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The effect of Anabolic Androgenic Steroid Hormonse Use by Body Builders on Sperm Count and Interleukin -10

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Abstract
Exercises done by body builders have a great benefit for the general health, muscle size and performance. Anabolic androgenic steroid (AAS) hormones are commonly used by body builders to increase muscle mass and strength depending on its mode of action to increase the level of nitrogen retention in muscle insulin like growth factor(IGF-1) increase in muscle tissue and in the liver.
Eighty male volunteers, their age groups are ranging from 21- 35 years. They were divided into three groups; the first group 27 age matched volunteers apparently healthy (healthy control group), Second group 27 Body builder players attending to a sport club in Baghdad with androgen anabolic steroid hormone (AAS) use (steroid group), and the third group 26 bodybuilders players attending the sport club without AAS use (fitness group). The serum was used for estimation of testosterone level by applying enzyme immuno-florescent technology and estimation Anti-sperm Ab test by heamo-agglutination method.
Sperm count was done for each seminal fluid sample, IL-10 cytokine was estimated by a sandwich ELISA method in seminal plasma.
A bodybuilders with AAS use shows a significant reduction in sperm count (P=0. 00),while fitness group sperm count shows slight non-significant decrease compared to the healthy control group (P=0. 7). Serum testosterone level in AAS use group shows a significant decline compared with fitness and healthy control groups (P= 0.00), IL-10 as an anti-inflammatory cytokine shows a high level in the AAS user group compared to the healthy group (0.04)
In general the final impact of AAS use on body builders immunity are; declining in phagocytosis and an increase in IL-10 which act as a suppressor to the immune response, these results suggest that AAS abuse players are more liable to infection and decrease in sperm count which may cause temporarily or permanently infertility
Key ward : Seminal Plasma IL-10, Serum Testosterone and Sperms count .

1.1 Introduction :

Athletes consume anabolic android steroids to gain muscle strength, shape and performance in the hope of gaining weight, strength, power, speed, endurance, and aggressiveness. They are widely used by athletes involved in different type of sports (Fahey 1998).

Nandrolone (19-nortestosterone) is anabolic steroid. Nandrolone is most commonly sold commercially as its decanoate ester (Deca-Durabolin) and less commonly as aphenyl propionate ester (Durabolin) used by body builders to increase muscle mass and strength, its mode of action is to increase the level of nitrogen retention in the muscle and increase the level insulin like growth factor -1(IGF-1) in muscle tissue. It has the ability to accelerate division of the cell- satellites which play an important role in restoring the damage muscle (Brueggemeier 2006). Primary abnormalities included either a complete lack of sperms or too few sperm to induce pregnancy due to android androgenic steroids use,other changes are included; cytoarchtacture changes in liver and prostate,Immune modulating properties by suppressing the expression of proinflammtory cytokines such TNF-alpha, IL1 beta, IL-6 while potentiating the expression of IL-10 by acting directly on CD4+ T cell to produce IL-10, and enhance production of adhesion molecules.

Lymphokines and monokines have an effect on sperm activity, administration of high dose of nandralon leads to deterioration of sperm parameters, DNA fragmentation and testicular apoptosis as well as lipid peroxidation and antioxidant enzyme activities (Meeker et al., 2007)

It was reported in U.S.Urologists survey that, when the testosterone is given from outside, the athletes will stop producing their own testosterone, which will temporarily or permanently stop sperm production and the shrinkage of the testicles. Exogenous testosterone or AAS abuse will lead to down regulate the action of hypothalamic - pituitary gonad (HPG) and may result in infertility (Ko et al., 2012)

Testosterone reduces macrophage expression in the mouse toll-like receptors- 4, a trigger for inflammation and innate immunity. Normal levels of testosterone maintain high level of cytokines, which in other tissues would promote inflammation, but in the testes testosterone controls testes function, including regulation of the sperm development by controlling their cell division and survival. (Gopichandranet al., 2014)

Still now there is a lack of information about the effect of AAS abuse on human since most of the scientific trials were carried out in animal models, we tried to shed a light on this area of research by dealing with human volunteer body builders with steroid use, so our aims are:-

To understand the impact of AAS on immunity by estimation of IL-10 in seminal plasma and to estimate the count of the sperms and finally to evaluate the correlations between all these factors.

2. Materials and methods:

2.1 Study subject:

This study was involved players who attend to the gym and looking for good body structure and performance by increasing the muscle size. Those players are two types either fatty (obese) trying to get fitness or fit persons attend to gym in order to gain weight with muscle size and good body performance, their number are 26 and called fitness group. Other players (27) are usually use course of anabolic steroid hormones orally or via intramuscular injection with exercise which is considered as a shortcut way to get big muscle in a short period(steroid group), 27 adult individual, apparently healthy, with age and gender matched with the other study groups. A letter of conscent was obtained form all volunteers involved in this study.

2.2 Samples:

a- Blood:
Form each case 5 ml of blood were obtained by venipuncture using 6ml disposable syringe between 4:00 -8:00 PM. Each blood sample was centerifuged in a plain tube to get serum for testosterone determination .
B - Seminal fluid samples:
For all individuals involved in this study ,sperm counts for the seminal fluid were done by microscopical examination, the remaining seminal fluid was centrifuged for 5 min., at 5000 rpm to separate seminal plasma, seminal plasma fluid was divided into 0.2-0.25 ml aliquates in eppendorf tube and stored at -20°C until used for determination of IL-10.

3.0 Materials
- Testosterone KIT BodiTech-Korea
-IL-10 ELISA KIT PeproTech- USA

3.1 Testosterone detection in serum:
According to manufacturer′s instruction.(BODITECH MED INC.), this test was applied by i-Chroma testosterone test. i-chroma testosterone is a fluorescence immunoassay for the quantitative determination of testosterone in human serum or plasma. i-chroma testosterone is used as an aid in the screening and monitoring of androgen level . The fluorescence intensity of the anti-testosterone antibody reflects the amount antigen captured and is processed in i-chroma reader to determine the testosterone concentration in the specimen. The test result was read on the display screen of the i-chroma reader.

3.2 Sperm counting:
All individuals(volunteers) involved in the study were asked to provide their seminal fluid. WHO recommended method was applied for sperm count(Ohl & Menge 1996). These samples were collected by applying the recommended collection procedure, samples were collected in wide mouth clean disposable container and incubated at 37C for 15 -30 minutes (monitored by mixing and shaking) to homogenize and liquify the seminal fluid.
The normal value of sperm count according WHO (2010) is more than 15 million/ml and less than this number is considered as an abnormal sperm count (Cooper et. al., 2010).
Counting was done under a light or phase-contrast microscope at a magnification of 40 x. (Only spermatozoa that are morphologically mature germinal cells with tails, are counted).

3.3 Human IL-10 in seminal plasma:
Human IL-10 ELISA Sandwich ABTS was applied. Manufacturer′s (PreproTech USA) manual instructions were followed for measurement of IL-10 in the seminal plasma..Reading was achieved after color development by ELISA plate reader at 405 nm with wavelength correction at 605 nm

4. Results :

4.1 Serum testosterone hormone:

Table .1: Testosterone concentration (ng/ml) with P value by ANOVA test for the studied groups

Result of serum Testosterone hormone:

Dependent Variable: Testosterone Conc. (ng/ml)

Study groups	Mean	No.	ANOVA (P value)
Healthy control	5.7407	27	F (0.015)
Steroid users	2.2278	27	
Fitness group	5.5577	26	
Total		80	

Table.1 figure 1;show the mean testosterone concentration of healthy control;5.74 Steroid users;2.23 Fitness group;5.56 ,Significant differences obtained between these groups with P values=0.015.

Table 2: Comparisons of testosterone concentration (ng/dl)of study group by T test.

Dependent Variable: Testosterone Conc. (ng/ml)		
Study groups	Study groups	Sig.
Healthy control	Steroid uses	.000
	Fitness group	.725
Steroid users	Healthy control	.000
	Fitness group	.000
Fitness group	Healthy control	.725
	Steroid users	.000

Table 2, figure 1: the comparison of testosterone between Healthy control and Steroid users show high significant difference (P value =0.000) and the comparison between steroid users with fitness group show high significant difference (P value =0.000) while healthy control with fitness group shows no significant difference (P value =0.725)

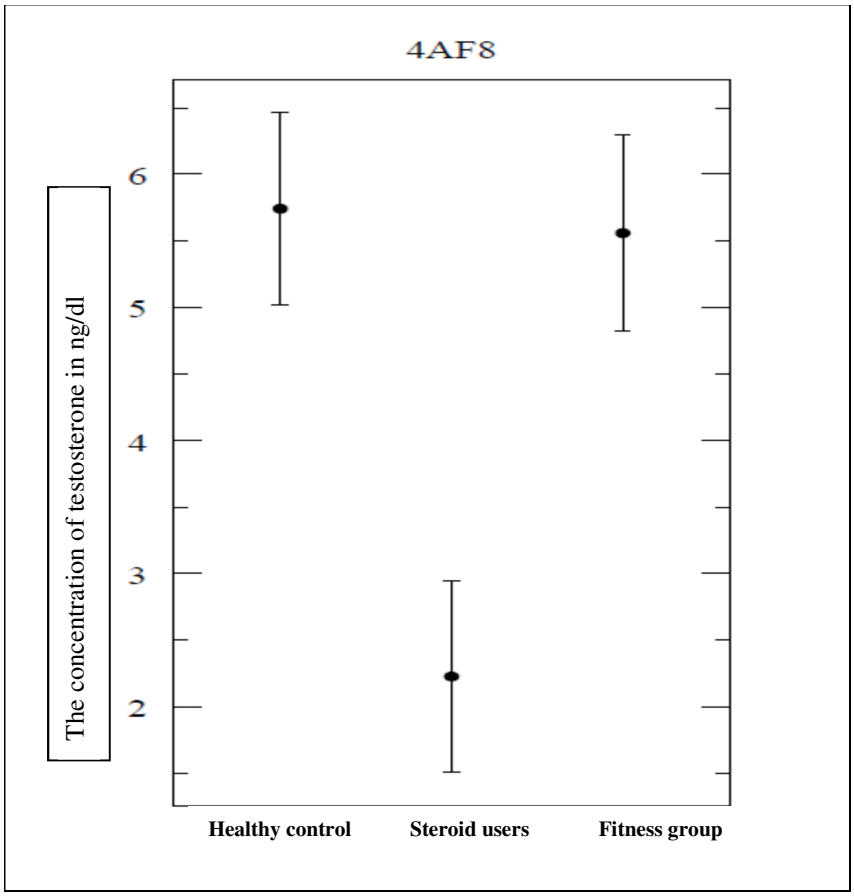

Figure. 1. Testosterone concentration (ng/dl) of study groups (Healthy control, steroid users and fitness group).

Reserve operating characteristic (ROC) analysis result of testosterone

Table 3: ROC test result for testosteron concentration in stroid users body builders

Area Under the Curve(AUC)	
Test Result Variable (s): testosterone (ng/ml)	
AUC	Asymptotic Sig.
0.709	0.003

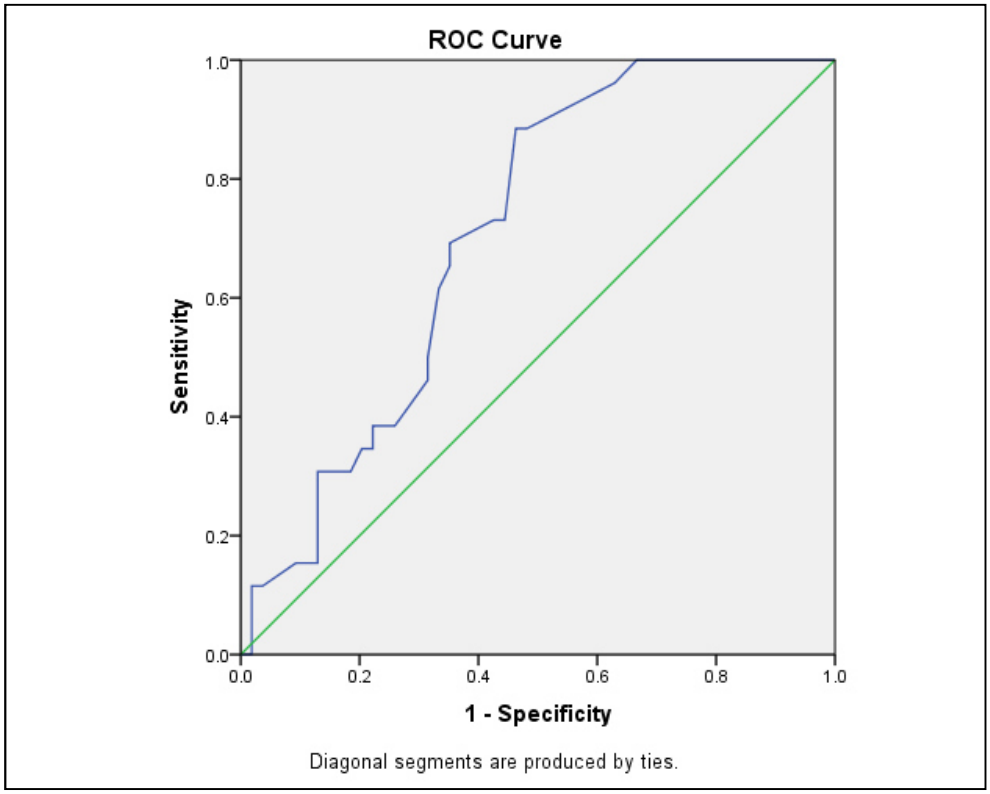

Figure 2 of testosterone area under the curve with high significant difference (P=0.03)
Table3 and figure 2 ; shows the ROC of serum testosterone level of AUC=0.709 with high significant difference (P=0.03).

4.2 Sperm count result:

Table 4:Comparisons between the perm count(million/ml) of the studied groups by ANOVA test

Dependent Variable: Sperm Count (million/ml)				
Study groups	Mean	Std. Deviation	No.	P value
Healthy control	48.1481	10.72633	27	0.21
Steroid usres	11.8778	17.40589	27	
Fitness group	47.4615	10.52703	26	
Total			80	

Table 4 shows the mean of Healthy control , Steroid usres and Fitness group with a concentration of 48.1481, 11.8778, 47.4615 (million/ml) respectively. The Steroid usres sperm count mean is every low compared with Healthy group and Fitness group

Table 5; shows comparisons of sperm count (million/ml) between the study groups by T test.

Dependent Variable: Sperm Count (million/ml)		
Study groups	Study groups	Sig.
Healthy control (A)	Steroid users (B)	.000
	Fitness group (C)	.852
Steroid usres (B)	Healthy control (A)	.000
	Fitness group (C)	.000
Fitness group(C)	Healthy control (A)	.852
	Steroid usres (B)	.000

Table 5 and figure 3; show the comparison between healthy control group and steroid usres group B has a high significant difference (P value =0.00) , also the comparisons between healthy , steroid usres with Fitness group have a high significant difference (P value =0.00) while the comparison between healthy control group with Fitness group shows no significant difference (P value = 0.852)

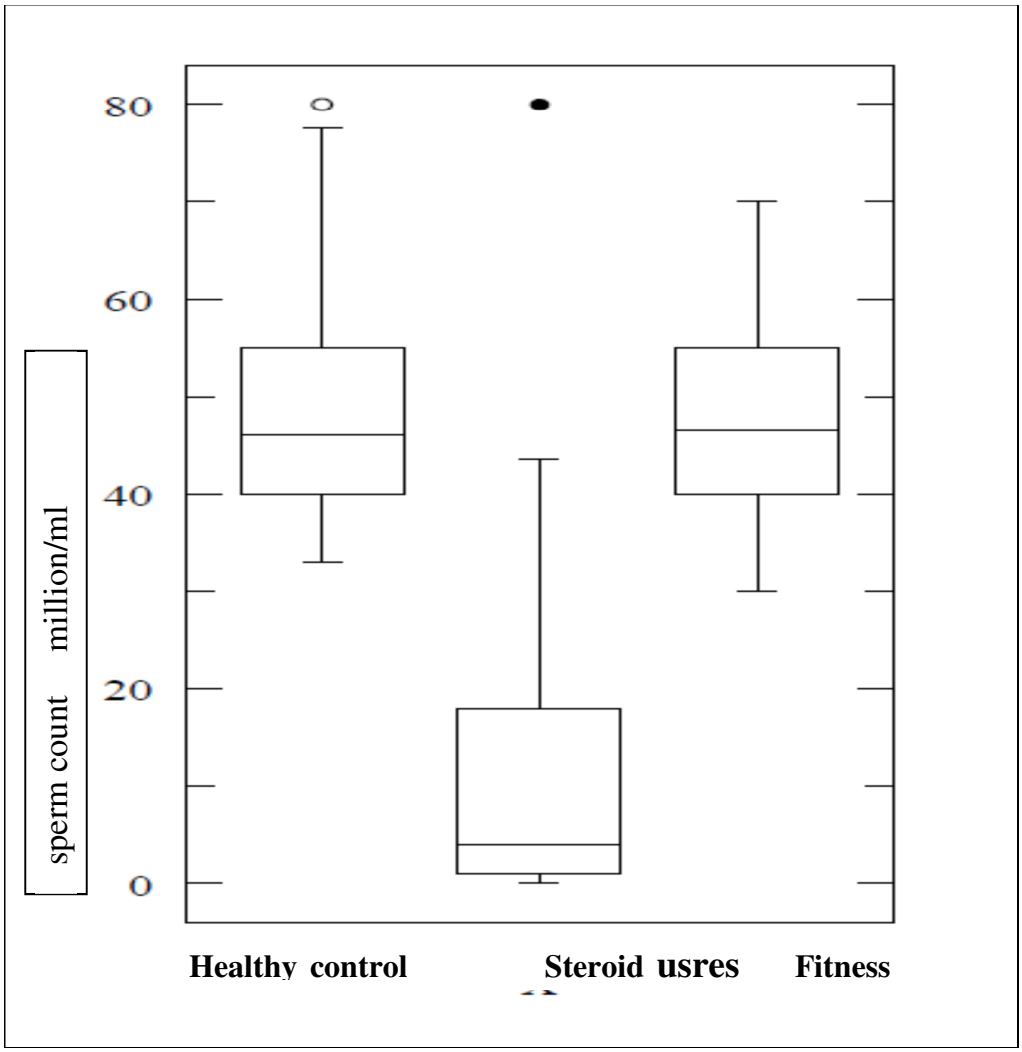

Figure 3: Sperm count (million/ml) of study groups (Healthy control, steroid users and fitness group).

Table 6: Reserve operating characteristic (ROC) analysis result of sperm count.

Area Under the Curve(AUC)	
Test Result Variable(s): Sperm count	
Area Under the Curve(AUC)	Asymptotic Sig.
0.726	.001

Figure 4.2.2 ROC analysis of sperm count in steroid users group

the Curve=0.72 with high significant difference (P=0.001)

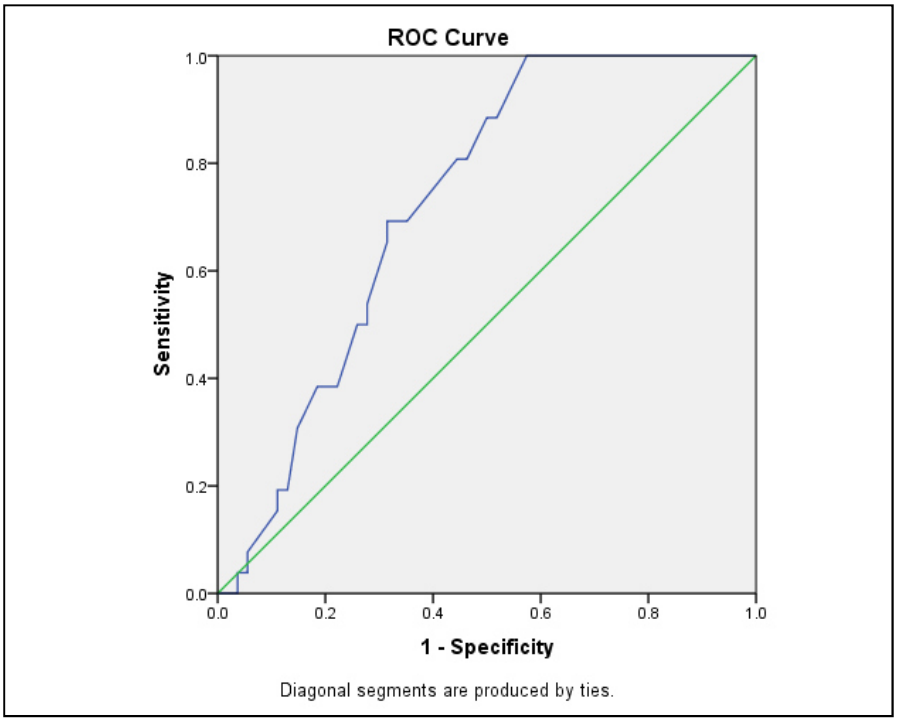

Figure 4 Sperm count area under the curve with high significant difference (P= 0.01).

Table 6 and figure 4 show the ROC analysis of sperm count(Area Under the curve= 0.72 with significant differences (P=0.001)

3. IL- 10 in seminal plasma:

Table 7: IL-10 concentration (pg/dl)in the seminal plasma by ANOVA test of the studied groups

Dependent Variable: IL-10 (pg/dl)			
	No.	Mean	P. value
Healthy control	27	53.33	0.032
Steroid users	27	78.404	
Fitness group	26	56.80	
Total	80		

Table 7, by applying ANOVA test, IL-10 mean of study groups were 53.33 , 78.404 and 56.8077 (pg/dl) respectively, the statistical analysis shows significant difference (P value =0.032).

Table 8: the comparisons of IL-10 concentration (pg/dl) of study group by T test

Dependent Variable: IL-10(pg/dl)		
Study groups	Study groups	Sig.
Healthy control	Steroid users	.040
	Fitness group	.938
Steroid users	Healthy control	.040
	Fitness group	.093
Fitness group	Healthy control	.938
	Steroid users	.093

Table 4.3.2, figure 4.3.1 show the comparison between Healthy control (A) with Steroid treated (B) have significant difference (P= 0.040) ,the comparison between Healthy control (A) with Fitness group (C) shows no significant difference (P=0.938), also the comparison between steroid uptake and fitness group (C)) shows no significant difference (P=0.093)

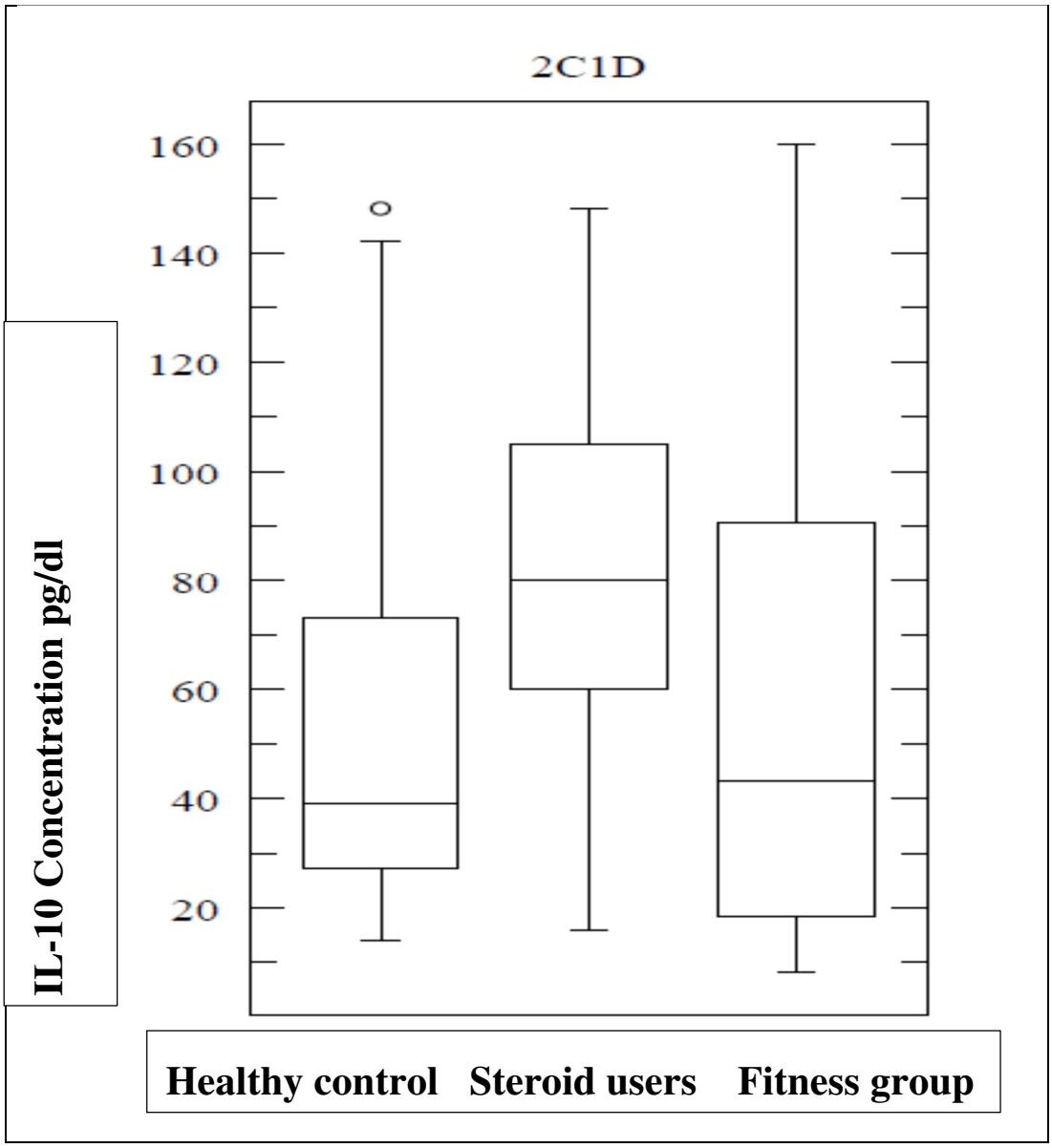

Figure 5 The comparisons of IL-10 concentration (pg/dl) of study groups by T test.

Table 9: Reserve operating characteristic (ROC) test result of IL-10

ROC test / Area Under the Curve(AUC)	
Test Result Variable(s): IL10 (ng/ml)	
Area Under the Curve(AUC)	Asymptotic Sig.
0.723	.001

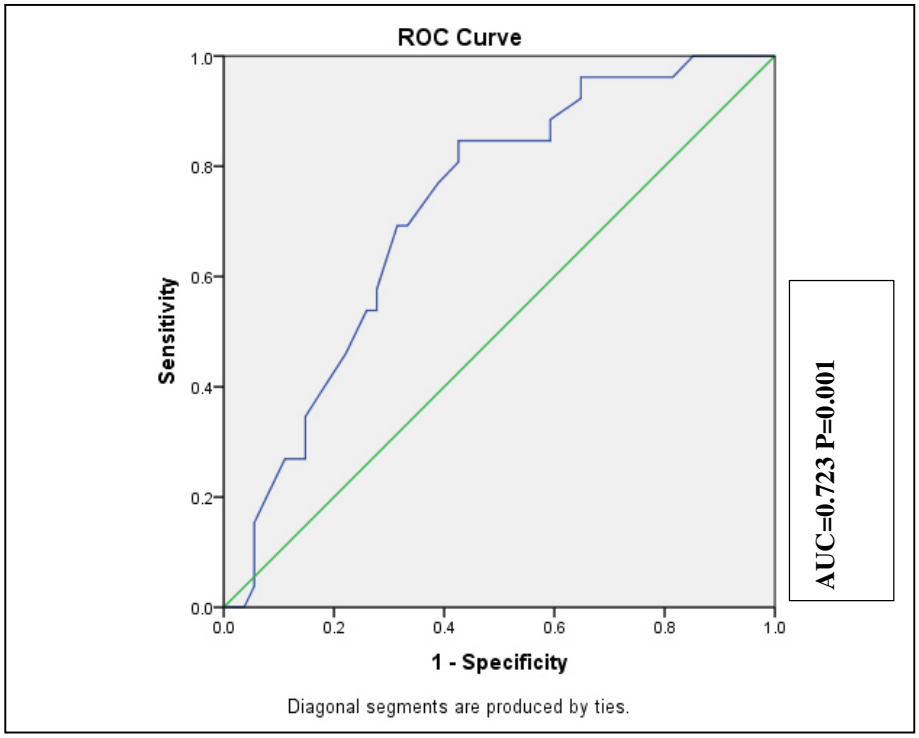

Figure 6:IL-10 area under the curve(0.723) with high significant difference (P=0.01)

Table 9 and figure 6 ; show the ROC of IL-10 level in seminal plasma which is =0.723 with high significant difference (P=0.001)

Discussion :

The sperm count of body builder steroid users group, fitness group and healthy control group show a significant difference by using ANOVA test (P value= 0.015)(table 1)

The steroid user group shows a high significant reduction in sperm count compared to both fitness and healthy control group (P value =0.00)(table 2 and figure 1) calculated by T test.

The AAS use by body building players have a direct effect towards the sperm count, the reduction of sperm count can be explained by the hormone imbalance occurred due to the AAS abuse, which indicate the impact of AAS abuse on testes by down regulation of production of sperms by these testes, while the fitness group, their sperm counts are not affected compared to control which suggests that fitness exercise has no significant effect on spermatogenesis compared to healthy control.

Ko et.al.at, 2012 reported in the U.S.Urologists survey that exogenous testosterone or AAS abuse will lead to down regulate the action of hypothalamic-pituitary gonad (HPG) and may result in infertility (Ko et al., 2012) which agreed with the present study.

Also other study displayed the effect of AAS on sperm count in 2013 they found that the exogenous testosterone or AAS abuse will cause a negative effect on HPG which lead to azoospermia or oligospermia (Jared et.al.,2013). Previously Bonetti et al., 2008 found that AAS inhibit the spermatogenesis and testicular atrophy, which also matched with the present study.
Oligospermia or azoospermia can occur to the body building player who abused AAS according to Bonetti et. al., (2008), which also agreed with the present study .
The comparisons between the study group, testosterone hormone level in serum shows significant difference by using ANOVA test (P value= 0.015),
The steroid user group shows a significant reduction in the serum testosterone level as compared with the healthy control group (P value = 0.00), suggesting the AAS injection will cause a decrease in serum testosterone level. Meanwhile the fitness group (exercises with using protein supplements) serum testosterone not affected compared to healthy control.
Other scientists studied the effect of AAS such as Deca-Durabolin (Nandrolone) injection also they agreed with the present study who they stated that AAS use will decrease level of testosterone in players who aimed to increase muscle's power and mass, (Purkaya and Mahanta 2012)

The AAS abuse able to deactivate the action of the pituitary- gonadal axis (Takahashi et al., 2004; Ramaswamy et.al., 2000). AAS use decreases testosterone level plus stimulation of the hypogonadotrophic hypogonadism (Jarow et al., 1990; Torres-Calleja et al., 2001)

The present study shows a significant difference of IL-10 level in seminal plasma of different groups with a P value = 0.032 by applying ANOVA test..
The steroid group shows significant increase in the IL-10 level of seminal plasma compared to a healthy control group (P value =0.040).
While, fitness group IL-10 level in seminal plasma shows no significant change compared to a healthy control group (P value =0.93).
IL-10 considered as an immune suppresser cytokine, which is able to down regulate the action of Th1- cell and also can activate B- cells. (Delves et al., 1998)
According to pervious study that found the steroid use was able to increase the level of an anti-inflammatory IL-10 (Corlett et al., 2002) which is matched with the present study.
Recently Havrylyuk et. al., at 2015 studied number of cytokines in an infertile patients they found a significant increase in seminal plasma IL-10 level compared with fertile control which is agreed with this study,

while, previous study found that cytokines including IL-10 act as immune-regulatory, which plays a role in infertility, they found that IL-10 in seminal plasma of infertile was lower than healthy fertile. (Huleihel et.al., 1999)
Another study found that IL-10 is one of the cytokines that had an impact on sperm production and infertility (Fossiez et al., 1996; Politch et al., 2007; Takaya et al., 2002)
Testes consider as an organ able to provide protection towards auto-immunity and interaction with systemic immunity (jacobo et. al., 2011).

Testes, Sertoli and lyding cells function are affected by cytokines network involved in these locations. (Havrylyuk, et. al., 2015).
ROC test revealed that testosterone, sperm count and IL-10 have a significant diagnostic ability to determine the impact of AAS use on bodybuilders bodies.

Generally it can be concluded that although AAS use has an advantages of body building,it suffers a disadvantage of decrease sperm production and downregulate immunity.

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