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Appraisal of Correlation of Sex Steroidal Hormones Interleukin 2 and 6 with soluble Fas in Seminal Fluid of infertile men

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***Abstract*:** *Approximately 50% of human infertility is attributable to male defects with the clinical presentation of abnormal sperm production, such as oligospermia, asthenospermia, teratospermia, or azoospermia. Numerous factors have been implicated in spermatogonial development. Several alternative hormonal mechanisms regulate the mitotic and meiotic dynamics of spermatogonia**.** Apoptosis is an important process in the context of germ cells since they undergo both mitosis and meiosis, and this process is affected by interleukins (IL6 and IL2).*

The aims of this study wasto assess the effect of sex steroidal hormones, seminal antiapoptotic factor soluble fibroblast associated surface antigen (sFas) and inflammatory markers Interleukin(IL-2, IL-6), with conventional semen parameters in infertile men.

This study involves One hundred and six male partners of infertile couples; Serum testosterone and estradiol$_2$were measured for all of them.

Semen sample was taken after 2-7 days of abstinence. Conventional semen analysis was done for each sample according to the protocol of (WHO) 2010, after incubation and liquefaction period (30-60 min). Semen plasma was collected for analysis of interleukins (2 and 6) and sFAS by specific kits.Patients with severe oligospermia (below million sperm/ml) were excluded from the study.

The results showed thatEstradiol was significantly higher in azoospermic and oligoasthenoteratospermic male partner of infertile couple. And the ratio of testosterone/estradiol is significantly lower in azoospermic group. A significant higher level of seminal plasma IL-2 and IL-6 in oligo,asthenoteratozoospermic group in comparison to normospermic group.Also there was a significant higher level of seminal plasma IL-2 in oligo,asthenoteratozoospermic group in comparison to azoospermic group.A significant higher expression of seminal plasma sFAS in azoospermic and oligoasthenoteratozoopermic groups in comparison to normospermic group.

From this study it's concluded that Testosterone level and Testosterone/E2 ratio affects male fertility;they were significantly low in azoospermic and oligoasthenoteratospermic,Apoptotic process has great effects on fertility since there was significant higher expression of SPsFAS in azoospermic and oligoasthenoteratozoopermic groups in comparison to normospermic group. On the contrary there was increase in proinflammatory markers IL2 and 6 in the same groups.

***Key words**: Testosterone, seminal plasma,IL2, IL6, s FAS*

I. INTRODUCTION

Infertility is defined as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse[1]

Spermatogenesis is defined as a dynamic and synchronized process of maturation of stem spermatogonia into mature spermatozoa that takes place in the seminiferous tubules of the testis. Cells in the seminiferous tubules of the testis are determined by a dynamic balance between cell proliferation and apoptotic cell death[2].

Spermatogenesis is controlled by testosterone and follicle-stimulating hormone (FSH). Testosterone and its immediate metabolite dihydrotestosterone (DHT) make their roles through the androgen receptor [3]

Tesariket al. in 1998 demonstrated that testosterone potentiates the effects of FSH in spermatogenesis by preventing sertoli cell apoptosis, and hence spermatogenesis will proceed[4].

Abnormal spermatozoa have a lower fertilizing potential, depending on the types of anomalies, Morphological defects have been associated with increased DNA fragmentation[5]and these abnormalities could be; head defects,Neck and midpiece defects,Principal piece defects: short, multiple, broken, smooth hairpin bends, sharply angulated bends, of irregular width, coiled, or any combination of these[6].

Round cells that are seen in seminal fluid are either round spermatids and spermatocytes or inflammatory cells (leukocytes)[7].

Androgens play a vital role in the control of spermatogenesis. The testis is not only the main source of androgens, but it is also a key target for androgen action[8].

Testosterone produced by the Leydig cells is the principal and most important testicular and circulating androgen. Luteinizing hormone (LH) stimulates testosterone biosynthesis by increasing mobilization and transport of cholesterol into the steroidogenic pathway, an action takes place within minutes; as well as by stimulating gene expression and activity of the steroidogenic enzymes (steroidogenic acute regulatory protein and P450), a slower process that requires several hours.[9]

FSH stimulates proliferation and secretory activity of the Sertoli cells, Testosterone stimulates spermatogenesis through receptor-mediated events in the Sertoli cells. [9]

Estrogens play important roles in male reproduction;they are present in the testis (spermatocytes, spermatids, Sertoli cells, Leydig cells, and rete testis), epididymides, prostate, seminal vesicles, and urinary bladder[10].

Over 80% of the 17β-estradiol in the plasma of adult men is formed by extragonadal and extraadrenal aromatization of circulating testosterone and androstenedione by the enzyme aromatase particularly in the adipose tissue. The remainder (20%) comes from the Leydig cells[11].

Estrogens affect male germ cell survival,estrogen receptor function is absolutely required for normal spermatogenesis and its disruption causes alteration of spermatogenesis and infertility[12].

testicular somatic cells and germ cells represent an additional source of estrogens,and the germ cells (both meiotic and post-meiotic cells) produce estrogens and they contain estrogen receptors this explain the role of estrogens in male germ cell development[13],Estrogens can cause alterations in the circulating concentrations of gonadotropins and testosterone and hence affect germ cell apoptosis indirectly [14].

Infertility affects around 1 in 7 couples of reproductive age, often causing substantial psychological distress[15], DNA damage reduces fertility in male ; DNA damage reduces fertility in male sperm, as caused by oxidative DNA damage, including reactive oxygen species, other like fever or high testicular temperature smoking, xenobiotic DNA damaging agents (such as drugs or chemotherapy)[16].

The pathophysiological significance of cytokines in sperm function is still controversial. The seminal plasma contains significant levels of several cytokines which are normally present in the male genital tract. It has been proposed that they are released by germ cells, Leydig cells, Sertoli cells, epididymis and prostate, their expression is modulated during the seminiferous cycle [17].

IL2is one of the major cytokines that exerts numerous immunological effects by stimulating the proliferation and growth of T, B and natural killer (NK) cells. Moreover, almost any cell possessing IL-2R will be stimulated to grow by IL-2 [18].

Interleukin-6 is capable of producing a variety of favorable and unfavorable biological effects to infertility, especially as related to defective sperm function [19]. IL-6 present in significantly higher levels in seminal plasma of infertile and immunoinfertile men compared to those of fertile men, and these levels demonstrated a significant inverse correlation with the sperm number in the ejaculate, the penetration rates, and with some sperm motion parameters. Also IL-6 increases the fertilizing capacity of human sperm by affecting capacitation and/or acrosome reaction[2o]

Apoptosisis a form of cell death in which the cells activate an intracellular death program and kill themselves in a controlled way, It's an essential physiological process that is required for the development and maintenance of tissue homeostasis, it eliminates cells that are useless or potentially dangerous to the host such as aged, infected, injured, or mutated cells, or cells that are produced in excessive amounts, such as germ cells in the testis.Testicular germ cell apoptosis is triggered by an internal clock or by extra-cellular mediators, such as cytokines, hormones, viruses, chemicals or physical factors [21],[22] .

In the human testis, spontaneous germ cell apoptosis involves all three classes of germ cell (spermatogonia), spermatocytes, and spermatids)In adult male germ cell apoptosis occurs only in spermatogonia and round spermatids ;which display the classical morphological and biochemical features of apoptosis, while apoptotic spermatocytes and elongated spermatids shows unusual morphology and DNA configuration [23].Inappropriate male germ cell apoptosis is associated with infertility, cryptorchidism, and testicular torsions [4].

FasL-induced signaling is suggested to play a major role in several types of physiological apoptosis [24].The expression of functional FasL by Sertoli cells accounts for the immune-privileged nature of the testis. [25].

Abortive apoptosis suggested that in some cases of infertility, the normal apoptotic mechanisms have malfunctioned, overridden or have not been completed and Fas positive sperm have failed to be eliminated. Men with abnormal semen had a higher percentage of Fas positive sperm than men with normal semen [26].

Soluble Fas (sFas) may be a marker of overall apoptosis triggering, at the same time regulating apoptosis by competing with the cell surface receptor. Previous reports have suggested that the Fas mediated system is implicated in the elimination of defective spermatozoa from the ejaculate and shows possible irregularities that could account for certain forms of male infertility [27][28]

II. Subjects And Methods

One hundred and six males of infertile couples attending the Infertility Clinic of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies were enrolled in this study. Their age ranged between 18-58 years with a mean of (33.67+8.52) The study involved those who had history of having free unprotected regular intercourse for at least 1 year without history of previous pregnancy or abortion.
A summary of study design for each subject is shown in figure (1).

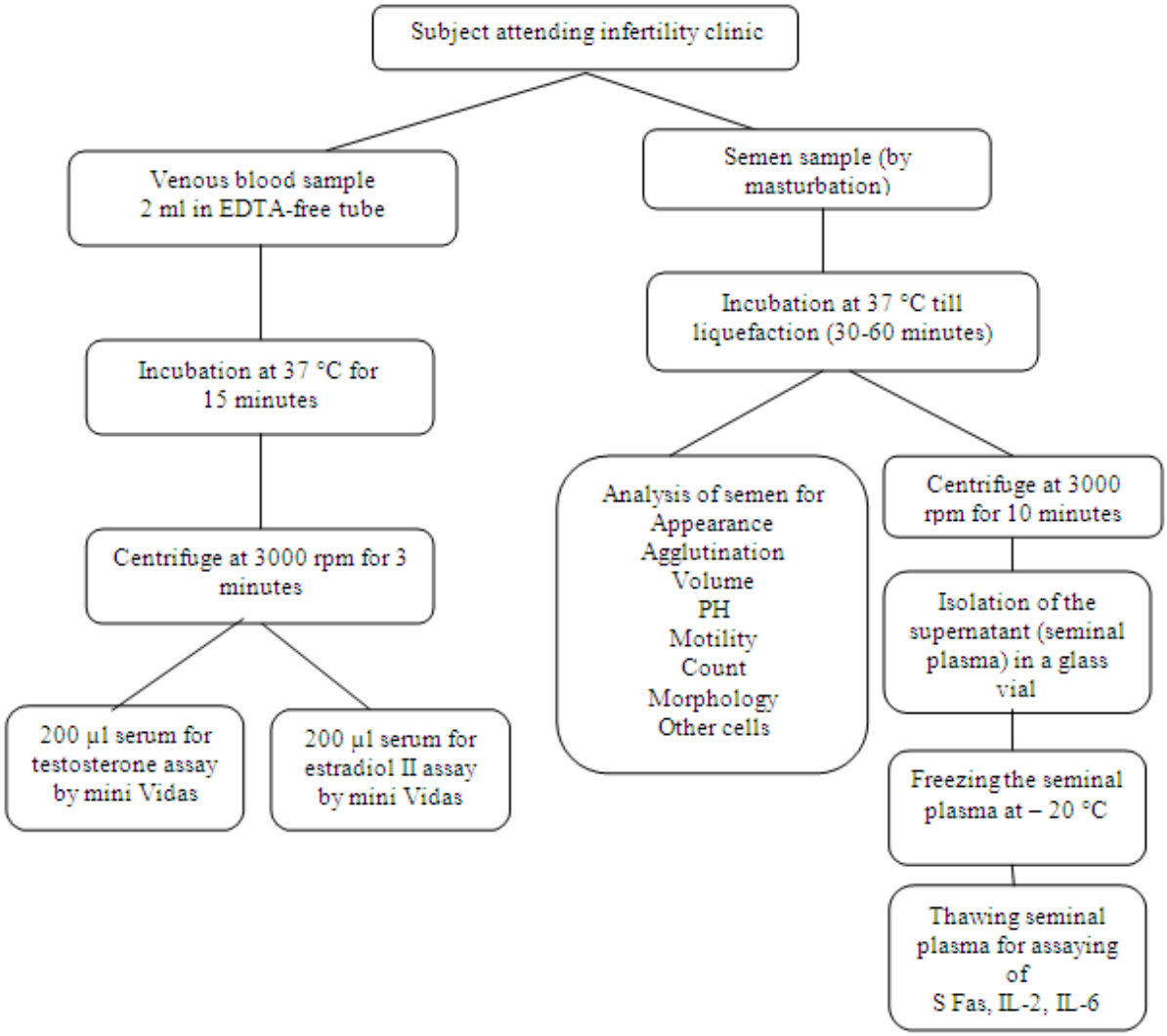

Figure (1): Summary of study design

Semen Analysiswas done according to(WHO, 2010) [29],then the seminal plasma was used for measurement of inflammatory and apoptotic markers.

Hormonal Assay: The serum level of estradiol II hormone was assayed using VIDAS® estradiol II kit (Ref. 30 431, BioMérieux® SA, France) and the serum level of testosterone hormone was assayed using VIDAS testosterone kit® (Ref. 30 418, BioMérieux® SA, France) using he VIDAS instruments for the enzyme immunoassay.

Measurement of seminal plasma (Interleukin 2 and 6 (IL-2, IL-6) and sAPO-1/Fas) using their specific kits Interleukin-2 Human (IL-2) bioAssay™ ELISA kit (USbiological, Catalog no. 17663-28E),(IL-6) bioAssay™ ELISA kit (USbiological, Catalog no. 18428-04). which depend on Enzyme Linked Immunosorbant Assay (ELISA) principle ,and sFas was measured using the kit of IBL International GMBH sAPO-1/Fas ELISA

Enzyme immunoassay for the quantitative determination of human sAPO-1/Fas in human cell culture supernatants, ref. BE51901 (Germany).

Statistical analysis: According to the semen analysis results the subjects were divided into 3 groups: Group 1: normal semen analysis (n=36),Group 2: azoospermic (n=21),Group 3: Oligoasthenoteratozoopermic (n=49)

The data were presented as mean±standard deviation (SD). except for concentrations of IL-2, IL-6 and sAPO/Fas were they presented as range and median (because they were not follow normal distribution curve).

Unpaired t-test was done to show the significance of data followed normal distribution curve, while those not follow normal distribution curve, Mann-Whitney U test was done for them and the p value (probability of chance factor) was calculated for both tests.

Pearson correlation was done and the r (correlation coefficient) was calculated with its p value.A p value less than 0.05 considered significant.

III. Results

The study sample (105 male partners of infertile couples) has been divided according to the results of the semen analysis into three groups based on lower reference limit (5^{th} centile, 95% confidence interval (CI)) of WHO Laboratory Manual for the Examination and Processing of Human Semen 2010. First group (NORM; normozoospermic) involved those whom their semen parameters were more than lower reference limit (n= 36); second group (AZO; azoospermic) involved those who had no sperm at all in their semen and confirmed by two previous semen analysis with same result (n= 21); and the third group (OAT; oligoasthenoteratozoospermic) involved all other cases whom one or more of their semen parameters was below the lower reference limit (n= 48). The 48 semen analysis results of the OAT group included the following categories (15 oligozoospermic, 17 asthenozoospermic, 5 oligoasthenozoospermic, 5 asthenoteratozoospermic, and 6 oligoasthenoteratozoospermic). "Table 3.1" shows the general descriptive data of the three study groups

Table (3.1): Descriptive data of the three study groups

Parameter	NORM(n= 36) Mean±SD	AZO(n= 21) Mean±SD	OAT(n= 48) Mean±SD
Age (yr)	30.36±5.56	32.05±8.33	33.81±7.96
Duration of Marriage (yr)	5.68±3.47	6.2±5.05	6.68±5.14
Body Mass Index (kg/m^2)	27.92±5.49	26.82±4.82	28.9±6.32

NORM= Normozoospermic, AZO= Azoospermic, OAT= Oligoasthenoteratozoospermic

The results of semen analysis of the three groups are displayed in "Table 3.2"; for NORM group, it is clear that all parameters were above lower reference limit mentioned by the WHO, 2010.

Table (3.2): Semen analysis of the three study groups

Parameter	NORM(n= 36) Mean±SD	AZO(n= 21) Mean±SD	OAT(n= 48) Mean±SD
Volume (ml)	2.34±0.72	1.75±0.68	2.07±0.78
Liquefaction duration (min)	41.39±16.06	42.86±15.13	44.06±18.15
pH	8.04±0.17	8.0±0.09	7.98±0.2
Sperm conc. (no./ml)	60.81±24.41	0.0	22.27±23.11
Progressive motile sperm %	45.08±9.29	--	16.35±14.12
Non-progressive motile sperms %	21.31±7.13	--	16.67±14.1
Immotile sperms %	33.64±8.17	--	62.81±28.19
Total/ejaculate (million)	137.82±76.21	0.0	45.39±52.35
Normal morphology %	33.0±7.88	--	11.58±12.44
Sperm agglutination %	1.97±4.38	--	0.13±0.87
Round cells (cell/HPF)	9.19±7.62	6.86±10.85	8.31±7.28

NORM= Normozoospermic, AZO= Azoospermic, OAT= Oligoasthenoteratozoospermic

On comparison between NORM group and AZO group by unpaired t-test "Table 3.3", the age, body mass index and duration of marriage was matched (no significant difference). However, for serum hormonal assay, there was significant higher level of E2 in AZO group compared to NORM group, (p= 0.0259). While the T/E2 ratio were significantly higher in NORM group (171.05±81.74) compared to AZO group, (p= 0.0118). Yet, there was no significant difference in serum T level between the two groups. The comparison between semen parameters between two groups show significant higher ejaculate volume in NORM group in comparison to AZO group (2.29±0.79 ml, 1.72±0.68 ml respectively; p= 0.0065), but no significant difference was shown in semen pH.

Table (3.3): Comparison between NORM group and AZO group by t-test

Parameter	NORM(n= 36) Mean±SD	AZO(n= 21) Mean±SD	P value
Age (yr)	30.36±5.56	32.05±8.33	0.4147
Duration of Marriage (yr)	5.68±3.47	6.2±5.05	0.6823
Body Mass Index (kg/m^2)	27.92±5.49	26.82±4.82	0.4335
Serum Testosterone (ng/ml)	5.39±2.88	5.37±3.87	0.984
Serum Estradiol (E2) (pg/ml)	32.68±13.05	54.44±40.59	0.0259
Testosterone/E2 ratio	171.05±81.74	115.36±74.49	0.0118
Ejaculate volume (ml)	2.29±0.79	1.72±0.68	0.0065
pH	8.04±0.17	8.0±0.09	0.2492

NORM= Normozoospermic, AZO= Azoospermic

The SP sFas showed a significant difference between AZO group and NORM group (median 102.0, 0.0 respectively, p= 0.035) using Mann-Whitney U test, "Table 3.4", whereas no significance found in SP IL-2 and SP IL-6 between the two groups.

Table (3.4): Comparison of seminal plasma inflammatory and apoptotic markers between NORM group and AZO group by Mann-Whitney U test

Parameter	NORM(n= 27) Median (Range)	AZO(n= 20) Median (Range)	P value
SP IL-2 (pg/ml)	0.0 (0.0-207.0)	51.5 (0.0-153.0)	0.216
SP IL-6 (pg/ml)	0.0 (0.0-96.5)	3.25 (0.0-321.0)	0.182
SP sFas (pg/ml)	0.0 (0.0-235.0)	102.0 (0.0-342.0)	0.035

NORM= Normozoospermic, AZO= Azoospermic

The comparison between NORM group and OAT group "Table 3.5" shows a significant higher mean of age in OAT group in relation to group one (33.81±7.96, 30.36±5.56 yrs respectively, p= 0.0218), while the means of duration of marriage and BMI displayed no significant difference. The mean serum level of E2 was significantly higher in OAT group in comparison to NORM group (45.93±28.98, 32.68±13.05 respectively, p= 0.0064) whereas both serum T and T/E2 ratio showed no significant difference between the two groups. Likewise, the pH and ejaculated volume in both groups showed no significant difference.

Table (3.5): Comparison between NORM group and OAT group by t-test

Parameter	NORM(n= 36) Mean±SD	OAT(n= 48) Mean±SD	P value
Age (yr)	30.36±5.56	33.81±7.96	0.0218
Duration of Marriage (yr)	5.68±3.47	6.68±5.14	0.2923
Body Mass Index (kg/m^2)	27.92±5.49	28.9±6.32	0.4509
Serum Testosterone (ng/ml)	5.39±2.88	5.86±3.01	0.4649
Serum Estradiol (E2) (pg/ml)	32.68±13.05	45.93±28.98	0.0064
Testosterone/E2 ratio	171.05±81.74	147.1±66.52	0.1555
Ejaculate volume (ml)	2.34±0.72	2.07±0.78	0.1099
pH	8.04±0.17	7.98±0.2	0.1862

NORM= Normozoospermic, AZO= Oligoasthenoteratozoospermic

"Table 3.6" shows a comparison between NORM group and OAT group by Mann-Whitney U test regarding SP IL-2, IL-6 and sFas. The SP IL-2 median was significantly higher in OAT group compared to NORM group (177.0, 0.0 respectively, $p < 0.001$). Concerning SP IL-6, its median was also higher in OAT group compared to NORM group (7.25, 0.0 respectively, $p = 0.024$). Likewise, the SP sFas was of higher median in OAT group compared to NORM group (40.0, 0.0 respectively, p= 0.048).

Table (3.6): Comparison of seminal plasma inflammatory and apoptotic markers between NORM group and OAT group by Mann-Whitney U test

Parameter	NORM(n= 27) Median (Range)	OAT(n= 41) Median (Range)	P value
SP IL-2 (pg/ml)	0.0 (0.0-207.0)	177.0 (40.5-351.0)	< 0.001
SP IL-6 (pg/ml)	0.0 (0.0-96.5)	7.25 (0.0-291.5)	0.024
SP sFas (pg/ml)	0.0 (0.0-235.0)	40.0 (0.0-712.0)	0.048

NORM= Normozoospermic, AZO= Oligoasthenoteratozoospermic

Concerning the comparison between AZO group and OAT group "Table 3.7" ; the age and BMI were matched, there was no significant difference between the two groups, the same for marriage duration. Also there was no significant difference between serum sex steroidal hormones levels and their ration between these two groups. Similarly, no significant difference regarding semen pH and volume was shown between the two groups.

Table (3.7): Comparison between AZO group and OAT group by t-test

Parameter	AZO(n= 21) Mean±SD	OAT(n= 48) Mean±SD	P value
Age (yr)	32.05±8.33	33.81±7.96	0.4171
Duration of Marriage (yr)	6.2±5.05	6.68±5.14	0.7183
Body Mass Index (kg/m^2)	26.82±4.82	28.9±6.32	0.1415
Serum Testosterone (ng/ml)	5.37±3.87	5.86±3.01	0.6058
Serum Estradiol (E2) (pg/ml)	54.44±40.59	45.93±28.98	0.3919
Testosterone/E2 ratio	115.36±74.49	147.1±66.52	0.1017
Ejaculate volume (ml)	1.72±0.68	2.07±0.78	0.0679
pH	8.0±0.09	7.98±0.2	0.6959

AZO= Azoospermic, OAT= Oligoasthenoteratozoospermic

The comparison between AZO and OAT groups in regard to median of SP IL-2 is shown in "Table 3.8" which displays a significant difference between the two groups (higher in OAT group) (177.0, 51.5 respectively, p< 0.001). While the median of SP IL-6 when compared between the two groups, showed no significant difference was found (7.25, 3.25 in OAT, AZO respectively, p=0.632).

Same result concerning SP sFas when compared between AZO and OAT groups was found, also there was no significant difference between the two groups (40.0, 102 in OAT, AZO respectively, p=0.7.23) as demonstrated in "Table 3.8".

Table (3.8): Comparison of seminal plasma inflammatory and apoptotic markers between AZO group and OAT group by Mann-Whitney U test

Parameter	AZO(n= 20) Range (median)	OAT(n= 41) Range (median)	P value
SP IL-2 (pg/ml)	51.5 (0.0-153.0)	177.0 (40.5-351.0)	< 0.001
SP IL-6 (pg/ml)	3.25 (0.0-321.0)	7.25 (0.0-291.5)	0.632
SP sFas (pg/ml)	102.0 (0.0-342.0)	40.0 (0.0-712.0)	0.723

AZO= Azoospermic, OAT= Oligoasthenoteratozoospermic

Correlation between parameters within each group

The correlation of age with hormonal, inflammatory and apoptotic markers, there was negative correlation with serum T and with T/E2 ratio in all three groups but only significant in AZO group (r= -0.587, p= 0.005, r= -0.465, p= 0.034 respectively) as shown in "Fig." (3.1 and 3.2). There was no significant correlation of age with any of SP cytokines (IL-2 and IL-6) or with SP sFas in all three study groups.

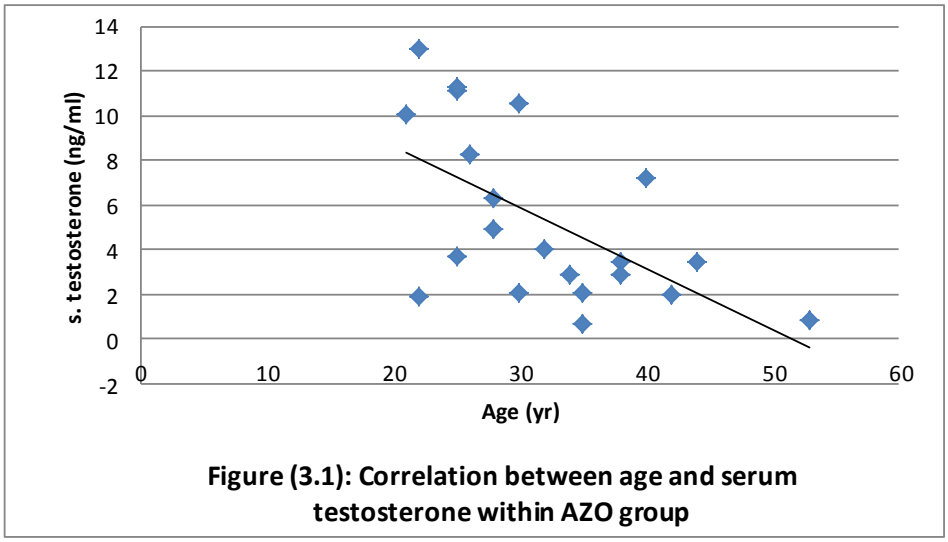

Figure (3.1): Correlation between age and serum testosterone within AZO group

AZO= Azoospermic, r= -0.587, p= 0.005

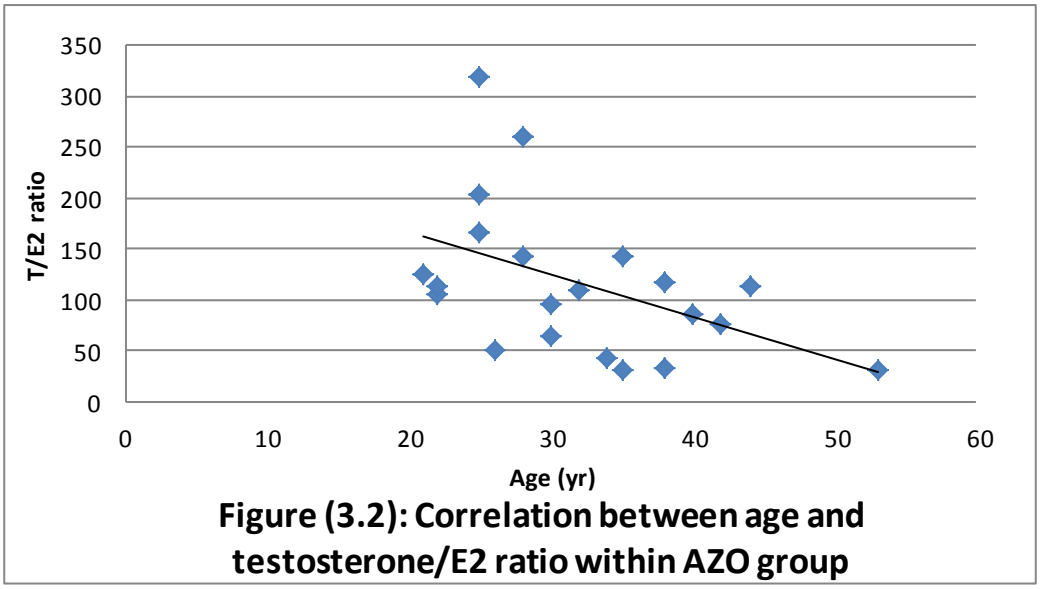

Figure (3.2): Correlation between age and testosterone/E2 ratio within AZO group

AZO= Azoospermic, r= -0.465, p= 0.034

Serum T was positively correlated with serum E2 in the three groups (NORM, r= 0.512, p= 0.001; AZO, r= 0.665, p= 0.001; OAT, r= 0.671, p< 0.001 respectively), also serum T was positively correlated with T/E2 ratio in all three groups, yet this correlation was only significant in NORM group (r= 0.694, p< 0.001) as it is demonstrated in "Table 3.9" and there was no significant correlation between serum T and seminal plasma cytokines (IL-2 and IL-6) in any of the three groups. However, serum T was positively correlated with SP sFas in NORM group (r= 0.642, p= 0.048) as shown in "Fig."(3.3).

Table (3.9): Correlation of serum testosterone with different parameters in the three groups

Parameter	NORM		AZO		OAT	
	r	p	r	p	r	p
S. E2	0.512	**0.001**	0.665	**0.001**	0.668	**< 0.001**
Testosterone/E2	0.694	**< 0.001**	0.293	0.198	0.197	0.118
SP IL-2	0.301	0.128	-0.227	0.335	-0.077	0.626
SP IL-6	0.094	0.642	-0.352	0.128	-0.056	0.729
SP sFas	0.383	**0.048**	0.108	0.649	-0.109	0.497

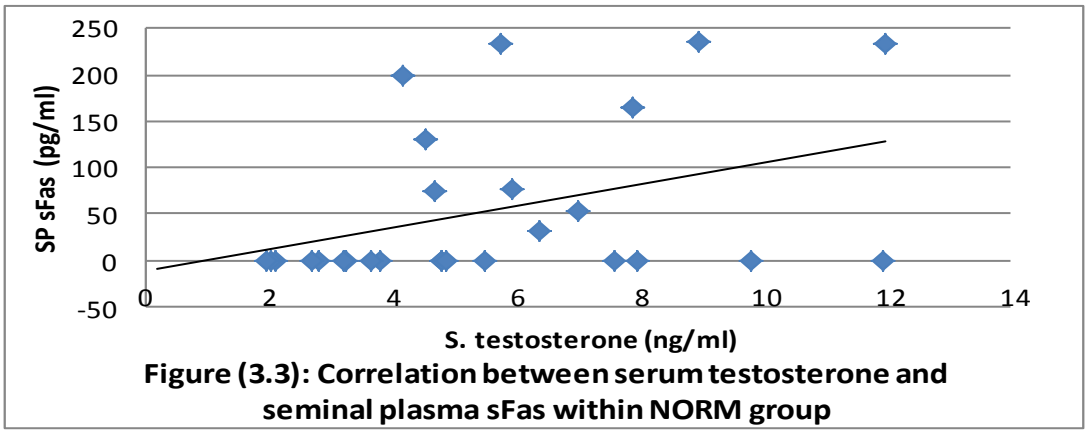

Figure (3.3): Correlation between serum testosterone and seminal plasma sFas within NORM group

NORM= Normozoospermic, r= 0.383, p= 0.048

Regarding correlation between serum E2 and other parameters, there was only significant negative correlation with T/E2 ratio in OAT group (r= -0.474, p= 0.001) as it is clear in "Fig." (3.4), while there was no significant correlation between E2 with any of SP interleukins or sFas in the three groups.

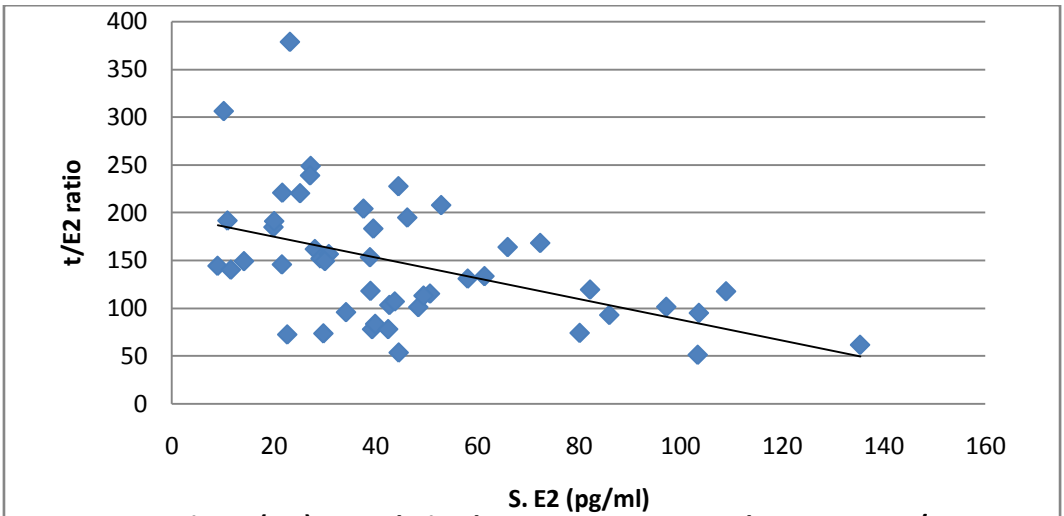

Figure (3.4): Correlation between serum E2 and testosterone/E2 ration within OAT group

OAT= Oligoasthenoteratozoospermia, r= -0.474, p= 0.001

T/E2 ratio was positively correlated with SP IL-2 and sFas in NORM group only (r= 0.426, p= 0.027; r= 0.507, p= 0.007 respectively) as it is demonstrated in "Fig." (3.5and 3.6), whereas no significant correlation between the same parameters was shown in AZO and OAT group.

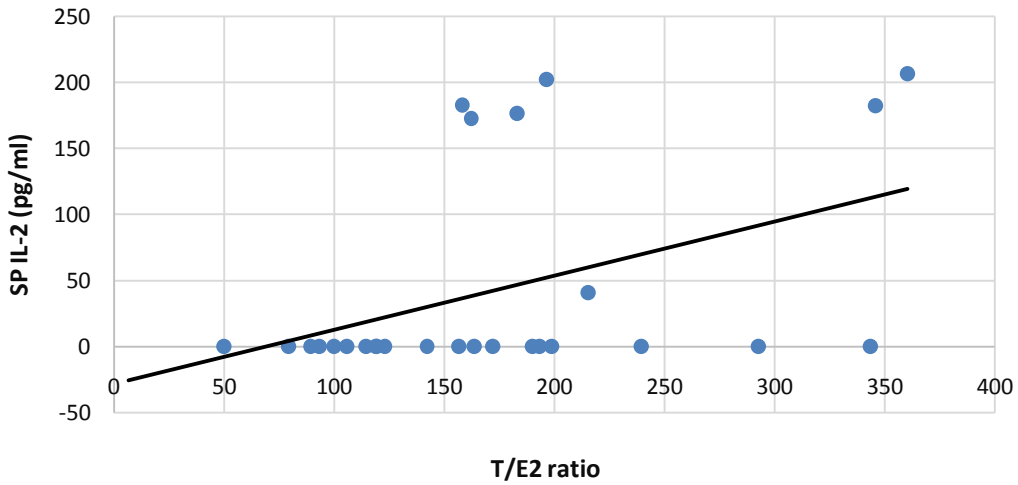

Figure (3.5): Correlation between testosterone/E2 ratio and seminal plasma IL-2 within NORM group

NORM= Normozoospermic, r= 0.426, p= 0.027

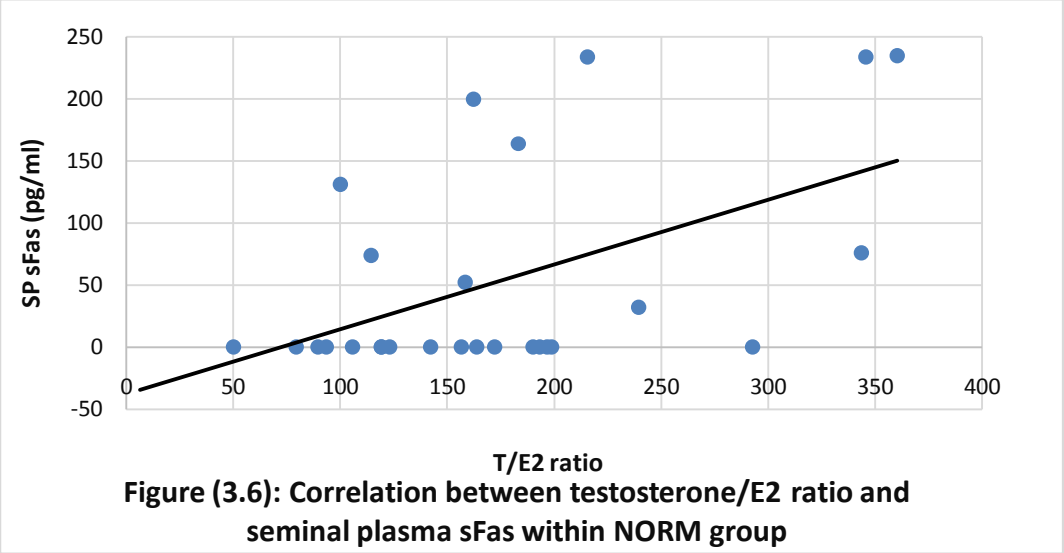

Figure (3.6): Correlation between testosterone/E2 ratio and seminal plasma sFas within NORM group

NORM= Normozoospermic, r= 0.507, p= 0.007

This study displayed a positive correlation between SP IL-2 and SP IL-6 and also with SP sFas in NORM group (r= 0.566, p= 0.002; r= 0.641, p< 0.001 respectively) ("Fig." 3.7and 3.8), yet no such correlation was shown AZO group and OAT group.

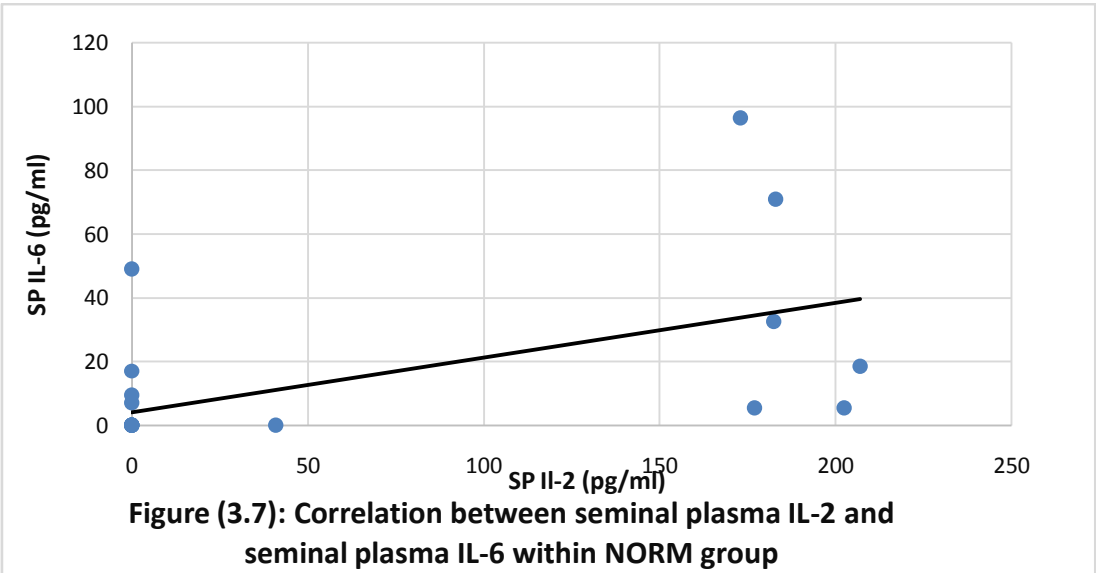

Figure (3.7): Correlation between seminal plasma IL-2 and seminal plasma IL-6 within NORM group

NORM= Normozoospermic, r= 0.566, p= 0.002

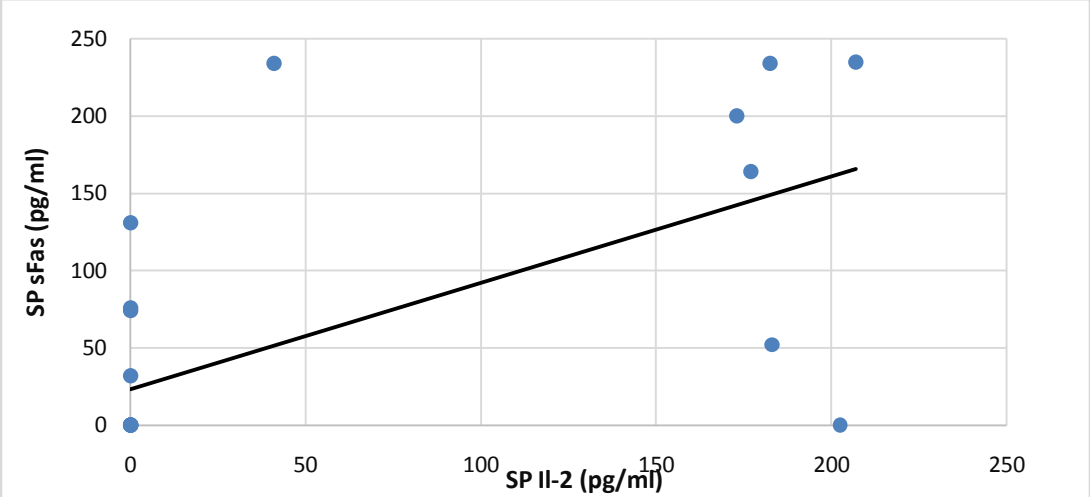

Figure (3.8): Correlation between seminal plasma IL-2 and seminal plasma sFas within NORM group

NORM= Normozoospermic, r= 0.641, p< 0.001

SP IL-2 have strong negative correlation with sperms morphology within NORM group (r= -0.844, p<0.001) as shown in "Fig." (3.9).

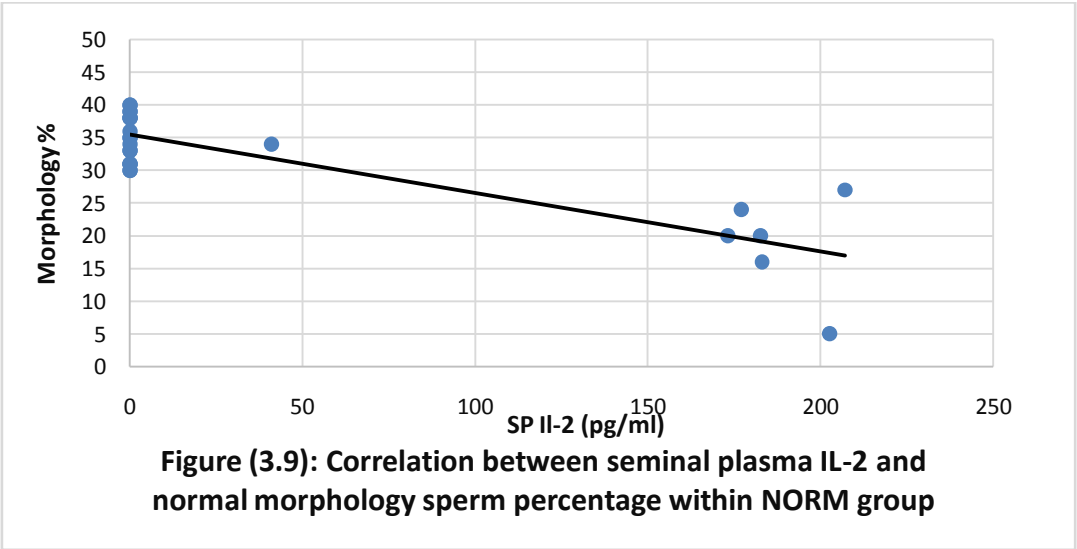

Figure (3.9): Correlation between seminal plasma IL-2 and normal morphology sperm percentage within NORM group

NORM= Normozoospermic, r= -0.844, p< 0.001

"Fig." (3.10) illustrates that SP IL-6 has a positive correlation with SP sFas in NORM group only (r= 0.405, p= 0.036), but this correlation was absent in AZO group and OAT group.

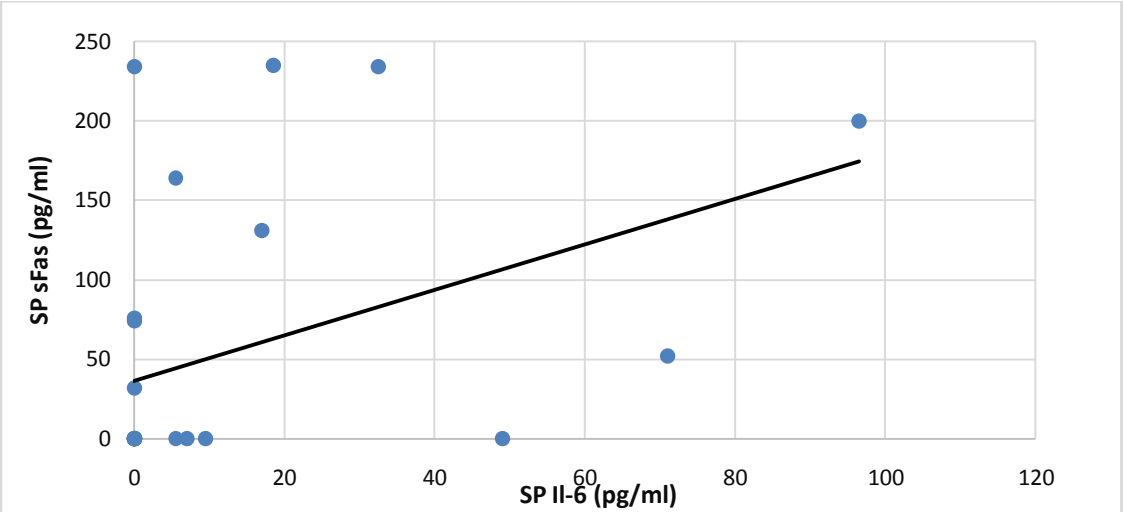

Figure (3.10): Correlation between seminal plasma IL-6 and seminal plasma sFas within NORM group

NORM= Normozoospermic, r= 0.405, p= 0.036

Moreover, SP IL-6 found to have positive correlation with immotile sperm percentage (r= 0.646, p< 0.001) and negative correlation with sperms morphology within NORM group (r= -0.474, p= 0.012) "Fig."(3.11 and 3.12) respectively.

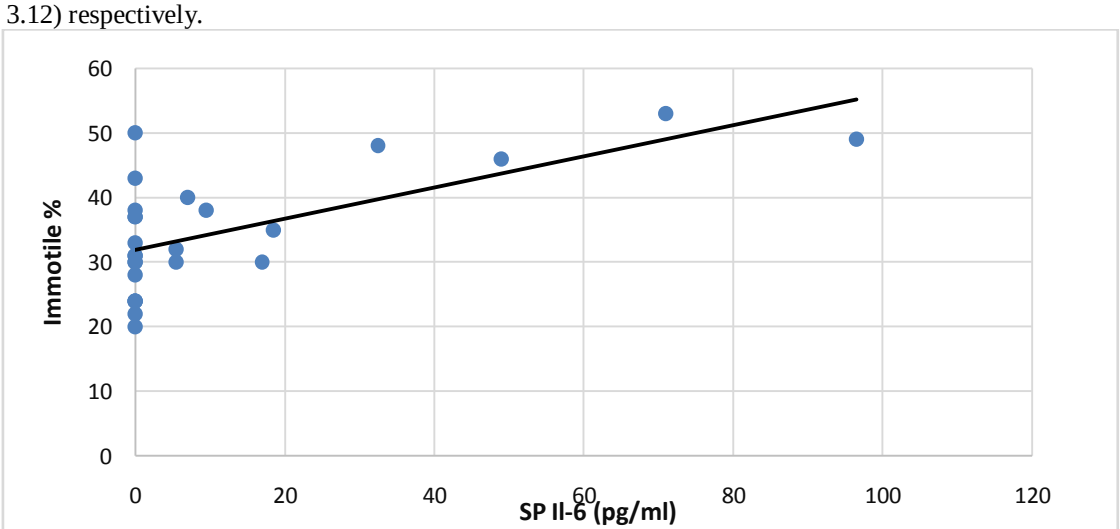

Figure (3.11): Correlation between seminal plasma IL-6 and immotile sperm percentage within NORM group

NORM= Normozoospermic, r= 0.646, p< 0.001

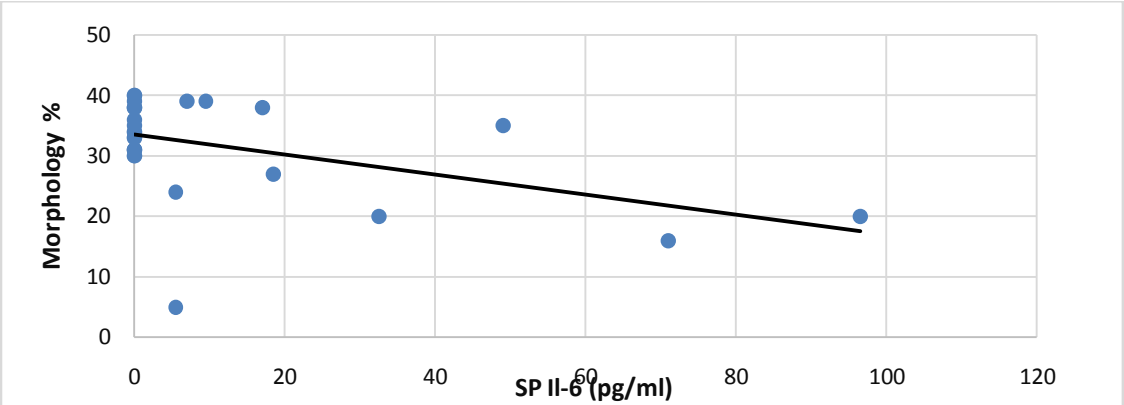

Figure (3.12): Correlation between seminal plasma IL-6 and normal morphology sperm percentage within NORM group

NORM= Normozoospermic, r= -0.474, p= 0.012

IV. Discussion

Although a consensus exists that the basic semen analysis is the most important tool in male fertility investigation, but there is an association between hormonal control of spermatogenesis, the inflammatory process at different parts of the reproductive system, and the process of apoptosis that naturally occurs in the testis, so the goal of this work was to gain more insight on the correlation between these different parameters.

In this study, G3 (Oligoasthenoteratospermia) was put altogether collectively because the no. of each abnormality whether in sperm no., motility, morphology or any combination of these abnormalities was very small. Likewise, G1 (normospermic) was considered as a control group that to be compared with other two groups. The classification of the groups in this study come in the same line with Guzick et al in 2001who stated that the threshold values for sperm concentration, motility, and morphology can be used to classify men as subfertile, of indeterminate fertility, or fertile [30].

The age and BMI was matched for the three study groups, the semen analysis results were not compared between the three groups as they were the basis of classification of subjects in the present study except for the semen volume which was significantly lower in the G2 compared to G1. These results could be expected as G2 is a collection of different pathologies that lead to azoospermia; of them, the ejaculatory duct dysfunction which may be due to either failure of emission or retrograde ejaculation, complete obstruction of both ejaculatory ducts (congenital or acquired. However, the secretions of bulbourethral gland, which normally comprise a small part of normal semen, may be ejaculated in an antegrade manner so that the patient may notice small semen which shows azoospermia [31]. So the mean of semen volume is affected by such pathologies and thus was lower than G1.

The sex steroidal hormones profile In this study was different in the three groups specifically the E2 level which was higher significantly in G2 and G3 when compared to G1 while testosterone level showed no significant difference among the three groups. The T/E2 ratio was only lower significantly in G2 when compared to G1.These results are in agreement with Pasquier et al in 2008 who found an increase in serum E2 in some types of azoospermia and a decrease in the testosterone/oestradiol ratio in azoospermic patients and theysuggested the hypothesis of greater conversion of testosterone to oestradiol in the testes. This could reflect increased aromatase activity in the absence of germ cells [32].

Estradiol plays a vital role in normal sperm cell development and function [11,14][33].Estrogens have a well-documented inhibitory effect on Leydig cell androgen secretion and RNA synthesis, [34], estradiol significantly but modestly enhances testosterone – induced suppression of spermatogenesis in human and increased proportions achieving azoopermia [35].Estrogen seems to have a separate function in maintaining epithelial morphology, particularly the apical cytoarchitecture of nonciliated cells [36].The primary function of estradiol is the expression of the NHE3 gene, which regulates the exchange of Na^+ and H^+ in mediating water transport and the concentration of sperm in the epididymis, and thus fertilizing ability of sperm. Interference with this physiological process leads to accumulation of luminal fluids or occlusion of these ductules, ductule dysfunction, may lead to seminiferous tubule degeneration, testicular atrophy, and infertility.[37]

A direct role of estradiol as a germ cell survival factor was then demonstrated in the human testis in vitro, where estradiol was shown to inhibit testicular apoptosis much more effectively (100 to 1,000-fold) than testosterone 17β-estradiol as a survival factor for male germ cells Estrogens have recently been shown to be essential for male reproduction but its exact role is unclear. [38]

Concerning the inflammatory markers in the semen, the current study a significant high level of SP IL-2 and IL-6 in G3 in comparison to G1, moreover, the SP IL-2 was even highly significant in comparison with G2. These results agree with Swatowskiand Jakielin 2002 who found significant higher level of SP IL-6 inoligoasthenozoospermia in comparison with normospermic and this may suggest the presence of subclinical inflammation in the genital tract.[39]high level cytokines may be considered as a marker of inflammatory process in the male reproductive system, they have decisive activities outside of the immune system where they acts as regulators of testicular steroid hormone production. Cytokines have also been implicated as novel growth and differentiation factors involved in the regulation of cells in both the endocrine and the tubular compartment of the testis [40].

The levels of IL-6 and IL2 in SP have been often demonstrated as the factors linked with a decrease in quality of semen parameters and presence of inflammatory process[41][42].

Testicular germ cell apoptosis is a fundamental and complex process required for testicular homeostasis during spermatogenesis and appears to have an essential role in the control of germ cell number in testis [43] . During spermatogenesis, germ cell death via apoptosis has been estimated to result in the loss of up to 75% of the potential number of mature sperm cells [44] . This apoptotic wave appears necessary for normal spermatogenesis to develop, probably because it maintains a proper cell number ratio between maturing germ cell stages and Sertoli cells.

The Fas system has been implicated as a key regulator of germ cell apoptosis in the mammalian testis. In the human testis, FasL constitutively expressed by Sertoli cells is suggested to bind to Fas of germ cells causing death of these Fas-bearing germ cells [43]. In addition, soluble forms of cell surface receptors such as sFas can be produced either by proteolytic cleavage of membrane-bound receptors or by alternative splicing, and was believed to inhibit Fas-FasL binding and thereby block Fas-mediated apoptosis [45] .

In the present study the expression of sFas in the seminal plasma was significantly higher in G2 and G3 in comparison with G1. i.e. decrease in apoptotic process mediated by Fas-FasL binding, as it is found that soluble Fas is supposed to act as a FasL inhibitor, "a survival factor", binding to Fas and preventing Fas-mediated apoptosis by binding to membrane-bound FasL, thus blocking binding of the ligand to the Fas receptor and preventing apoptosis induction in the target cell [46].

These results go with the hypothesis of presence of a subclinical inflammatory process especially in G3 and the role of FasL as a promoter of immunoregulation that prevent inflammation thus the increased SP sFas in G3 may be due to continuation of the inflammatory process in the testis,since the Fas-mediated pathways are activated during infection and inflammation [46][47].

in this study a negative correlation was found between age and serum testosterone level especially noticed in G2, increasing male age has an impact on every level of the hypothalamo-pituitary-testicular axis, leading to decreased circulating androgen levels and ultimately to reduced androgenic effects at target organs[48] .The impact of age may be caused by aging per se, or by mediators generated secondarily by age-related cofactors, as for example, vascular diseases, accumulation of toxic substances or infections of the reproductive accessory glands [49] .

Regarding the positive correlation of serum testosterone and E2 which was observed in the 3 study groups; these results are expected as E2 is a product of aromatase enzyme activity on androgens (testosterone and androstendione) in the testis (20%) and other tissues (mainly adipose tissue) so the more testosterone the more E2.

Serum T showed a positive correlation with SP sFas in group 1, i.e. increase testosterone is associated with decrease apoptosis process in the testis of normozoospermic subjects. The same finding mentioned by Yang et al in 2006 who stated that low concentration of sexual hormones may increase the apoptosis of germ cells, which can induce male infertility[50].

The results showed that T/E2 ratio was positively correlated with seminal plasma IL-2 and sFas in group 1 only but not in other study groups, and this may be due to group 1 represent the normospermic while other two groups (azoospermicand oligoasthenoteratozoospermic) are a collection of different pathologies which has different level of T/E2 ratio and SP IL-2 and sFas, so no clear correlation was shown in the other groups.

Regarding correlation of SP IL-2 and IL-6 with SP sFas only was evident in group 1 but not in other 2 groups. This finding agrees with Pentikäinen, 2002who showed that Fas system is a mediator of human inflammatory responses.[38]

V. Conclusion

Inflammatory process is important in infertility as indicated byhigh level of IL-2 and IL-6 in the seminal plasma in both azoospermic andoligoasthenoteratozoospermic groups in comparison to normozoospermicgroup, Apoptosis plays an important role in cell selection process of spermatozoa as indicated by decrease process of apoptosis in both azoospermic and oligoasthenoteratozoospermic groups in comparison

to normozoospermic group hence the use of apoptotic marker in evaluationof male infertility is essential.The inflammatory cytokines IL-2 and IL-6 may manipulate apoptoticprocess in the seminal plasma,regarding the hormonesE2 and T/E2 ratio are important parameters in male infertility.

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