RESEARCH
O&G Forum 2024; 34 – 3s: 1491-1497

CORRELATION OF SERUM AND SEMINAL PLASMA ANTI-MULLERIAN HORMONE WITH FERTILITY HORMONES PROFILE AND SEMEN PARAMETERS

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Abstract
Background: Anti-Müllerian hormone (AMH) is produced by the Sertoli cells and present in the blood serum and seminal fluid of adult males. While it has been proposed as an indicator of spermatogenesis, the precise role of this marker in adult males remains mainly unidentified.
Objective: The aimed of this study was to explore the potential correlation between AMH levels in serum and seminal plasma with sperm parameters and fertility hormones profile such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (T), and prolactin hormone (PRL) in infertile men
Study design, setting, size, duration: This study included 100 males who were classified according to seminal fluid analysis into 25 patients with normozoospermia, 26 patients with oligozoospermia, 21 patients with asthenozoospermia, and 28 patients with oligoasthenoteratozoospermia (OAT). Individuals who visited a private andrology clinic in Al Najaf, Iraq, for male infertility consultations between October 2023 and November 2023 submitted specimens.
Participants/materials, methods: The mean age of the participants was 30.85 ± 0.70 years, and their BMI was 27.60 ± 0.58 kg/m2. Semen quality was evaluated by semen analysis according to the guidelines of the World Health Organization for semen analysis in 2021. AMH levels in seminal plasma and serum, along with levels of FSH, LH, T, and PRL, were analyzed using the ELISA technique. We first examined total AMH in semen and blood with total values of semen characteristics and hormones profile, then the association between serum and semen AMH was assessed with sperms parameters and FSH, LH, T and PRL according to the four study groups separately.
Results: Total seminal plasma AMH showed a positive correlation with the total values for sperm concentration, sperm count, sperm motility, and sperm morphology. Additionally, there was a positive correlation between total serum AMH and total FSH. However, serum and semen AMH were not associated with semen parameters nor with hormones profile in all study groups, except for a positive association between testosterone and semen AMH in the oligospermia group.
Conclusion: levels of seminal plasma in this study, AMH showed a significant inter-individual difference. AMH in seminal plasma may serve as a marker for immature sertoli cells and spermatogenesis.
Keywords: anti-Mullerian hormone; seminal plasma; sperm count; sperm motility; serum; sertoli cells.

INTRODUCTION

Infertility refers to the condition of being unable to conceive or reproduce. Infertility is a medical condition affecting the reproductive system of either males or females, characterized by the inability to conceive a pregnancy after having frequent unprotected sexual intercourse for a period of 12 months or more (1). Infertility has a global impact on millions of individuals in their reproductive years, as well as their families and communities. Global estimates indicate that there are between 48 million couples and 186 million individuals who experience infertility. The majority of couples experience infertility due to a specific cause, whereas the rest have infertility that is that cannot be explained. Female partners account for 40% to 55% of infertility cases, whereas male partners contribute approximately 20% to 40% (2).

AMH, part of the TGF-β superfamily, has a molecular weight of 140 kD (Aksglaede et al. 2018). Its principal role is to cause regression of the Müllerian ducts in male fetuses during sexual differentiation (3). Immature Sertoli cells release AMH (4). Following the determination of fetal sex, Sertoli cells identify AMH as one of their first secretion products in the testis (5). AMH secretion is bidirectional, with Sertoli cells secreting it both apically into the seminiferous tubules and basally toward the interstitium and circulation (6). Because the Sertoli cells are still immature, serum AMH concentrations in boys remain high throughout the entire prenatal and prepubertal periods (6, 7). Puberty causes a significant decrease in serum AMH levels,

which coincides with the emergence of meiotic germ cells, the testosterone sensitivity of Sertoli cells, and its final maturation (8). As a result, AMH secretion in semen is higher than in blood. Although FSH initially regulates AMH positively, testosterone has a stronger down-regulating effect. If the levels of AMH in adult blood are higher than in semen, this may indicate the immaturity of sertoli cells (9). Humans commonly use AMH to identify immature Sertoli cells during spermatogenesis (10). Spermatozoa are produced in the epithelium of the seminiferous tubules of the testes. This epithelium has Sertoli cells that release AMH. Consequently, we investigated the correlation between the production of AMH and parameters linked to sperm. The objective of our study was to compare the AMH levels in serum and seminal plasma with sperms parameters and fertility hormones profile among groups of patients's men with normozoospermia, oligozoospermia, asthenozoospermia, and oligoasthenoteratozoospermia (OAT).

PATIENTS, MATERIALS AND METHODS

Ethical approval:
This cross-sectional study was authorized by the ethical council of Al-Nahrain University's High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Iraq.

Patients:
This study included 100 infertile men who ranged in age from 20 to 45 years. Age, weight, height, smoking, and the type and duration of infertility are all part of the history of infertile men. Individuals who visited a private andrology clinic in Al Najaf, Iraq, for consultations on male infertility between October 2023 and November 2023 submitted the specimens. Participants in the study provided blood and semen samples. Based on the results of the semen analysis, we categorized the patients into four groups: normozoospermia (n = 25), oligozoospermia (n = 26), asthenozoospermia (n = 21), and oligoasthenoteratozoospermia (n = 28). This study excluded males with azoospermia, cryptozoopermia, and endocrine diseases.

Semen Analysis:
Patients were selected for this investigation based on their confirmed seminal fluid analysis results, according to the World Health Organization Laboratory Manual (12). Semen samples were obtained through masturbation after abstinence from intercourse for a period of 2–7 days. Following liquefaction, we manually analyzed the semen for both macroscopic and microscopic examinations. These include the semen volume, sperm concentration, sperm count, sperm motility, sperm morphology, and round cells.

Hormone Analysis:
After analyzing the seminal fluid, the semen samples were centrifuged at 2500 rpm for 20 minutes to separate the seminal plasma from the cell fraction. We stored aliquots of seminal plasma at -20° until the day of analysis. Each patient had five milliliters of peripheral venous blood aspirated. We collected blood samples using gel tubes and centrifuged them at a speed of 2500 rpm per minute for 10 minutes. The serum was separated and stored in aliquots at a temperature of -20 °C until the day of analysis. We measured the levels of all hormones using the ELISA kits according to the manufacturer's instructions (Biotechnology, USA). AMH (ELK2336), FSH (ELK1386), LH (ELK9280), Testosterone (ELK 8525), and Prolactin (ELK1224).

Statistical Analysis:
Microsoft Office 2010 and the Statistical Package for Social Sciences (SPSS) version 23.0 were used to analyze the data. The frequency, range, mean, and standard error of the descriptive statistics were measured in order to characterize the data. Analysis of variance (ANOVA test employed to compare more than two separate groups) was used to compare the groupings. When the p value was equal to or less than 0.05, the results were deemed statistically significant. Pearson's correlation coefficient (r) was used to evaluate the degree of relationship between continuous variables.

RESULTS

We included 100 males infertile in this cross-sectional study, and we expressed the results as the mean, plus or minus the standard error of the mean. The mean patients' age was 30.85 ± 0.70 years, and the mean body mass indices were 27.60 ± 0.58 kg/m2. 41 patients (41.0%) had primary infertility, while 59 (59.0%) had secondary infertility, with a duration of infertility equal to 4.79 ± 0.38 years. Fifty-three (52.0%) of the patients were smokers, and 47 (47.0%) were non-smokers. The baseline mean seminal fluids volume was 3.77 ± 0.20 ml, mean sperm concentration was 12.67 ± 0.74 (10^6/ml), mean sperm count was 44.49 ± 3.21 (10^6), mean progressively motile sperms percent 32.78 ± 1.19, non-progressively motile sperms percent 31.15 ± 0.74, immotile sperms percent 36.00 ± 1.45, morphologically normal sperms percent was 7.60 ± 0.47, round cells were 2.26 ± 1.14. The baseline hormonal levels were: mean FSH was 4.99 ± 0.37 mIU/ml, mean LH was 6.59 ± 0.25 mIU/ml, mean testosterone was 5.22 ± 0.26 ng/ml, and mean prolactin level was 26.04 ± 1.47 mIU/ml. The mean serum AMH level was 2.08 ± 0.11 ng/ml, and the mean seminal plasma AMH level was 0.97 ± 0.09 ng/ml.

Table 3.1 shows no significant differences between the four study groups in terms of age (p=0.113), body mass index (p=0.460), duration of infertility (p=0.096), type of infertility (p=0.986), and smoking status (p=0.250).

Table 3.1: Comparison of demographic characteristics between the studied groups

Parameters (Mean±SE)	Normozoo-spermia N.=25	Oligozoo-spermia N.=26	Asthenozoo-spermia N.=21	AOT N.=28	p value
Age (Years)	33.24 ± 1.87	30.69 ± 0.85	33.00 ± 1.25	30.04 ± 1.23	0.113 ∀ NS
BMI (Kg/m²)	28.33 ± 1.57	27.22 ± 0.58	26.07 ± 0.91	28.47 ± 1.25	0.460 ∀ NS

Parameters (Mean±SE)		Normozoo-spermia N.=25	Oligozoo-spermia N.=26	Asthenozoo-spermia N.=21	AOT N.=28	p value
Duration of infertility (years)		5.24 ± 0.78	3.21 ± 0.50	5.77 ± 0.99	5.10 ± 0.74	0.096 ∀ NS
Type of infertility	Primary infertility	15 (60.0%)	15 (57.7%)	13 (61.9 %)	13 (52.0%)	0.986 ∀ NS
	Secondary infertility	10 (40.0%)	11 (42.3%)	8 (38.1%)	16 (57.1%)	
Smoking status	Smokers	13 (52.0%)	18 (69.2%)	9 (42.9%)	12 (42.9%)	0.250 ∀ NS
	Non-Smokers	12 (48.0%)	8 (38.2%)	12 (57.1%)	15 (53.6%)	

NS: Not significant (p > 0.05); S: Significant (p ≤0.05); ∀: Analysis of variance (ANOVA)

Table 3.2 reveals a comparison of hormonal levels between normozoospermia, oligozoospermia, asthenozoospermia, and AOT groups. There were significant differences in testosterone levels (6.80 ±0.50 vs. 4.60 ± 0.42 vs. 5.06 ± 0.52 vs. 4.50 ± 0.52; *p* =0.004), but no significant differences in FSH (p=0.379), LH (p=0.438), and prolactin levels (p=0.153).

Table 3.2: Comparison of hormonal levels between the studied groups

Parameters (Mean±SE)	Normozoo-spermia N.=25	Oligozoo-spermia N.=26	Asthenozoo-spermia N.=21	AOT N.=28	p value
FSH (mIU/ml)	5.71 ±1.33	4.14± 0.49	5.23±0.76	5.85±0.90	0.379 ∀ NS
LH (mIU/ml)	7.14± 0.76	5.95 ± 0.42	6.68 ± 0.39	6.64 ± 0.38	0.438 ∀ NS
Testosterone (ng/ml)	6.80 ±0.50	4.60 ± 0.42	5.06 ± 0.52	4.50± 0.52	0.004 ∀ S
Prolactin (mIU/ml)	20.9± 3.27	20.3 ± 2.54	28.2± 3.25	27.9± 2.62	0.153 ∀ NS

NS: Not significant (p > 0.05); S: Significant (p ≤0.05); ∀: Analysis of variance (ANOVA)

Table 3.3 presents patients with normozoospermia had significantly higher seminal plasma AMH levels compared to those with asthenozoospermia, oligozoospermia, and AOT (1.96 ± 0.13 vs. 0.53 ± 0.10 vs. 0.92 ± 0.26 vs. 0.52 ± 0.10; $p < 0.001$), but there were no significant differences in serum AMH levels (p= 0.288).

Table 3.3: Comparison of AMH levels between the studied groups

Parameters (Mean±SE)	Normozoo-spermia N.=25	Oligozoo-spermia N.=26	Asthenozoo-spermia N.=21	AOT N.=28	p value
Serum AMH (ng/ml)	2.33 ± 0.24	1.73 ± 0.23	1.81± 0.22	2.05 ± 0.23	0.288 ∀ NS
Seminal AMH (ng/ml)	1.96 ± 0.13	0.53 ± 0.10	0.92 ± 0.26	0.52 ± 0.10	< 0.001 ∀S

S: Significant (p ≤0.05); AMH: Anti mullerian hormone; ∀: Analysis of variance (ANOVA)

Table 3.4 reports a strong positive correlation of total serum AMH with total serum FSH ($r = 272$, $p = 0.006$). However, there was no significant association between total serum and seminal AMH with other reproductive hormones profile.

Table 3.4: Correlations between total serum and seminal AMH total with hormones Profile(N=100)

Parameters (Mean±SE)		Serum AMH	Seminal AMH
FSH (mIU/ml)	r	**0.272**	0.004
	p value	**0.006 S**	0.969 NS
LH (mIU/ml)	r	- 0.145	0.013
	p value	0.150 NS	0.898 NS
Testosterone (ng/ml)	r	- 0.075	- 0.184
	p value	0.461 NS	0.067 NS
Prolactin(mIU/ml)	r	-0.084	0.072
	p value	0.408 NS	0.475 NS

r: Pearson's correlation coefficient; S: Significant (p ≤ 0.05); NS: Not significant (p > 0.05)

Table 3.5 illustrates strong positive association between total seminal plasma AMH and total sperm concentration (r=0.298 & p=0.003), sperm count (r=0.456 & p<0.001), progressively motile sperms percent (r=0.215 & p=0.031), and morphologically sperms percent (r=0.417 & p <0.001). While total serum AMH no associated with semen parameters.

Table 3.5: Correlations between total serum and seminal plasma AMH with total semen parameters (N=100)

Parameters (Mean±SE)		Serum AMH	Seminal AMH
Volume	r	- 0.084	- 0.124
	p value	0.404 NS	0.219 NS
Sperm's concentration	r	- 0.028	**0.298**
	p value	0.785 NS	**0.003 S**
Sperm's count	r	- 0.017	**0.456**
	p value	0.867 NS	**< 0.001 S**
Progressively motile sperms (PMS) %	r	- 0.001	**0.215**
	p value	0.898 NS	**0.031 S**
Non-Progressively motile sperms %	r	0.042	0.121
	p value	0676 NS	0.232 NS
Immotile sperms %	r	0.005	- 0.201
	p value	0.959	0.119 NS
Normal morphology %	r	0.017	**0.417**
	p value	0.867 NS	**< 0.001 S**
Round cells	R	- 0.004	0.136
	p value	0.971 NS	0.177 NS

r: Pearson's correlation coefficient; S: Significant ($p \leq 0.05$); NS: Not significant ($p > 0.05$)

DISCUSSION

First, the total seminal plasma AMH was evaluated, along with the total fertility hormones and the total semen parameters. This is due 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 (13). As a result, hormone analysis in the semen reveals high individual differences due to the contributions of these glands (14). Following this, we separately analyzed the AHM serum and seminal plasma for each of the four study groups: normozoospermia (n = 25), oligozoospermia (n = 26), asthenozoospermia (n = 21), and OAT (n = 28). The current study found no significant differences among groups in terms of age, body mass index, duration of infertility, type of infertility, or smoking status. This suggests that the demographic variables under investigation had little influence on this study. However, the study's nature, small sample size, one-time conduct, and the fact that the participants did not represent the population of infertile men limit these results. Most studies have confirmed the influence of demographic parameters on spermatogenesis, which in turn links to male infertility (15).

Comparison of Hormonal Levels among Studied Groups

We found that serum AMH levels are higher than those in seminal plasma. Our results are consistent with another study (16). However, our findings differ from those of the authors (17), who discovered that the concentrations of AMH in plasma seminal fluid high those in blood. There are significant differences between the four groups in terms of seminal plasma AMH, and the group of men with normal sperm was higher compared to the other groups; these results are similar to others (18). But there were no statistically significant differences among the groups regarding serum AMH. These findings agree with reference (19). High levels of AMH in the blood that are compared to seminal plasma may indicate that Sertoli cells are immature or defective (20). Because it is a characteristic of fully developed Sertoli cells secrete AMH in high quantities from their apical pole toward the seminiferous tubules, compared to those produced by their lower pole toward the interstitium and circulation. (21). There are several factors that affect the proliferation, differentiation, and maturation of Sertoli cell, some of which are hormonal, such as gonadotropins (FSH, LH and testosterone), or non-hormonal, for example, growth factors, cytokines, WNT and bone morphogenetic proteins (BMPs) signaling pathways and normal androgen receptors (ARs). Weakness or absence of one or more of these factors causes problems in the Sertoli cell and thus it does not reach its final maturity and functions required to facilitate the process of spermatogenesis (22 ,23).

We conducted comparisons between the study groups in relation to FSH, LH, T, and PRL. The results indicated significant variations in testosterone among groups, with normospermic men exhibiting higher levels of testosterone compared to the other groups. The results of this study agree with other research's (36, 37, 38), but are in contrast to the conclusions drawn by authors (16, 35). The decline in testosterone levels may be associated with the psychological state of infertile individuals, as elevated cortisol levels lead to a reduction in testosterone levels (39). Additionally, lifestyle factors such as smoking, consumption of unhealthy food, inadequate sleep, deficiencies in vitamins (specifically zinc, magnesium, and vitamin D), and the use of plastic materials can contribute to this decrease. These factors disrupt the balance of reproductive hormones, particularly testosterone. As a result, it has an impact on male fertility (40). The groups showed no statistically significant differences in FSH, LH, and PR.

Correlations of Serum and Seminal AMH with Fertility Hormones profile and sperms parameters (N=100)

In this study was observed positive significant correlations between seminal plasma AMH concentration, count, and motility of sperm. Other investigations (14, 24) support these results. However, other studies didn't show consistent data for this association (16). Neither these studies (14, 16, 24) nor any other study found that there is a significant positive correlation between semen AMH and sperm morphology, in contrast to our results, which showed this association. More studies are needed to understand the effect of AMH on the structure and shape of sperm. The presence of high levels of AMH in the testis may support germ cell proliferation (25) and the early stages of sperms maturation (16); thus, it is a good marker for spermatogenesis, but the mechanisms behind such an association remain unknown. The effect of AMH on sperm motility is still unknown, but an interesting study demonstrated that recombinant AMH added to seminal plasma increased sperm motility and longevity (26). Our results did not reveal any significant correlation between serum AMH and semen characteristics. Others (27) reported the same results. But there are previous studies that confirmed the association of AMH serum with semen quality (28, 29, 30).

Spermatogenesis is a sequence of spermatogenic cell maturation and differentiation processes. The hypothalamic-pituitary-gonadal axis, regulates it. The process involves hormones such as gonadotropin (GnRH) secreted by the hypothalamus, FSH and LH secreted by the pituitary gland, and T secreted by interstitial cells. Instead of working directly with spermatogenic cells, FSH and T first bind to receptors on Sertoli cells and then feed them through a paracrine mechanism (35, 36). AMH is secreted by Sertoli cells into the seminal plasma and blood. Therefore, in this study, we also examined the association of both AMH in seminal plasma and blood with fertility hormone profiles. The study's findings showed that, with the exception of a strong positive association between serum AMH and serum FSH, there are no other correlations between levels of seminal plasma a serum AMH with levels of FSH, LH, testosterone, and prolactin. According to research by Aksglaede et al. (27), serum AMH had no correlation with total testosterone but a substantial negative correlation with FSH and LH. However, serum and seminal AMH levels did not significantly correlate with hormones profile, according to Andersen et al. (14). While FSH initially has a favorable regulatory role in AMH, testosterone has a powerful down-regulating effect (31).

Correlations of Serum and Seminal AMH with Fertility Hormones and sperms parameters among groups.

There was no significant correlation of semen parameters with serum and seminal plasma AMH in the normozoospermia, oligozoospermia, asthenozoospermia, and OAT groups, which agrees with investigators (31, 32) but does not correspond with studies (33, 34). Compared to other study groups, men with normal sperm exhibited higher seminal plasma AMH levels, potentially indicative of their sperm quality. Additionally, there was no correlation between fertility hormone profiles and serum or seminal plasma AMH, except for a positive correlation between seminal AMH and testosterone in oligozoospermic men. These findings are in line with previous research (18, 41). According to reports, AMH regulates steroidogenesis and Leydig cell proliferation (42).

CONCLUSION

We found a correlation between total semen characteristics and total seminal plasma AMH, suggesting a role for AMH in spermatogenesis. To elucidate the mechanisms behind AMH control in spermatogenesis, more studies and molecular pathways are required. Significant variations in AMH levels in the seminal plasma were observed. Low concentrations of semen AMH are a predictive marker that Sertoli cells are still immature and have poor spermatogenesis production.

Conflict of interest: None declared.

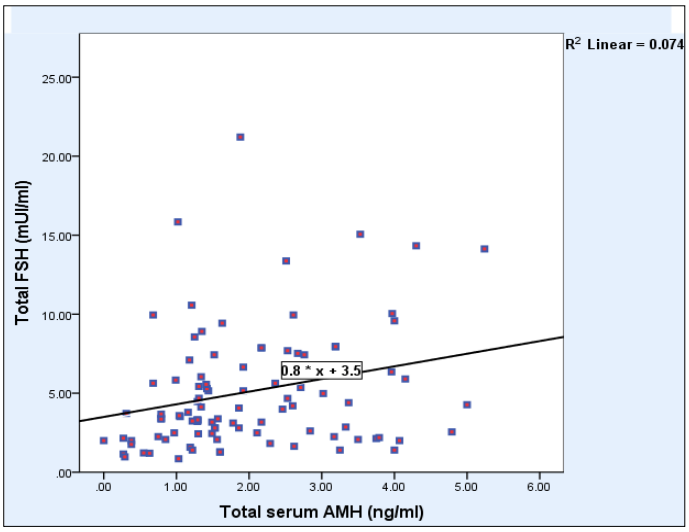

Figure 1: Correlations between total serum AMH & total serum FSH

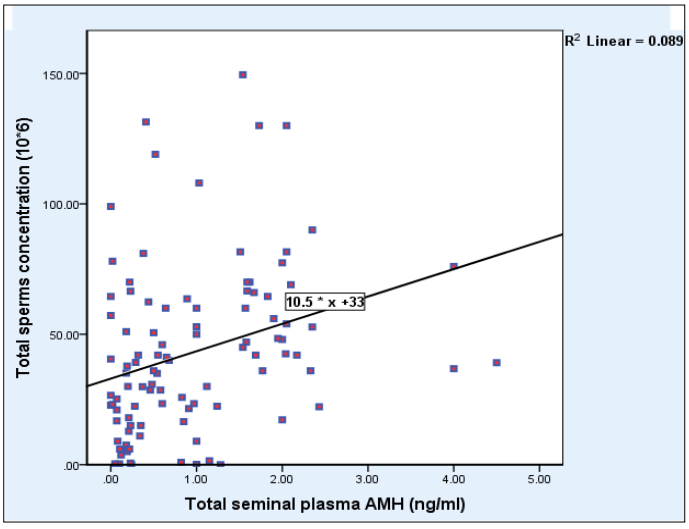

Figure 2: Correlations between total seminal plasma AMH & total sperms concentration

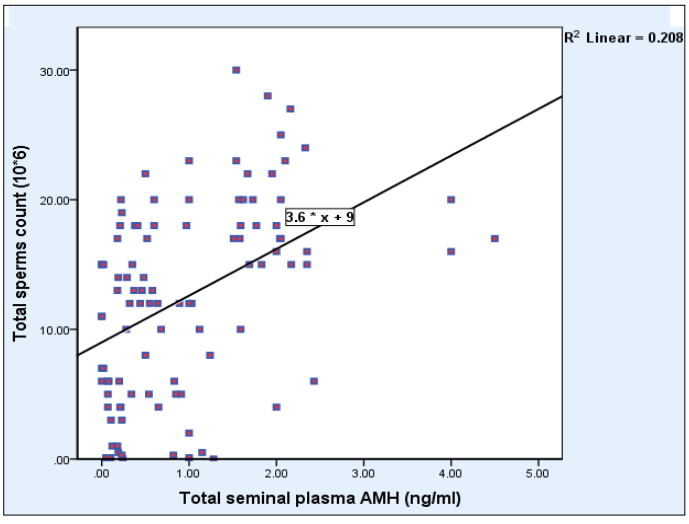

Figure 3: Correlations between total seminal plasma AMH & total sperms count

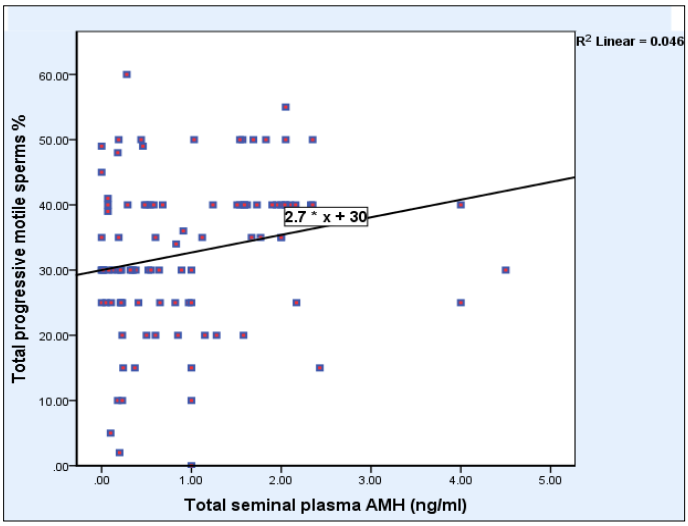

Figure 4: Correlations between total seminal plasma AMH & total progressively motile sperms

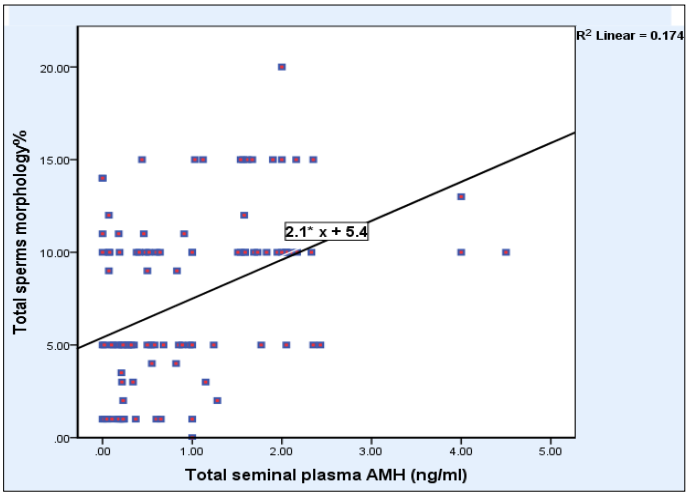

Figure 5: Correlations between total seminal plasma AMH & total sperms morphology

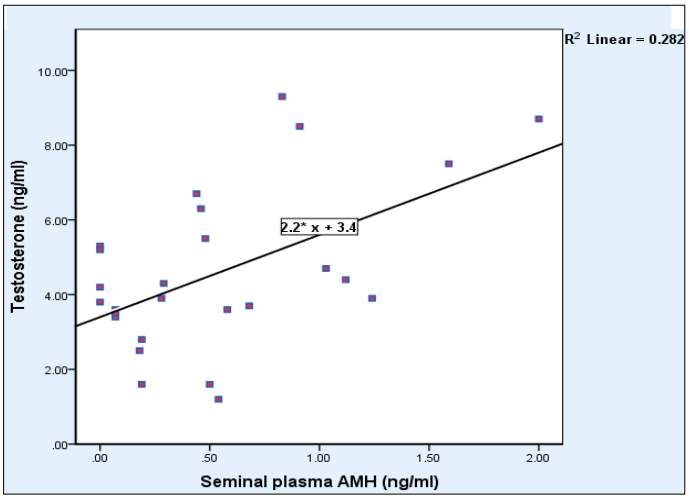

Figure 6: Correlations between semen AMH & serum testosterone in oligozoospermia group r=0.531, p=0.005

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