

Relationship of FSH, LH, DHEA and Testosterone Levels in Serum with Sperm Function Parameters in Infertile Men

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Abstract

Background: Treatment of infertility related hormonal dysfunction in men requires an understanding of the hormonal basis of spermatogenesis. The best method for accurately determining male androgenization status remains elusive.

Objectives: This study is designed to study levels of follicle-stimulating hormone (FSH), Luteinizing hormone (LH), Dehydroepiandrosterone acetate (DHEA), Testosterone (T) in blood serum, and their relation to seminal fluid functional parameters in different groups of infertile patients.

Subjects, Materials and Methods: Blood and semen samples were collected from 44 subjects, 34 were infertile patients (12 Asthenozoospermic, 10 Oligozoospermic, 12 Azoospermic) and 10 Normozoospermic subjects as fertile (control group). Hormones levels were measured by using Enzyme Linked Immunosorbent Assay (ELISA).

Results: There was a highly significant difference ($P < 0.001$) in the levels of FSH and LH between fertile and infertile men subgroups. There was a significant increase ($P < 0.05$) in the levels of DHEA and Testosterone in comparison between fertile men and infertile men subgroups. Significant correlations between the reproductive hormones levels in serum (FSH, LH, DHEA, and Testosterone) and main seminal fluid parameters as sperm concentration, sperm motility and morphologically normal sperm.

Conclusion: The determination of serum FSH, LH, DHEA and Testosterone levels are beneficial in determining the type of infertility.

Keywords: FSH, LH, DHEA, Testosterone, Infertility.

INTRODUCTION:

Infertility, defined as the inability to achieve pregnancy after 12 months of unprotected intercourse (1). Male infertility is found in 50% of infertile couples (2). When reviewed, 55% of the reasons for infertility are found to be male-related and 35% to be female-related, while 10% constitutes infertility of unknown origin (3). The etiology of declining male fertility can be related to falling androgen levels, decreased sexual activity, alterations in sperm motility and morphology, and deterioration in sperm quality and DNA integrity (4). Gonadotropin releasing hormone (GnRH) secreted by the hypothalamus elicits the release of gonadotropins i.e. follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland (5). LH hormone is glycoprotein regulates the Testosterone synthesis by the extratubular Leydig cells. The other gonadotropic hormone, FSH controls spermiocytogenesis and spermiogenesis by affecting both the germinal epithelium and Sertoli cells (6). The levels of these hormones are under negative feedback control by the gonada (7). Dehydroepiandrosterone acetate (DHEA) is a precursor sex steroid hormone synthesized from cholesterol in the zona reticularis of the adrenal cortex, the gonads, adipose tissue, brain, and skin (8). The physiological importance and mechanisms of action of these steroid precursors are only partially understood. In fact, DHEA do not possess intrinsic androgenic or estrogenic activity. Some authors have suggested a receptor mediated pathway for explaining the activity of DHEA in the immune and central nervous systems but a specific DHEA receptor has

not yet been described (9). Testosterone is responsible for normal growth, development of male sex organs, and maintenance of secondary sex characteristics. A high intratesticular levels of Testosterone is an absolute prerequisite for sperm production, and function. Testosterone improve sperm motility & epididymis function (10). Failure of pituitary gland to secrete FSH and LH will result in disruption of testicular function leading to infertility (11). Semen is an organic fluid that may contain spermatozoa. It is secreted by the gonads (sexual glands) and other sexual organs of male, and can fertilize female ova. In humans, semen contains several components besides spermatozoa: proteolytic and other enzymes as well as fructose which are the elements of seminal fluid that promote the survival of spermatozoa, and provide a medium through which they can move or "swim" (12). Male infertility can be assessed through semen analysis and hormonal profile (13). The aim of this study is to explore the relation of Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), Dehydroepiandrosterone acetate (DHEA), and Testosterone (T) levels in serum to seminal fluid functional parameters in different groups of infertile patients.

SUBJECTS, MATERIALS AND METHODS:

This prospective case control study was carried out in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University during the period from November 2016 to May 2017. It included collection of serum of 44 patients attended the male

infertility outpatient clinic in the institute. The total number of infertile couples enrolled in this study was 34 as study group (GA) which classified into three subgroups as: (Azoospermia (GA1=12), Oligozoospermia (GA2=10), and Asthenozoospermia (GA3=12)) . Ten healthy fertile men were considered as control group (GB=10). The collected sera was stored at -36°C until use.

Assessment of seminal fluid functional parameters

Concentration:

Sperm concentration was measured from the mean number of sperm in five high power fields under magnification of 400 X. This number was multiplied by a factor of one million. The total sperm count were obtained by multiplying the sperm concentration with a sample volume. Sperm concentration (million/ml) = number of sperm/HPF $\times 10^6$.

Total sperm count (million/ejaculate) = sperm concentration \times volume.

Sperm concentration is considered normal if equal or more than 15×10^6 sperm/ml according to (WHO,2010)⁽¹⁴⁾.

Sperm Motility:

The prepared slides were examined for the determination of sperm motility. It was examined immediately in order to prevent the effect of the heat of the microscope light source. The number of motile sperm in five randomly selected fields away from the cover slip edge was counted. At least one hundred spermatozoa were counted. One hundred spermatozoa on a plain slide were examined, the number of progressively motile and immotile sperm were documented. Sperm were classified in 3 categories ⁽¹⁵⁾, **Non-motile:** these sperm were not moving, **Non-progressive motile:** sperm that were moving but not going anywhere just wiggling, and **Progressive motile:** sperm were moving and actually getting somewhere.

Sperm Morphology: The examination of morphologically normal sperm was performed by using the same prepared slides for sperm motility. At least 100 spermatozoa were counted. The percentage of morphologically normal sperm were calculated by using the following formula (15).

$$\text{Morphologically Normal Sperm\%} = \frac{\text{No. normal sperms}}{\text{Total No. sperms(normal and abnormal)}} \times 100$$

Blood Collection and Samples Preparation : Venous blood samples were taken from the cubital vein. The samples were left for 30 min. at room temperature, then centrifuged at 3000rpm for 15 minutes at room temperature. The sera were transported to small Eppendorf tubes and stored at - 36°C.

Hormonal Assay: The hormonal levels in serum of FSH (IU/L), LH (IU/L), DHEA (ng/ml) and Testosterone (ng/ml) were measured by using ELISA(enzyme-linked immunosorbent assay).

Statistical Analysis: The Statistical Analysis System- SAS (2016) program was used to measure the effect of different factors in study parameters. Least significant difference – LSD test (ANOVA) was used to significantly compare between means. A p-value <0.05 was considered statistically significant. The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) (16).

RESULTS:

Comparison of hormones between subgroup: The comparison of FSH, LH, DHEA and Testosterone levels in the serum between normozoospermic fertile men and infertile men subgroups: oligozoospermic, asthenozoospermic and azoospermic men are shown in table (1).

A-Comparison of serum FSH levels between fertile men group (normozoospermic) and infertile men subgroups:

The mean and standard error of FSH in azoospermic men (30.47 ± 9.70), were significantly ($p < 0.001$) higher than that of oligozoospermic men (18.00 ± 7.51), asthenozoospermic men (6.96 ± 1.46), and normozoospermic fertile men (4.92 ± 0.51), as shown in table (1), figure (1).

B-Comparison of serum LH levels between fertile men group (normozoospermic) and infertile men subgroups:

The mean and standard error of LH in azoospermic men were (14.35 ± 4.63), which was significantly ($p < 0.001$) higher than that of oligozoospermic men (9.05 ± 2.52), asthenozoospermic men (6.00 ± 1.04), and normozoospermic fertile men (7.72 ± 1.29), as shown in table (1), figure (2).

Table 1: Comparison of serum FSH, LH, DHEA and Testosterone levels between fertile men group(normozoospermic) and infertile men subgroups.

The group	Mean \pm SE			
	FSH	LH	DHEA	Testosterone
Normo.	4.92 ± 0.51 c	7.72 ± 1.29 b	4.67 ± 0.52 b	2.73 ± 0.36 ab
Azoo.	30.47 ± 9.70 a	14.35 ± 4.63 a	6.80 ± 1.80 a	2.40 ± 0.41 b
Oligo.	18.00 ± 7.51 b	9.05 ± 2.52 b	7.26 ± 2.35 a	3.03 ± 0.57 a
Astheno	6.96 ± 1.46 c	6.00 ± 1.04 b	6.17 ± 0.85 a	2.77 ± 0.50 ab
LSD value	8.943 **	4.012 **	1.273 *	0.549 *
P-value	0.0021	0.0037	0.0463	0.0318

* (P<0.05), ** (P<0.001).
Means having different letters in same column differ significantly. M \pm SE = Mean \pm Standard Error

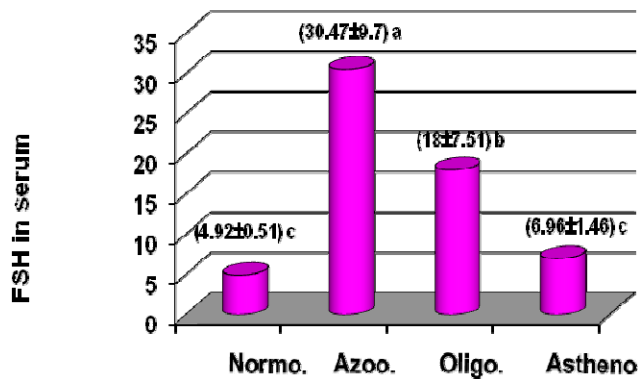


Figure (1): Comparison of serum FSH levels between fertile men group (normozoospermic) and infertile men subgroups

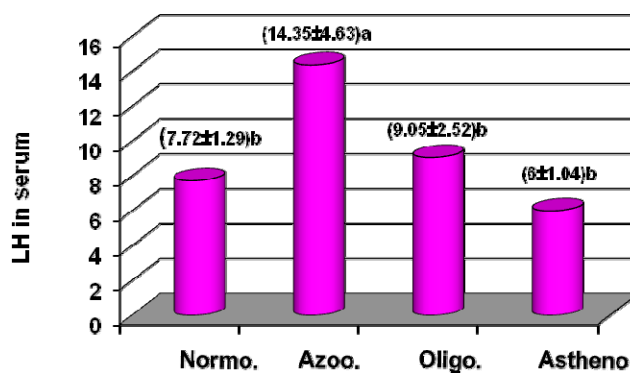


Figure (2): Comparison of serum LH levels between fertile men group (normozoospermic) and infertile men subgroups

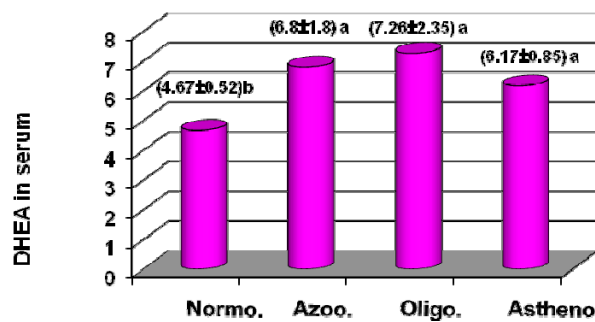


Figure (3): Comparison of serum DHEA levels between fertile men group (normozoospermic) and infertile men subgroups.

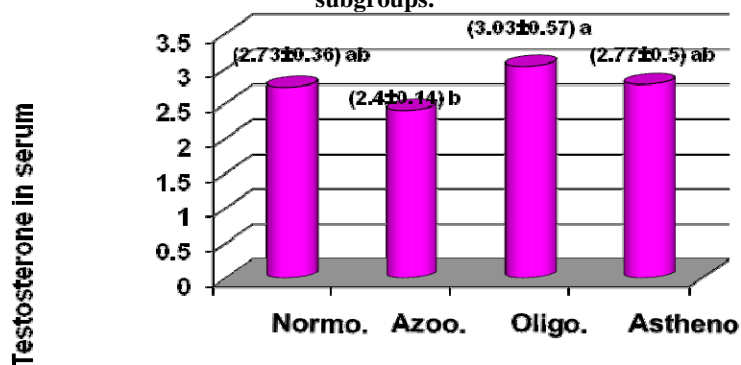


Figure (4): Comparison of serum Testosterone levels between fertile men group (normozoospermic) and infertile men subgroups.

Table (2): Comparison of hormones between fertile and infertile (main group).

The group	Mean \pm SE of hormones			
	Testosterone	DHEA	LH	FSH
Fertile men	2.73 \pm 0.36	4.67 \pm 0.52	7.72 \pm 1.29	4.92 \pm 0.51
Infertile men	2.71 \pm 0.28	6.71 \pm 0.96	9.85 \pm 1.88	18.50 \pm 4.33
T-Test value	0.531 NS	1.855 *	3.924 NS	5.659 *

* (P<0.05), NS: Non-Significant

Table (3): Correlations between hormones in serum seminal fluid functional parameters

Hormones in serum	Correlation coefficient-r		
	Concentration	Motility	MNS
Testosterone	0.04 NS	0.28 *	0.28 *
DHEA	0.08 NS	-0.03 NS	0.33 *
LH	-0.21 NS	-0.29 *	0.02 NS
FSH	-0.29 *	-0.34 *	0.08 NS

* (P<0.05), NS: Non-Significant.

C-Comparison of serum DHEA levels between fertile men group (normozoospermic) and infertile men subgroups: The mean and standard error of DHEA in oligozoospermic men (7.26 \pm 2.35) was significantly (P<0.05) higher than that of azoospermic men (6.80 \pm 1.80), significantly (P<0.05) higher than that of asthenozoospermic men (6.17 \pm 0.85), and was significantly (P<0.05) higher than that of normozoospermic fertile men (4.67 \pm 0.52), as shown in table (1), figure (3).

D-Comparison of serum Testosterone levels between fertile men group (normozoospermic) and infertile men subgroups: The mean and standard error of Testosterone in oligozoospermic men (3.03 \pm 0.57) were significantly (P<0.05) higher than that of normozoospermic fertile men (2.73 \pm 0.36), asthenozoospermic men (2.77 \pm 0.50), and azoospermic men (2.40 \pm 0.41), as shown in table (1), figure (4).

Comparison of hormones between fertile and infertile group (main group):

The mean and standard error of Testosterone in fertile men (2.73 \pm 0.36) was not significant (p>0.05) in comparison with Testosterone in infertile men (2.71 \pm 0.28) as shown in table (2). The mean and standard error of DHEA in infertile group (6.71 \pm 0.96) was significantly (P<0.05) higher than that of DHEA of fertile men (4.67 \pm 0.52), as shown in table (2). The mean and standard error of LH in infertile groups men (9.85 \pm 1.88) was not significant (p>0.05) in comparison with those of LH in fertile men (7.72 \pm 1.29), as shown in table (2). The mean and standard error of FSH in infertile men (18.50 \pm 4.33) was significantly (P<0.05) higher than that of FSH in fertile men (4.92 \pm 0.51) as shown in table (2).

Correlations between hormones in serum and seminal fluid functional parameters:- The FSH levels showed significant negative correlation (p <0.05) (r=-0.29) with sperm concentration. Testosterone, DHEA assays was not significantly correlated with concentration (p>0.05) (r=0.04), (p>0.05) (r=0.08) respectively. LH assay showed no significant negative correlation (p>0.05) (r=-0.21) with sperm concentration as shown in table (3). Regarding motility, Testosterone assay revealed significant (p <0.05) positive correlation (r=0.28), DHEA assay exposed non significant negative correlation (p>0.05) (r=-0.03). Both serum LH assay, and serum FSH assay revealed significant negative correlations (p <0.05) (r=-0.29), (p <0.05) (r=-0.34) respectively, as shown in table (3). With regard to morphologically normal sperm, serum Testosterone, and serum DHEA assays showed significant positive correlations (p >0.05) (r=0.28), (p >0.05) (r=0.33) respectively. Serum LH assay, and serum FSH assays were not significantly correlated to morphologically normal sperm (p>0.05) (r=0.02), (p>0.05) (r=0.08, respectively), as shown in table (3).

DISCUSSION:

1-Comparison of hormones between subgroups:

It is extremely important in the evaluation of male infertility to consider the reproductive hormone levels. It was reported that these hormones have a major role in male spermatogenesis (17). It has been reported that the relationship between hormone concentration and parameters of testicular functions are quite variable, and abnormal spermatogenesis sometimes occur concurrently with endocrine abnormalities (18). FSH, LH and Testosterone are prime regulators of germ cell development. The quantitative production of spermatozoa generally requires the presence of FSH, LH and Testosterone. FSH acts directly on the seminiferous tubules whereas luteinizing hormone stimulates spermatogenesis indirectly via Testosterone (19).

A- Comparison of serum FSH levels between fertile men group (normozoospermic) and infertile men subgroups:

In the present study, elevated mean serum levels of FSH were observed in (azoospermia ,oligazoospermia), when compared with levels in control group (normozoospermic men) as shown in table (1) , figure (1).These results were in agreement with De Kretser *et al.* and Babu *et al.* who found higher concentration of serum FSH with increasing severity of seminiferous epithelial destruction. (20, 21) Subhan *et al.* proved that increase in FSH levels, in azoospermia, may reflect decreased testicular activity resulting in an alteration of the normal feedback mechanism between the testes and the hypothalamic pituitary axis, through an impairment of Sertoli cells, and decreased inhibin secretion (22). Mann *et al.* found that tubular damage accompanied with a rise in serum FSH (23). Yanam *et al.* found in infertile males with abnormal histopathology (Sertoli cell only syndrome, hypo spermatogenesis, and spermatid arrest), the mean FSH levels were significantly elevated compared to the control group (24).

B-Comparison of serum LH levels between fertile men group (normozoospermic) and infertile men subgroups:

In the present study, elevated mean serum levels of LH were observed in (azoospermia ,oligazoospermia), when compared with the levels in control group (normozoospermic men) ,as shown in table (1), figure (2).These results were in agreement with Turek *et al.* and Reyes *et al.* who found the increase in serum levels of gonadotropins (FSH and LH) might have disrupted the spermatogenic process leading to decline in sperm count and infertility (25,26). The primary role of LH in the male is to stimulate the production of Testosterone by the Leydig cells (27). Our findings were disagreed with R. Dale *et al.* who found that most infertile men with seminiferous tubule abnormalities have no detectable endocrinopathy and have normal serum LH, FSH and Testosterone levels. (28).

C-Comparison of DHEA levels in the serum between fertile men group (normozoospermic) and infertile men subgroups:

In the present study, elevated mean serum levels of DHEA were observed in (azoospermia ,oligazoospermia ,asthenozoospermia), when compared with their levels in control group (normozoospermic men) as shown in table (1), figure (3). These results were in agreement with Charlton *et al.* who found high chromatin damage in men who have impaired or low androgen status. DHEA and its sulfate ester (DHEA-S) are the most abundant steroid hormones in humans (29).

D-Comparison of serum Testosterone levels between fertile men group (normozoospermic) and infertile men subgroups:

mean serum Testosterone levels in males with (azoospermia ,oligazoospermia)and their levels in control group (normozoospermic men) were within permissible levels as shown in table (1) and figure (4). These results were in agreement with Smith *et al.* and Nistal *et al.*, who

found normal levels of Testosterone in infertile men with Sertoli cell syndrome when compared with control group.(30,31) Our findings were disagreed with Mohammad Shoaib *et al.* who found significant decrease in the Testosterone levels in azoospermic and oligozoospermic males when compared with fertile controls, the increase in the levels of Testosterone might have disrupted the spermatogenic process leading to decline in sperm count and infertility(32).

2- Comparison of hormones levels between fertile and infertile group (main group):

In the present study, significant ($p < 0.05$) elevation in the levels of (FSH and LH) in serum within infertile men compared to fertile controls men as shown in table (2). These results were in agreement with Samal *et al.* who found higher concentration of serum FSH and LH levels are to stimulate the Sertoli and Leydig cells for proportionate synthesis and secretion of Testosterone thereby enhancing spermatogenesis. With advancement of age, decline in Testosterone and inflation in gonadotropins are associated with a decrease in sperm production and number of normal sperm(33). In the present study, a significant ($p < 0.05$) elevation in the levels of DHEA in serum of infertile men when compared to fertile controls men as shown in table (2). These results were in agreement with Charlton *et al.* who found high chromatin damage in male factor infertility in men who have impaired or low androgen status.

3- Correlations between hormones levels in serum and seminal fluid functional parameters

Regarding concentration, Testosterone- was not significantly correlated , LH assay showed no significant negative correlations and FSH assay showed significant negative correlations. Matzkin *et al.* found serum hormones levels in oligozoospermic and normozoospermic men showed increase in FSH levels, with no significant change in LH and Testosterone levels. A significant, but inverse, correlation between FSH levels and sperm concentration.(34) DHEA assay was not significantly correlated, but there was no previous study for comparison. With regard to sperm motility, Testosterone showed significant positive correlations and FSH revealed significantly negative correlations. Sherwood *et al.* found patients with abnormalities in both semen and hormonal levels showed an elevation in serum FSH and a reduction in serum Testosterone with decline in sperm concentration & active sperm motility(35). DHEA assay showed significantly negative correlation ($p < 0.05$) with motility. Regarding morphologically normal sperm, DHEA assay exposed significant positive correlation ($p < 0.05$) but there was no previous study for comparison. John *et al.* suggested that the relation of testosterone with semen parameters (increased sperm motility) and relation between LH and semen parameters (decreased sperm concentration, motility and morphology) are related to disruptions and compensatory mechanisms in the hypothalamo-pituitary-gonadal axis(36).

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