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Dose-Dependent Reproductive Toxicity of Oxandrolone in Adult Male Wistar Rats: Endocrine Disruption, Spermatogenic Impairment, and Histopathological Alterations

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ABSTRACT: **Objective**: This study aimed to investigate the effects of repeated intraperitoneal administration of oxandrolone on reproductive hormones, epididymal sperm parameters, and testicular histopathology in adult male Wistar rats.

Materials and Methods: Twenty-four adult male Wistar rats, aged 10–12 months, were randomly divided into four groups (n = 6 per group). The control groups received distilled water or dimethyl sulfoxide (DMSO), whereas the treatment groups received oxandrolone at doses of 0.82 and 1.62 mg/kg body weight by intraperitoneal injection once daily for four weeks. Serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels were determined using enzyme-linked immunosorbent assay (ELISA). Epididymal sperm count was assessed using a Neubauer hemocytometer, and sperm abnormalities were evaluated following eosin staining. Testicular tissues were examined histologically using hematoxylin and eosin (H&E) staining.

Results: Oxandrolone administration resulted in a significant, dose-dependent reduction in serum testosterone, LH, and FSH levels compared with the control groups Oxandrolone-treated rats also exhibited a reduction in epididymal sperm count and an increase in the frequency of sperm morphological abnormalities. Histopathological examination revealed degeneration of the seminiferous tubules, sloughing of the germinal epithelium, depletion of spermatogenic cells, nuclear hypochromasia, and a reduction in the number of mature spermatozoa. These histological alterations were more pronounced in rats receiving the higher oxandrolone dose.

Conclusions: Repeated intraperitoneal administration of oxandrolone adversely affected male reproductive function by suppressing reproductive hormone levels, impairing sperm production and morphology, and inducing structural alterations in the testes. The findings suggest that prolonged or inappropriate oxandrolone exposure may compromise male fertility and highlight the importance of appropriate medical supervision during its therapeutic use

1. INTRODUCTION

Anabolic-androgenic steroids (AAS) are synthetic derivatives of testosterone that have been developed to enhance protein synthesis, increase skeletal muscle mass, and improve physical performance while retaining varying degrees of androgenic activity. Although these compounds have recognized therapeutic applications in the management of muscle wasting, severe burns, chronic catabolic disorders, and delayed puberty their non-medical use has increased markedly among athletes, bodybuilders, and recreational exercisers seeking rapid improvements in muscular appearance and athletic performance. The

widespread misuse of AAS has become an important public health concern because supraphysiological doses are associated with serious adverse effects involving the cardiovascular, hepatic, endocrine, and reproductive systems. Moreover, growing evidence indicates that chronic AAS exposure may profoundly impair male reproductive function by disrupting hormonal homeostasis and testicular physiology **[1,2]** Oxandrolone is a synthetic anabolic-androgenic steroid derived from 5α-dihydrotestosterone (DHT). Structural modifications, including oxygen substitution at the C2 position and 17α-methylation, increase its anabolic activity while reducing its androgenic effects and improving oral bioavailability Because of these pharmacological characteristics, oxandrolone has been prescribed for the treatment of severe weight loss, extensive burns, chronic catabolic conditions, and muscle wasting associated with prolonged illness. Despite these therapeutic applications, its misuse at supraphysiological doses has become increasingly common among athletes and recreational bodybuilders. Accumulating evidence indicates that prolonged oxandrolone exposure can adversely affect endocrine homeostasis and male reproductive function through suppression of the hypothalamic–pituitary–gonadal (HPG) axis and impairment of testicular physiology **[3]** The hypothalamic–pituitary–gonadal (HPG) axis is the principal endocrine pathway responsible for regulating male reproductive function. Under physiological conditions gonadotropin-releasing hormone (GnRH) released from the hypothalamus stimulates the anterior pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH) Luteinizing hormone promotes testosterone synthesis by Leydig cells, whereas FSH acts on Sertoli cells to support spermatogenesis and maintain the maturation of germ cells within the seminiferous tubules. Administration of exogenous anabolic-androgenic steroids suppresses the HPG axis through a negative feedback mechanism, leading to reduced secretion of LH and FSH, decreased intratesticular testosterone concentration, and impairment of normal spermatogenesis. Consequently, prolonged steroid exposure may result in endocrine dysfunction, testicular atrophy, and reduced male fertility **[3,4]**. Spermatogenesis is a highly coordinated biological process that occurs within the seminiferous tubules and involves the proliferation, meiotic division, and differentiation of germ cells into mature spermatozoa. This process requires the integrated action of testosterone, LH, and FSH to maintain normal germ cell development and sperm maturation. Disruption of hormonal regulation or direct injury to the seminiferous epithelium can impair spermatogenesis, resulting in reduced sperm count and increased morphological abnormalities. Experimental and clinical studies have demonstrated that prolonged exposure to anabolic-androgenic steroids adversely affects sperm quality by decreasing sperm concentration and increasing abnormalities of the head, midpiece, and tail. These structural defects compromise sperm motility and fertilizing capacity, thereby contributing to male infertility **[5,6]**.

The present study aimed to evaluate the effects of intraperitoneal oxandrolone administration on male reproductive function in adult Wistar rats through the assessment of reproductive hormones (testosterone, LH, and FSH), sperm count, sperm morphological abnormalities, and testicular histopathological changes.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Wistar rats were used in the present study. A total of 24 male white laboratory rats were obtained from the BALB/C College of Veterinary Medicine, University of Basra. They ranged in age from 10 to 12 weeks and weighed 200–250 g. They were housed in the animal facility of the Department of Life Sciences at the College of Education in Al-Qurna from November 1, 2025, through January 1, 2026, under controlled temperature conditions, good ventilation, and a consistent 12-hour light/12-hour dark schedule. Then, the rats were placed in standard-sized breeding cages lined with wood shavings, which were replaced every 2–3 days. The animals were allowed 10 days to acclimate before the start of the experiment, and clean water was provided continuously throughout the experiment.

2.2 Materials Used

In this experiment, oxandrolone was used as the primary test substance (Figure 1). It was obtained from a reliable source and stored under appropriate conditions (2–8°C) away from light and moisture to ensure its chemical and pharmacological stability throughout the experiment. In addition to the hormone (Figure 2), distilled water was used to prepare the solutions. Dimethyl sulfoxide (DMSO) was used as the solvent, for injection to ensure complete dissolution of the hormone

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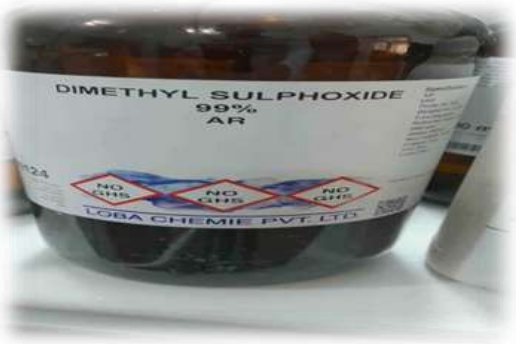


Figure 1: A di-methyl solvent



Figure 2: Oxandrolone Hormone

2.3 Experimental Design

After the acclimatization period, the animals were randomly allocated into four experimental groups (n = 6 rats per group).

Group T1 (Control): received 1 mL distilled water by intraperitoneal (IP) injection.

Group T2 (Vehicle control): received 1 mL dimethyl sulfoxide (DMSO) by intraperitoneal injection.

Group T3 (Low-dose oxandrolone): received oxandrolone at a dose of 0.82 mg/kg body weight, dissolved in DMSO and administered by intraperitoneal injection.

Group T4 (High-dose oxandrolone): received oxandrolone at a dose of 1.62 mg/kg body weight, dissolved in DMSO and administered by intraperitoneal injection.

All treatments were administered once daily for 30 consecutive days, with an injection volume of 1 mL per animal.

2.4 Blood Collection and Hormonal Analysis

Blood samples were collected from all animals before treatment and weekly throughout the experimental period. Samples were allowed to clot at room temperature and centrifuged at 3000 rpm for 15 min to obtain serum. Serum samples were stored at −20°C until hormonal analysis.

Serum concentrations of testosterone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured using rat-specific enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Hormone concentrations were calculated from the standard calibration curves supplied with each kit.

2.5 Determination of Epididymal Sperm Count

At the end of the experimental period, the animals were euthanized, and the cauda epididymis was carefully dissected and transferred into physiological saline. The epididymal tissue was finely minced to release spermatozoa into the suspension. Following adequate dilution, sperm concentration was determined using a Neubauer hemocytometer under a light microscope according to standard laboratory procedures. Sperm counts were expressed as the number of spermatozoa per milliliter of epididymal suspension.

2.6 Determination of Epididymal Sperm Count

Sperm morphological abnormalities were evaluated using epididymal sperm obtained from the cauda epididymis. A drop of sperm suspension was mixed with 1% eosin stain, smeared onto clean glass slides, and allowed to air dry. The stained smears were examined under a light microscope at ×400 magnification. At least 200 spermatozoa were examined for each animal. Morphological abnormalities, including detached heads, hookless heads, banana-shaped heads, bent tails, coiled tails, and headless spermatozoa, were identified and recorded, and the percentage of abnormal spermatozoa was calculated.

2.7 Determination of Epididymal Sperm Count

Following euthanasia, both testes were immediately excised, washed with normal saline, and fixed in 10% neutral buffered formalin for 24–48 h. Histological processing was carried out in the Histopathology Laboratory, Department of Biology,

College of Education Al-Qurna, University of Basrah. Fixed tissues were dehydrated through ascending grades of ethanol, cleared in xylene, embedded in paraffin wax, and sectioned at 5 µm thickness using a rotary microtome. Tissue sections were mounted on glass slides and stained with hematoxylin and eosin (H&E).

Histopathological alterations were examined under a light microscope, and representative photomicrographs were captured using a digital microscope camera.

2.8 Determination of Epididymal Sperm Count

Statistical analysis was performed using IBM SPSS Statistics version 26.0. Data were expressed as Mean ± Standard Error (Mean ± SE). The effects of treatment and experimental period were analysed using two-way analysis of variance (Two-way ANOVA) followed by the Least Significant Difference (LSD) post hoc test. Differences were considered statistically significant at $P < 0.05$

3. RESULTS AND DISCUSSION

3.1 Effect of Oxandrolone on Serum Testosterone Concentration

Table 1 presents the effect of repeated intraperitoneal administration of oxandrolone on serum testosterone concentration in adult male Wistar rats No significant differences ($P > 0.05$) were observed among the experimental groups before treatment, indicating comparable baseline testosterone levels. Following oxandrolone administration, serum testosterone concentrations decreased significantly ($P \leq 0.05$) in both treated groups compared with the control and vehicle-treated groups. The reduction was dose- and time-dependent, with the greatest decrease observed in rats treated with 1.62 mg/kg body weight (T4) during the fourth week of the experiment. In contrast, testosterone concentrations remained relatively stable in the control (T1) and DMSO-treated (T2) groups throughout the study.

Table 1. presents the effect of repeated intraperitoneal administration of oxandrolone on serum testosterone concentration in adult male

average periods	T1	T2	T3	T4	Transaction Period
A 3.49 ± 0.35	b 2.41 ± 0.35 A	a 3.92 ± 0.34 A	a3.42 ± 0.36 A	a 4.19 ± 0.40 A	Before using oxandrolone
A 3.53 ± 0.38	b 2.69 ± 0.34 A	a 4.21 ± 0.39 A	a 3.23 ± 0.38 A	a 3.98 ± 0.38 A	the first week of use
A 3.19 ± 0.36	a 3.11 ± 0.32 A	a 3.82 ± 0.37 A	b 2.15 ± 0.31 B	a 3.69 ± 0.35 A	the second week
B 2.99 ± 0.33	b 2.84 ± 0.33A	a 4.11 ± 0.41 A	b 2.26 ± 0.30 B	b 2.74 ± 0.31 B	the third week
B 2.97 ± 0.39	a 3.37 ±0.31 A	a 4.71 ± 0.43 A	b 1.94 ± 0.29 C	b 1.86 ± 0.33 C	the fourth week
	2.88 ± 0.45 b	4.13 ± 0.56 a	2.60 ± 0.47 b	3.29 ± 0.49 ab	Average transactions

Horizontal capital letters indicate the presence of significant differences at the level of ($p \leq 0.05$) among the different transactions. The small letters vertically indicate the presence of significant differences at the level ($p \leq 0.05$)

between the time intervals

T1: The first treatment was injected with distilled water only (negative control group)

T2: The second case, inoculated with the solvent. (DMSO), with distilled water (solvent group)

T3: The third treatment, it was injected with oxandrolone hormone at a dose of (0.82 mg/kg of body weight) dissolved in the solvent.

T4: The fourth treatment, oxandrolone hormone was injected at a dose of)1.62 mg/kg body weight (dissolved in the solvent.

3.2 Effect of Oxandrolone on Serum Luteinizing Hormone (LH)

Table 2 shows the effect of repeated intraperitoneal administration of oxandrolone on serum luteinizing hormone (LH) concentration in adult male Wistar rats. Serum LH concentrations did not differ significantly ($P > 0.05$) among the experimental groups before treatment. However, repeated administration of oxandrolone resulted in a significant reduction ($P \leq 0.05$) in LH concentrations in both treated groups compared with the control groups. The reduction became progressively greater throughout the experimental period and was most pronounced in the high-dose group (T4). No significant changes were detected in the control or vehicle-treated groups during the study.

Table 2. shows the effect of repeated intraperitoneal administration of oxandrolone on serum luteinizing hormone (LH) concentration in adult male

average periods	T1	T2	T3	T4	the transaction the period
A1.33 ± 0.12	b 0.72 ± 0.05 A	a 1.32 ± 0.11 A	a 1.92 ± 0.17 A	a 1.42 ± 0.15 A	Before using oxandrolone
A 1.25 ± 0.10	b 0.92 ± 0.06 A	a 1.42 ± 0.14 A	a 1.61 ± 0.14 A	ab 1.04 ± 0.09 A	the first week of use
A 1.11 ± 0.11	b 0.87 ± 0.05 A	a 1.15 ± 0.15 A	a 1.10 ± 0.08 A	ab 0.96 ± 0.09 A	the second week
AB 0.96 ± 0.09	a 0.94 ± 0.04 A	a 1.35 ± 0.12 A	ab 0.82 ± 0.07 B	b 0.73 ± 0.06 B	the third week
B 0.79 ± 0.06	b 0.76 ± 0.09 A	a 1.48 ± 0.1 A	b 0.52 ± 0.09 C	c 0.39 ± 0.02 C	the fourth week
	0.84 ± 0.13 b	1.42 ± 0.12 a	1.91 ± 0.17 a	0.91 ± 0.12 b	Average transactions

3.3 Effect of Oxandrolone on Serum Follicle-Stimulating Hormone (FSH)

Table 3 illustrates the effect of repeated intraperitoneal administration of oxandrolone on serum follicle-stimulating hormone (FSH) concentration in adult male Wistar rats. No significant differences ($P > 0.05$) were detected among the experimental groups before treatment. Oxandrolone-treated rats exhibited a significant decrease ($P \leq 0.05$) in serum FSH concentrations compared with the control and DMSO-treated groups. This reduction became more evident with increasing treatment duration and dose, with the lowest FSH concentration observed in the high-dose oxandrolone group during the final week of the experiment.

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Table 3. the effect of repeated intraperitoneal administration of oxandrolone on serum follicle-stimulating hormone (FSH) concentration in adult male

average periods	T1	T2	T3	T4	the transaction the period
A 4.80 ± 0.42	b 4.15 ± 0.49 B	a 5.11 ± 0.46 B	b 4.41 ± 0.48 A	a 5.51 ± 0.50	Before using oxandrolone
A 4.76 ± 0.44	b 4.42 ± 0.44 A	a 5.13 ± 0.48 B	b 4.38 ± 0.42 A	a 5.10 ± 0.49 A	the first week of use
B 4.47 ± 0.40	a 4.35 ± 0.43 B	a 5.21 ± 0.48 A	a 4.11 ± 0.41 AB	a 4.22 ± 0.47 AB	the second week
C 4.19 ± 0.45	a 4.01 ± 0.43 A	a 5.41 ± 0.46 A	b 3.21 ± 0.38 B	a 4.11 ± 0.44 AB	the third week
B 4.22 ± 0.41	b 4.81 ± 0.46 A	a 5.37 ± 0.42 A	b 3.61 ± 0.38 B	c 0.39 ± 0.02 C	the fourth week
	4.35 ± 0.51 ab	5.25 ± 0.54 a	3.84 ± 0.50 b	4.51 ± 0.49 a	Average transactions

3.4 Effect of Oxandrolone on Epididymal Sperm Count

Table 4 shows the effect of repeated intraperitoneal administration of oxandrolone on epididymal sperm count in adult male Wistar rats during the experimental period. Before treatment, no statistically significant differences ($P > 0.05$) were observed among the experimental groups, indicating that the animals were homogeneous at the beginning of the experiment. Following oxandrolone administration, significant differences ($P \leq 0.05$) were observed among the experimental groups according to the Least Significant Difference (LSD) test. The control group (T1) maintained relatively stable sperm counts throughout the study, whereas the vehicle-treated group (T2) showed only slight fluctuations with no marked reduction. In contrast, oxandrolone-treated rats exhibited a dose- and time-dependent decrease in epididymal sperm count. The low-dose group (T3) showed a progressive reduction beginning during the treatment period, while the high-dose group (T4) exhibited the greatest decline, reaching the lowest sperm count during the fourth week of treatment. These findings indicate that repeated intraperitoneal administration of oxandrolone adversely affects spermatogenesis in a dose-dependent manner.

Table 4. the effect of repeated intraperitoneal administration of oxandrolone on epididymal sperm count in adult male

T1	T2	T3	T4	the transaction the period
300 B	200 C	300 A	320 A	Before using oxandrolone
280 C	155 D	300 A	250 B	the first week of use
250 D	200 C	200 B	310 AB	the second week

320 A	310 A	210 B	110 C	the third week
292 BC	230 B	117 C	102 C	the fourth week
16.65	36.90	49.47	68.09	LSD

3.5 Effect of Oxandrolone on Sperm Morphological Abnormalities

presents This study examines the effect of repeated intraperitoneal oxandrolone administration on sperm morphological abnormalities in adult male Wistar rats throughout the experimental period. No differences were observed between the experimental groups prior to treatment, indicating that sperm morphology was normal at baseline. Following oxandrolone administration, the percentage of abnormal sperm increased significantly in both treated groups compared to the control group (T1) and the solvent-treated group (T2). Figure (3) The increase in sperm abnormalities was gradual throughout the experiment and was more pronounced in mice treated with a higher dose of oxandrolone (1.62 mg/kg body weight). The observed abnormalities included detached heads, heads without hooks, banana-shaped heads, curved tails, coiled tails, and headless spermatozoa. In contrast, the control group and the DMSO-treated group showed only a low rate of sperm abnormalities throughout the study (Figures 4, 5, and 6).



Figure 3: A sperm with a head, midpiece, and tail

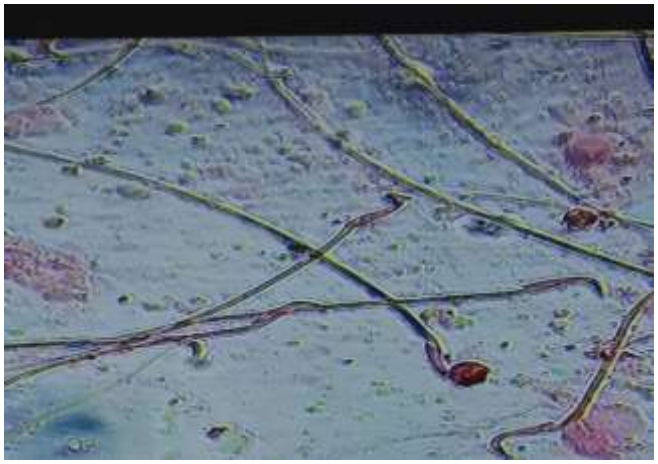


Figure 4: Sperm with a Deformed Head



Figure 5: Curvature of the Mid-Section and Tail

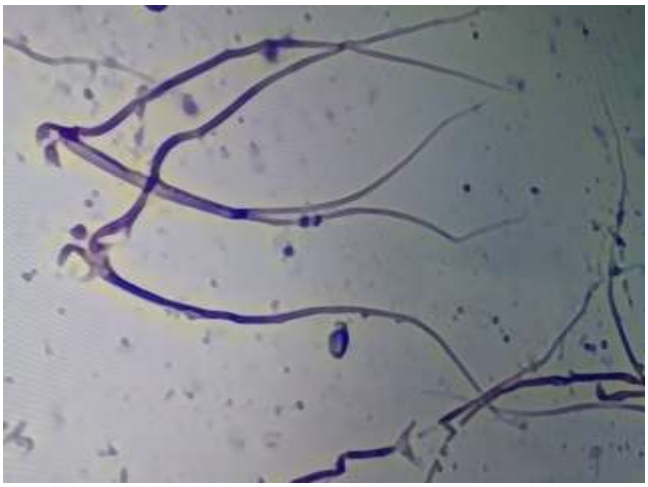


Figure 6: Splitting of the Sperm Tail

3.6. Histopathological Findings of the Testis

Histopathological examination of the testes revealed normal architecture of the seminiferous tubules in both the control (T1) and vehicle-treated (T2) groups. The seminiferous tubules were regularly arranged and lined by intact germinal epithelium containing all stages of spermatogenic cells, with abundant mature spermatozoa occupying the tubular lumen. Sertoli cells and interstitial Leydig cells appeared normal, and no histopathological abnormalities were observed.

In contrast, rats treated with oxandrolone exhibited progressive histopathological alterations that increased with treatment dose. The low-dose group (T3) showed mild to moderate degenerative changes characterized by partial sloughing of the germinal epithelium, degeneration and disorganization of seminiferous tubules, nuclear hypochromasia of spermatogenic cells, reduced numbers of developing germ cells, and a noticeable decrease in mature spermatozoa within the tubular lumen. The high-dose group (T4) demonstrated more severe histopathological lesions, including marked degeneration and distortion of the seminiferous tubules, extensive sloughing of the germinal epithelium, depletion of spermatogenic cell layers, pronounced nuclear hypochromasia, severe reduction or absence of mature spermatozoa in the lumen of many seminiferous tubules, and disruption of the normal testicular architecture. Overall, the severity of the histopathological changes increased in a dose-dependent manner following repeated intraperitoneal administration of oxandrolone.

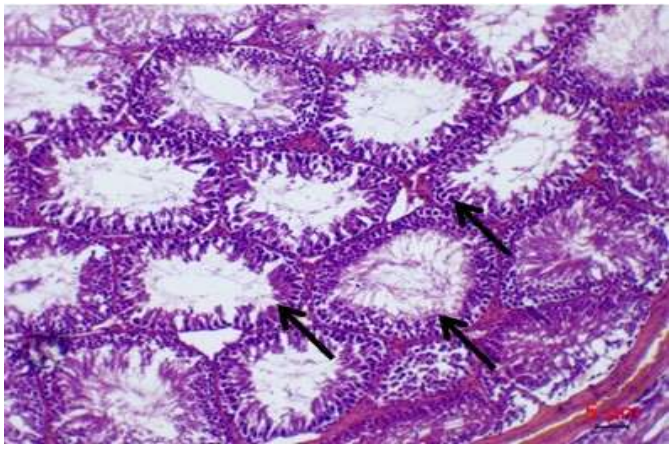


Figure 7: Testis showing some preserved seminiferous tubules with intact spermatogenic layers and mature spermatozoa (arrows). H&E stain, ×10 (T1)

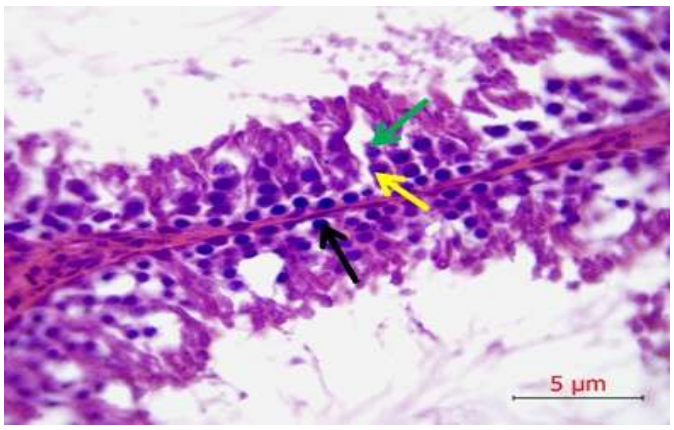


Figure 8: Testis showing some preserved seminiferous tubules with intact spermatogenic layers and mature spermatozoa spermatogonia (black arrow), primary spermatocytes (yellow arrows), spermatids (green arrow. H&E stain, ×40 (T2)

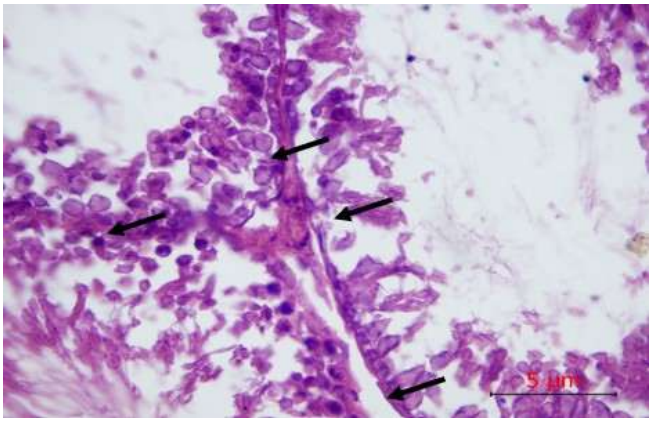


Figure 9: Testis showing nuclear hypochromasia (arrows) of spermatogenic cells with reduced spermatogonia and mature spermatozoa, indicating seminiferous epithelial degeneration. H&E stain, ×40 (T3)

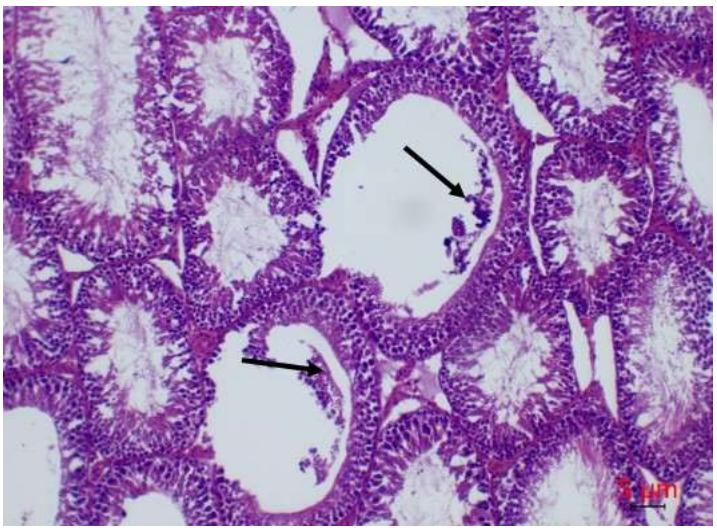


Figure 10: Testis showing sloughing of the seminiferous germinal epithelium (arrows) into the tubular lumen with disrupted spermatogenic organization. H&E stain, ×10 (T4)

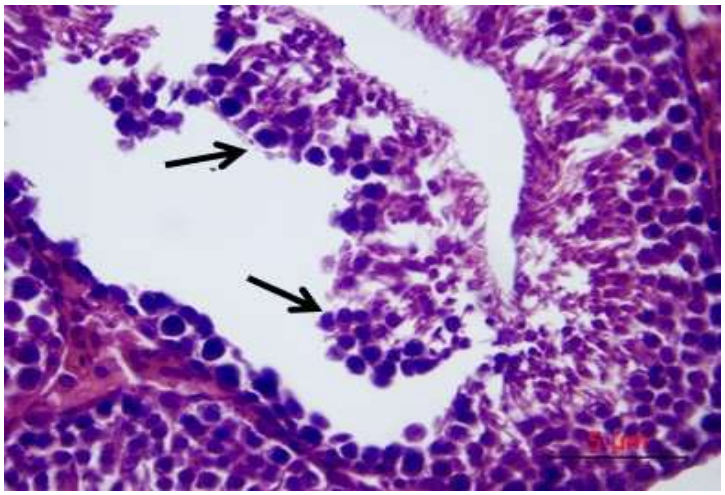


Figure 11: Testis showing sloughing of the seminiferous germinal epithelium(arrows) into the tubular lumen with disrupted spermatogenic organization. H&E stain, ×40(T4)

3.7. Discussion of Testosterone

The present study demonstrated a significant dose-dependent reduction ($P \leq 0.05$) in serum testosterone concentrations following repeated intraperitoneal administration of oxandrolone in adult male Wistar rats. The greatest reduction was observed in the high-dose treatment group, indicating that prolonged oxandrolone exposure markedly suppresses endogenous androgen production and disrupts normal reproductive endocrine function.

Testosterone is the principal androgen responsible for the development and maintenance of male reproductive function. It is synthesized by Leydig cells under the stimulation of luteinizing hormone (LH) and is indispensable for the initiation and maintenance of spermatogenesis, maturation of germ cells, and preservation of normal seminiferous tubule structure. Therefore, any reduction in testosterone production directly compromises testicular function and male fertility **[5]**. The decline in testosterone observed in the present study is mainly attributed to suppression of the hypothalamic–pituitary–gonadal (HPG) axis by exogenous oxandrolone. Administration of anabolic-androgenic steroids suppresses hypothalamic secretion of gonadotropin-releasing hormone (GnRH), which subsequently reduces pituitary LH secretion. Reduced LH stimulation decreases Leydig cell steroidogenesis and ultimately lowers endogenous testosterone production. This endocrine suppression has been recognized as the primary mechanism responsible for anabolic steroid-induced hypogonadism **[7].**

In addition to endocrine suppression, oxandrolone may directly impair Leydig cell function via oxidative stress. Excessive production of reactive oxygen species (ROS) damages mitochondrial membranes and inhibits steroidogenic enzymes involved in testosterone biosynthesis, including steroidogenic acute regulatory protein (StAR), CYP11A1, and 17β-hydroxysteroid dehydrogenase. Impairment of these enzymes decreases cholesterol transport into mitochondria and reduces testosterone synthesis. Furthermore, oxidative stress activates inflammatory pathways and apoptosis within Leydig cells, thereby aggravating steroidogenic dysfunction **[8].**

The reduction in testosterone observed in the present study was accompanied by a significant decrease in sperm count, increased sperm morphological abnormalities, and marked histopathological degeneration of seminiferous tubules. These findings indicate that testosterone deficiency adversely affected Sertoli cell function and impaired the microenvironment required for normal spermatogenesis. Since intratesticular testosterone concentrations are essential for germ-cell differentiation and maturation, suppression of testosterone secretion likely contributed to the reproductive alterations observed in oxandrolone-treated animals.
The findings of the present study are consistent with those reported by **[7]** who concluded that anabolic-androgenic steroid use suppresses endogenous testosterone production through inhibition of the HPG axis. Similarly, **[9]** demonstrated that exogenous anabolic steroids reduce Leydig cell activity and impair reproductive endocrine function. Furthermore **[8]** reported that oxidative stress-mediated damage to steroidogenic cells represents an important mechanism underlying reduced testosterone biosynthesis and impaired male fertility.

Overall, the present findings demonstrate that repeated intraperitoneal administration of oxandrolone significantly suppresses endogenous testosterone production through endocrine inhibition and oxidative damage to Leydig cells, thereby contributing to impaired spermatogenesis and progressive deterioration of male reproductive function

3.8. Discussion of Luteinizing Hormone (LH)

The present study demonstrated a significant dose-dependent decrease ($P \leq 0.05$) in serum luteinizing hormone (LH) concentrations following repeated intraperitoneal administration of oxandrolone in adult male Wistar rats. The reduction was more pronounced in the high-dose group, indicating that prolonged oxandrolone exposure markedly suppresses pituitary gonadotropin secretion and disrupts the normal endocrine regulation of male reproduction.
Luteinizing hormone is a key gonadotropin secreted by the anterior pituitary gland in response to gonadotropin-releasing hormone (GnRH). Its primary physiological function is to stimulate Leydig cells within the testes to synthesize testosterone, which is essential for maintaining spermatogenesis and normal reproductive function. Consequently, suppression of LH secretion results in decreased testosterone production and impaired testicular activity **[3].** The decline in LH observed in the present study can be explained by the negative feedback inhibition induced by exogenous oxandrolone. Administration of anabolic-androgenic steroids elevates circulating androgenic activity, which suppresses hypothalamic GnRH secretion and subsequently reduces pituitary LH release. This hormonal suppression decreases Leydig cell stimulation and contributes to the marked reduction in endogenous testosterone observed in the treated animals. Similar endocrine alterations have been described as the principal mechanism responsible for anabolic steroid-induced hypogonadotropic hypogonadism **[9].**

Experimental evidence also indicates that prolonged anabolic steroid exposure may alter pituitary responsiveness to GnRH and reduce the expression of genes involved in gonadotropin synthesis. Furthermore, persistent suppression of LH may indirectly promote degeneration of seminiferous tubules through reduced intratesticular testosterone concentrations. Since testosterone is indispensable for germ-cell differentiation and maturation, decreased LH secretion may accelerate spermatogenic failure and impair male fertility **[10].**

In the present study, suppression of LH was associated with decreased serum testosterone concentrations, reduced epididymal sperm count, increased sperm morphological abnormalities, and marked histopathological degeneration of the testes. These findings indicate that inhibition of LH secretion represents one of the earliest endocrine events leading to reproductive dysfunction following oxandrolone administration.

The findings of this study agree with those of **[10],** who reported that chronic administration of anabolic-androgenic steroids suppresses pituitary gonadotropin secretion through negative feedback mechanisms. Likewise, **[9]** demonstrated that suppression of LH is a characteristic feature of anabolic steroid-induced hypogonadism and is directly associated with impaired

Leydig cell function and reduced testosterone biosynthesis. Furthermore, **[3]** emphasized the central role of LH in regulating male reproductive endocrinology and maintaining normal spermatogenesis.

Overall, the present findings indicate that repeated intraperitoneal administration of oxandrolone significantly suppresses serum LH concentrations through inhibition of the hypothalamic–pituitary axis, thereby reducing Leydig cell activity, decreasing endogenous testosterone production, and contributing to impaired spermatogenesis and testicular degeneration

3.9. Discussion of Follicle-Stimulating Hormone (FSH)

The present study demonstrated a significant dose-dependent decrease ($P \leq 0.05$) in serum follicle-stimulating hormone (FSH) concentrations following repeated intraperitoneal administration of oxandrolone in adult male Wistar rats. The reduction was most evident in the high-dose group, suggesting that oxandrolone markedly suppresses pituitary gonadotropin secretion and disrupts the endocrine mechanisms responsible for maintaining normal spermatogenesis. Follicle-stimulating hormone is essential for the regulation of spermatogenesis through its direct action on Sertoli cells within the seminiferous tubules. Sertoli cells provide nutritional and structural support for developing germ cells and regulate the production of androgen-binding protein (ABP), inhibin B, growth factors, and other molecules necessary for germ-cell differentiation and maturation. Consequently, suppression of FSH secretion leads to impaired Sertoli cell activity, reduced germ-cell survival, and defective sperm production **[11]**.

The reduction in FSH observed in the present study is likely a consequence of negative feedback inhibition induced by exogenous oxandrolone on the hypothalamic–pituitary–gonadal axis. Increased circulating androgen levels suppress hypothalamic gonadotropin-releasing hormone (GnRH) secretion, thereby reducing pituitary release of FSH. Persistent suppression of FSH compromises Sertoli cell function and disrupts the microenvironment required for normal spermatogenesis, ultimately resulting in decreased sperm production and impaired reproductive capacity **[3]**.

In addition to endocrine suppression, experimental studies have suggested that anabolic-androgenic steroids may induce oxidative stress and inflammatory responses within the seminiferous epithelium, leading to dysfunction of Sertoli cells and apoptosis of developing germ cells. These pathological alterations interfere with the maturation of spermatocytes and spermatids, resulting in reduced sperm output and deterioration of semen quality. This mechanism may explain the significant reduction in sperm count and the increase in sperm abnormalities observed in the oxandrolone-treated groups of the present study **[8].**

Furthermore, the histopathological findings observed in this investigation, including degeneration of seminiferous tubules, sloughing of the germinal epithelium, depletion of spermatogenic cells, and marked reduction of mature spermatozoa within the tubular lumen, strongly support the functional consequences of FSH suppression. Since Sertoli cells are indispensable for maintaining the integrity of the seminiferous epithelium, reduced FSH stimulation may have contributed directly to the structural deterioration observed in the testes.

The present findings are consistent with those reported by **[11]**, who emphasized the indispensable role of FSH in maintaining Sertoli cell function and normal spermatogenesis. Likewise, **[3]** reported that disruption of FSH signalling adversely affects germ-cell differentiation and sperm maturation. Moreover, **[8]** demonstrated that oxidative stress associated with reproductive toxicants impairs Sertoli cell function and contributes to defective spermatogenesis.

Overall, the present findings indicate that repeated intraperitoneal administration of oxandrolone significantly suppresses serum FSH concentrations, thereby impairing Sertoli cell activity, disrupting spermatogenesis, reducing sperm production, and contributing to progressive histopathological damage of the testes.

3.10. Discussion of Epididymal Sperm Count)

The present study demonstrated a statistically significant dose-dependent reduction ($P \leq 0.05$) in epididymal sperm count following repeated intraperitoneal administration of oxandrolone in adult male Wistar rats. The Least Significant Difference (LSD) analysis confirmed significant differences among the experimental groups, with the high-dose oxandrolone group exhibiting the greatest reduction in sperm count compared with the control and vehicle-treated groups. These findings indicate that oxandrolone exerts cumulative adverse effects on spermatogenesis in a dose-dependent manner **[12]**.

The decline in sperm count observed in the present study can primarily be attributed to suppression of the hypothalamic–pituitary–gonadal (HPG) axis. Administration of exogenous anabolic-androgenic steroids inhibits hypothalamic gonadotropin-releasing hormone (GnRH) secretion, resulting in decreased release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), followed by reduced endogenous testosterone production. Since intratesticular testosterone and FSH are essential for maintaining Sertoli cell function and normal spermatogenesis, suppression of these hormones disrupts germ-cell proliferation, differentiation, and maturation, ultimately leading to a marked reduction in sperm production **[12]**.

Another important mechanism responsible for the reduction in sperm count is oxidative stress induced by anabolic-androgenic steroids. Excessive production of reactive oxygen species (ROS) damages germ-cell membranes, induces lipid peroxidation, disrupts mitochondrial function, and causes DNA fragmentation and apoptosis of spermatogenic cells. Because germ cells are characterized by rapid proliferation and high metabolic activity, they are particularly susceptible to oxidative injury, resulting in decreased sperm output and impaired semen quality **[2,13]**.

The histopathological findings observed in the present study strongly support these mechanisms. Rats treated with oxandrolone exhibited degeneration and disorganization of seminiferous tubules, sloughing of the germinal epithelium, depletion of spermatogenic cell layers, and a marked reduction in mature spermatozoa within the tubular lumen. These structural alterations directly impair spermatogenesis and explain the significant reduction in epididymal sperm count observed in the treated groups. Furthermore, the severity of these lesions increased with increasing oxandrolone dose, indicating a clear dose-dependent reproductive toxic effect **[14].**

The present findings agree with previous studies demonstrating that chronic abuse of anabolic-androgenic steroids is associated with oligozoospermia, severe suppression of spermatogenesis, and in some cases azoospermia. Recent systematic reviews have also indicated that prolonged exposure to anabolic steroids significantly decreases sperm concentration through endocrine suppression, oxidative stress, and direct testicular toxicity, while recovery of spermatogenesis may require several months after discontinuation of steroid use **[12,13]**

Overall, the findings of the present study indicate that repeated intraperitoneal administration of oxandrolone significantly reduces epididymal sperm count through combined endocrine suppression, oxidative stress, and progressive degeneration of seminiferous tubules, ultimately impairing spermatogenesis and male fertility

3.11. Discussion of Sperm Morphological Abnormalities

The present study demonstrated a significant increase ($P \leq 0.05$) in sperm morphological abnormalities following repeated intraperitoneal administration of oxandrolone in adult male Wistar rats. with the highest percentage of abnormal spermatozoa recorded in the high-dose oxandrolone-treated group. These findings indicate that prolonged oxandrolone exposure adversely affects sperm quality in a dose-dependent manner **[15]** similarly reported that abuse of anabolic-androgenic steroids is associated with deterioration of semen quality, including increased sperm morphological abnormalities and impaired fertility .The sperm abnormalities observed in the present study, including detached heads, hookless heads, banana-shaped heads, bent tails, coiled tails, and headless spermatozoa, may be attributed to disruption of the normal process of spermatogenesis and epididymal sperm maturation. Proper sperm morphology depends on adequate intratesticular testosterone concentrations, normal Sertoli cell function, and coordinated differentiation of germ cells. Therefore, suppression of testosterone, LH, and FSH observed in the oxandrolone-treated groups likely contributed to the production of structurally abnormal spermatozoa **[5]** concluded that anabolic-androgenic steroid abuse results in hormonal imbalance accompanied by impaired spermatogenesis and increased abnormalities in sperm morphology.

Oxidative stress is considered one of the principal mechanisms responsible for sperm morphological defects induced by anabolic-androgenic steroids. Excessive production of reactive oxygen species (ROS) promotes lipid peroxidation of sperm plasma membranes, mitochondrial dysfunction, DNA fragmentation, and oxidation of structural proteins, ultimately impairing sperm head and tail formation. Because spermatozoa possess limited endogenous antioxidant defenses, they are particularly vulnerable to oxidative injury **[16]** emphasized that oxidative stress is strongly associated with abnormal sperm morphology, impaired sperm function, and reduced male fertility.

The histopathological alterations observed in the testes of oxandrolone-treated rats in the present study further support these findings. Degeneration of seminiferous tubules, depletion of spermatogenic cell layers, and sloughing of the germinal epithelium interfere with normal germ-cell differentiation and maturation, resulting in the release of immature and morphologically

abnormal spermatozoa into the epididymis. Similar observations have been described in recent reviews investigating the reproductive toxicity of anabolic-androgenic steroids The findings of the present study are also supported by the systematic review of **[14]**, which demonstrated that anabolic-androgenic steroid use significantly impairs semen quality by increasing abnormal sperm morphology while adversely affecting reproductive hormone levels and testicular function **[17]** reported that prolonged anabolic steroid abuse suppresses spermatogenesis and may require several months or longer for recovery after discontinuation of steroid use. Overall, the findings of the present study indicate that repeated intraperitoneal administration of oxandrolone significantly increases sperm morphological abnormalities through endocrine disruption, oxidative stress, and progressive histopathological degeneration of the testes, ultimately impairing sperm quality and male reproductive function.

3.12. Discussion of Histopathological Findings

The histopathological examination of the testes in the present study revealed that repeated intraperitoneal administration of oxandrolone induced marked dose-dependent degenerative changes in the testicular tissue of adult male Wistar rats. In contrast to the control (T1) and vehicle-treated (T2) groups, which exhibited normal seminiferous tubules with intact germinal epithelium and abundant mature spermatozoa, the oxandrolone-treated groups showed progressive structural damage characterized by degeneration and disorganization of seminiferous tubules, sloughing of the germinal epithelium, depletion of spermatogenic cell layers, nuclear hypochromia, and a marked reduction in mature spermatozoa. These findings indicate that oxandrolone exerts a direct toxic effect on the testicular tissue, and the severity of these lesions increases with increasing dose **[2]**.

The observed histopathological alterations can be attributed primarily to suppression of the hypothalamic–pituitary–gonadal (HPG) axis following administration of exogenous anabolic-androgenic steroids. Inhibition of LH and FSH secretion reduces intratesticular testosterone concentrations, resulting in impaired Sertoli cell function and disruption of germ-cell proliferation and differentiation. Consequently, degeneration of the seminiferous epithelium and reduced spermatogenesis become evident in the testicular tissue **[3]**.

Oxidative stress also appears to play a major role in the development of testicular injury. Increased production of reactive oxygen species (ROS) induces lipid peroxidation, mitochondrial dysfunction, DNA damage, and apoptosis in germ cells, ultimately leading to the destruction of seminiferous tubules and the deterioration of normal testicular architecture. Similar mechanisms have been reported in recent experimental studies evaluating anabolic steroid-induced reproductive toxicity in laboratory animals **[5]**.

The dose-dependent increase in testicular lesions observed in the present study is consistent with recent experimental investigations. A recent rat study evaluating the anabolic steroid stanozolol demonstrated significant degeneration of seminiferous tubules, reduced germinal epithelium thickness, decreased sperm quality, and increased sperm abnormalities, with the severity of tissue damage increasing with higher administered doses **[9].**

Furthermore, experimental studies on anabolic steroids such as nandrolone decanoate have demonstrated persistent reductions in seminiferous tubule diameter, epithelial height, and testicular volume, even after discontinuation of treatment, suggesting that structural recovery of the testes may be incomplete following prolonged exposure to anabolic steroids **[4]**.

Overall, the histopathological findings of the present study strongly support the biochemical and reproductive alterations observed in oxandrolone-treated rats. The degeneration of seminiferous tubules and depletion of spermatogenic cells explain the significant reductions in sperm count and the increase in sperm abnormalities, confirming that repeated intraperitoneal administration of oxandrolone impairs male reproductive function through endocrine suppression, oxidative stress, and direct testicular toxicity

5. CONCLUSION

The present study demonstrated that repeated intraperitoneal administration of oxandrolone induced significant dose-dependent adverse effects on the reproductive system of adult male Wistar rats. Oxandrolone treatment significantly reduced serum testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations, indicating suppression of the hypothalamic–pituitary–gonadal (HPG) axis and disruption of normal endocrine regulation.

The hormonal alterations were accompanied by a marked reduction in epididymal sperm count and a significant increase in sperm morphological abnormalities, including detached heads, hookless heads, banana-shaped heads, bent tails, coiled tails, and

headless spermatozoa. These findings indicate impaired spermatogenesis and deterioration of sperm quality following oxandrolone administration Histopathological examination further confirmed the reproductive toxicity of oxandrolone by revealing progressive dose-dependent degeneration of the seminiferous tubules, sloughing of the germinal epithelium, depletion of spermatogenic cell layers, nuclear hypochromia, and a marked reduction in mature spermatozoa within the tubular lumen. The severity of these lesions increased with increasing oxandrolone dose, demonstrating a clear relationship between dose and testicular damage. Collectively, the findings of the present study indicate that repeated oxandrolone administration impairs male reproductive function through endocrine disruption, suppression of spermatogenesis, oxidative stress-mediated testicular injury, and progressive histopathological alterations. These results highlight the potential reproductive risks associated with the misuse or prolonged administration of oxandrolone and emphasize the importance of careful medical supervision when this anabolic-androgenic steroid is used for therapeutic purposes.

AUTHOR CONTRIBUTIONS

Rusel K. Waheed: Conceptualization, Investigation. Writing original draft Resources.

Alaa S. Kadhim: Methodology, Laboratory Analysis.

Murtadha F. Abdul-Hussein: Data Curation, Formal Analysis.

Alaa S. Kadhim and Murtadha F. Abdul-Hussein: Supervision, Writing—Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

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