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Original Research Article

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

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Abstract

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. This study is designed to study levels of follicle-stimulating hormone (FSH), Luteinizing hormone (LH), Dehydroepiandrosterone (DHEA) and Testosterone (T) in seminal plasma and microscopic examination of seminal fluid have been measured, in different groups of infertile patients. Semen samples were collected from the infertile and fertile subjects. The samples will be collected from (44) subjects (34) as infertile patients (12 Asthenozoospermic, 10 Oligozoospermic, and 12 Azoospermic) and 10 Normozoospermic subjects as fertile (control group). Then the hormones levels were measured by using Enzyme Linked Immunosorbent Assay (ELISA). The results of hormones levels in seminal plasma showed that there was a highly significant increase ($p<0.001$) in the levels of FSH and LH by comparison between fertile and infertile men subgroups. Hormones levels showed that there was a significant increase ($p<0.05$) in the levels of DHEA and Testosterone in comparison between fertile men and infertile men subgroups. The Significant correlations between the reproductive hormones (FSH, LH, DHEA, Testosterone) in seminal plasma and main seminal fluid parameters were found with sperm concentration, sperm motility and morphologically normal sperm in fertile and in the infertile group as a whole. From the results obtained, it can be concluded that: the determination of hormones (FSH, LH, DHEA, and Testosterone) of infertile men are of great importance to determine the degree of infertility.

Keywords: Seminal plasma FSH, Seminal plasma LH, Seminal plasma Testosterone, Seminal plasma DHEA and male infertility

INTRODUCTION

Infertility, defined as the inability to achieve pregnancy after 12 months of unprotected intercourse (Agarwal et al., 2016). Male infertility is found in 50% of infertile couples (Cooper et al., 2010). 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 (Speroff and Fritz, 2010). The etiology of declining male fertility is related to falling androgen levels, decreased sexual activity, alterations in sperm motility and morphology, and deterioration in sperm quality and DNA integrity (Sartorius and Nieschlag, 2010). Gonadotropins 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 (De Krester, 1979). LH hormone is glycoprotein regulates the Testosterone synthesis of the extratubular Leydig cells. The other gonadotropic hormone, FSH controls spermiocytogenesis and spermiogenesis by affecting both the germinal epithelium and Sertoli cells (Amelar,

1966). The levels of these hormones are under negative feedback control by the gonada (Jarow, 2003). Dehydroepiandrosterone (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 (Orentreich et al., 1984). The physiological importance and mechanisms of action of these precursor steroids 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 (Svec and Porter, 1998). Testosterone is responsible for normal growth, development of male sex organs, and maintenance of secondary sex characteristics. A high intratesticular level of Testosterone is an absolute prerequisite for sperm production, and function. Testosterone improves sperm motility & epididymis function (Gray et al., 2005). The failure of pituitary to secrete FSH and LH will result in disruption of testicular function leading to infertility (Weinbauer and Nieschlag, 1995). 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 human, semen contains several components besides spermatozoa: proteolytic and other enzymes as well as fructose which are the elements of seminal fluid which promote the survival of spermatozoa, and provide a medium through which they can move or “swim” (Ali et al., 2015). Male infertility can be assessed through semen analysis and reproductive hormonal profile (Jungwirth et al., 2012).

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 seminal fluid 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), Asthenozoospermia (GA3=12)) . Ten healthy fertile men were considered as control group (GB=10). The seminal plasma was separated by centrifugation of semen at 3000 rpm for 15 minutes and stored at -36° C until hormonal analysis.

Assessment of seminal fluid functional parameters

Sperm 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 × volume.

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

Assessment of Sperm Motility

The prepared slide was 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 on the result. 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 and the number of progressively motile and immotile sperm was documented, then sperm were classified in 3 categories (WHO, 1999).

a) Non-motile: these sperm were not moving.

b) Non-progressive motile: sperm that were moving but not going anywhere; they are just wiggling.

c) Progressive motile: sperm were moving and actually getting somewhere.

Assessment of 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 using the following formula (WHO, 1999).

Morphologically Normal Sperm % =

$$\frac{\text{No. normal sperms}}{\text{Total no. sperms (normal and abnormal)}} \times 100$$

Hormonal Assay

Seminal plasma levels 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

Table 1. Comparison of DHEA, Testosterone, LH and FSH in the seminal plasma between fertile men group (normozoospermic) and infertile men subgroups.

The group	Mean ± SE			
	FSH	LH	DHEA	Testosterone
Normo.	2.26 ± 0.37 b	1.39 ± 0.44 c	8.07 ± 0.64 b	1.74 ± 0.17ab
Azoo.	9.04 ± 6.52 a	9.45 ± 5.26 b	7.41 ± 0.80 b	1.78 ± 0.33ab
Oligo.	2.15 ± 0.70 b	14.87±12.24a	11.67 ± 1.90a	1.92 ± 0.25 a
Astheno	0.958 ± 0.48b	1.38 ± 0.26 c	12.32 ± 2.12a	1.32 ± 0.10 b
LSD value	4.155 **	6.837 **	2.318 *	0.562 *
P-value	0.00082	0.0026	0.0394	0.0447

* (P<0.05), ** (P<0.001).
Means having with the different letters in same column differed significantly.

M±SE = Mean ± Standard Error

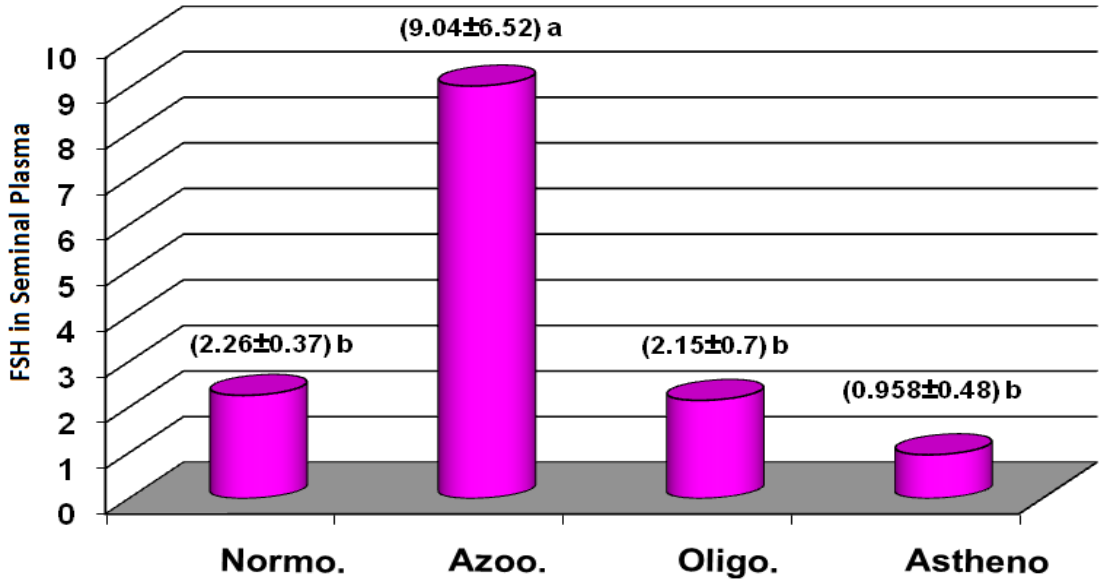

Figure 1. Comparison of FSH in the seminal plasma between fertile men group (normozoospermic) and infertile men subgroups.

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) (Statistical Analysis System, User's Guide, 2016).

This study was ethically approved by the ethics committee in the High Institute for Infertility Diagnosis and ART's, Al-Nahrain University.

RESULTS

Comparison of hormones in seminal plasma between subgroups in this study

The comparison of FSH, LH, DHEA and Testosterone, in the seminal plasma between normozoospermic fertile men and infertile men subgroups: oligozoospermic, asthenozoospermic and azoospermic men can be shown in Table(1).

Comparison of FSH in the seminal plasma between fertile men group (normozoospermic) and infertile men subgroups

The mean and standard error of FSH in azoospermic men (9.04 ± 6.52), was significantly (p<0.001) higher than that of normozoospermic fertile men (2.26 ± 0.37), oligozoospermic men (2.15 ± 0.70), and asthenozoospermic men (0.958 ± 0.48), as shown in Table (1) and Figure (1).

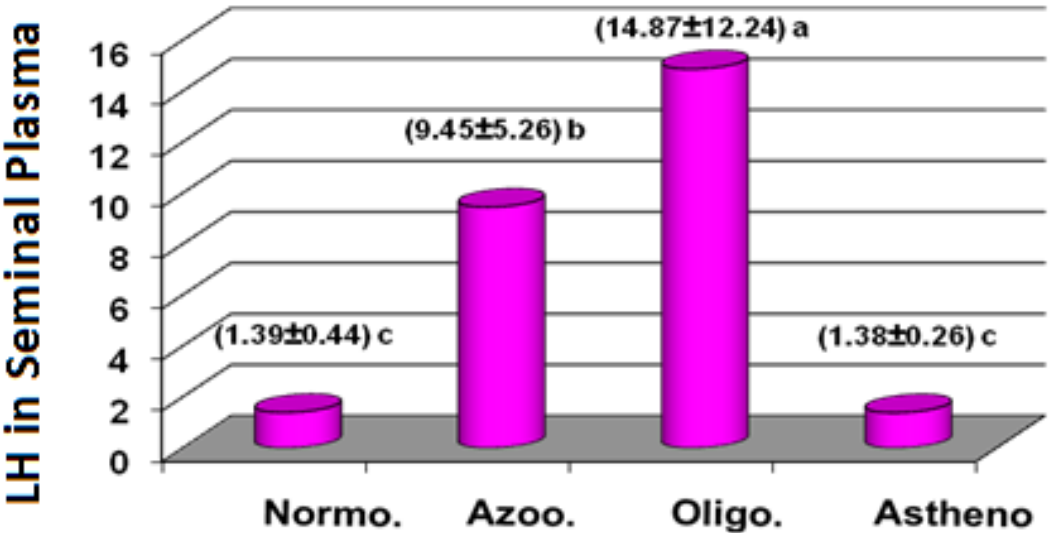

Figure 2. Comparison of LH in the seminal plasma between fertile men group (normozoospermic) and infertile men subgroups

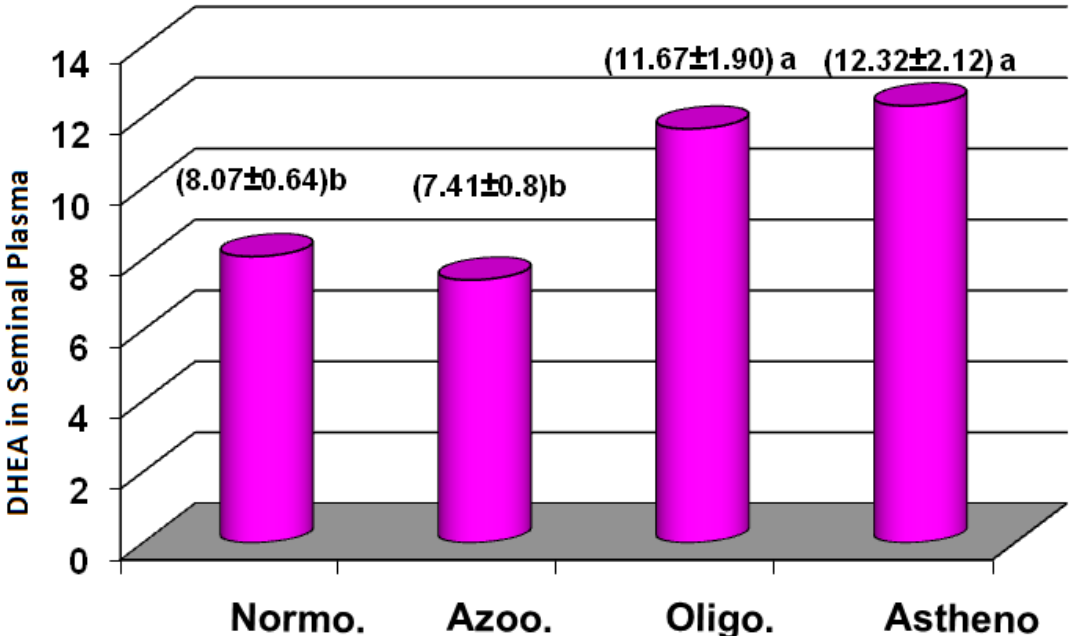

Figure 3. Comparison of DHEA in the seminal plasma between fertile men group (normozoospermic) and infertile men subgroups.

Comparison of LH in the seminal plasma between fertile men group (normozoospermic) and infertile men subgroups

The mean and standard error of LH in oligozoospermic men (14.87 ± 12.24), was significantly ($p<0.001$) higher than that of azoospermic men (9.45 ± 5.26), normozoospermic fertile men (1.39 ± 0.44), and asthenozoospermic men (1.38 ± 0.26), as shown in Table (1) and Figure (2).

Comparison of DHEA in the seminal plasma between fertile men group (normozoospermic) and infertile men subgroups

The mean and standard error of DHEA in asthenozoospermic men (12.32 ± 2.12) and oligozoospermic men (11.67 ± 1.90) were significantly ($p<0.05$) higher than that of normozoospermic fertile men (8.07 ± 0.64), and azoospermic men (7.41 ± 0.80), as shown in Table (1) and Figure (3).

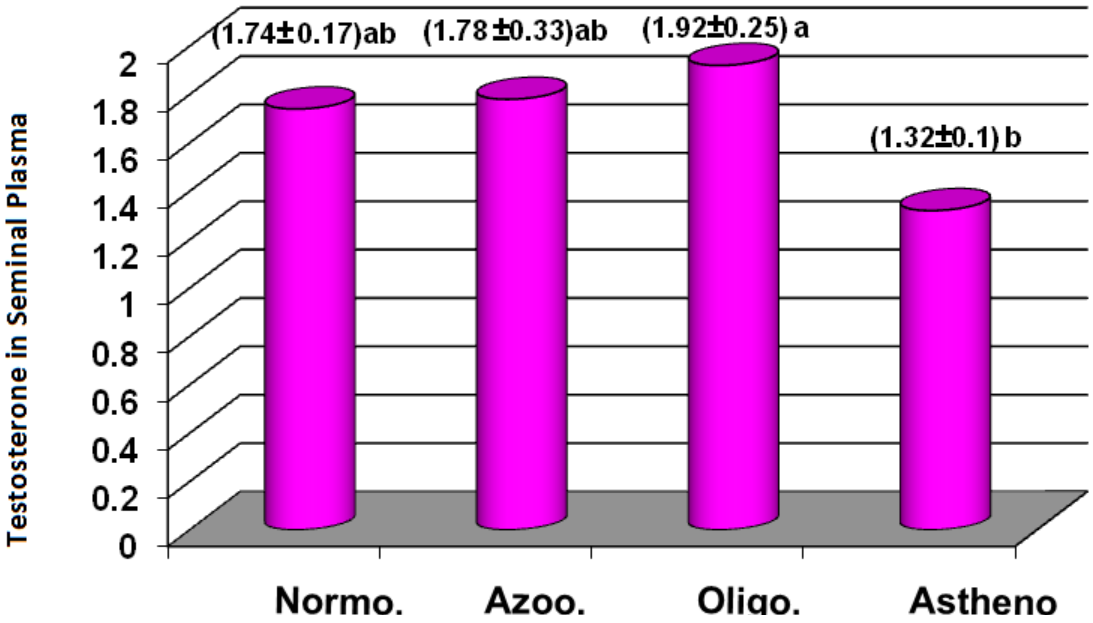

Figure 4. Comparison of Testosterone in seminal plasma between fertile men group (normozoospermic) and infertile men subgroups

Table 2. Comparison of hormones levels in seminal plasma between fertile and infertile groups

The group	Mean ± SE of hormones			
	Testosterone	DHEA	LH	FSH
Fertility	1.74 ± 0.17	8.07 ± 0.64	1.39 ± 0.44	2.26 ± 0.37
Infertile	1.66 ± 0.14	10.39 ± 1.02	8.19 ± 4.02	4.16 ± 2.33
T-Test value	0.438 NS	3.072 NS	3.975 *	2.169 NS
* (P<0.05), NS: Non-Significant.				

Comparison of Testosterone in seminal plasma between fertile men group (normozoospermic) and infertile men subgroups

The mean and standard error of Testosterone in oligozoospermic men (1.92 ± 0.25) was significantly (p<0.05) higher than that of azoospermic men (1.78 ± 0.33), normozoospermic fertile men (1.74 ± 0.17), and of asthenozoospermic men (1.32 ± 0.10), as shown in Table (1) and Figure (4).

Comparison of hormones in seminal plasma between fertile and infertile groups (main groups)

The comparison between fertile and infertile groups (main groups) in seminal plasma was interpreted and included the followings: Testosterone, DHEA, FSH, LH hormones. The mean and standard error of Testosterone in fertile men (1.74 ± 0.17) was not significant (p>0.05) in comparison with Testosterone in infertile groups men (1.66 ± 0.14) as shown in Table (2). The mean and standard error of DHEA in infertile group (10.39 ± 1.02) was not significantly differ in comparison with DHEA of fertile men (8.07 ± 0.64), as shown in Table (2). The mean and standard error of LH in infertile groups (8.19 ± 4.02) was significantly (p<0.05) higher than LH of fertile men (1.39 ± 0.44) as shown in table (2). The mean and standard error of FSH in infertile groups men (4.16 ± 2.33) was not significantly different in comparison with FSH of fertile men (2.26 ± 0.37), as shown in Table (2).

Correlations between hormones levels in seminal plasma and main seminal fluid parameters

Correlations were done of hormones levels in seminal plasma with main seminal fluid parameters (table (3)). In concentration, as shown that Testosterone assay showed significantly negative correlation (p<0.05) (r=-0.45), DHEA assay was not significantly correlated(r=0.22), LH assay showed no significant negative correlation (p <0.05) (r=-0.15) and FSH assay showed significantly negative correlation (p <0.05) (r=-0.31) as shown in table (3). Regarding motility, as shown that Testosterone assay was significantly negatively correlated (p >0.05)

Table 3. Correlations between hormones in seminal plasma and main seminal fluid parameters

Hormones in plasma	Correlation coefficient-r		
	Concentration	Motility	MNS
Testosterone	-0.45 *	-0.33 *	-0.31 *
DHEA	0.22 NS	0.31 *	0.14 NS
LH	-0.15 NS	-0.15 NS	-0.19 NS
FSH	-0.31 *	0.30 *	-0.20 NS

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

(r=-0.33), DHEA assay showed significant positive correlation (p <0.05) (r=0.31), with LH assay it showed no significant negative correlation (p <0.05) (r=-0.15), and FSH assay showed significantly positive correlation (p <0.05) (r=0.30), as shown in table (3).Concerning morphologically normal sperm, as shown that Testosterone assay showed significant negative correlation (p >0.05) (r=-0.31),DHEA assay showed not significant positive correlation (r=0.14), LH assay showed not significant negative correlation (p <0.05) (r=-0.19), and FSH assay showed not significant negative correlation (r=-0.20), as shown in Table (3).

DISCUSSION

Comparison of hormones in seminal plasma between subgroups in this study

Comparison of FSH in the seminal plasma between fertile men group (normozoospermic) and infertile men subgroups

In the present study, elevated mean seminal plasma levels of FSH were observed in the male patients group (azoospermic men) when compared with the levels in control group (normozoospermic men) as shown in table (1) and figure (1). These results are in agreement with Fakhrildin *et al.* who found that the elevation and/or sharp fluctuation of FSH and/or LH have direct impact on certain sperm parameters (Fakhrildin, 2006). Hussain ZA, who found in infertile Iraqi men that elevated FSH is a reliable indicator for germinal epithelial damage, decreased Sertoli cells function and azoospermia (Hussain, 2008). Steinberger *et al.* found that high levels of FSH could be explained by the following hypotheses:

1. Germ cell cooperation is necessary for inhibin production.

2. Sertoli cell damage co-exists with germ cell damage.

3. Leydig cells are unable to provide enough Testosterone for optimal Sertoli function (Steinberger and Steinberger, 1976).

Comparison of LH in the seminal plasma between fertile men group (normozoospermic) and infertile men subgroups

In the present study, elevated mean seminal plasma levels of LH were observed in the male patients group (azoospermia ,oligozoospermia), when compared with the levels in control group (normozoospermic men) as shown in Table (1) and Figure (2). These results are in agreement with Beyler *et al* who found higher concentration of luteinizing hormone and Testosterone in seminal plasma in fertile men (Beyler and Zaneveld, 1982), Niknam *et al.* who found sperm chromatin damage has a significant positive relationship to LH, FSH in all infertile men (Niknam et al., 2012).

Comparison of DHEA in the seminal plasma between fertile men group (normozoospermic) and infertile men subgroups

In the present study, decrease mean seminal plasma of DHEA were observed in the male patients group (azoospermia), when compared with the levels in oligozoospermia, asthenozoospermic and normozoospermic men as shown in Table (1) and Figure (3). These results are in agreement with Thualfeqar *et al*., who found the lack of effects of the pre-hormone DHEA on the sperm production as well as testes weight can be attributed partially to a relatively weak androgen effect of this substance (Thualfeqar et al., 2012). Our findings are disagreed with Niknam *et al.* who found sperm chromatin damage has a non significant positive relationship to DHEA in all infertile men. However, these relationships were not showed when patients were classified as male factor infertility (Niknam et al., 2012).

Comparison of Testosterone in seminal plasma between fertile men group (normozoospermic) and infertile men subgroups

In the present study, the mean seminal plasma Testosterone levels in both the male patients group (azoospermia, oligozoospermia), and the levels in control group (normozoospermic men) were within acceptable

levels as shown in Table (1) and Figure (4). These results are in agreement with Beyler *et al*., who found higher concentration of the levels of luteinizing hormone and Testosterone in seminal plasma, their possible relationships with the spermiatogenic and/or spermatozoal functions have yield conflicting results (Beyler and Zaneveld, 1982). Bremner *et al*., who found Testosterone deficiency in men is manifested typically by symptoms of hypogonadism, including decreases in erectile function and libido (Bremner et al., 1994).

Comparison of hormones levels in seminal plasma between fertile and infertile groups (main groups)

In the present study, not significance in the levels of FSH in seminal plasma within in infertile men compared to fertile controls men as shown in Table (2). These results are disagreed with Fakhrildin *et al*. who found that the elevation and/or sharp fluctuation of FSH and/or LH have direct impact on certain sperm parameters (Fakhrildin, 2006). In the present study, significant levels of LH in seminal plasma within in infertile men compared to fertile controls men as shown in Table (2). These results are in agreement Niknam *et al.* who found sperm chromatin damage has a significant positive relationship to LH, FSH in all infertile men (Niknam et al., 2012). In the present study, non significance in the levels of DHEA in seminal plasma within in infertile men when compared to fertile controls men as shown in Table (2). Our findings are agreed with Niknam *et al.* who found sperm chromatin damage has a not significant positive relationship to DHEA in all infertile men. However, these relationships were not showed when patients were classified as male factor infertility (Niknam et al., 2012). In the present study, non significance in the levels of Testosterone in seminal plasma within infertile men compared to fertile controls men as shown in Table (2). These results are in agreed with Beyler *et al*., who found higher concentration of levels of luteinizing hormone and Testosterone in seminal plasma and to evaluate their possible relationships with the spermiatogenic and/or spermatozoal functions have yield conflicting results (Beyler and Zaneveld, 1982).

Correlations between hormones in seminal plasma and main seminal fluid parameters

In the present study, correlations were done of hormones levels in seminal plasma and main seminal fluid parameters Table (3). In concentration, Testosterone assay revealed significant negative correlation, in motility, Testosterone assay showed significant negative correlation, in morphologically normal sperm, Testosterone assay revealed significant negative correlation. These results are in agreement with Moreno *et al*, and Chan *et al*. who found dose dependent inhibition of spermatozoal fertilizing capacity by exogenous androgens suggest a preventive role for seminal plasma Testosterone to protect premature spermatozoa capacitation before the spermatozoa reach the site of fertility (Moreno et al., 1980; Chan et al., 1983). In concentration, LH assay revealed no significant negative correlation, and for FSH assay was significant negative correlation. In motility, with LH assay was not significant negative correlation, for FSH assay was significant positive correlation. In morphologically normal sperm, for LH assay was not significant negative correlation, and for FSH assay was not significant negative correlation. These results are in agreement with Wijeratna *et al*., who found these higher levels of these hormones are found in infertile men. The mechanism by which the semen hormones bring about negative effects on sperm will also affect their fertilization ability. High levels of semen hormones could occur due to dysfunction or impairment of the fluid reabsorption in the epididymis. Malabsorption may result in high levels of hormones in the seminal plasma, the high concentrations of which would be detrimental to spermatozoa (Wijeratna et al., 2005). In concentration, DHEA assay was not significant correlations, in motility, as shown that for DHEA assay revealed significantly positive correlation, in morphologically normal sperm, DHEA assay was not significant but positive correlation there was no previous study for comparison. Hier *et al*, found androgens may have a permanent organizing effect on some cognitive abilities because, men with idiopathic form of hypogonadism have markedly impaired spatial ability that does not improve with Testosterone therapy, whereas visuospatial ability in men with acquired hypogonadism is similar to controls (Hier and Crowley, 1982).

CONCLUSION

In conclusion in the present study, in relevance to measured seminal plasma levels of gonadotropins and testosterone in the normozoospermic, asthenozoo-spermic, oligozoospermic and azoospermic groups and their correlations with parameters of seminal fluid analysis. The results suggest that measurements of seminal plasma hormones have predictive value of the fertility potential of the semen samples and it is not doubtful if they may yield additional information to the conventional seminal fluid analysis (SFA).

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