Alani GT, El Yaseen HD. Creatine kinase activity and malondialdehyde in the seminal plasma of normospermic infertile males. *Journal of the Faculty of Medicine Baghdad*. 2009;51(3):336-40. <https://doi.org/10.32007/jfacmedbaghdad.5131146>
- Aziz ASA, Elyaseen HD, Kadhemi HK. Correlation between MDA Level and Chitotriosidase-I Activity in Seminal Fluid of Iraqi Infertile Males. *Journal of the Faculty of Medicine Baghdad*. 2024;66(4):479-86. <https://doi.org/10.32007/jfacmedbaghdad.6642395>
- Pereira R, Sousa M. Morphological and molecular bases of male infertility: a closer look at sperm flagellum. *Genes*. 2023;14(2):383. <https://doi.org/10.3390/genes14020383>
- Salman FS, Al-Qadhi HI, Al Kareem BA. N-acetyl cysteine's effect on semen parameters in a sample of Iraqi men with oligoasthenoteratozoospermia. *Journal of the Faculty of Medicine Baghdad*. 2022;64(3):170-4. <https://doi.org/10.32007/jfacmedbaghdad.6431938>
- Xu H, Zhang M, Zhang H, Alpadi K, Wang L, Li R, et al. Clinical Applications of Serum Anti-Müllerian Hormone Measurements in Both Males and Females: An Update. *Innovation*. 2021;2(1):100091. <https://doi.org/10.1016/j.xinn.2021.100091>
- Josso N, Picard JY. Genetics of anti-Müllerian hormone and its signaling pathway. *Best Practice & Research Clinical Endocrinology & Metabolism*. 2022;36(1):101634. <https://doi.org/10.1016/j.beem.2022.101634>
- Howard JA, Hart KN, Thompson TB. Molecular mechanisms of AMH signaling. *Frontiers in Endocrinology*. 2022;13:927824. <https://doi.org/10.3389/fendo.2022.927824>
- Olumide OB, Godwin AI, Titilayo JO, Christian IO, Etukudoh NS, Uchejeso OM, et al. Assessment of Serum Anti-Müllerian Hormone (AMH) as an Independent Marker for Oligozoospermia and Non-Obstructive Azoospermia in Infertile Nigerian Men. *Biomedical and Pharmacology Journal*. 2023;16(1):35-42. <https://doi.org/10.13005/bpj/2585>
- Yuanyuan Z, Guicheng Z, Yi Z, Jiang X. From biological marker to clinical application: the role of anti-Müllerian hormone (AMH) for delayed puberty and idiopathic non-obstructive azoospermia in males. *Endocrine connections*. 2025; EC-24. <https://doi.org/10.1530/EC-24-0630>
- Vannaprasedh B. WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction 4th Edition. *The Australian Journal of Medical Science*. 2005;26:100. <https://api.semanticscholar.org/CorpusID:57556090>
- Poulet PE, Tran M, Tezenas du Montcel S, Dubois B, Durrleman S, Jedynak B. Prediction-powered inference for clinical trials. *medRxiv*. 2025;2001-25. <https://doi.org/10.1101/2025.01.15.25320578>
- Ramlau-Hansen CH, Thulstrup AM, Aggerholm AS, Jensen MS, Toft G, Bonde JP. Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. *Human Reproduction*. 2007;22(1):188-96. <https://doi.org/10.1093/humrep/del364>
- Irteimah T, Al Dubais A, Al Rubaihat R. The Impact of Hookah on Anti-müllerian Hormone Level. *Journal of the Royal Medical Services*. 2022;29(1):28-35. <https://doi.org/10.12816/0060310>
- Banihani SA, Shefa'M A. Men with oligozoospermia had lower level of seminal plasma pyridoxine compared to normozoospermic men. *Heliyon*. 2022;8(12). <https://doi.org/10.1016/j.heliyon.2022.e11983>
- Melnyk O V, Vorobets MZ, Fafula R V, Onufrovych OK, Vorobets ZD. Features of spermogram indicators in idiopathic infertility in men. *Bulletin of Problems in Biology and Medicine*. 2022;167:187-92. <https://doi.org/10.29254/2077-4214-2022-4-167-187-192>
- Ahmad SS, Al-Murshidi SY. Evaluation the level of antisperm antibodies, some cytokines and zinc for oligozoospermia, asthenozoospermia patients compared with normozoospermia. *International journal of health sciences*. 2022;6(S6):5324-31. <https://doi.org/10.53730/ijhs.v6nS6.10907>
- Bonanno O, Romeo G, Asero P, Pezzino FM, Castiglione R, Burrello N, et al. Sperm of patients with severe asthenozoospermia show biochemical, molecular and genomic alterations. *Reproduction*. 2016;152(6):695-704. <https://doi.org/10.1530/REP-16-0342>
- Al-Nedaw IA, Hassan SY. A Comparison of ICSI Outcomes and Reactive Oxygen Species Levels in Seminal Plasma between Normozoospermia and Sperm Abnormalities Groups for Infertile Men. *Kufa Medical Journal*. 2023;19(2). <https://doi.org/10.36330/kmj.v19i2.12866>
- Jaber ZA, Mohammed AA, Rahim AI, Technologies AR. Correlation of serum and seminal plasma anti-müllerian hormone with fertility hormones profile and semen parameters. *Obstetrics & Gynaecology Forum*. 2024;(3):1491-7.

<https://obstetricsandgynaecologyforum.com/index.php/ogf/article/view/516>

22. Holt R, Yahyavi SK, Kooij I, Andreassen CH, Andersson AM, Juul A, et al. Low serum anti-Müllerian hormone is associated with semen quality in infertile men and not influenced by vitamin D supplementation. *BMC Medicine*. 2023;21(1):1-10. <https://doi.org/10.1186/s12916-023-02782-1>

23. Castellini C, Totaro M, Parisi A, D'Andrea S, Lucente L, Cordeschi G, et al. Bisphenol A and male fertility: Myths and realities. *Frontiers in endocrinology*. 2020;11:540459. <https://doi.org/10.3389/fendo.2020.00353>

24. Turhan G, Çil N, Kabukçu C, Turan T, Fenkçi İV, Abban MG. Relationship of seminal plasma anti-Müllerian hormone concentration with sperm morphology and sperm DNA damage. *Journal of Urological Surgery*. 2022; <https://doi.org/10.4274/jus.galenos.2022.2021.0121>

25. Xiao H, Ding YL, Yang P, Chen Q, Huang HL, Chen X, et al. Association between anti-Müllerian hormone concentrations and sperm retrieval outcomes in patients with idiopathic nonobstructive azoospermia: a systematic review and meta-analysis. *Asian Journal of Andrology*. 2024;26(5):522-7. <https://doi.org/10.4103/aja202419>

26. Kang-sheng LIU, Hui YAO, Xiao-dong MAO, Xiao-yong T, Ya-jun C. Application of Anti-Müllerian Hormone in Diagnosis of Male Infertility. *Journal of International Translational Medicine*. 2017;5(1):19-22. <https://doi.org/10.11910/2227-6394.2017.05.01.04>

27. Andersen JM, Herning H, Witczak O, Haugen TB. Anti-Müllerian hormone in seminal plasma and

serum: association with sperm count and sperm motility. *Human Reproduction*. 2016;31(8):1662-7. <https://doi.org/10.1093/humrep/dew121>

28. Benderradji H, Barbotin AL, Leroy-Billiard M, Prasivoravong J, Marcelli F, Decanter C, et al. Defining reference ranges for serum anti-Müllerian hormone on a large cohort of normozoospermic adult men highlights new potential physiological functions of AMH on FSH secretion and sperm motility. *The Journal of clinical endocrinology & metabolism*. 2022;107(7):1878-87. <https://doi.org/10.1210/clinem/dgac218>

29. Sabir FNK. Association of Anti-Müllerian Hormone with some physiological and immunological parameters of infertile males. *Cellular and Molecular Biology*. 2022;68(9):171-8. <https://doi.org/10.14715/cmb/2022.68.9.27>

30. Buratini J, Dellaqua TT, Dal Canto M, La Marca A, Carone D, Mignini Renzini M, et al. The putative roles of FSH and AMH in the regulation of oocyte developmental competence: From fertility prognosis to mechanisms underlying age-related subfertility. *Human Reproduction Update*. 2022;28(2):232-54. <https://doi.org/10.1093/humupd/dmab044>

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### دور الهرمون المضاد للمولر في مصل الدم والسائل المنوي في تمييز الأنماط الفرعية لعقم الذكور

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#### الخلاصة:

**خلفية البحث:** العقم هو عدم القدرة على الإنجاب بعد سنة واحدة من الجماع غير المحمي، وتعزى حوالي 50% من الحالات إلى عوامل ذكرية. يعد الهرمون المضاد للمولر، وهو بروتين سكري ينتمي إلى عائلة عوامل النمو المحولة بيتا، أحد الهرمونات التي تفرز من خلايا سيرتولي ويلعب دوراً مهماً في التمايز الجنسي لدى الذكور.

**الاهداف:** تهدف الدراسة إلى فحص الدور المحتمل للهرمون المضاد لمولر في التمييز بين أنواع العقم الذكري المختلفة. **المرضى والمنهجية:** أجريت دراسة الحالات-الشواهد هذه في مركز العقم بمستشفى البتول التعليمي في محافظة ديالى، العراق، من قبل قسم الكيمياء الحيوية / كلية الطب / جامعة بغداد، خلال الفترة من نيسان 2024 إلى كانون الثاني 2025. شملت الدراسة 111 رجلاً تتراوح أعمارهم بين 20-55 عاماً، حيث تم تقسيم المشاركين إلى أربع مجموعات وفقاً لتحليل السائل المنوي: المجموعة الضابطة (النورموزوسبيرميا): 30 رجلاً يتمتعون بمستويات طبيعية من الحيوانات المنوية. مجموعة الوهن النطفي (أستينوزوسبيرميا): 29 رجلاً يعانون من ضعف في حركة الحيوانات المنوية. مجموعة قلة النطاف (أوليغوزوسبيرميا): 25 مريضاً يعانون من انخفاض عدد الحيوانات المنوية. مجموعة العقم مجهول السبب: 27 رجلاً يعانون من العقم دون سبب واضح.

تم قياس مستويات الهرمون المضاد للمولر في كل من مصل الدم والسائل المنوي باستخدام تقنية المقايضة المناعية المرتبطة بالإنزيم. **النتائج:** أظهرت النتائج أن المتوسط  $\pm$  الانحراف المعياري لتركيز الهرمون المضاد لمولر كانت أعلى بشكل ملحوظ في مصل الدم مقارنة بالسائل المنوي في جميع المجموعات، باستثناء مجموعة الأوليغوزوسبيرميا التي كان فيها تركيز الهرمون أعلى في السائل المنوي منه في المصل. أظهر تحليل منحنى الخصائص التشغيلية للمستقبل (ROC) أن الهرمون المضاد للمولر في المصل يمتلك أداء تشخيصياً يتراوح بين الجيد والمقبول عند قيمة قطع تتراوح بين (2.5-3.2 نانوغرام/مل)، في حين أظهر AMH في السائل المنوي دقة تشخيصية أقل في التمييز بين أنواع العقم الذكري، حيث تراوحت قيمة القطع بين (1.75-3.83 نانوغرام/مل).



**الاستنتاجات:** أظهرت مستويات الهرمون المضاد للمولر في المصل أداء تشخيصيا جيدا إلى مقبول في التمييز بين الأنواع الفرعية للعقم عند الذكور. بينما أظهر هذا الهرمون في السائل المنوي دقة تشخيصية أقل في جميع الفئات، مع ارتفاع مستوياته لدى الأشخاص الذين يعانون من قلة الحيوانات المنوية. تشير هذه النتائج إلى أن الهرمون المضاد للمولر في المصل قد يكون بمثابة مؤشر حيوي قيم في تقييم الأنواع الفرعية للعقم عند الذكور.

**مفتاح الكلمات:** الهرمون المضاد للمولر، وهن النطاف، قلة النطاف، بلازما السائل المنوي، العقم مجهول السبب.