

Impact of Testosterone propionate on semen quality and endocrine profile in male Wistar Rats

ABSTRACT

Aim: This study investigated the impact of testosterone propionate on semen quality and endocrine profile in male wistar rats.

Study design: Thirty-five (35) male wistar rats were used for this study. The rats were divided into seven groups (n =7) of five rats per group. Group 1, the normal control received feed and water only. The test groups 2-7 received different doses of testosterone propionate for varying periods. Groups 2 and 3 received 3mg/kg b. wt of TP for 11 days and 22 days respectively. Groups 4 and 5 received 6mg/kg b. wt of TP for 11 days and 22 days respectively. Groups 6 and 7 received 9mg/kg b. wt of TP for 11 days and 22 days respectively.

Place and duration of study: The study was conducted in the Faculty of basic medical sciences animal house, University of Port Harcourt, Choba, Rivers state, Nigeria. Between October 2024 to March 2025

Methodology: The rats were subjected to 24 hours fasting prior to sacrifice. Blood samples were collected and used to determine hormonal assay. Seminal fluid was extracted from the rats' epididymis and was examined microscopically for spermatozoa evaluation.

Results: the induced groups with testosterone propionate showed a significant($P = .05$) decrease in sperm motility and total sperm count when compared to the normal control group 1. Whereas the concentration of testosterone was elevated in all the testosterone induced groups when compared to the normal control group1, FSH and LH levels decreased in the induced groups when compared with the normal control group1 ($P = .05$) The histology of the testis shows varied degrees of histological distortions in the testosterone induced groups when compared with normal control group 1.

Conclusion: The rat's induction with testosterone propionate resulted in dysregulation of both semen quality and hormonal parameters.

Keywords: Testosterone, follicle stimulating hormone, luteinizing hormone, semen fluid, hormonal assay.

1. INTRODUCTION

Infertility is one of the most serious and rising problems in the world, it is a significant health challenge affecting millions of couples worldwide with male infertility accounting to approximately 50% of cases [1]. Numerous external and internal factors including environmental, genetic, and lifestyle factors can contribute to male infertility, including hormonal imbalances, oxidative stress, infections, and exposure to endocrine disrupting chemicals [2]. Testosterone plays a crucial role in male reproductive health, influencing sperm production, libido, and overall endocrine function [3]. Aging is characterized by testosterone deficiency due to decreasing testosterone levels associated with low testicular production, genetic factors, adiposity, and illness [4]. Low testosterone levels in men are associated with sexual dysfunction (low sexual desire, erectile dysfunction), reduced skeletal muscle mass and strength, decreased bone mineral density, increased cardiovascular risk and alterations of the glycometabolic profile [5]. Testosterone replacement therapy (TRT) has gained attention as a treatment for hypogonadism and other testosterone deficiencies. While TRT offers benefit such as improved libido, muscle mass and bone density, its long term impact on male fertility remains controversial, it shows several therapeutic effects while maintaining a good safety profile in hypogonadal men [6]. TRT restores normal levels of serum testosterone in men, increasing libido and energy level and producing beneficial effects on bone density, strength and muscle as well as yielding cardio protective effects [7]. Nevertheless, TRT could be contraindicated in men with untreated prostate cancer, different potential side effects, such as polycythemia, cardiac events and obstructive sleep apnea, should be monitored [8]. Testosterone has a positive correlation with semen parameters, meaning higher testosterone levels are generally associated with better sperm count and motility [9]. However, when testosterone levels are artificially elevated through exogenous administration (like testosterone therapy), it can suppress the hypothalamic-pituitary-gonadal (HPG) axis, which can negatively impact on sperm production by suppressing the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are necessary for spermatogenesis, potentially leading to decreased semen quality and infertility [10]. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are critical regulators of male reproductive function, primarily through their actions on the testes. These gonadotropins are secreted by the anterior pituitary gland under the control of gonadotropin-releasing hormone (GnRH) from the hypothalamus. LH plays a key role in stimulating the Leydig cells of the testes to produce testosterone, which is essential for spermatogenesis, libido, and secondary sexual characteristics. On the other hand, FSH primarily acts on the Sertoli cells within the seminiferous tubules, promoting sperm maturation and supporting spermatogenesis.[11].

The intricate balance between LH and FSH is crucial for maintaining normal reproductive function. Elevated testosterone levels, whether due to endogenous production or exogenous administration, exert negative feedback on the hypothalamic-pituitary-gonadal (HPG) axis, leading to reduced secretion of both LH and FSH. This suppression disrupts spermatogenesis, potentially leading to decreased sperm count and impaired fertility. In the context of testosterone propionate administration, the suppression of LH and FSH is expected, which may explain the observed alterations in semen quality and testicular histology in the study. Understanding the mechanistic role of these hormones provides insight into the complex interplay between exogenous testosterone and male reproductive health, emphasizing the importance of maintaining hormonal balance to preserve fertility.[11]

Long-term use of testosterone propionate causes enlarged prostate, decrease in sperm production and inconsistency in hormonal parameters [12]. The adverse effect of this is increasing on daily basis, making

it a health concern as causes of infertility are on the rise. Despite the prevalence of male infertility in Nigeria, not much effort has been made at tackling the problem; therefore, if adequate measures are not taken there will be an increasing impact of male infertility[13]

1.1 Aim of Study

This study investigates the impact of testosterone propionate on semen quality and hormonal parameters in male wistar rats, aiming to provide insights into its potential risk concerning fertility.

1.2 Objectives

- i. Determination of Hormonal Parameters
- ii. Semen Analysis
- iii. Histological Assessment of Testicular Structures

2. Materials and Methods

2.1. Study Animals

The animal model employed for this study were thirty –five (35) adult male Wistar rats which were eight weeks' old weighing 180-250g). They were housed in clean plastic cages with controlled environmental temperature of 25°C-28°C, 12-hour light/dark cycle. The rats were allowed access to water and normal rat chow freely throughout the duration of the study. The rats were also acclimatized for 2 weeks before the commencement of the study. The procedure for animal handling and the study methodology conformed to the ethical Guideline on animal research as stipulated by guideline for Laboratory Animal Care (NIH Publication 1996, No 85-23).

2.2. Experimental Design

Rats were induced daily through subcutaneous injection (S.C) with testosterone-propionate adopting the method laid forth by [14] .They were randomly selected into seven groups of five animals each(n=5) ,Group 1 (normal control group) received feed and water only , Group 2 received TP (3mg/kg b.wt) for 11days , Group 3 received TP (3mg/kg b.wt) for 22days, Group 4 were induced with TP(6mg/kg b.wt) for 11 days , Group 5 were given TP (6mg/kg b.wt) for 22 days, Group 6 were administered with TP (9mg/kg b.wt) for 11 days, Group 7 were induced with TP (9mg/kg b.wt) for 22 days . The animal grouping and induction protocol is summarized in Table 1.

GROUPS	INDUCED AND NON INDUCED GROUPS
Group 1	Normal rats received feed and water only
Group 2	TP (3mg /kg. b. wt) for 11 days
Group 3	TP (3mg/kg. b. wt) for 22 days
Group 4	TP (6mg/kg. b. wt) for 11 days
Group 5	TP (6mg/kg. b. wt) for 22 days
Group 6	TP (9mg/kg. b. wt) for 11 days
Group 7	TP (9mg/kg. b. wt) for 22 days

Table 1: indicates the research design from group 1 to group 7 which were induced with the highest concentration of testosterone propionate

2.3. Determination of Hormonal Parameters

The rats were subjected to 24- hours fasting period before sacrifice, and blood samples were meticulously collected in plain bottles. The blood samples were centrifuged at 3000 rpm for 20mins to separate the serum, which was then use to determine testosterone, FSH and LH concentration levels were estimated using bio check, ELIZA kit, with catalog number: BC-1115 FOR TESTOSTERONE, FSH catalog number: BC-1029 and LH with catalog number: BC-1031, following manufacturer's instructions.

2.4. Semen Analysis

Assessment of semen parameters, which include, the percentage of motile cells, dead cells, and morphological appearance of the sperm cells of the spermatozoa and total sperm count were determined by adopting method of Ogheneochuko et al.,2024. [15]. Semen fluid was extracted from the rat's epididymis onto a clean microscope slide, followed by the addition of few drops of normal saline. This preparation was used to evaluate sperm quality, including total sperm count, motility, and morphology.

The total sperm cells were counted in the five larger squares of the counting chamber using a high-magnification of the objective lens of the microscope. The motility assessment after sample preparation, the active sperm cells and dead cells were recorded accordingly

2.5. Histological Assessment of Testicular Structures

The testes were rapidly removed from the rats and fixed in 10% formalin for 24 hrs. The testicular tissue was then dehydrated and embedded in paraffin by standard procedures. Sections were deparaffinized and rehydrated. After hematoxylin and eosin (HE) staining, the histopathology of the testicle was observed with microscopic assessment (Leica Microsystems DM1000, Wetzlar, Germany).

2.6. Statistical Analysis

Data collected were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's (HSD) multiple comparison. The significance of the data was assessed using a *P*-value of less than 0.05 and reported as Mean \pm Standard Error of Mean (SEM).

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1. Effect of TP on Semen Quality Parameters

Individual results of seminal fluid analyzed is given in Table 2. The analysis of semen quality parameters showed that rats induced with testosterone propionate (TP) showed a significant ($P = .05$) decrease in sperm motility(mot), viability(viab) and total sperm count(TSC) for the experimental groups when compared to the normal control group 1. Higher doses and prolonged exposure to TP resulted to more pronounced negative effects with Group 7 induced with 9mg/kg b. wt of the TP for 22 days showed a more Significant decrease in the number of active sperm cells to $4.67 \pm 4.16\%$ when compared to the normal control group with active cells of 24.67 ± 24.03 . However, the morphology showed an increase in the head defect, midpiece defect and tail defect which was statistically significant at ($P = .05$).

3.1.2. Effect of TP on Hormonal Parameters

Hormonal analysis as shown in Fig 1. Shows the levels of testosterone(TEST),follicle stimulating hormone(FSH), and luteinizing hormone(LH) in testosterone propionate(TP) treated rats as shown in Fig 1. The concentration of TEST in the normal control was 3.67 ± 0.81 ng/ml which significantly ($P = .05$) increased to 10.07ng/ml (group 6) and 8.20ng/ml (group 7) indicating a significant increase in serum testosterone in all TP-treated groups. However, this was accompanied by a significant ($P = .05$) decrease in the levels of FSH and LH for the treated groups when compared to the normal control.

3.1.3. Effect of TP on Testes Histoarchitecture

The testes of the control shows normal histological features. The experimental groups showed varied degrees of histological distortions.

Table 2: Semen Analysis of Testosterone Propionate Treated Normal Male Wistar Rats

Grp	Mot(%)	Act(%)	Slu(%)	Dead(%)	TSC(* 10 ⁶ cells /mL)	Viab(%)	Hd Df(%)	Mid Df(%)	Tail Df(%)
1	85.00±13.23 ^a	73.33±11.55 ^a	11.67±2.89 ^a	15.00±13.23 ^a	113.33±31.53 ^a	94.33±2.89 ^a	2.33±2.31 ^a	2.00±0.00 ^a	0.67±0.57 ^a
2	30.00±26.46 ^b	18.33±18.93 ^b	11.67±7.64 ^a	70.00±26.46 ^b	30.00±4.00 ^b	54.00±47.15 ^b	8.00±3.46 ^b	9.00±9.64 ^b	6.33±3.21 ^b
3	15.00±13.23 ^c	11.67±10.40 ^b	3.33±2.89 ^b	51.67±44.81 ^c	22.67±25.32 ^b	51.00±44.17 ^b	2.67±4.62 ^a	4.00±5.29 ^b	2.33±3.21 ^c
4	48.67±8.08 ^b	24.33±8.15 ^c	24.33±4.04 ^c	51.33±8.08 ^c	39.33±14.01 ^b	79.00±6.08 ^c	5.67±1.53 ^b	5.67±2.08 ^c	5.67±1.53 ^b
5	21.67±7.53 ^b	16.67±28.87 ^c	5.00±8.66 ^b	11.67±20.21 ^d	13.33±23.09 ^c	28.67±49.65 ^d	1.67±2.89 ^a	1.33±2.31 ^a	1.67±2.89 ^a
6	21.67±18.93 ^b	6.67±7.64 ^d	15.00±13.23 ^d	78.33±18.93 ^b	26.00±7.21 ^b	89.00±3.40 ^c	5.33±5.58 ^b	3.33±2.52 ^c	2.33±1.53 ^c
7	24.67±24.03 ^b	4.67±4.16 ^d	20.00±20.00 ^c	75.33±24.03 ^b	25.00±4.58 ^b	86.67±5.77 ^c	4.33±6.58 ^b	2.67±3.06 ^a	9.67±5.51 ^b

^a Mot – Percentage of sperm cell motility, ^b Slu – Percentage of sluggish sperm cells

^c Act – Percentage of active sperm cells, ^d TSC – Total sperm cell count

^e Viab – Percentage of viable sperm cells, ^f Hd Df – Percentage of sperm cells with head defect

^g Mid Df – Percentage of sperm cells with mid piece defect

^h Tail Df – percentage of sperm cells with tail defect

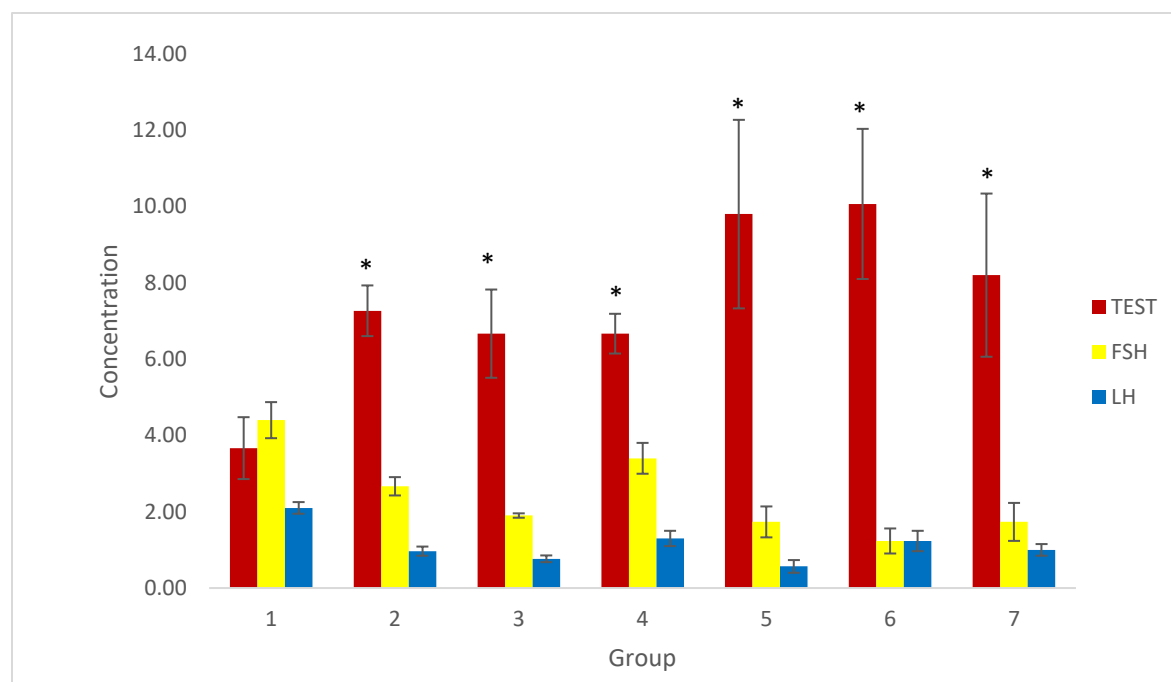


Fig 1: Graphical representation of testosterone, FSH and LH concentrations of both the experimental group and the normal control group

3.1.4 HISTOLOGY OF THE TESTES

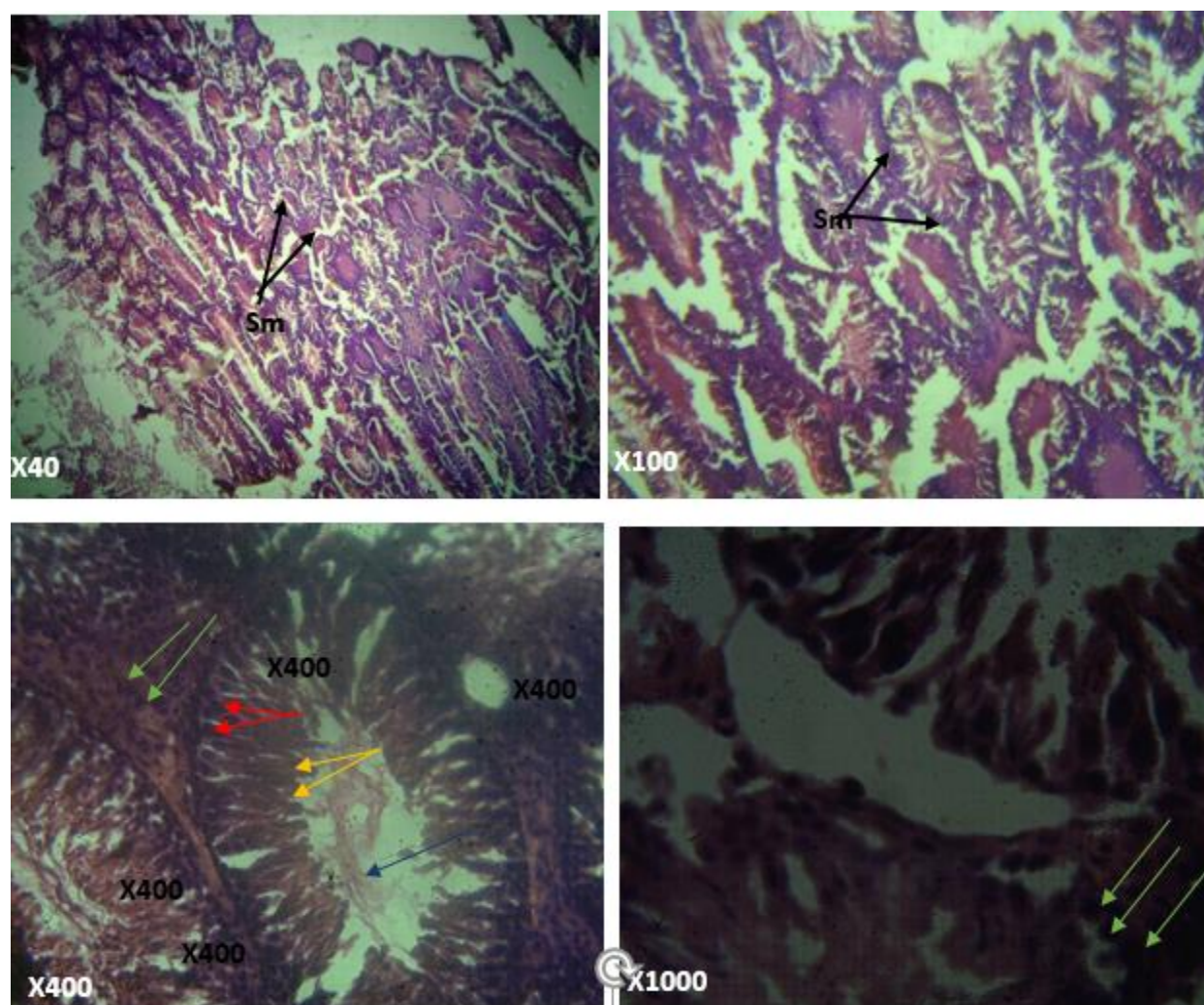


Plate 1: Photomicrograph of normal control(Group 1)

The testes shows normal histological features with active spermatogenic activities occurring in the seminiferous tubules (Sm). Spermatogonia (red arrow), spermatocytes (yellow arrow), spermatids in the lumen (blue arrow) and interstitial cells of Leydig (green arrow)

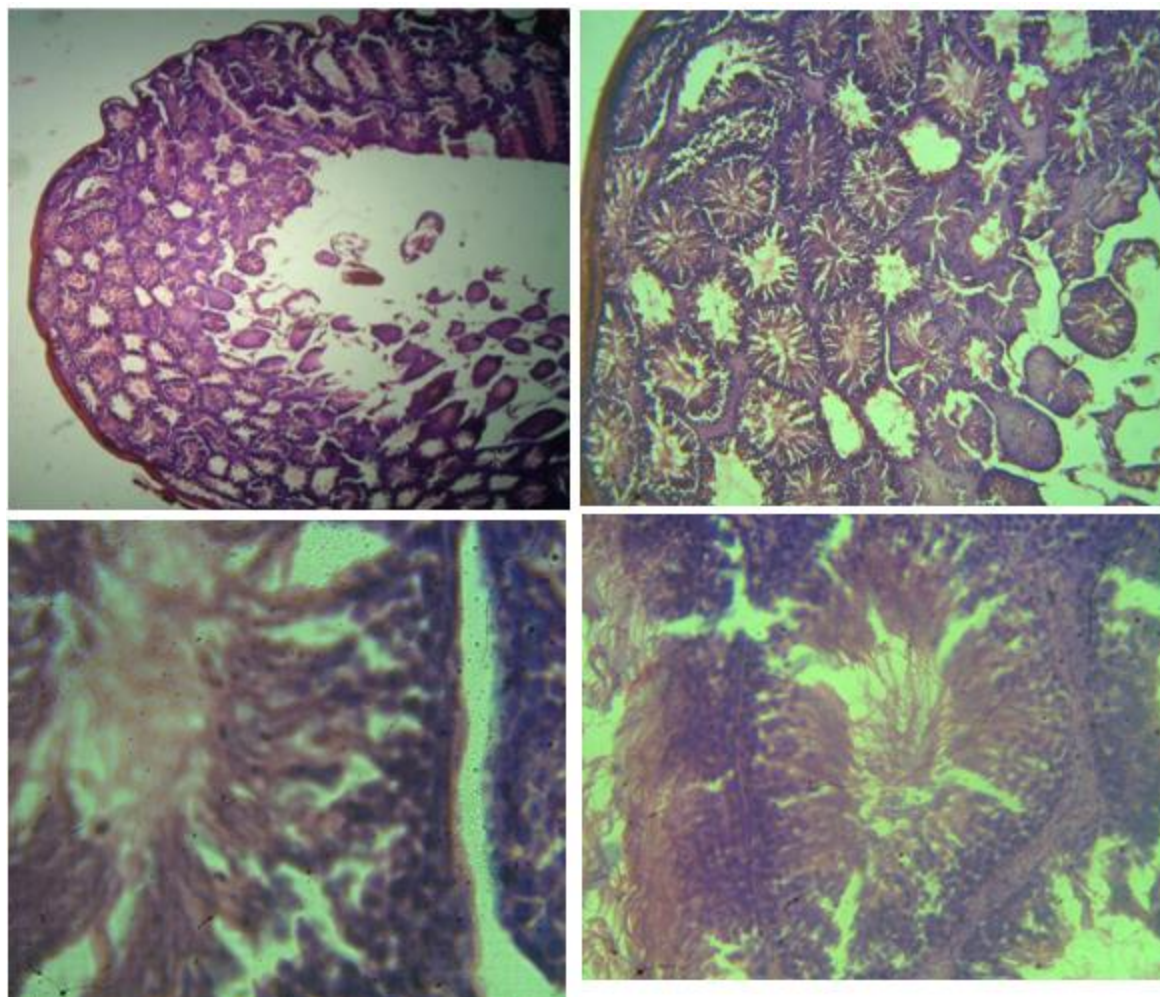


Plate 2: Photomicrograph of Group 2: 3mg/kg b. wt TP 11 days- The testes shows normal spermatogenic activities occurring in the seminiferous tubules (black arrow). Spermatogonia (red arrow), spermatids in the lumen (blue arrow)

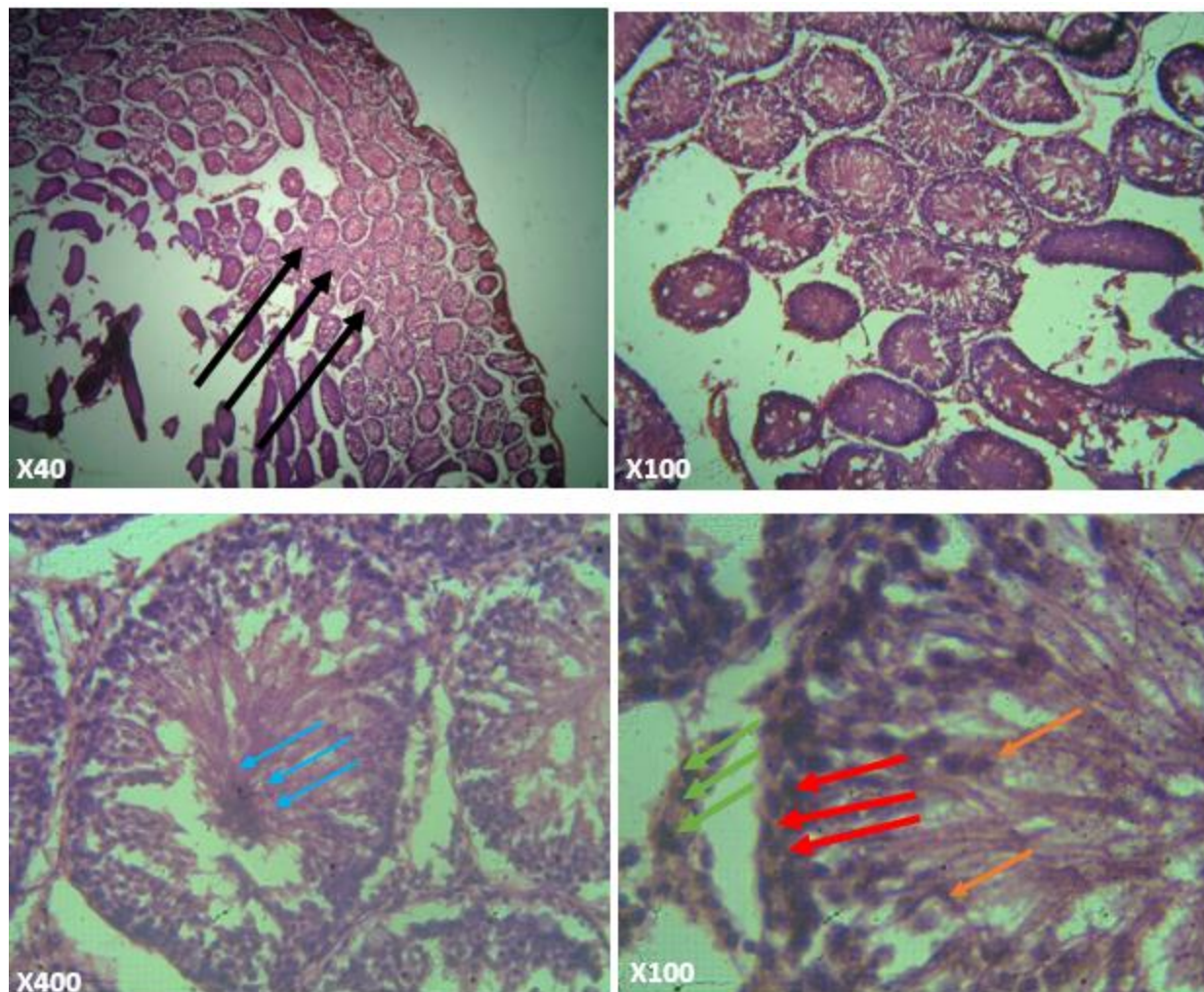


Plate 3: Photomicrograph of Group 3: 3mg/kg b. wt 22days- The testes shows recovery with normal spermatogenic activities occurring in the seminiferous tubules (black arrow). Spermatogonia (red arrow), spermatocytes (yellow arrow), spermatids in the lumen (blue arrow) and interstitial cells of Leydig (green arrow)

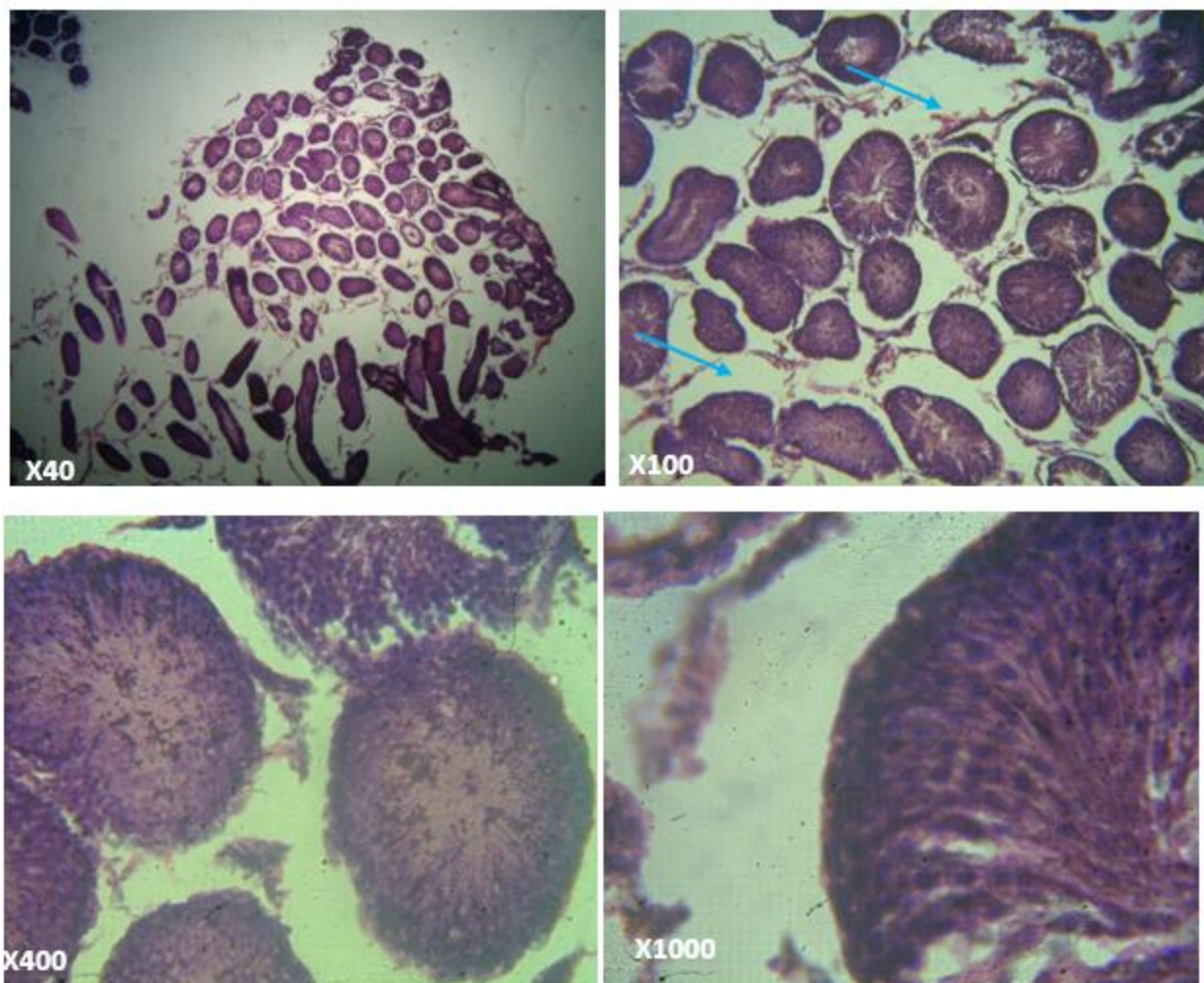


Plate 4: Photomicrograph of Group 4: 6mg/kg b. wt TP 11days- Seminiferous tubules (Sm) are sparse, there is poor spermatogonia, spermatocytes spermatids differentiation.

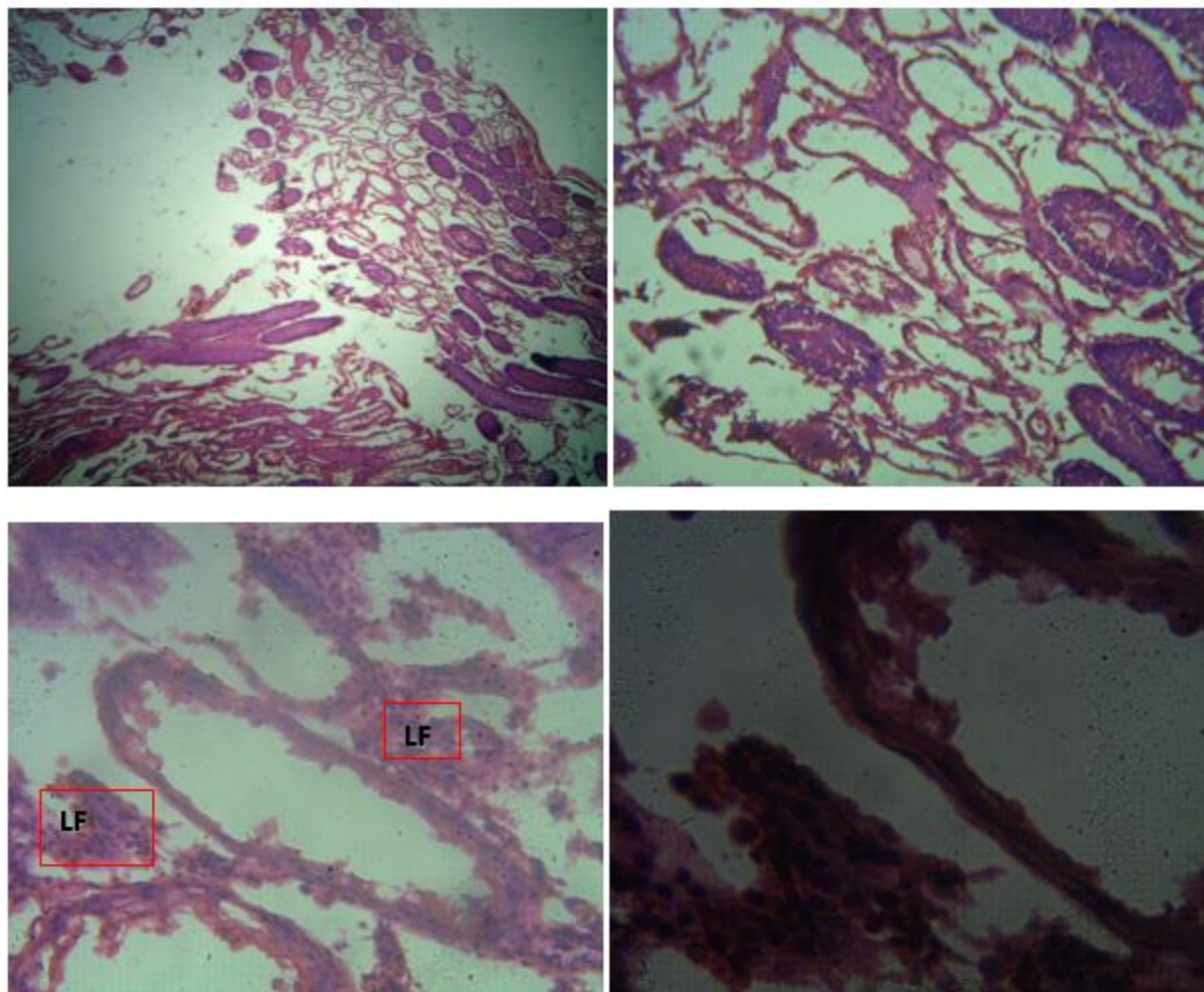


Plate 5: Photomicrograph of Group 5: 6mg/kg b. wt 22days- The testes shows eroded spermatogenic epithelium indicating arrested spermatogenesis. There is mild lymphocytic cell infiltration [LF] of the interstitium indicating inflammation of tubules (Sm). Interstitial cells of Leydig are not distinguishable (green). There is lack of spermatogonia and spermatocytes and no spermatids in the lumen

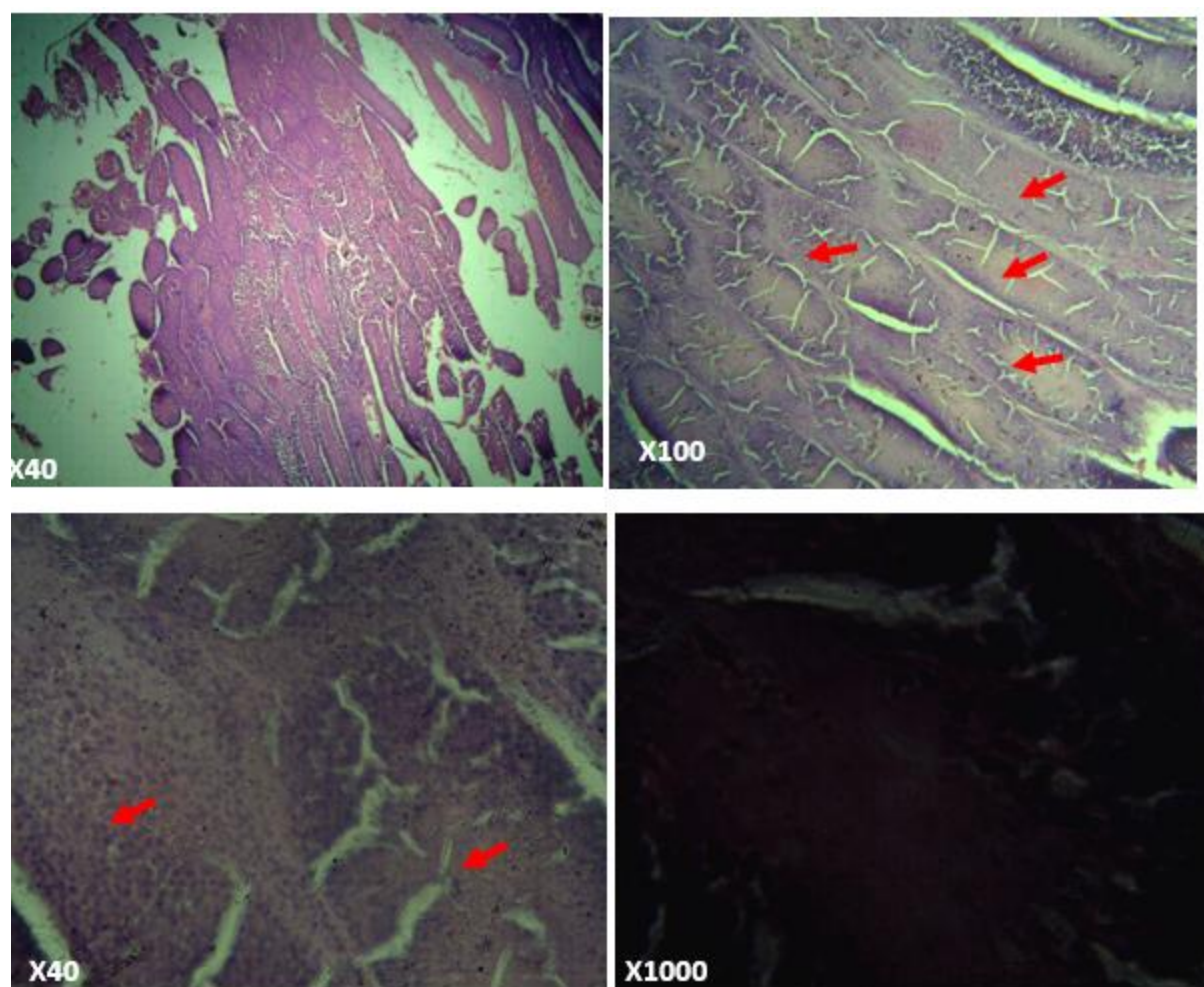


Plate 6 :Photomicrograph of Group 6, 9mg/kg b. wt 11days- The testes shows hyalinization of the seminiferous tubules (red arrow) indicating distortion of spermatogenic activities. The Spermatogonia, spermatocytes, spermatids and interstitial cells of Leydig are obliterated.

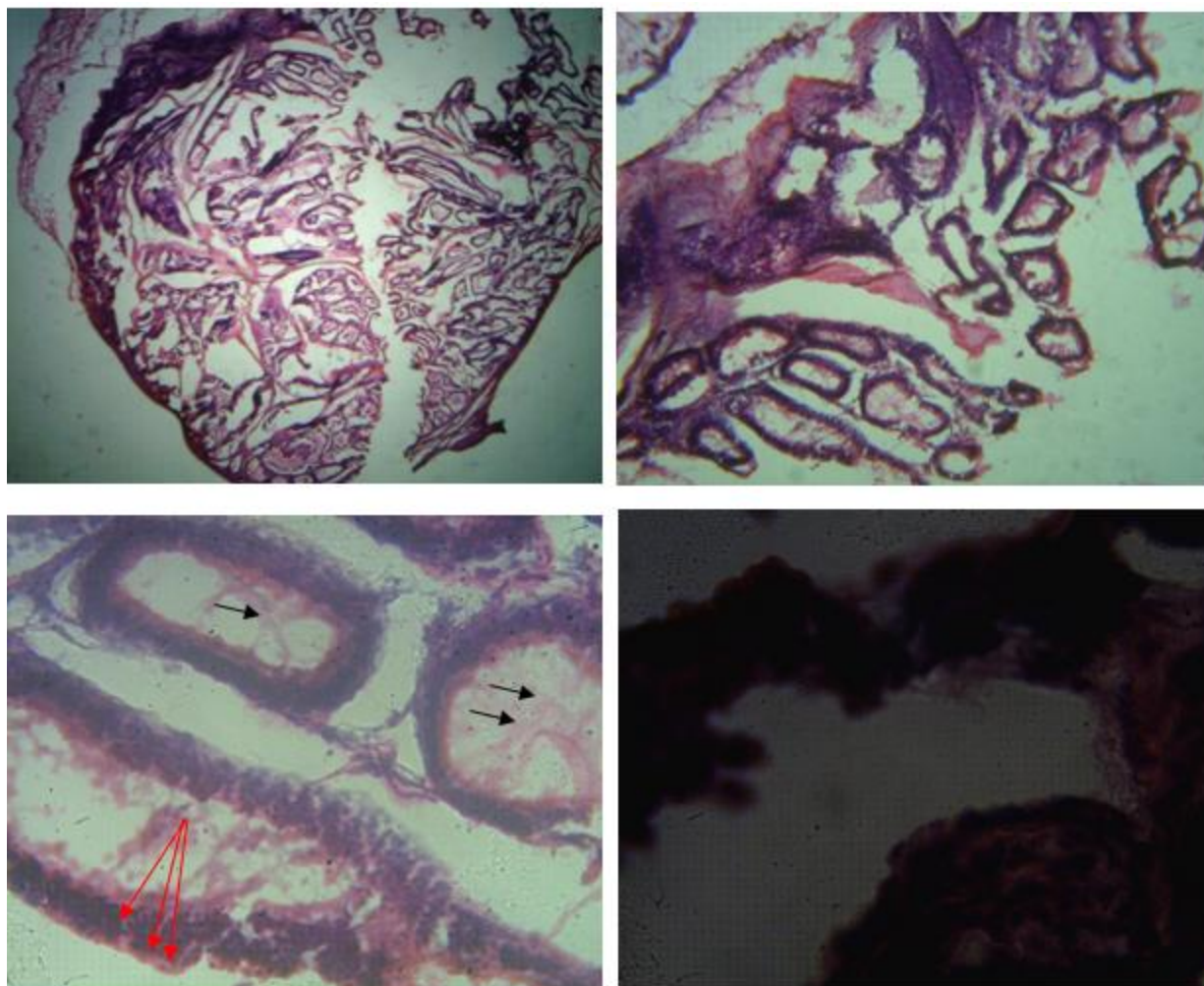


Plate 7: Photomicrograph of Group 7: 9mg/kg b. wt 22 days- The testes shows altered spermatogenic epithelium with more of spermatogonia (red arrow) indicating distorted spermatogenesis. Spermatids in the lumen are few (black arrow)

3.2 DISCUSSION

3.2.1. Semen Motility and Active Cells

The experimental groups treated with testosterone propionate (TP) exhibited a significant ($P = .05$) decline in sperm motility and active cells compared to the normal control (Group 1). In Group 1, sperm motility was $85.00 \pm 13.23\%$, while in Group 7 (highest TP dose for 22 days), it dropped drastically to $24.67 \pm 24.03\%$. Similarly, the percentage of active sperm cells decreased from $73.33 \pm 11.55\%$ (control) to $4.67 \pm 4.16\%$ (Group 7).

This aligns with Yassin et al. (2025) [10], who reported that exogenous testosterone administration suppresses spermatogenesis by reducing follicle-stimulating hormone (FSH) and luteinizing hormone (LH), leading to impaired sperm motility. Silveira et al. (2023) [12] also noted that long-term testosterone administration negatively affects sperm function.

3.2.2. Sluggish and Dead Cells

A marked increase in sluggish and dead sperm cells was observed across the TP-induced groups. In Group 1, dead sperm cells were $15.00 \pm 13.23\%$, whereas in Group 7, the percentage rose to $75.33 \pm 24.03\%$. The increase in sluggish sperm suggests a deteriorating sperm quality, which has been previously linked to testosterone therapy-induced gonadal suppression [9]

3.3.3. Total Sperm Count and Viability

The total sperm count (TSC) and sperm viability followed a decreasing trend. The control group had a TSC of $113.33 \pm 31.53 \times 10^6$ cells/mL, but in Group 7, it plummeted to $25.00 \pm 4.58 \times 10^6$ cells/mL. Viability also reduced from $94.33 \pm 2.89\%$ (control) to $86.67 \pm 5.77\%$ (Group 7). According to Agarwal et al. (2021), [13], testosterone therapy leads to oligospermia due to suppression of gonadotropins.

3.3.4. Testosterone, LH, and FSH Levels

A paradoxical trend was observed: testosterone concentration increased significantly ($(P = .05)$) in TP-induced groups, with Group 7 showing 8.20 ± 0.79 ng/mL compared to 3.67 ± 0.81 ng/mL in the control. Conversely, FSH and LH levels were significantly lower in TP-treated groups, indicating HPG axis suppression, confirming Yassin et al. (2025).[10] findings that exogenous testosterone inhibits the hypothalamic-pituitary-gonadal axis, reducing endogenous FSH and LH levels. These findings suggest that exogenous hormone regulation, leading to impaired spermatogenesis and potential infertility. The suppression of LH and FSH highlights the negative feedback effect of exogenous testosterone on gonadotropin secretion

3.3.5. Histological Alterations

Testicular histology showed normal spermatogenesis in the control group as shown in plate 1, whereas testosterone-treated groups exhibited varying degrees of degeneration and structural distortions such as hyalinization of seminiferous tubules and depletion of spermatogenic cells, especially in the high dose as shown in plate 7 with 9mg/kg b.wt of testosterone propionate for 22 days, this is similar to findings by Awarajih, U. C et al., (2024) [14]

4. Conclusion

The results confirm that exogenous testosterone administration leads to a decline in sperm quality, suppression of FSH and LH, and testicular structural damage, which are consistent with previous literature. This study demonstrates that testosterone propionate administration significantly alters semen quality, hormonal balance and testicular histology in male wistar rats. The observed suppression of FSH and LH levels suggests an inhibitory effect on endogenous testosterone production leading to impaired spermatogenesis and potential infertility. These findings align with clinical reports indicating that exogenous testosterone therapy may contribute to male fertility. Future research should explore the reversibility of these effects following the cessation of testosterone administration. Additionally, further studies on the molecular mechanisms underlying testosterone –induced testicular dysfunction are necessary to develop potential therapeutic strategies for mitigating the adverse reproductive effects of exogenous testosterone use.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

CONSENT

It is not applicable

ETHICAL APPROVAL

The experimental protocol of the present study was thoroughly reviewed and approved by the University of Port Harcourt ethical Committee with Reference no: UPH/CEREMAD/REC/MM98/048, University of Port Harcourt, Choba, Rivers State, Nigeria

REFERENCES

1. Liang, Y., Huang, J., Zhao, Q., Mo, H., Su, Z., Feng, S., ... & Ruan, X. (2025). Global, regional, and national prevalence and trends of infertility among individuals of reproductive age (15–49 years) from 1990 to 2021, with projections to 2040. *Human Reproduction*, deae292.
2. Eisenberg, M. L., Esteves, S. C., Lamb, D. J., Hotaling, J. M., Giwercman, A., Hwang, K., & Cheng, Y. S. (2023). Male infertility. *Nature Reviews Disease Primers*, 9(1), 49.
3. Feeley, R. J., Saad, F., Guay, A., & Traish, A. M. (2009). Testosterone in men's health: a new role for an old hormone. *Journal of Men's Health*, 6(3), 169-176.
4. Cohen, J., Nassau, D. E., Patel, P., & Ramasamy, R. (2020). Low testosterone in adolescents & young adults. *Frontiers in endocrinology*, 10, 916.
5. Marberger, M., Wilson, T. H., & Rittmaster, R. S. (2011). Low serum testosterone levels are poor predictors of sexual dysfunction. *BJU international*, 108(2), 256-262.
6. Barbonetti, A., D'Andrea, S., & Francavilla, S. (2020). Testosterone replacement therapy. *Andrology*, 8(6), 1551-1566.
7. Lawton, J., & Amjad, M. (2023). Weighing the Benefits and Drawbacks of Testosterone Replacement Therapy. *Sciential-McMaster Undergraduate Science Journal*, (10).
8. Basheer, B., Ila, V., Barros, R., Mesquita, F., Lopes, L. S., Lima, V. F. N., ... & Ramasamy, R. (2025). Management of Adverse Effects in Testosterone Replacement Therapy. *International braz j urol: official journal of the Brazilian Society of Urology*, 51(3)
9. Di Guardo, F., Vloeberghs, V., Bardhi, E., Blockeel, C., Verheyen, G., Tournaye, H., & Drakopoulos, P. (2020). Low testosterone and semen parameters in male partners of infertile couples undergoing IVF with a total sperm count greater than 5 million. *Journal of clinical medicine*, 9(12), 3824
10. Yassin, A., Al-Zoubi, R. M., Alzubaidi, R. T., Kamkoum, H., Zarour, A. A., Garada, K., ... & Al-Ansari, A. A. (2025). Testosterone and men's health: An in-depth exploration of their relationship. *UroPrecision*
11. Al-Suhaimi, E. A., Khan, F. A., & Homeida, A. M. (2022). Regulation of male and female reproductive functions. In *Emerging concepts in endocrine structure and functions* (pp. 287-347). Singapore: Springer Nature Singapore.
12. Silveira, L. F. G. (2023). of Testosterone Therapy. *Testosterone: From Basic to Clinical Aspects*, 331.
13. Agarwal, A., Baskaran, S., Parekh, N., Cho, C. L., Henkel, R., Vij, S., ... & Shah, R. (2021). Male infertility. *The Lancet*, 397(10271), 319-333.
14. Awarajih, U. C., Onyegeme-Okerenta, B. M., Amadi, B. A., & ThankGod, A. O. (2024). Biochemical evaluation of testosterone propionate-induced benign prostatic hyperplasia in Wistar rat. *Scientia Africana*, 23(5), 49-58.
15. Oghenechuko, E. H., Chinazo, M. I. C., & Emmanuel, P. D. (2024). Spermatogenic and Androgenic Potential of Nephrolepis biserrata on Testosterone-Propionate Induced Benign Prostatic Hyperplasia In Male Wistar Rats. *J. Appl. Health Sci. Med*, 4(10), 21-37.

