

Correlation between Seminal Fluid Analysis and Levels of Gonadotropins in Serum and Seminal Plasma of Normozoospermic Men and Infertile Patients

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Abstract

Background: Levels of serum gonadotropins have direct effects on testicular functions and spermatogenesis. Assessment of levels of serum gonadotropins from fathered subjects and infertile patients indicates wide range diversity. In this study, we tried to find out whether the levels of seminal FSH and LH affect the parameters of seminal fluid analysis (SFA) and if there is any correlation between levels of serum FSH and LH in healthy men and infertile patients.

Materials and Methods: Levels of FSH and LH in serum and seminal plasma were assessed randomly, in addition to examination of seminal fluid analysis from 12 normozoospermic subjects (age range: 33-56 years) and 66 infertile patients (age range: 20-62 years) with duration of infertility (15-201 months). Macroscopic and microscopic parameters of semen specimens were determined. Data were statistically analyzed using multiple correlation and regression, and MANOVA tests.

Results: Result of the present study observed significant positive correlation between FSH levels in serum and seminal plasma ($r=0.984$; $p<0.001$) of normozoospermic subjects as compared to other groups of infertile patients. No correlations were noticed between LH levels in serum and seminal plasma of normozoospermic subjects and groups of infertile patients. Significant and positive correlation was assessed between sperm concentration and levels of seminal FSH ($r=0.822$; $p<0.05$ and $r=0.940$; $p<0.01$) and seminal LH ($r=0.989$; $p<0.001$ and $r=0.999$; $p<0.001$) of asthenozoospermic and OAT patients respectively. In asthenozoospermic patients, significant and positive correlations were observed between seminal FSH and percentages of sperm motility, progressive motility, sperm normal morphology and total progressive motile sperm/ejaculate.

Conclusion: This study shows a strong association and effect between seminal FSH and serum FSH and parameters of SFA for normozoospermic men and different groups of infertile patients. These finding may call for large cohorts being executed with larger population of patients that is required for this analysis to be more accurate.

Keywords: Gonadotropins, Serum, Seminal Plasma, Infertility

Introduction

The management of infertility problems has become an increasingly important part of health services during the past two decades in the most countries. Recent studies have focused on semen quality of men in the general population. However, most studies of semen quality and reproductive hormones in unselected populations have little previously reported (1).

Gonadotropins comprise follicle stimulating

hormone (FSH) and luteinizing hormone (LH) produced by the pituitary gland. In men, they are essential for spermatogenesis and testosterone secretion (2). Circulating FSH has long been considered a valuable marker for Sertoli cell function and spermatogenesis, but FSH is influenced by hypothalamic function, as well as testicular factors and steroidal hormones (3). However, an increased concentration of

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LH in circulation accompanying idiopathic oligozoospermia suggests that LH secretion may be linked to the factors regulation spermatogenesis (4, 5).

It has generally been observed that testis size and function is associated with gonadotropic activities that are necessary for initiation and maintenance of spermatogenesis (6). Previous studies had shown no specific pattern in the serum or seminal plasma hormone profiles of men with infertility and it is debatable whether there is a need to perform routine seminal hormone assays in the management of men with infertility (7). Furthermore, low concentration of sexual hormones may increase the apoptosis of germ cells, which can induce male infertility (8).

The significance of the correlation between the levels of LH and FSH in seminal plasma and sperm concentration and motility is unknown (4). Although, seminal sex hormones might be more sensitive indices to assess the direct effect on sperm functions and extent of feedback inhibition on hypothalamus-pituitary-testis axis (9). Thus, to investigate the possible causes of the deterioration of male fertility and make attempts to prevent further decay, they can be studied. Therefore, the present study was designed: 1. to evaluate the levels of serum and seminal FSH and LH, 2. to find out whether gonadotropins affect the parameters of seminal fluid analysis (SFA) and 3. to assess correlation between levels of gonadotropins in serum and seminal plasma of healthy men and infertile patients.

Materials and Methods

Subjects

Seventy eight men were enrolled in the present study during their attendance to IVF Institute of Embryo Research and Infertility Treatment at Al-Nahrain University. Presence or absence of varicocele, hydrocele, cryptorchidism, hernia and other congenital abnormalities for all men were assessed by a consultant urologist. History of pre-

pubertal and pubertal mumps, orchitis, alcohol consumption and smoking habit are reported. Complete history regarding venereal diseases, systemic chronic diseases or febrile disease were undertaken. Men suffering from any chronic disease were rejected in advance.

Seminal fluid analysis (SFA)

The sample of the seminal fluid was collected after three days of abstinence directly into a clean, dry, sterile, disposable Petri dish by masturbation, in a room near the laboratory. The container labeled with the necessary information including name and age of couple, file number, abstinence period and time of sample collection. Macroscopic and microscopic examinations were performed according to WHO methodology mentioned in details in WHO manual (10). Classification of subjects into normozoospermic and infertile men was dependent on WHO criteria for normal semen values presented in manual of NAFA-ESHRE (11).

Gonadotropins assay

A blood sample (3 ml) was drawn and collected in a clean, disposable plastic tube. Serum was aspirated and isolated from precipitated blood after 8 minutes centrifugation (at 2500 rpm). Also, seminal plasma free of spermatozoa was prepared for gonadotropins assay after centrifugation (at 2500 rpm for 10 minutes) and upper layer of seminal plasma (1.0 ml) was aspirated and isolated in a clean, disposable plastic tube. Prepared serum and seminal plasma samples were stored at -20°C until later use for gonadotropins assay (12).

Serum and seminal plasma levels of gonadotropins (FSH and LH) were measured for all subjects using immunoradiometric assay (IRMA) technique and Wallac apparatus (HVD Life Sciences; LKB Wallac, Turku, Finland). The sensitivity of FSH was 0.02 mIU/mL, intra- and inter-assay coefficients of variation were 3.07%

and 2.4%; respectively. The sensitivity of LH was 0.02 mIU/mL, intra- and inter-assay coefficients of variation were 3.1% and 5.27%; respectively.

Statistics

Results were presented as mean \pm standard error of mean (SEM) for parameters of SFA and levels of serum and seminal plasma prolactin. The data were statistically analyzed by multiple analysis of variance (MANOVA) and multiple correlation and regression tests to compare among different means of groups using statistical computerized package SPSS (Statistical package of Social Science, version-12).

Results

The results of this study appeared according to the parameters of SFA in subjects that were classified into 12 normozoospermic men (age mean: 34.2 year) and 66 infertile patients (age mean: 35.6 year). Subjects with abnormal semen had significant impairment of at least one parameter of their SFA. Therefore, infertile patients were divided into azoospermic group (No. 52), asthenozoospermic group (No. 6) and oligoasthenoteratozoospermic group (OATzoospermic; No. 8).

However, all men with normal and abnormal SFA had no significant point in their medical history and comparable age as presented in the figure (1).

The duration of infertility was significantly ($p < 0.01$) increased for azoospermic patients compared to the other two groups of infertile patients (Figure 1).

In the present work, the level of seminal FSH for normozoospermic and asthenozoospermic samples shows positive and highly significant correlation with the age ($r = 0.825$; $p = 0.001$ and $r = 0.907$; $p = 0.013$, respectively). Meanwhile, weak and non significant ($p > 0.05$) correlations were assessed for azoospermic and OATzoospermic patients. In general, seminal plasma LH levels shows weak and non

significant ($p > 0.05$) correlations with age for groups of normozoospermic, azoospermic and asthenozoospermic men, meanwhile, negative and significant correlation ($r = -0.736$; $p = 0.01$) was achieved between level of seminal plasma LH and age of OATzoospermic patients.

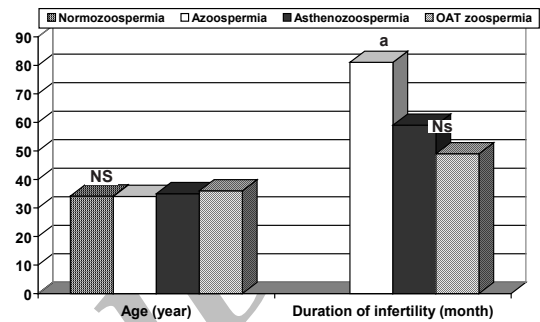


Fig 1: Mean age and duration of infertility for normozoospermic men and different groups of infertile patients

No. of normozoospermic men=12

Total no. of infertile patients=66

No. of azoospermic patients=52

No. of asthenozoospermic patients=6

No. of OATzoospermic patients=8

NS: means non significant ($p > 0.05$) differences among all groups

a: means significant ($p < 0.05$) differences between azoospermic and other two groups of infertile patients

Ns: means non significant ($p > 0.05$) differences between asthenozoospermic and OATzoospermic groups.

Positive and highly significant correlation ($r = 0.984$; $p = 0.001$) was demonstrated between levels of FSH in serum and seminal plasma of normozoospermic men. In contrast, all groups of infertile patients, in this study, have weak and non significant ($p > 0.05$) correlations reported between serum and seminal plasma levels of FSH (Table 1).

From same table, levels of LH in seminal plasma and serum have weak and non significant ($p > 0.05$) correlations were detected for normozoospermic men and the other groups of infertile patients. A significant ($p < 0.05$) reduction in the levels of seminal plasma gonadotropins was noticed as compared to levels of serum gonadotropins. Furthermore, levels of FSH in serum and seminal plasma for

azoospermic patients have significant ($p<0.05$) elevation when compared to the other groups. Levels of serum LH were increased significantly ($p<0.05$) in azoospermic patients as compared to the other groups of the present work (Table 1). Negative and significant correlations between level of seminal LH and semen volume for normozoospermic men ($r=-0.664$; $p=0.05$) and OAT zoospermic patients ($r=-0.649$; $p=0.05$). While highly significant correlations were reported between semen acidity (pH) and levels of seminal LH for azoospermic ($r=-0.420$; $p=0.002$) and asthenozoospermic ($r=0.942$; $p=0.005$) patients (Table 2). In the same table (2), highly significant correlations were obtained between seminal FSH and semen liquefaction time of normozoospermic men ($r=0.631$; $p=0.02$) and azoospermic patients ($r=-0.336$; $p=0.01$). However, seminal FSH was highly significant correlated with semen pH for asthenozoospermic ($r=0.961$; $p=0.002$) and OATzoospermic ($r=-0.778$; $p=0.02$) patients.

Microscopic parameters of SFA for normozoospermic men and different groups of infertile patients were shown in the table (2).

In normozoospermic men, levels of seminal

FSH and LH were non significantly ($p>0.05$) correlated with all sperm parameters were examined, with except to percentage of normal sperm morphology that was negatively and significantly ($r=-0.646$; $p=0.02$) correlated with seminal LH. However, levels of seminal FSH have positive and highly significant correlations with sperm parameters, with except to percentage of immotile spermatozoa, for asthenozoospermic patients. On the other hand, within same group of infertile patients, highly positive and significant correlations were observed between seminal LH level and sperm concentration ($r=0.989$; $p=0.001$), percentage of sperm activity grade C ($r=0.988$; $p=0.001$), total progressive motile spermatozoa/ ejaculate ($r=0.930$; $p=0.007$) and percentage of sperm agglutination ($r=0.942$; $p=0.005$). In the OATzoospermic group, positive and highly significant correlations was reported between levels of seminal FSH and LH and sperm concentration ($r=0.940$; $p=0.001$ and $r=0.999$; $p=0.001$; respectively), percentage of sperm activity grade C ($r=0.982$; $p=0.001$ and $r=0.954$; $p=0.001$; respectively) and percentage of sperm agglutination ($r=0.982$; $p=0.001$ and $r=0.954$; $p=0.001$; respectively).

Table 1: Levels of serum and seminal plasma gonadotropins and its correlations for normozoospermic men and different groups of infertile patients (Mean±S.E.M.)

Parameters	Gonadotropins					
	FSH(mIU/ml)			LH(mIU/ml)		
	Serum	Seminal plasma	Correlation (p value)	Serum	Seminal plasma	Correlation (p value)
Normozoo spermia	5.843 ±1.717	0.478±0.051 ^b	0.984(0.001)	5.295±0.387	1.290±0.11 ^b	0.286(0.366)
Azoo sepermia	16.231±1.581	1.485±0.637 ^b	-0.074(0.600)	12.033±1.847	0.677±0.075 ^b	0.012(0.934)
Asthenozoo spermia	2.723±0.297	0.515±0.044 ^a	-0.258(0.621)	3.008±0.312	1.359±0.032 ^a	0.152(0.774)
OATzoo spermia	7.230±2.87	0.409±0.309 ^b	-0.418(0.309)	5.903±0.752	0.582±0.056 ^b	0.456(0.256)

No. of normozoospermic men = 12.

Total no. of infertile patients = 66.

No. of azoospermic patients = 52.

No. of asthenozoospermic patients = 6.

No. of OATzoospermic patients = 8.

a: means significant ($p<0.05$) differences between seminal plasma and serum within same hormone.

b: means significant ($p<0.01$) differences between seminal plasma and serum within same hormone.

c: means significant ($p<0.001$) differences between seminal plasma and serum within same hormone.

Table 2: Macroscopic and microscopic parameters of seminal fluid analysis for normozoospermic men and different groups of infertile patients (Mean±S.E.M.)

Parameters	Number of Subjects	SFA-macroscopic examination		
		Semen volume (ml)	Liquefaction time (min)	Semen acidity (pH)
Normozoo Spermia	12	2.166±0.195 ^d	28.333±2.47 ^a	7.850±0.051
Azoo Spermia	52	2.076±0.145	44.807±2.059 ^c	7.750±0.029 ^d
Asthenozoo Spermia	6	2.433±0.358	45.010±3.958	7.866±0.042 ^b
OATzoo Spermia	8	1.450±0.172 ^d	28.750±2.72	7.675±0.049 ^c

Parameters	SFA-macroscopic examination							
	Sperm Covc. (X10 ⁶ /ml)	Sperm motility (%)	Sperm grade activity			Total progr sperm/ejacul.	Normal sperm morph. (%)	Sperm agglut. (%)
			Progres. (A+B) (%)	Non Progres. (C) (%)	Immot. (D) (%)			
Normozoo Spermia	122.103±12.85	61.021±3.020	45.166±3.169	13.833b±1.353	39.001±3.020	125.785±12.47	35.081 ^d ±2.611	6.166±1.460
Azoo Spermia	-	-	-	-	-	-	-	-
Asthenozoo Spermia	51.02 ^{ab} ±9.681	43.101±8.951	26.108 ^a ±6.937	16.804 ^{ab} ±2.898	57.088 ^c ±8.95	55.06 ^{ab} ±11.29	19.036 ^a ±7.33	10.666 ^{ab} ±1.054
OATzoo Spermia	5.08 ^{ab±} 1.138	34.750±3.876	29.75±4.270	5.00 ^{ab±} 2.073	65.25±3.876	1.715±0.177	6.250±1.091	7.507 ^{ab} ±1.909

a: means positive and significant correlation with the seminal plasma FSH

b: means positive and significant correlation with the seminal plasma LH

c: means negative and significant correlation with the seminal plasma FSH

d: means negative and significant correlation with the seminal plasma LH

Discussion

Gonadotropins have the main role in sperm production and presence of male characteristics (13). Furthermore, assessment levels of gonadotropins are considered most important in cases of infertility investigation (5). It has been reported that the relationship between hormone concentration and parameters of testicular functions are quite variable, and abnormal spermatogenesis sometimes occurs concurrently with endocrine abnormalities (14). Therefore, this study gave us an opportunity to compare gonadotropins in two types of samples from normozoospermic men and patients with different groups of male infertility factors. Not surprisingly, the healthy males with known fertility are included in the present study. Although, semen samples are difficult to obtain within sampling frames that allow comparisons. Blood samples are easier to obtain than semen ejaculates. Same finding was reported by Jensen *et al* (3).

In the present study, significant ($p < 0.05$) reduction in the levels of gonadotropins within seminal plasma compared to serum.

Although, we do not believe that the observed differences in serum and seminal plasma levels of gonadotropins can be explained by differences in the hour of sampling. Several factors may affect this result including different abnormalities in the functions and distribution of receptors (15), differences in pharmacokinetics and pharmacodynamics (16), presence of special serum antibodies against FSH activity (17), disturbances in the 5 α -reductase activity (18), abnormal function of Sertoli cells (19) and genital diseases (1). However, it was reported that the concentration of hormones in the blood stream of male infertile patients (particularly gonadotropins and testosterone) is likely to be abnormal, but it is unclear whether this is a secondary change that reflects testicular injury or a factor that contributes to further derangement of testicular function (14).

Non significant correlation was observed between levels of FSH and LH in serum and seminal plasma of men with abnormal SFA. Also, same result was obtained for correlation between levels of LH in serum and seminal plasma of normozoospermic

men. A clinical study demonstrated same result (20), which reported that FSH and LH levels in serum and seminal plasma are not correlated. However, seminal FSH concentrations were positively correlated with sperm output but not sperm motility (4). It has been proposed that, 5 α -reductase activity in the mature adult testis is low when the concentration of testosterone produced by the Leydig cells are high enough to maintain spermatogenesis (18). Interestingly, seminal gonadotropins levels may be more sensitive indices to assess the extent of spermatogenic suppression resulting from inhibition of gonadotropin secretion (9).

Negative and significant correlations between level of seminal LH and semen volume were assessed for normozoospermic men and OATzoospermic patients. Our data confirm that the normal level of testosterone, in response to LH action on Leydig cells, positively affect growth of male accessory glands and its secretions which constitute main volume of seminal plasma (21). However, it was reported that a significant positive correlation observed between seminal plasma FSH concentration and seminal plasma volume in normal and infertile subjects (7).

In normozoospermic men, levels of seminal FSH and LH were non significantly ($p > 0.05$) correlated with all sperm parameters in our assessments. It was indicated that the FSH and LH levels have inverse/negative correlation to sperm concentration (22). Low levels of seminal plasma testosterone are associated with defects in sperm morphology (23). It has been demonstrated that transferrin, as a specific marker to assess the function of Sertoli cells and spermatogenic status of seminiferous tubule, is more sensitive than other parameters such as FSH and testosterone (19). Therefore, the nature of seminiferous tubule dysfunction can be precisely defined by examining seminal fluid transferrin in combination with other biological values usually used to explore testicular function

(24).

Serum and seminal plasma levels of gonadotropins were significantly elevated in azoospermic patients compared with two other groups of patients in this study. Conversely to another study (9), it was mentioned that the seminal FSH and LH levels were significantly lower in group azoospermia than in group oligozoospermia. Furthermore, within asthenozoospermic patients of the present study, levels of seminal FSH have positive and highly significant correlation with sperm concentration, sperm motility, sperm progressive grade activity and sperm morphology. It was indicated that no major differences have been identified in physical or hormonal characteristics or pharmacokinetics and pharmacodynamics between azoospermia and oligozoospermia subjects (16). It was reported that the elevation and/or sharp fluctuation of FSH and/or LH have direct impact on certain sperm parameters (5). It was suggested that the loss of germinal epithelium causes lower sperm concentration and motility and higher sperm abnormalities (6). In accordance with Vasquez *et al* (4), we observed positive and highly significant correlations between seminal LH level and sperm concentration, total progressive motile spermatozoa/ ejaculate and percentage of sperm agglutination. In normozoospermic men, significant correlation was found only between LH concentration and sperm motility. However, in males with abnormal SFA, LH concentration had significant positive correlation with sperm motility only (7).

Conclusion

It can be concluded from the present study that there is a strong association and interactive effect between seminal FSH and serum FSH and parameters of SFA in men with either normal or abnormal SFA. These findings may call for large cohorts with a larger population of patients that is required to make a more accurate analysis.

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