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EFFECTS OF THE ANABOLIC STEROID, NANDROLONE-DECANOATE ON REPRODUCTIVE PERFORMANCE OF MALE RABBITS

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ABSTRACT

Nandrolone decanoate (ND) is one of the most commonly abused anabolic-androgenic steroid compounds in the world. Owing to the abuse of ND among rabbit breeders to increase body weight together with the shortage in studies that deal with ND administration in rabbits, the present study was designed to evaluate the effects of ND on reproductive performance of rabbits. Four groups of adult male rabbits were used; the first one was control, while other groups were injected with ND in two different doses at day one and at day 15 of the experimental period (8 weeks). The obtained results cleared that ND had, in general, dose-dependent harmful effects. Administration of ND led to significant decrease of rabbit body weight; arrested spermatogenesis that was accompanied by sever reduction of inseminated spermatozoa and their motility reached to oligospermia; reduction of seminal plasma biomarkers, alkaline phosphatase and fructose; and markedsuppression of testicular gene expressions,androgen receptor(AR) andcytochrome P450(CYP3A6)genes. It could be concluded that ND administration, specifically in high dose, is not recommended for use as an anabolic in male rabbit breeding. Further studies are needed to determine ND tissue residues and safety limits for use.

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INTRODUCTION

Fertility of male rabbitsis considered the most important economic factors in rabbit production. That may be affected by many diverse factors including drugs (**1**). The most relevant parameters correlated with the fertility rate are the number of inseminated spermatozoa and their motility, but the use of a single attribute is not sufficiently accurate to predict the fertilizing ability of the semen **(2-3).** So

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the biochemical analysis of secretion components from prostate, seminal vesicles and epididymis in semen evaluation is required as markers to givea good mirror about the functional state of these organs. These markers include fructose as seminal vesicles marker, and other markers are measured using enzymatic assays **(4).**

Anabolic Androgenic Steroids (AAS) are synthetic derivatives of the endogenous primarily male steroid hormone, testosterone. They have both anabolic and androgenic properties **(5)**. Due to theiranabolic effects AAS have not always been used for pure medical purposes **(6**). One of the most commonly abused AAS in the world is the 19-nor-testosterone derivative, nandrolonedecanoate (ND**) (7).** In humans, when ND isintramuscularly administered, it has a half-life in the muscle of approximately six days and the duration of its effect is approximately three weeks (**8).** It has been used to treat chronic debilitating diseases (human immunodeficiency virus (HIV), acquired immunodeficiency syndrome (AIDS)**(9)** and to be applied in clinical practice (trauma, burns and various forms of anemia) **(10).**However, due to its beneficial properties it has become very popular among athletes in their attempt to increase their strength, to accelerate muscle development, to promote recovery or to enhance aggression (**11).** Supra-pharmacological doses of AAS that are administered by abusers are 10 to 100 times the therapeutic dose **(12).**

As in other tissues, Androgen action in the testis is mediated through androgen receptor (AR) transcriptional activation**.** The androgen receptor (AR) (NR3C4, nuclear receptor subfamily 3, group C, gene 4) belongs to the steroid hormone group of nuclear receptors. The binding of the AR to its native ligand 5α-dihydrotestosterone (DHT) and testosterone initiates male sexual development and differentiation **(13).** As endogenous testicular androgens maintain AR expression, testosterone withdrawal leads to disruption of spermatogenesis **(14).**Side effects of AAS abuse at supra physiological dosesaffect almost all body systems as almost all major tissues in the body have androgen receptors**(15).**

Cytochrome P450 enzymes play crucial roles in the metabolism of drugs and the biosynthesis or degradation of endogenous substrates, such as steroids. 3 Members of the cytochrome P450 3A (CYP3A) subfamily are arguably the most important enzymes that participate in the oxidative metabolism of drugs **(16).** They are predominantly expressed in the liver; however, many steroid sensitive extra hepatic tissues expressed this enzyme. Altered CYP3A metabolism impacts the presentation of prostate cancer and androgen-mediated prostate carcinogenesis **(17)**.

The use of ND among rabbit breeders in their attempt to increase body weight together with the shortage in studies that deal with ND administration in rabbits paid us to carry out this work aiming to evaluate the effects of low and high doses of ND on reproductive performance of rabbits. Such evaluation was through the investigations of libido and semen properties; seminal plasma biomarkers ALP, AST and fructose; testicular gene expression of AR and CYP3A6; and testicular histopathological properties as well as body weight values.

MATERIAL AND METHODS

Experimental animals: A total number of 40 sexual mature bucks and 2 monoparous does of New Zealand White (NZW) rabbits were used in the present work. Rabbits raised on rabbit unit of **Animal Reproduction Research Institute, Agricultural Research Centre**. At beginning of the study, the rabbits age were 8to 10 months and their weigh

ranged from 2500 to3000 gm. Animals were fed *ad libitum* a commercial diet which was covering the nutritional requirements of the buck and different physiological status of doe rabbits according to **National Research Council** (NRC)**(18)** recommendations. All animals were kept under the same managerial and hygienic conditions and were raised in wired batteries in a windowed Rabbi try with natural ventilation.

Drug: Nandrolone decanoate was available in 1 ml ampoule: Nandurabolin 25 mg & 50 mg / ml, oily solution for I.M. injection (Nile Pharmaceutical Co.).

Experimental design: The animals were randomly divided into four different groups, each of 10 animals. Group A: control rabbits received 0.5 ml saline, groups B and C: received 1 and 3 mg/kg b.w.t. respectively (low doses) of ND **(19),** group D: received 10 mg/kg b.w.t (high doses) of ND **(19).** The injection of each, saline or drug was intramuscularly at day one and at day 15 of the experimental period (8 weeks). The rabbits were then left untreated for 6 weeks .Body weight (g) was recorded weekly till the end the experimental period. Libido (reaction time /seconds) was calculated according to the method described by **Daader et al (20-21).**

Semen collection and evaluation: Semen was collected artificially using an artificial vagina twice a week for up to 4 weeks before experimental period and 8 weeks (experimental period) as described by(**22)**. Two ejaculates were collected for each buck, with an interval of 30 min in between. One served as row semen sample for semen analysis. Ejaculate volume /ml, percentages of sperm-cell concentration (N x 10^6/ ml); advanced sperm motility, dead and abnormal spermatozoa were estimated according to **(23).** The other consecutive semen was centrifuged at 6000 g for 15 minutes and the supernatant was removed and stored at –20 ^{0}C until enzymatic assay.

Enzymatic assay: Alkaline phosphates (ALP) and aspartate aminotransferase (AST) activities were determined according to **(24)**, and **(25)**, respectively. Fructose utilization was determined according to **(26)**.

Gene expression analysis:

The gene expression of AR and CYP 3A6 genes were determined in testicular tissue isolated and kept in liquid nitrogen using Real Time-PCR technique using suitable kits provided by RNeasy mini kit (Cat. No. 74104, Qiagen, Heidelberg, Germany) for RNA extraction, Nano Drop® Technologies, Wilmington, Delaware, USA for detection of purity, QIAGEN 2 Step RT-PCR KitQiagen , Heidelberg , Germany(Cat. No. 11146040)for cDNAsynthesisand SYBR ® Green PCR mix with ROX (Bio-Rad, California, USACat. No.0252)for real time PCR.The quantitative folds changes in mRNA expression were determined relative to the housekeeping controls ß-actin mRNA levels in each corresponding group and calculated using the 2-DD CT method. The primer sequences were listed in Table (I).

Histopathological analysis:

The other testicle was harvested, fixed in 10 % formalin, prepared for histopathological examination, stained by H &E dyes **(27),** graded and scored according to **(11)**.

Statistical analysis:

A repeated measures analysis of variance (ANOVA) model with SPSS (IBM SPSS, version 23)was used for statistical analysis of the obtained data.The Kruskal-Wallis non-parametric ANOVA with a Dunn’s multiple comparison tests was used to evaluate statistical differences in histpathological scores of the testis. Correlations were assessed using the Pearson's correlation coefficient.Correlation is significant at the 0.01 level (2-tailed).

RESULTS

Effect of ND on body weight:

As illustrated in Table (II):Nandrolone decanoate administration showed significant decreases in the body weight regardless of the dose and the duration used.

Effect of ND on semen quality:

Ejaculate volume/ml, sperm individual motility, sperm concentration (count), and live sperm percentages in all ND-treated groups were significantly decreased ($P< 0.05$). In group D (10mg /kg) showed marked oligospermia at the 8^{th} week. However the reaction time and sperm abnormality percentage were increased ($P < 0.05$) in treated groups (Table III &Fig. 2-4).

Effect of ND on Seminal plasma biochemical analysis:

Positively correlation was detected between seminal plasma ALP and fructose concentration. Both were decreased significantly ($P<0.05$) in all ND-treated groups, B, C and D. In contrast, SP-AST activity was increased gradually till the 3^{rd} week of the experiment then it drastically declined to values lower than the control ($P < 0.05$) and under the base line (Table IV).

Effect of ND on molecular investigation:

Marked suppression was detected in relative gene expression of both AR and CYP 3A6 genes in testicular rabbit tissue given ND by low and higher doses (Fig: 5&6) .Moreover, an inverse correlation between AR expression and spermatogenesis was observed.

Histopathological study:

Nandrolone decanoate had a dose dependent effect on histological abnormalities in the testis including arrested spermatogenesis, seminiferous tubular distortion, giant cell formation and interstitial fibrosis (Figs.7-10).

DISCUSSION

The abuse of steroidal preparations became field problem;thus strict studies are required to clarify the hazards of misusing of these. This work was planned to study the molecular-biochemical effects of administration of nandrolonedecanoate in different doses (1, 3 and 10 mg/Kg BW) for different periods (1 to 8 weeks) especially on bucksemen.

Unfortunately, the gross body weight was significantly decreased in ND-administedrabbits even in low doses.This decrease may be due to the lipolytic effect of testosterone (**28**) and consequently the decrease in subcutaneous and intramuscular fat mass as previously reported in rats by **(29).** Moreover, **(30)** found that the use of supra-physiological doses of AAS can inhibit body growth and weight gain in rat. Other studies, **(31-32)** established that ND treatment in rat usually causes a significant decrease of total body weight. In horse, 6.8% decrease in the body weight was obtained under the effect of another anabolic steroid, Boldenone (BOL) **(33).**

Contradictory, an increase in body weight of ND-treated female rabbit, was reported by **(34)** based on the appetizer and anabolic effect of ND which stimulate protein synthesis **(35)**. And no significant effect on body weight was obtained in ND treated rat **(36-37)** or in male rabbit administered ND, BOL and ND + BOL **(38**).

Multiple factors, including dose, administration time-course, composition, and target-tissue metabolism have been contributed the AAS effects on body weight gain. Furthermore, androgens are known to cause alterations in many physiological processes whish act as predisposing factors to change animal weight gain during the treatment period **(39).**

Reproductive performance:

Negative effect of Nandrolone decanoate administration on libido(reaction time) and semen quality evaluation may be due to the testis dysfunction and/or the decreased serum testosterone. Moreover, hypogonadotrophic-hypogonadism coupled with decreased serum testosterone concentrations represents the main adverse effect result from AAS on the pituitary-gonad axis **(31&40-41)**. Meanwhile ejaculate volume, sperm motility, sperm count and live sperm % of ND-treated adult male rabbits showed significant reduction that may be considered an oligospermia. This decrease in sperm count may reflect the inhibition effect on germ cell development which is related to the degree of FSH, LH and testosterone suppression (**37&42).**

Sperm morphological abnormalities may result in the generation of ROS including superoxide anion (O2.) and H_2O_2 which are thought to be responsible for loss of sperm motility and the other sperm functions **(43-45)**

Seminal plasma ALP and SP fructose were decreased in ND-treated groups that may indicate the close relationship between semen fructose, ALP and the androgenic activity of the male (**46-47).**Alkaline phosphatase is essential for fructose synthesis in the accessory genital glands through dephosphorylation of fructose–6–phosphate **(48).**

On the other hand, the observed increase of SP AST result from the loss of sperm membrane integrity and sperm damage (**4**). By the 4^{th} week SP AST decreased to an oligospermic extent.

The decrease in the gene expression of AR and CYP3A6 can be explained in the light of obtained results matched with histological degenerative changes and atrophy of testis. In support,(**37)** found that, ND-administration in male rat caused a reduction in the number of Sertoli cells expressing AR gene**.**

Histopathologyical findings clearly demonstrated that ND showed retrogressive histological abnormalities in the testis in a dose-dependent manner including arrested spermatogenesis, seminiferous tubular distortion, giant cell formation and interstitial fibrosis. These findings can explain the decreased serum testosterone concentrations, the negative adverse effect ofAAS administration on the pituitary-gonad axis **(31&40-41)**

In conclusion, this study has provided new evidences that pointed to the harmful effects of the random use of ND on adult male rabbits. Administration of ND not only decreased body weight but also severely reduced fertility. So, ND specifically in high doses is not recommended for use as anabolic in male rabbit breeding. Further studies are needed to determine the ND tissue residues and to investigate the safety limits for use in rabbits.

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Table (I): Primer oligonucleotide sequences AR, CYP3A6 and ß-actin genes and expected product sizes for the expected amplified genes

Gene name	Forward primer	Reverse primer	Size (bp)	Reference
AR	TCCACCTCCTCCAAGGACAGT	CCAACGCCTCCACACCCAA	112	
CYP3A6	TCCTTCATTATGCATTTGTTGGCC	ACCACCATGTCCAGATATTCCATC	137	**(16)**
β-Actin	TCCTTCCTGGGCATGGAGTC	GGATGTCCACGTCGCACTTC	76	

Table (II): Body weight (g) in male rabbits administered ND by 1, 3 or 10 mg/kg:

Parameters	Duration / Dose		0 w	1st w	2nd w	3rd w	4th w	5th w	6th w	7th w	8th w
Body weight (g)	*A 0.5ml saline		2770 ± 50^{a}	2785 ± 56^{a}	2810 ± 49^{a}	2790 ± 45^{a}	2780 ± 43^{a}	2810± 46^{a}	2780 ± 48^{a}	2770 ± 48^{a}	2800 ± 49^{a}
	B 1mg/kg	ND	2750 ± 61^{a}	2700 ± 61^{b}	2690 ± 66^{b*}	2680 ± 62^{b*}	2670 ± 65^{b*}	2670 ± 65^{b*}	2670 ± 65^{b*}	2670 ± 65^{b*}	2670 ± 65^{b*}
	C 3mg/kg		2760 ± 50^{a}	2660 ± 50^{bc*}	2630 ± 50^{c**}	2600 ± 47^{c**}	2590 ± 44^{c**}	2580 ± 42^{c**}	2580 ± 42^{c**}	2580 ±42^{c**}	2580 ± 42^{c**}
	D 10mg/kg		2780 ± 76^{a}	2640 ± 78^{c**}	2580 ± 83^{c**}	2560 ± 72^{c**}	2550 ± 75^{c**}	2540 ± 78^{c**}	2530 ± 76^{c**}	2530 ± 76^{c**}	2530 ± 76^{c**}

* A control ND Nandrolone decanoate W week

Means ± S.E within the same raw carrying different superscripts are significantly different at ($P < 0.05$) based on LSD test. Significant (*) and highly significant (**) difference at $p< 0.005$ & $P< 0.001$, respectively compared to pre-treated rabbits (week 0) in the same group

Table (III): Libido (reaction time), semen volume (ml), and live sperm % of adult male rabbits administered ND by 1, 3 or 10 mg/kg:

Semen analysis	Duration / Dose		0 w	1st w	2nd w	3rd w	4th w	5th w	6th w	7th W	8th w
Reaction time (seconds)	*A 0.5ml saline		9.8 ± 0.1^{a}	9.7 ± 0.2^{c}	9.6 ± 0.2^{c}	9.7 ± 0.2^{d}	9.8 ± 0.2^{d}	9.7 ± 0.2^{d}	9.8 ± 0.1^{d}	9.6 ± 0.2^{d}	9.7 ± 0.2^{d}
	B 1mg/kg	ND	9.7 ± 0.3^{a}	10.0 ± 0.3^{bc}	10.2 ± 0.3^{c}	10.4 ± 0.2^{c*}	10.7 ± 0.2^{c*}	11.1 ± 0.3^{c**}	11.3 ± 0.2^{c**}	11.7 ± 0.2^{c**}	12.0 ± 0.1^{c**}
	C 3mg/kg		9.6 ± 0.2^{a}	10.4 ± 0.2^{ab*}	10.9 ± 0.2^{b**}	11.4 ± 0.2^{b**}	11.9 ± 0.2^{b**}	12.4 ± 0.2^{b**}	13.0 ± 0.2^{b**}	13.5 ± 0.2^{b**}	14.0 ± 0.2^{b**}
	D 10mg/kg		9.7 ± 0.2^{a}	10.9 ± 0.2^{a**}	11.7 ± 0.2^{a**}	12.3 ± 0.2^{a**}	11.9 ± 0.2^{a**}	13.5 ± 0.2^{a**}	14.4 ± 0.2^{a**}	15.1 ± 0.2^{a**}	15.5 ± 0.2^{a**}
Semen	*A 0.5ml		0.7	0.78	0.78	0.79	0.78 ±	0.78 ±	0.78 ±	0.79	0.78

volume (ml)	saline		8± 0.0 3[a]	± 0.02[a]	± 0.02[a]	± 0.02[a]	0.01[a]	0.01[a]	0.02[a]	± 0.01[a]	± 0.02[a]
	B 1mg/kg	ND	0.77± 0.02[a]	0.76 ± 0.03[ab]	0.75 ± 0.03[a]	0.74± 0.03[ab]	0.72 ± 0.03[b]	0.71 ± 0.03[b]	0.71 ± 0.03[b]	0.71 ± 0.02[b]	0.71 ± 0.02[b]
	C 3mg/kg		0.76± 0.02[a]	0.74 ± 0.02[ab]	0.72± 0.02[ab]	0.70± 0.02[bc]	0.68± 0.01b[bc]*	0.67± 0.02b[bc]*	0.66± 0.02b[bc]*	0.65± 0.02[bc]*	0.64± 0.02[c]**
	D 10mg/kg		0.78± 0.02[a]	0.71 ± 0.02[b]*	0.66± 0.02[b]*	0.64± 0.02[c]*	0.63± 0.02[c]**	0.62± 0.01[c]**	0.61± 0.02[c]**	0.60± 0.02[c]*	0.59± 0.02[c]**
Live sperm %	*A 0.5ml saline		92.8 ± 0.6[a]	92.8 ± 0.6[a]	92.7 ± 0.6[a]	92.8 ± 0.4[a]	92.3 ± 0.5[a]	92.4 ± 0.6[a]	92.5 ± 0.3[a]	92.4 ± 0.4[a]	92.8 ± 0.5[a]
	B 1mg/kg		92.6 ± 0.3[a]	88.6 ± 0.8[b]*	86.6 ± 0.5[b]*	85.2 ±1.0[b]*	83.8 ± 0.9[b]**	78.2 ± 0.5[b]**	76.0 ± 0.5[b]**	71.0 ±0.5[b]*	56.6 ±1.0[b]*
	C 3mg/kg		92.8 ± 0.5[a]	92.8 ± 0.5[c]	79.6 ±0.5[c]*	73.7 ± 0.5[c]**	71.3 ± 0.7[c]**	65.5 ± 0.9[c]**	58.4 ± 0.7[c]**	51.6 ± 0.9[c]**	42.6 ± 1.4[c]**
	D10 mg/kg		92.7 ± 0.8[a]	92.7 ± 0.8[a]	73.4 ±0.8[d]*	65.0 ±0.9[d]*	61.4 ± 1.1[d]**	54.0 ± 1.2[d]**	44.1 ± 2.0[d]**	27.4 ±2.1[d]*	------ §

* A control ND Nandrolone decanoate § Oligospermia
W week
Means ± S.E within the same raw carrying different superscripts are significantly different at (P < 0.05) based on Least Significant Difference (LSD) test. Significant (*) and highly significant (**) difference at p< 0.005 & P< 0.001, respectively compared to pre-treated rabbits (week 0) in the same group.

Table (IV): Seminal plasma ALP, AST activities (U/L) and fructose concentration of adult male rabbits administered ND by 1, 3 or 10 mg/kg:

Parameters	**Duration / Dose**		**0 w**	**1st w**	**2nd w**	**3rd w**	**4th w**	**5th w**	**6th w**	**7th w**	**8th w**
SP ALP (U/L)	*A 0.5ml saline		1.1[a] ± 48.9	48.8 ± 1.2a	48.6 ± 1.2[a]	48.5 ± 1.4[a]	48.6 ± 1.4[a]	48.6 ± 1.3[a]	48.4 ± 1.4[a]	48.7 ± 1.0[a]	48.9 ± 1.3[a]
	B 1mg/kg	ND	48.2 ± 0.5[a]	45.7 ± 0.6b	42.9 ± 0.6[b]*	39.3 ± 0.8[b]*	36.7 ± 1.4[b]*	33.7 ± 1.9[b]**	31.1 ± 2.2[b]*	29.6 ± 2.2[b]**	27.7 ± 1.5[b]**
	C 3mg/kg		48.8 ± 2.2[a]	44.7± 2.4bc*	40.4 ± 2.6[b]**	36.2 ± 2.5[c]**	33.0 ± 2.0[c]**	29.7 ± 1.9[c]**	26.3 ± 1.2[c]**	25.0 ± 1.0[c]**	22.2 ± 0.6[c]**

	D 10mg/kg		48.2 ± 1.4[a]	41.8± 1.6c[**]	35.5 ± 1.3[c**]	29.1 ± 1.3[d*] *	26.7 ± 1.4[d*] *	24.4 ± 1.3[d**]	21.2 ± 0.7[d*] *	17.9 ± 0.6[d**]	17.5 ± 1.1[d**]
SP AST (U/L)	*A 0.5ml saline		31.4 ± 0.6[a]	31.4 ± 0.5c	31.3 ± 0.5[d]	30.9 ± 0.5[d*] *	31.2 ± 0.6[d]	31.1 ± 0.5[b]	30.9 ± 0.5[b]	31.0 ± 0.4[a]	31.3 ± 0.4[a]
	B 1mg/kg	ND	31.2 ± 0.4[a]	31.6 ± 0.7c	39.9 ± 1.4[c**]	50.1 ± 3.2[c**]	38.1 ± 1.8[c**]	25.3 ± 1.4[c*]	23.8 ± 1.7[c**]	22.3 ± 0.8[b**]	20.2 ± 0.3[b**]
	C 3mg/kg		31.7 ± 1.2[a]	38.± 1.2b[**]	46.8 ± 2.4[b**]	61.8 ± 0.9[b*] *	50.8 ± 1.4[b*] *	30.7 ± 1.8[b]	22.9 ± 2.1[c**]	16.2 ± 1.6[c**]	13.0 ± 1.2[c**]
	D 10mg/kg		31.6 ± 0.9[a]	41.8± 2.1a[**]	55.1 ± 1.7[a**]	78.0 ± 2.7[a**]	60.4 ± 1.6[a**]	49.3 ± 2.2[a**]	38.3 ± 1.7[a**]	16.2 ± 1.3[c**]	9.9 ± 0.9[d**]
Fructose (mg/dL)	*A 0.5ml saline		198.7 ± 6[a]	198.5 ± 7[a]	198.6 ± 5[a]	198.5 ± 4[a]	198.0 ± 6[a]	198.2 ± 3[a**]	198.5 ± 4[a]	198.2 ± 4[a]	198.5 ± 5[a]
	B 1mg/kg	ND	198.0 ± 6[a]	191.9 ± 6[a]	170.7 ± 9[b**]	150.7 ± 8[b**]	136.5 ± 7[b**]	125.5 ± 5[b**]	119.5 ± 4[b**]	111.0 ± 4[b**]	105.0 ± 3[b**]
	C 3mg/kg		198.5 ± 7[a]	179.0 ± 8[b*]	148.0 ± 9[c**]	125.0 ± 9[c**]	106.0 ± 8[c**]	85.0 ± 8[c**]	71.5 ± 8[c**]	61.0 ± 6[c**]	51.0 ± 4[c**]
	D 10mg/kg		198.7 ± 4[a]	162.0 ± 5[c**]	116.0 ± 3[d**]	94.6 ± 3[d**]	74.5 ± 2[d**]	61.5 ± 1[d**]	53.5 ± 2[d**]	44.7 ± 2[d**]	29.5 ± 2[d**]

* A: control ND: Nandrolone decanoate W: week

Means ± S.E within the same raw carrying different superscripts are significantly different at ($P < 0.05$) based on Least Significant Difference (LSD) test. Significant (*) and highly significant (**) difference at $p< 0.005$ & $P< 0.001$ respectively compared to pre-treated rabbits (week 0) in the same group.

SP ALP Seminal plasma alkaline phosphatase

SP AST Seminal plasma aspartate aminotransferase

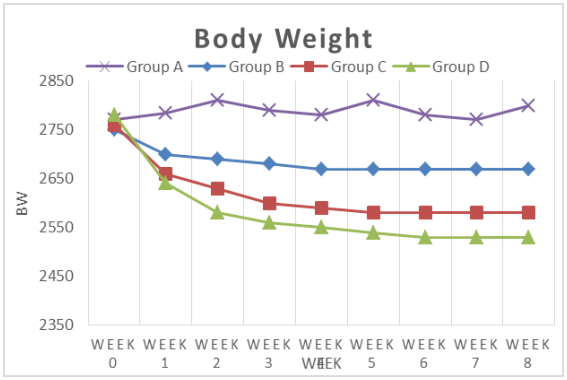

Fig. (1): Body weight (g) in male rabbits administered ND by 1, 3 or 10

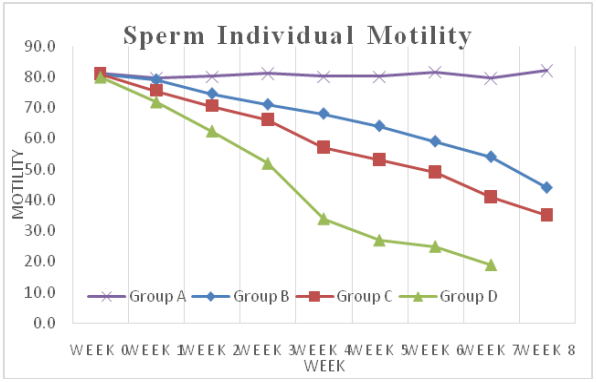

Fig. (2):Effect of ND injection (1, 3 and 10 mg/kg) on sperm individual motility (%) of adult male rabbits

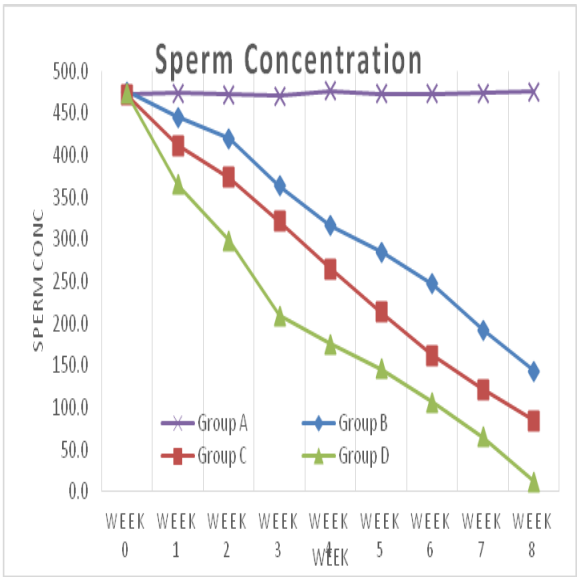

Fig. (3): Effect of ND injection (1, 3 and 10 mg/kg) on sperm concentration (×106) of adult male rabbits (n=10)

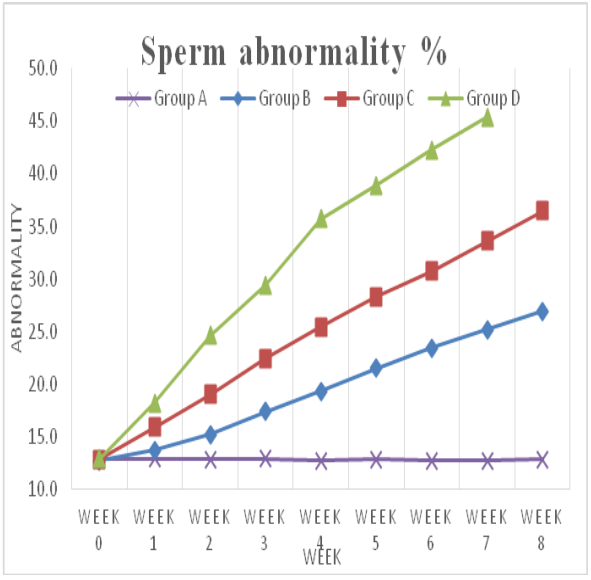

Fig. (4):Effect of ND injection (1, 3 and 10 mg/kg) on sperm abnormality percentage (%) of adult male rabbits (n=10)

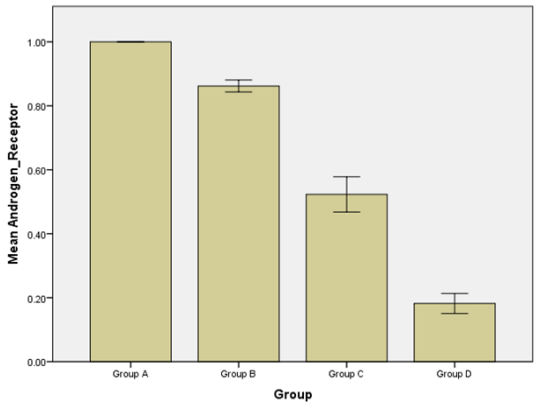

Fig. (5): Relative gene expression of androgen receptor (AR) gene of adult male rabbits under the effect of nandrolone decanoate injection (1, 3 and 10 mg/kg) (n=10)

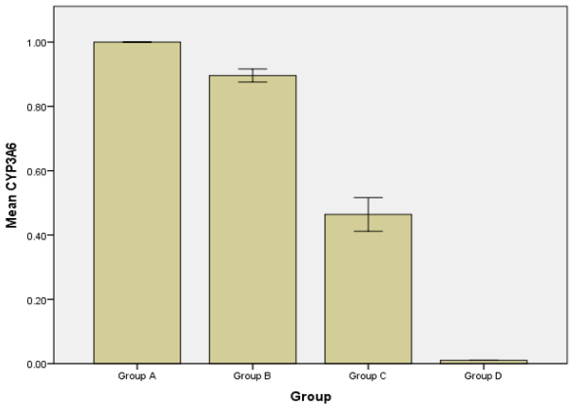

Fig. (6): Relative gene expression of cytochrome P450 3A6 (CYP 3A6) gene of adult male rabbits under the effect of Nandrolone decanoate injection (1, 3 and 10 mg/kg) (n=10)

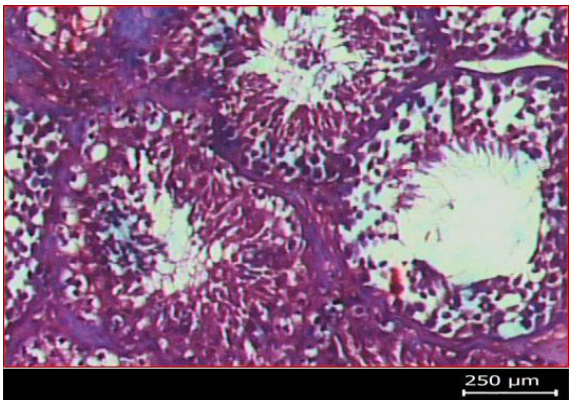

Fig.(7): Photograph of cross section of the testis of group A. showed normal spermatogenesis, normal architecture and had no abnormality

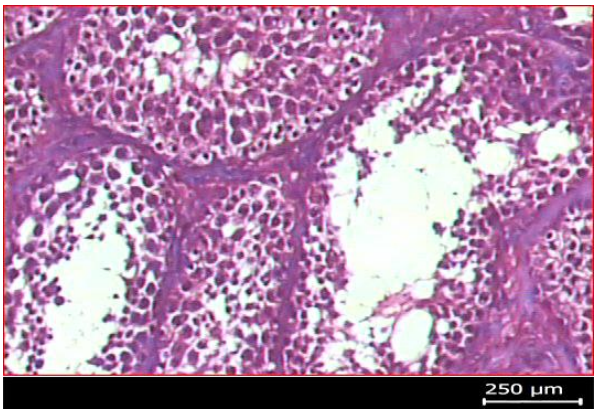

Fig. (8): Photograph of cross section of the testis of group B. showed complete spermatogenesis with disorganization, sloughing, and low to moderate sperm scores with normal Leydig cells, mild tubular

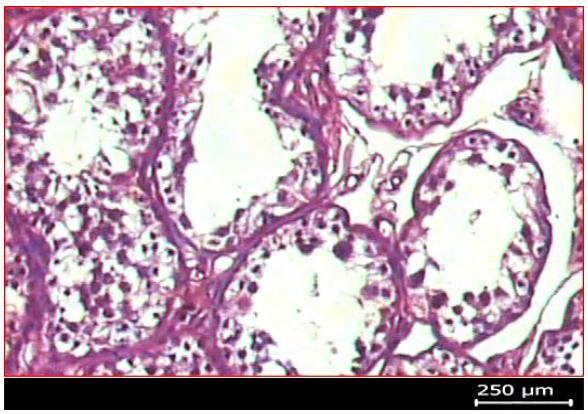

Fig.(9):Photograph of cross section of the testis of group C. showed arrested spermatogenesis or interruption of normal germ cell maturation at the level of spermatogonia, moderate tubular distortion, and moderate interstitial fibrosis.

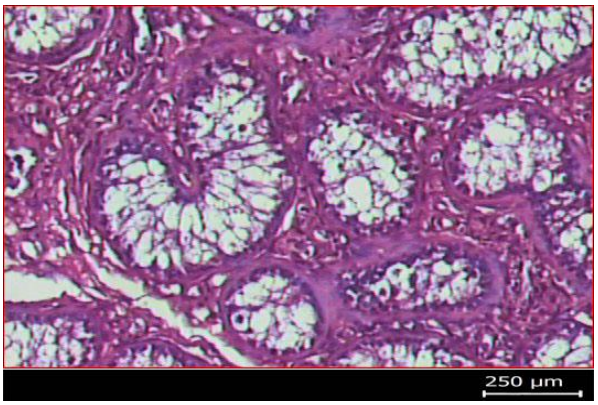

Fig.(10): Photograph of cross section of the testis of group D showed germ cell aplasia and sertoli cell only (SCO) pattern, severe tubular distortion, and severe interstitial fibrosis