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EFFECT OF SERUM AND SEMINAL AMH ON SPERM PRODUCTION FOR OLIGOZOOSPERMIC AND AZOOSPERMIC MEN

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ABSTRACT
This study was aimed to investigate the clinical value of serum and seminal AMH levels in normozoospermia and infertile men particularly in those oligozoospermic (O) and azoospermic (NOA) males. Three groups of infertile males (n=59): normozoospermia (n=38), oligozoospermia (n=8) and NOA (n=13), were subjected to study at High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq. The levels of serum and seminal plasma AMH were measured by ELISA. Results showed a significant decrement ($P \leq 0.05$) in the serum AMH for males with oligozoospermia as compared to the other groups of male infertility factors. Meanwhile, non- significant difference ($P > 0.05$) was observed in the level of AMH for normozoospermia as compared to azoospermia. A significant decrement ($P \leq 0.05$) was observed in the level of seminal AMH for males with normozoospermia as compared to the males complaining of oligozoospermia. Similarly, significant increment ($P \leq 0.05$) in the level of seminal AMH for males suffering from oligozoospermia as compared to normospermia and azoospermia. Moreover, there was a significant decrement ($P \leq 0.05$) for males suffering from azoospermia as compared to the normozoospermia and oligozoospermia. The AMH levels were not indicative of spermatogenesis and could not differentiate between fertile and infertile males. Seminal plasma AMH is an absolute testicular marker of testicular function; AMH concentrations are specific markers in seminal plasma to definitely evaluate the status of spermatogenesis.

KEY WORDS: Anti-mullerian hormone, oligozoospermia, non-obstructive azoospermia (NOA),

INTRODUCTION

The gonads are differentiated as testes; that secrete two distinct hormones involved in normal male sexual differentiation: anti-Mullerian hormone (AMH) and testosterone. AMH, also called Mullerian inhibiting substance (MIS) or factor is Sertoli cell glycoprotein that causes regression of the Mullerian ducts (Lasala *et al*., 2004). Anti-Mullerian hormone (AMH), is a member of the transforming growth factor-B family expressed in the Sertoli cells, exerts paracrine inhibition of Muullerian derivatives during fetal life (Teixeira *et al.*, 2001). AMH in the testis is secreted by SC both apically into seminiferous tubules and basally towards the interstitium and circulation. After puberty, AMH is released preferentially by the apical pole of the SC towards the lumen of the seminiferous tubules, resulting in higher concentrations in the seminal plasma than in the serum (Sinisi *et al*., 2008). AMH is measurable in human serum and has diagnostic applications as a specific marker of immature Sertoli cell number and function (Josso, 1995: Rey *et al.*, 1996). There is no information on the expression of anti-Mullerian hormone receptor type (AMHR2) in appendix testis (AT), although it is possible that the cells of AT express this receptor during embryonic development and most likely after birth as well. Torsion of the AT is painful, and patients with this condition show similar symptoms to those with torsion of the testis. Therefore, removal of the AT is medically indicated when it appears during intrascrotal operations (Kistama´ *et al.*, 2013). seminal fluid and, being a specific marker of Sertoli cell function; its measurement may be useful to obtain information on spermatogenesis in infertile men (Omabe, 2013). This study was aimed to investigate the clinical value of serum and seminal AMH levels in normozoospermia and infertile men particularly in those complaining from oligozoospermia (O) and azoospermia (NOA).

MATERIALS & METHODS

The infertility case-control study was carried out in High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, during the period from March, 2014 to December, 2014. The study involved of 38 normozoospermic, 8 oligozoospermic men and 13 azoospermic men. Semen analyses were done according to the World Health Organization standard criteria (2010). The ejaculates were collected after abstinence period of (3-5days). In a sterile, non-toxic, disposable Petri-dish by masturbation achieved in a private room near the laboratory prepared for this purpose in order to minimize the exposure of the semen to inconstancies in temperature and to control the time between collection and analysis. Specimen was labeled with patient's name and lab number. Containers were placed in an incubator at 37°C allowed for liquefaction (Nafa and ESHRE, 2002). The liquefied semen was carefully mixed by glass Pasteur pipette for few seconds, and then the specimen was examined in detail by macroscopic and microscopic examination.

Seminal plasma preparation and storage

Semen samples were centrifuged for 15 minutes at 3000 rpm. The supernatant of seminal plasma was quickly and carefully recovered and put to freeze at -20°*C* for later measurements. Concentrations of AMH were measured by Enzyme-Linked Immunosorbent Assay (ELISA) technique.

Blood Collection

Five milliliters of peripheral venous blood was aspirated from each male. Blood samples were collected in plain tubes, allow clotting and then centrifuged at 2500 rpm for 10 minutes. The specimens were categorized into two groups according to the results of sperm analysis. Concentrations of AMH were measured by Enzyme-Linked Immunosorbent Assay (ELISA) technique.

Statistical analysis

The data were statistically analyzed using SPSS/PC version 18 soft ware (SPSS, Chicago). Sperm parameters, levels of seminal and serum AMH were analyzed using complete randomized design (CRD) (one way ANOVA). Differences among means were tested using the Duncan multiple ranges test (Duncan, 1955).

RESULTS

Table(1) explains semen parameters for normozoospermic, oligozoospermic and azoospermic males participated in this study. The macroscopic examination of semen parameters revealed that the semen volume, semen liquefaction time and semen pH were within normal values when compared with the criteria of WHO (2010). Also, the microscopic examination which include the sperm concentration, sperm grade motility, total progressive sperm, normal sperm morphology, sperm agglutination and round cells were within the normal values when compared with the criteria of WHO (2010).

TABLE 1: Semen parameters for Normozoospermic, Oligozoospermic and Azoospermic males participated in this study.

Semen parameters		Normozoospermia (no. 38)	Infertile patients		WHO(2010)criteria
			Oligozoospermia (no.8)	Azoospermia (no.13)	
Macroscopic Examination					
Semen volume(mL)		2.589 ± 0.17	2.775 ± 0.53	2.162 ± 0.22	1.5-5 mL
Semen liquefaction(min)		44.026 ± 1.94	49.375 ± 3.95	44.620 ± 2.97	Within 60 Minutes
Semen viscosity		Normal	Normal	Normal	Drops/≤2cm thread
Semen pH		7.711 ± 0.04	7.488 ± 0.14	7.508 ± 0.08	7.2-8.0
Microscopic Examination					
Sperm Concentration		48.824 ± 3.32	2.729 ± 1.53	0.000	≥15millions/ml
Sperm motility (%)		73.324 ± 1.09	67.625 ± 2.24	0.000	Progressive motile
Sperm grade activity (%)	Progressive sperm motility	41.750 ± 0.67	41.750 ± 0.67	0.000	sperm (32%)Within 60 minutes
	Non Progressive sperm motility	25.875 ± 2.61	25.875 ± 2.61	0.000	
	Immotile sperm	32.750 ± 2.84	32.750 ± 2.84	0.000	
Total Progressive sperm (millions/ejaculate)		48.969 ± 4.31	3.588 ± 2.63	0.000	≥8.2 millions/ejaculate
Normal sperm morphology (%)		37.921 ± 0.51	36.250 ±1.75	0.00	≥30%
Sperm Agglutination (%)		3.079 ± 1.03	0.000	0.00	≤10%
Round cells count (HPF)		5.500 ± 0.55	5.375 ± 1.73	0.00	≤5 cells/HPF

From the same table macroscopic examination of semen parameters for oligozoospermic males showed normal values as compared with the criteria of WHO (2010). Moreover, the sperm concentration of the microscopic examination was lower than the normal values when compared with the criteria of WHO (2010). Whereas, the other parameters of microscopic examination were within normal values when compared with the criteria of WHO (2010). Additionally, the macroscopic examination of semen parameters for azoospermic males revealed that the semen volume, semen liquefaction and pH were within the normal values when compared with the criteria of WHO (2010). On the other hand, the sperm concentration was zero when compared with the standard criteria of WHO (2010), However, the round cell count was within the normal values. Figure (1) shows the level of serum AMH classified according to study groups. A significant decrement ($P \leq 0.05$) was observed in the serum AMH for males with oligozoospermia as compared to the other groups of present study. Meanwhile, non-significant difference ($P > 0.05$) was observed in the serum level of AMH for normozoospermia as compared to azoospermia. Levels of seminal AMH for male were represented in figure (2) classified according to study groups. A significant defferences ($P \leq 0.05$) was observed in the level of seminal AMH for males complaining from normozoospermia as compared to the males complaining of oligozoospermia and azoospemia. Similarly, significant increment ($P \leq 0.05$) in the level of seminal AMH for males suffering from oligozoospermia as compared to the other groups. Moreover, there was a significant decrement ($P \leq 0.05$) for males with azoospermia as compared to the other groups of infertility.

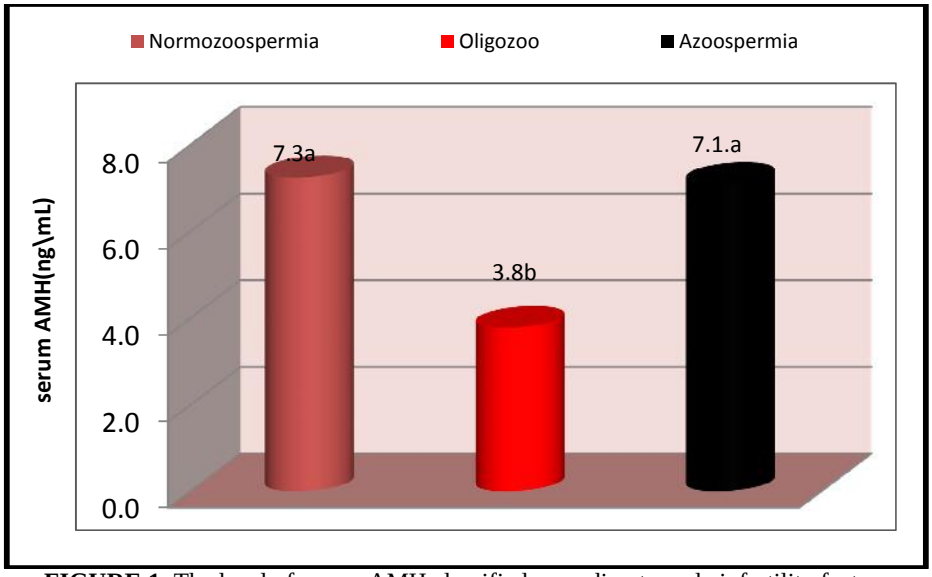

FIGURE 1: The level of serum AMH classified according to male infertility factor.
Means with different letters are significantly different (P<0.05).
Means with similar letters are not significantly different (P>0.05).

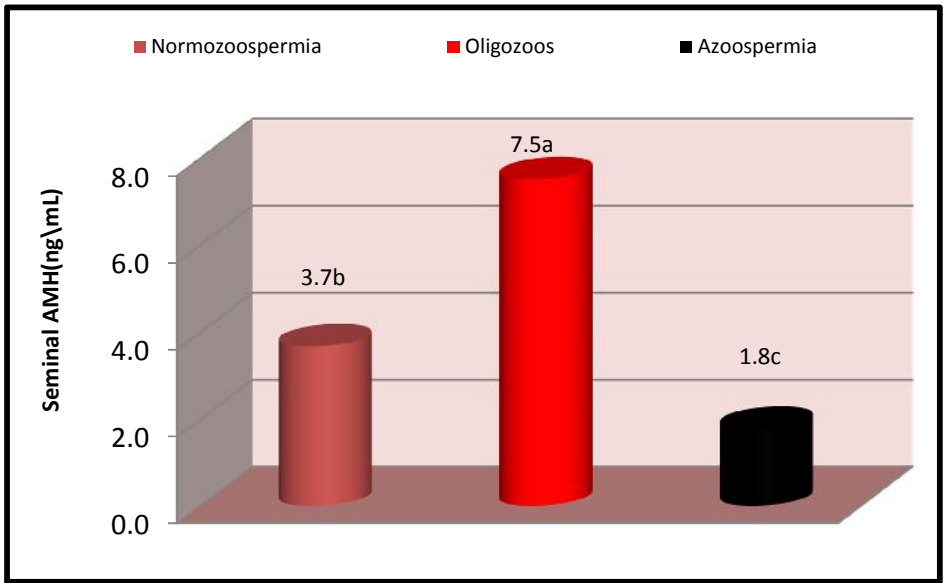

FIGURE 2: The level of seminal AMH classified according to male infertility factor
Means with different letters are significantly different (P<0.05).
Means with similar letters are not significantly different (P>0.05).

DISCUSSION

A significant decrement (P≤0.05) was observed in the serum AMH for males with oligozoospermia as compared to the other study groups. This result agreed with Sweeney *et al*. (1997). They reported that in humans, large amounts of AMH are produced during fetal and post-natal testicular development. The expression and production of AMH is principally reduced at onset of puberty and this may reflects terminal differentiation of Sertoli cells (Saleh *et al.*, 2014). Serum AMH was found to be significantly lower in men with oligozoospermia as compared with other groups of infertility and this is in accordance with results of obtained by (Al- Qahtani *et al*., 2005). The regulation of AMH after birth is complex; basal levels of AMH are independent of gonadotropin regulation, for example, during childhood and in patients with hypogonadotropic hypogonadism (Young *et al*., 1999; Al – Chalabi *et al.*, 2012). A significant decrement (P≤0.05) in the level seminal AMH was observed for males with normozoospermia as compared to the males complaining of oligozoospermia. This result is consistent with Sabetian *et al*. (2012). Who revealed that AMH is preferentially secreted by Sertoli cells into the seminiferous lumen, resulting in higher concentrations in the seminal plasma than in the serum (Fujisawa *et al*., 2002; Al-Qahtani *et al*., 2005). Although this evidence suggests that seminal AMH might be a marker for Sertoli cell functional maturation and spermatogenesis progression (Chang *et al*., 2004). So, in this work all the samples collected have different types

of infertility factors and that could be attributed to a defect in sperm formation process or a defect in which Sertoli cells led to a decrease in AMH semen compared with serum (Matuszczak *et al.,* 2013).

However, the presence of more advanced spermatogenic cells may increase AMH secretion that is related to specific stages of the seminiferous epithelium. Higher concentration of seminal AMH in normospermic men compared with azoospermic subjects is also in agreement with previous studies (Duvilla *et al.,* 2008; Fénichel *et al.,* 1999). Lower AMH levels were also found in infertile men compared with controls (Goulis *et al*., 2008; Bensalem *et al.,* 2013 Fujisawa *et al*., 2002).

CONCLUSION

According to our results could be considered the levels of serum AMH were not indicative of spermatogenesis and could not differentiate between fertile and infertile males**.** Seminal plasma AMH is an absolute testicular marker for testicular function; AMH concentrations are specific markers in seminal plasma to evaluate the status of spermatogenesis

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