Al-Humidi, A. & Abdulsalam, M.; Yemeni J Med Sci. 2025; 19(4):8 -14
doi: https://10.20428/yjms.v19i4.2793
https://journals.ust.edu/index.php/yjms

OPEN ACCESS ORIGINAL ARTICLE

Significant Association between Sleep Deprivation and Somato Testicular Index: an experimental study on Albino Rats

Ameera Al-Humidi [1, 2*], Mohammed Abdulsalam [1]

[1] *Basic Science Department, Faculty of Medicine and Health Sciences, University of Science and Technology, Aden, Yemen*
[2] *Faculty of Medicine, National University, Sudan*

ABSTRACT

Background: Sleep deprivation is total or partial absence of sleep. It is a risk factor for many diseases including disorders of reproductive system**.**
Objective: This study aimed to investigate the correlation between sleep deprivation and testicular morphology in albino rat model.
Method: Twelve adult albino rats with average weight 300±20 grams divided randomly into 6 as a control group and 6 as a study group. Study group was exposed to sleep deprivation by continuous light 24 h and intermittent noisy alarming shuffled daily regarding duration and timing. Weight of rats was reported at the beginning and at the end of the experiment. After general anesthesia and scarification, the testes were retrieved, fixed in Bouin's solution, dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin. Sections of 5 µ thicknesses were stained by hematoxylin and eosin. Relative testis weight, testicular volume, were determined and somato-teticular index was computed using SPSS version 24.
Results: in the study group significant weight reduction was observed, however significant increased testicular volume and subsequent somato-testicular index STI ($p < 0.05$) were observed. Study group showed swollen edematous testes with redness appearance and apparently dilated vessels on the surface. Light microscopy showed slightly separated seminiferous tubules, reduced number of Leydig's cells containing apoptotic vacuoles as well as vacuoles within cells of Sertoli, atrophied seminiferous tubules with mild fluid accumulation within the stroma and reduced sperm cell count in the center.
Conclusion: a statistically significant association between sleep deprivation and testicular damage had been detected together with alteration in somato-testicular index.

Key Words: sleep deprivation, testicular damage, infertility, testicular interstitial stroma.

**** Corresponding author address:*** *asalam62@gmail.com*

2025 University of Science and Technology, Main Campus Aden, Yemen. This article can be unrestrictedly used, distributed or produced in any medium, provided that credit is given to the authors and the journal. Online ISSN: 2227-961X.

INTRODUCTION

Sleep is a crucial biological process linked to changes in neuroendocrine function and immunity. Sufficient sleep is essential for maintaining overall health, including proper fertility. Studies have demonstrated a strong connection between adequate sleep and the secretion of gonadotropin-releasing hormone (GnRH), which plays a fundamental role in reproductive functions (1).

Sleep deprivation is identified when there is either a complete absence of sleep for a certain period or an insufficient amount of the recommended optimal sleep duration. It is recognized as a form of stress, with recent studies highlighting its high morbidity. Sleep deprivation has been shown to impact endocrine and reproductive functions, as well as the central and peripheral nervous systems (1, 2).

The testes, or testicles, are male reproductive organs responsible for sperm production and the synthesis of the hormone testosterone. They have a unique anatomy that the testes are oval-shaped and located within the scrotum, which is a pouch of skin below and behind the penis. The testes consist of lobules containing seminiferous tubules, where sperm production occurs. These tubules are lined by Sertoli cells that aid in sperm maturation. The interstitial tissue contains Leydig cells, which are responsible for testosterone production (3).

The testes (testicles) are paired, oval-shaped male gonads located within the scrotum, a sac of skin that provides a cooler environment essential for sperm production. Each testis is approximately 4-5 cm in length and 2.5 cm in diameter. They are suspended within the scrotum by the spermatic cord, which contains blood vessels, nerves, and the vas deferens (4).

Each testis is covered by three layers: Tunica vaginalis: The outer serous membrane derived from the peritoneum, Tunica albuginea: A tough, fibrous capsule that extends inward to form septa and Tunica vasculosa: A thin vascular layer beneath the tunica albuginea (5).

Inside each testis, the septa divide the testis into about 250-300 lobules. Each lobule contains 1-4 seminiferous tubules, where spermatogenesis (sperm production) occurs. These tubules are lined with Spermatogenic cells (germ cells in various stages of development) (4).

Sertoli cells, which provide support, nutrients, and hormonal signals to developing sperm. Between the seminiferous tubules, the interstitial tissue contains: Leydig cells, which synthesize and secrete testosterone, the primary male sex hormone (4).

Microscopic structure of the seminiferous tubule is uniformly composed of two layers of fibrous tissue of equal thickness. The total connective tissue content of the testes is less than 10% (4). The rete testis is lined with low cuboidal epithelium and is divided into two sections: intratesticular and extra testicular, which are joined by various channels that enter the tunica albuginea obliquely. The intratesticular component of the rete is located on the craniodorsal side of the testis, beneath the tunica. The extra testicular component of the rete is made up of a thin cord-like segment with anastomosing tubules that open into a large cap-shaped area with a diameter of approximately 1 mm (7, 8).

Testicular tissue is particularly vulnerable to oxidative damage due to its distinct structural composition, which includes polyunsaturated fatty acids. The excessive generation of reactive oxygen species (ROS) in semen can lead to the loss of membrane integrity, DNA damage, and apoptosis of spermatozoa (3).

The testes have two main functions: Spermatogenesis that takes place in the seminiferous tubules and regulated by follicle-stimulating hormone (FSH) from the pituitary gland and testosterone from the Leydig cells and Hormone Production by Leydig cells produce testosterone, regulated by luteinizing hormone (LH) from the pituitary gland. Testosterone is essential for spermatogenesis, male secondary sexual characteristics, and libido (9, 10).

This study aimed to investigate the effects of sleep deprivation on the structure of the testes in albino rat model through subjecting a study group of albino rats to continuous light and noisy alarm compared to a matching control group which is allowed to have a normal day – night sleeping rhythm life.

METHODOLOGY

Study design

This was an experimental study registered in the Faculty of Medicine, National University in Sudan at 2023. The experiment was performed in the animal

© 2025 University of Science and Technology, Main Campus Aden, Yemen. This article can be unrestrictedly used, distributed or reproduced in any medium, provided that credit is given to the authors and the journal. Online ISSN: 2227-961X.

house Al Azahar University in Cairo Egypt. 12 male albino rats of average weight of 300±20 gm were divided into equally to control group and study group. The duration of the experiment was eight weeks.

Animals

All the rats were obtained from Theodor Bilharz Animal House in Cairo, Egypt. They were housed in stainless steel cages with wood shaving beddings, kept at a constant temperature of 25 ± 2◦ C, relative humidity of approximately 50%, and fed a standard diet in heavy pot containers. The experimental procedures were conducted in adherence to the Guiding Principles in the Use and Care of Animals published by the National Institutes of Health (NIH Publication No 85–23, Revised 1996). The rats were kept for 10 days prior to the start of the study to allow proper acclimatization. The animals were fed standard laboratory chow and allowed free access to water in an air-conditioned room with a 12 h light-dark cycle. Ethical approval was obtained from the Ethical Committee of the Faculty of Medicine, National University, Sudan.

Animal groups

After the acclimatization period, the rats were randomly divided into two experimental groups, each consisting of six rats.

Group I: (Control group)

The rats were maintained at room temperature under a natural day-night cycle and normal conditions throughout the entire experiment, with unrestricted access to water and a balanced diet.

Group II: (Sleep deprivation group)

Maintaining a similar feeding and temperature condition of the control group, the study group was subjected to a sleep deprivation process through exposure to continuous light and irregular noisy alarm. The selected rats were transferred to a separate room. The noisy alarming was programed to be 15 minutes ringing phase followed by 10 minutes silent phase throughout the day. The light source was light-emitting diode (LED) bulbs.

Specimen collection

All the rats were weighed immediately before and after the experiment. The testes were retrieved after scarification under general anesthesia. Length (L), width (W), and thickness (T) of testes were calculated with help of vernier caliper. Volume (V) of the testes was obtained applying the formula mentioned by (11).

$V = 4/3 \times \pi \times L/2 \times W/2 \times T/2$

Where pi (π) is a constant figure and its value is 3.141. In addition, Somato-testicular index (STI) was computed by the following formula:

STI = (testis weight / body weight) x 100 (12).

Histopathology examination

The right testes were preserved in 10% formol saline for 5–7 days. The specimens were rinsed in tap water for 10 minutes and then dehydrated using graded ethanol solutions (70% and 90% overnight, followed by three changes of 100% ethanol for 1 hour each). They were then cleared in xylene (20–30 times). After that, the specimens were impregnated with soft paraffin wax at 55–60°C for 2 hours, followed by embedding in hard paraffin wax at room temperature using molds. Tissue blocks were sectioned into 5 µm thick slices using a rotary microtome. The sections were placed in a warm water bath, transferred onto clean slides, and placed on a hot plate for 2 minutes. Finally, the tissue sections were stained with hematoxylin and eosin (H&E) to examine the general architecture of the studied tissues. All procedures were conducted by an experienced technician under the supervision of the researcher. The histological examination was carried out by the researcher in consultation with a senior histopathologist.

Statistical analysis

The relative testis weight, testicular volume, and somato-testicular index were computed using SPSS version 24, SPSS Inc, Chicago, Illinois, USA. Dual comparisons between groups exhibiting significant values were evaluated with a Mann-Whitney U test. These differences were considered significant when p value was less than or equal to 0.05.

RESULTS

The result showed no significant difference in body weight between the two groups at the start of the experiment whereas significant reduction was observed among the study group was observed after

© 2025 University of Science and Technology, Main Campus Aden, Yemen. This article can be unrestrictedly used, distributed or reproduced in any medium, provided that credit is given to the authors and the journal. Online ISSN: 2227-961X.

doi: https://10.20428/yjms.v19i4.2793
https://journals.ust.edu/index.php/yjms

the end of the experiment. (340±30.50 g vs. 223.3±173.94 g, p <0.05) (Table 1). Interestingly, there was significant increase in the somato-testicular index in the study group when compared to the control group (0.3±0.23 vs. 0.7±0.01, p <0.05) (Table 2). The gross appearance of testes in the study group was swollen, edematous and reddish with dilated vessels on the surface of the testes when compared to the testes of the control group.

Histopathologic examination

The control group showed the following features:

1. Many seminiferous tubules separated from each other by narrow interfollicular spaces.
2. Each tubule surrounded by well-defined basal lamina, lined by stratification of spermatogenic cells and Sertoli cells
3. Mature spermatozoa in the lumen.
4. Clusters of the interstitial cells of Leydig with acidophilic cytoplasm were found in the triangular areas between the seminiferous tubules (Figure 1).
5. While the study group showed the following features:
6. Massive destructive changes including distortion of the seminiferous tubules.
7. Loss of germ cells.
8. Spermatogenic cells showed apoptotic figures as pyknotic nuclei and degenerated ones, decreased height of seminiferous epithelium,
9. Total absence of spermatozoa in some tubules.
10. Cytoplasmic vacuolations of spermatogenic cells and detachment of the germ cells from the basement membrane in some tubules.
11. The interstitial tissue showed dilated congested blood vessel.
12. Vacuolation, hyalinized acidophilic material and degeneration of the interstitial Leydig cells with deeply stained pyknotic nuclei with a mild accumulation of fluid within the stroma. (Figure 2).

Table 1: Mean and standard deviation of the body weight in grams of the control group and study group before and after the experiment

	Control group	Study group	P value
Before experiment	342.5±32.37	362±35.03	-
After experiment	340±30.50	223.3±173.94	0.001
P value	0.6	0.001	

Table 2: Difference in STI between study group and control group after the end of the experiment

	Mean ± SD		P-Value
STI	Study group	Control group	0.002
	0.7±0.01	0.3±0.23	

Table 3: difference in Volume of testes in cc between control group and study group after the end of the experiment

	Volume in cc (mean +/- SD)	P-Value
Study group	352.1±39.2	0.004
Control group	249.9±19.7	

© 2025 University of Science and Technology, Main Campus Aden, Yemen. This article can be unrestrictedly used, distributed or reproduced in any medium, provided that credit is given to the authors and the journal. Online ISSN: 2227-961X.

doi: https://10.20428/yjms.v19i4.2793
https://journals.ust.edu/index.php/yjms

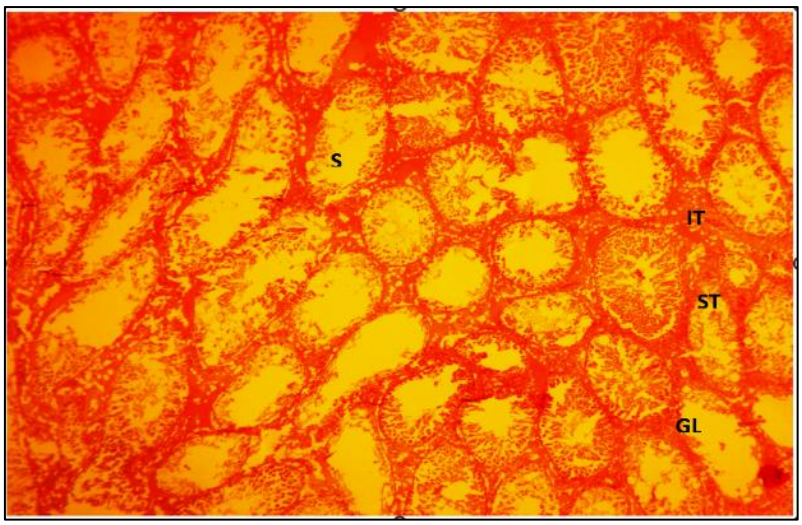

Figure (1): H & E stain x200) of the testis in control group (seminiferous tubules (ST) germinal layers (GL) sperms (S) interstitial tissue (IT))

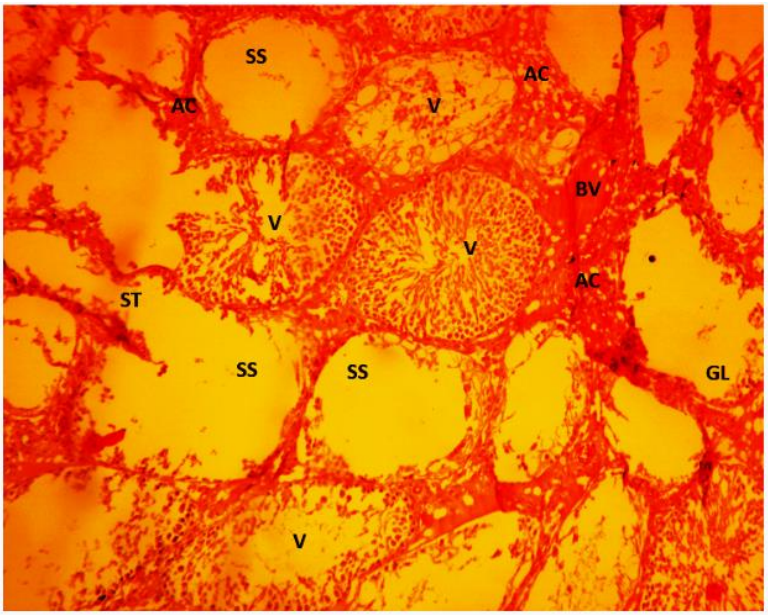

Figure (2): Hx. & E stain x200 of the testes of the study group (seminiferous tubules (ST) germ layers (GL) vacuolation (V) scanty sperms (SS) acidophilic vacuoles (AC)

DISCUSSION

This study investigated the correlation between sleep deprivation and testicular morphology via experimental study using an albino rats model. Concerning the general results to best of our knowledge, studies on testiculo-somatic index correlation with sleep deprivation are scant. Yet the present study showed a highly significant altered of testiculo-somatic index manifested with weight reduction in the study group (p-value< 0.05). This agrees with (13, 14) who explained the weight-reducing effect of sleep deprivation may be due to the alteration of hormonal activity that interferes with metabolism and dietary components.

Interestingly, the current study showed swollen, red and edematous testes, with apparently dilated

© 2025 University of Science and Technology, Main Campus Aden, Yemen. This article can be unrestrictedly used, distributed or reproduced in any medium, provided that credit is given to the authors and the journal. Online ISSN: 2227-961X.

vessels indicating clear evidence of inflammatory reactions. Almost all the retrieved studies did not comment on the gross appearance of the testes. Significant increase in testicular volume is observed in the current study among study group compared with the controls ($p< 0.05$). This could be attributed to fluid accumulation resulted from inflammatory process accompanying the oxidative stress similar to what was mentioned by (14).

In addition, Rizk et al 2020 concluded in his study that histopathological analysis of the testes from the study group demonstrated that sleep deprivation had harmful effects on the seminiferous epithelium (14). The consequences included a significant reduction in the number and viability of spermatogenic cells, with several cells displaying features of apoptosis. The diminished seminiferous epithelium exhibited a considerable quantity of degraded cells, presumably due to the detrimental impact of reactive oxygen species (ROS). This finding (apoptosis) is in agreement with who states that apoptosis in germ cells can be caused by acute or chronic stress (15).

Scanty literature reporting on the influence of sleep deprivation per se in testicular destruction; yet evidences of altered microstructures of seminiferous tubules is reported in association with stress in general. Cytoplasmic vacuolation and degeneration reported in the present study could be considered as a sign of cell necrosis. This is in coincidence with reports that correlate the presence of vacuoles in the seminiferous tubules with the digestion of the necrotic germ cells (15). Injecting in the same vein of poor sperm quality had been reported in the current study showing agreement with (16).

CONCLUSION

sleep deprivation has detrimental effects on male reproductive system. This is manifested by our findings which showed inflammatory changes as well as alteration in the testiculo-somatic index.

ACKNOWLEDGMENTS

Authors wish to thank the Faculty of Medicine, National University, Sudan and Prof. Dr Qurashi for providing facilities. Also, thanks for the Faculty of Medicine and Faculty of Pharmacy, Azhar University for providing facilities. Authors would also like to acknowledge the valuable assistance of Prof. Dr. Ahmed Mansour and Dr. Amjad and members of the Animal House Lab, Faculty of Medicine, Al-Azhar University.

FUNDING

This research was conducted without any specific financial support from public, commercial, or non-profit funding agencies.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

[1] Lateef OM, Akintubosun MO. Sleep and reproductive health. J Circadian Rhythms. 2020 Mar 23;18:1.

[2] Crosswell AD, Lockwood KG. Best practices for stress measurement: how to measure psychological stress in health research. Health Psychol Open. 2020;7:2055102920933072.

[3] Walke G, Gaurkar SS, Prasad R, Lohakare T, Wanjari M. The impact of oxidative stress on male reproductive function: exploring the role of antioxidant supplementation. Cureus. 2023 Jul 27;15(7):e42587.

[4] Junqueira LC, Carneiro J. Basic histology: text & atlas. 16th ed. McGraw Hill; 2020.

[5] Standring S. Gray's anatomy: the anatomical basis of clinical practice. 42nd ed. Elsevier; 2020.

[6] Cheng CY, Mruk DD. The blood-testis barrier and its implications for male contraception. Pharmacol Rev. 2012;64(1):16-64.

[7] Nistal M, Paniagua R. Non-neoplastic diseases of the testis. Urol Surg Pathol. 2020 Jun 22;614.

[8] Sadler TW. Langman's medical embryology. 14th ed. Wolters Kluwer; 2019.

[9] Choi JH, Lee SH, Bae JH, Shim JS, Park HS, Kim YS, et al. Effect of sleep deprivation on the male reproductive system in rats. J Korean Med Sci. 2016;31(10):1624-30.

[10] Moore KL, Dalley AF, Agur AMR. Clinically oriented anatomy. 9th ed. Wolters Kluwer; 2023.

© 2025 University of Science and Technology, Main Campus Aden, Yemen. This article can be unrestrictedly used, distributed or reproduced in any medium, provided that credit is given to the authors and the journal. Online ISSN: 2227-961X.

[11] Khan H, Rafique M, Jiskani AR. Testicular volume: indicator for male fertility in albino rats. Prof Med J. 2015 Oct 10;22(10):1356-61.
[12] Kurowicka B, Chrusciel M, Zmijewska A, Doroszko M, Kotwica G, Rahman NA. Distinct testicular steroidogenic response mechanisms between neonatal and adult heat-acclimated male rats. Cell Physiol Biochem. 2015 Apr 1;35(5):1729-43.
[13] Tajik R, Heshmati H, Zahedi M, Yousefi P, Asadi-Noghabi F. Coronavirus and sexual transmission: a systematic review. Hormozgan Med J. 2023 Sep 28;27(3):162-7.
[14] Rizk NI, Rizk MS, Mohamed AS, Naguib YM. Attenuation of sleep deprivation-dependent deterioration in male fertility parameters by vitamin C. Reprod Biol Endocrinol. 2020 Dec;18(1):1-3.
[15] Du CQ, Yang YY, Chen J, Feng L, Lin WQ. Association between sleep quality and semen parameters and reproductive hormones: a cross-sectional study in Zhejiang, China. Nat Sci Sleep. 2020;12:11-8.
[16] Tortora GJ, Derrickson B. Principles of anatomy and physiology. 16th ed. Wiley; 2021.

© 2025 University of Science and Technology, Main Campus Aden, Yemen. This article can be unrestrictedly used, distributed or reproduced in any medium, provided that credit is given to the authors and the journal. Online ISSN: 2227-961X.