

# The Effect of Abuse of Some Anabolic Steroids on Hormonal and Testicular Function in Male Rats

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## Abstract

Anabolic androgenic steroids are complex molecules contain innate male hormone, the prolonged uses of these molecule cause destructive effects on health, thus, the investigation goals of this study to clarify the effect of high doses of anabolic steroids on follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone hormone, prolactin and estrogen hormones levels, sperm parameters (sperm count, motility, abnormality and viability), also to clarify the effect on body ,Epididymis and test weight. This study was carried out by treating rats with a high doses of anabolic steroids (testosterone and nandrolone) hormones. After 35 days of treatment ,the result indicated that ,there was a significant decrease in FSH,LH and testosterone hormones level while prolactin and estrogen will be increased, Although there was a significant decrease in sperm count ,motility and viability while abnormality will be increased ,body and test weight show statistically decrease.

**Key words:** Anabolic androgenic steroids, testosterone, prolactin

## Introduction

Anabolic androgenic steroids (AAS), are a complex group of molecules that include both steroidal androgens such as testosterone and its related-precursors (dehydroepiandrosterone, androstenediol and androstenedione) as well as many different synthetic compounds, physiologically produced by adrenal cortex and Leydig cells in men and by corpus luteum, preovulatory follicles and adrenal cortex in women, testosterone is the major anabolic hormone in the human body [1-3], and original AAS. It functions by penetrating the cellular membrane of target cells and binding to an intracytosolic molecule [4]. The testosterone effects can be divided into two types: anabolic and androgenic. The anabolic effects include increased bone metabolism, muscular growth, collagen synthesis and nitrogen fixation. The androgenic effects include effects that correlated with male puberty such as effect on the hair growth, reproductive

tract function, and sebaceous gland activity [5].

Beside the use of AAS in disease conditions, they are most frequently used in higher doses and for long durations among athletes [6,7], to improve body image (through an increase in muscle and/or decrease in fat mass) and also exercise performance [8]. The prolonged administration and/or using high doses of AAS were shown to compromise the function and structure of different organs such as, testis, kidney, heart and liver [9,10]. Exogenous testosterone provides in various preparations and each form carries various risks. Many men who take AASs are infertile because chronic AAS use suppresses spermatogenesis, this may relate to deficient of estrogen effect on the brain. Testosterone is aromatized to estradiol, and estradiol effects on the brain are very important for normal sexual function. Many AAS compounds are not aromatizable. In addition to oligomenorrhea and anovulatory infertility, women often develop clitoromegaly with chronic high-dosage of anabolic steroid use [11,12]. Testosterone is associated with hepatotoxic effects, the derivatives of alkylated testosterone are hepatotoxic especially when taken orally [13]. Regarding the effects of endogenous sex hormones

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on the urinary markers of nephrotoxicity, an experimental study in rats investigate that, there was a significant association between testosterone and urinary excretion of leucine aminopeptidase,  $\gamma$ -glutamyl transpeptidase, alkaline phosphatase and  $\beta$ 2-microglobulin, as biomarkers of kidney's proximal tubule<sup>[14]</sup>. The current study was designed to evaluate the effect of high doses of testosterone and nandrolone hormones on males sex hormones and sperm parameters in male rats as well as evaluate the effect on body, testes and epididymis weight after 35 days of treatment.

## Materials and Method

### 1. Blood collection

After 35 days of treatment by AAS, animals were anesthetized by intramuscular injection of ketamine 90 mg/Kg BW and xylazine 40 mg/kg BW. Blood samples were obtained via cardiac puncture technique from each anesthetized animals using disposable insulin needles. Sample were centrifuge at 2500 round per minute (rpm) for 15 minutes and then sera sample were stored in freeze at -18 c° till there use..

### 2. Hormonal estimation

Serum testosterone hormone concentration was determined by using Elisa kit(catlogMBS282195 from MyBiosource/USA),

Serum follicle stimulating hormone and Serum luteinizing hormone concentration were measured by using Elisa kits provide by( Human-Germany)

### 3. preparation of Epididymal tail suspension

The tail of epididymis of both sides were taken and embedded in one drop of phosphate buffer solution at 37 c° in a watch glass, and then the tail was cut into at least 200 section by micro-surgical scissors to perform the following microscopically examination on sperm characters<sup>[15]</sup>.

### 4. Sperm function test

#### 4.1.Sperm count

Sperm count was done according to<sup>[15]</sup>, by using hemocytometer (neubauer type). The hemocytometer slide were filled with 5 microliter of sperm suspension

and covered by cover slide, the sperm were counted in twenty-five small squares of the chamber. Estimation of sperm was made according to the following formula

$$\text{Sperm con.} = \text{No. of sperm} * 10$$

### 4.2Sperm motility

Sperm motility was assessed by methods described by [16]. Sperm motility percentage was assessed according to the method reported in this measurement is motility grade where the motility of sperm are divided into four different grade

**Grade A:** Sperm with progressive motility. These are the strongest and swim fast in a straight line.

**Grade B:** (non liner motility). These also move forward but tend to travel in a curved or crooked motion.

**Grade c:** These have non-progressive motility because they do not move forward despite the fact that they move their tails

**Grade D:** These are immotile and fail to move at all

### 4.3 Abnormal sperm morphology

To evaluate the abnormal sperm morphology, a drop of sperm suspension prepared was placed over the edge of slide and then a drop of easion-nigrosin stain was added and mixed, then two smear of each sample were prepared on microscopic slide. The smear of sperms were examined for abnormal morphology, 200 sperm were counted in each smears. The final percentage was calculated by estimating the average of two smears. Abnormal sperm morphology was calculated according to the following equation

$$\text{Percentage of abnormal sperm} = \frac{\text{No. of morphologically abnormal sperm}}{\text{Total sperm no.}} * 100$$

The morphologically abnormal sperms were estimated depending on sperm abnormality which had tapered head, tailless, coiled tail, bifurcated tail and broken tail<sup>[17]</sup>

### 4.4 Sperm viability

The assessment of alive and dead sperms was carried out by putting one drop of sperm suspension on a slide and a drop of easion-nigrosin stain was added and mixed.

Viable sperms repel the vital stain (easion-nigrosin), while dead sperm had lost the structural integrity of their plasma membrane and there for absorbed the dye ,for each sample two smears were made and 200 sperms were examined in each smear .Sperm viability was calculated as in the following equation

Percentage of dead sperms= No. of dead sperms/ total sperm No.\* 100

### 5. Testicular weight to body weight ratio

After the end of treatment period (35 days), rats were weighed, anesthetized by intramuscular injection of ketamine 90 mg/kg BW and xylazine 40 mg/kg BW), and tests were excised and weighted by sensitive balanced after being cleaned from accessory connective and adipose tissue testicular weight to body weight as in the following equation

Relative weight = testicular weight (gm)/body weight (gm) \*100

### Statistical Analysis

All data of the experiment were analyzed with one-way (ANOVA) by SPSS for windows (version 15) followed by LSD test. P<0.05 was assumed as statistically significant.

### Results

The results indicated that, the FSH, LH and testosterone hormones level showed statistically decrease while prolactin and estrogen hormones showed statistically increase after treatment by high doses of anabolic steroids compared with control, as shown in Table 1

**Table(1) the effect of some steroid hormone of body building on some sex hormones in male rats**

Groups	FSH MIU/ l	LH MIU/ l	Testosterone ng/ml	prolactin ng/ml	Estrogen mlu/ml
Control	3.10±0.32 a	2.88±0.23 a	3.06±0.47 a	10.86±1.42 b	8.34 ±1.57 c
Testosterone cypionate	1.53±0.45 b	0.92±0.11 c	2.96±0.87 b	15.59±2.08 a	14.65± 3.37 a
Nandrolone decanoate	1.56±0.09 b	1.02±0.02 b	2.52±0.09 b	14.25±1.55 a	12.25±2.54 b

Table 2 illustrate a significant decrease in total count ,motility and viability in male rats after treatment by high doses of anabolic steroids while abnormality showed statistically increase compared with control groups

**Table(2) the effect of some steroid hormone of body building on sperm characterization in male rats**

Group	Total count	Motility	Abnormality	Viability	MDA
control	44.23 ±5.84 a	92.14±4.72 a	7.39±1.36 c	90.12±5.62 a	4.35±1.43 b
Testosterone cypionate	35.46±4.55 b	83.31±4.32 b	18.37±0.95 b	80.45±6.34 b	6.93±1.58 a
Nandrolone decanoate	30.76±3.52 c	79.43±1.67 c	20.38±2.62 a	75.33±5.28 c	7.43±0.96 a

According to the body weight, the results indicated that, the high dose of steroid hormone cause a decrease in body, testes and epididymis weight as compared to the control group as shown in table 3

**Table(3) the effect of some steroid hormone of body building on weight in male rats**

groups	Body weight (g)	Testes weight (g)	Epididymis weight (g)
Control	240.94±3.07 a	5.56±0.40 a	2.81±0.13 a
Testosterone cypionate	239.43±4.22 a	3.34±0.28 b	1.23±0.40 b
Nandrolone decanoate	237.58±2.64 a	3.92±0.68 b	1.09±0.60 b

### Discussion

The objective of this paper is to evaluate the effect of high doses of AAS on FSH, LH, testosterone, prolactin and estrogen hormones, as well as estimate the effect on some sperm parameters and body, test and epididymis weight in male rats after 35 days of treatment. The present finding showed a significant reduction in FSH, LH and testosterone hormones levels while prolactin and estrogen showed significantly increase compared with the control groups. This may be due to the temporary impairment of AAS in the pituitary function reflected in decreased LH and FSH hormones levels and hence reduced testosterone levels [18]. The use of exogenous hormone is thought to inhibit the production of gonadotropin-releasing hormone, which reduces the release of luteinizing hormones and subsequently reduces the production of testosterone [19]. It was again supported by an animal model study conducted in India, which showed adverse effects on testes caused by intraperitoneal injection of AAS, also these results investigated detrimental changes in the seminiferous epithelium of the testes and reduction in tubular diameter, resulting in lower pituitary gonadotropin (LH and FSH) and serum testosterone levels [20]. Furthermore, these effects result from that, high doses of AAS derivatives induces negative feedback on the hypothalamic-pituitary axis and reducing the secretion of LH and FSH. Spermatogenesis is dependent on the action of intratesticular testosterone concentration and FSH on Sertoli cells. Within the seminiferous tubules, only Sertoli cells possess receptors for FSH and testosterone. Numerous

signaling pathways are activated when FSH binds to FSH receptors on these cells, and acts synergistically with testosterone to increase the efficiency of spermatogenesis and fertility [21]. Infertility after AAS abuse commonly presents as azoospermia and oligozoospermia with abnormalities in sperm morphology and motility [22]. The hyperprolactinemia could also be the results of elevation in the prolactin levels through increased sensitivity to prolactin-releasing factor and prolactin inhibitory factor and from altered regulation of serotonergic and oradrenergic neurons which in turn modulate dopamine release. Adding to that, seizures induce discharges in the neuron cell which stimulate the hypothalamus and increased prolactin secretion by the pituitary gland [23]. According to the present study there was a significant decrease in sperm count, motility and viability, the best explanation of this result may be related to apoptosis processes. Apoptosis has been reported to play an important role in the regulation of germ cell populations in the adult testis. The main basic cells which interrupted spermatogenesis are the Leydig, Sertoli cells and the germ cells. Each of these cell types in animals can be selectively affected by high doses of AAS leading to apoptosis [24]. Recently, the correlation between apoptosis and high AAS doses has been assessed in animal studies. AAS led to a deficient in the Sertoli cells activities, which lead to incomplete meiosis, failure of spermatogenesis, and stop of transition of spermatocytes to spermatids [25]. Shokri et al. report a significant increase in the rate of apoptosis of spermatogenic cells after nandrolone administration. Moreover, AAS

adversely affected sperm parameters by reducing sperm concentration and motility, increasing abnormal sperm morphology, sperm DNA damage and causing damage in the testicular structure of male rats, the mechanism of AAS toxicity in the male reproductive system arises from the alterations in hormonal status and promotion of oxidative stress [26].

Our finding clarified that, the body, test and epididymis weight decreased after 35 days of treatment by AAS, these results may be related to that, an excessive concentration of testosterone can inhibit growth and weight gain due to decreased appetite, electrolyte imbalance and increased lipid oxidation [27]. A study reported by [28] indicated that androgens inhibit the ability of some fat cells to store lipids by blocking a signal transduction pathway that normally supports adipocyte function. This might reduce the body weight gain. Also, AAS can decrease fat by increasing basal metabolic rate. The present work illustrated the extensive effects of AAS on male reproductive system of the rats. For this reason, drug-prevention counseling to athletes and teenagers is focused on the use of anabolic steroids.

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**Ethical Clearance:** Not required

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