African Scientist Vol. 24, No. 3 September 30, 2023
Printed in Nigeria

1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

AFS2023044/24305

Testes Histo-Morphological Changes in Laboratory Rats, *Rattus norvegicus*, Exposed to Various Spectral of Artificial Light at Night (ALAN)

Festus Olasehinde Kehinde[1], Gabriel Adewunmi. Dedeke[2], Matthew Ayotunde Olude[3], Kehinde Oluwatoyin Ademolu[2], Adeyinka A. Aladesida[2] and Folarin Ojo Owagboriaye[4]

[1]Department of Animal and Environmental Biology, Faculty of Natural Science, Prince Abubakar Audu University, Anyigba, Nigeria
[2]Department of Pure and Applied Zoology, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria
[3]Department Anatomy, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria
[4]Department of Zoology and Environmental Biology, Faculty of Science, Olabisi Onabanjo University, Ago-Iwoye, Nigeria

*Corresponding author Email: kehinde.fo@ksu.edu.ng, Tel: +234 (0) 803 819 2339

(Received August 25, 2023; Accepted in revised form September 11, 2023)

ABSTRACT: Exposure to various spectral of light at night is presently inevitable, hence the need to examine its spectral effect on male reproductive function. This study aimed at evaluating the periodical change in testicular histomorphology of albino rats exposed to various lights spectral {Blue (BL), Green (GL), Yellow (YL), Red (RL), and White (WL)} at night for 126 days. Compact fluorescence bulbs, 11 watts, maintained at 300 Lux, were used. At d_{63}, d_{91}, and d_{126}, four rats per treatment were euthanized and the testes removed, weighed, and fixed in 10 % formalin for histological examination. Testicular weight (TW) was significant ($p < 0.05$) with the highest values under BL at d_{63}, d_{91}, and d_{126}. Rats exposed to YL had the least significant ($p < 0.05$) TW at day 126. TW increased significantly ($p < 0.05$) under GL and significantly reduced ($p < 0.05$) under YL considering the difference between d_{63} and d_{126}. Non-specific atrophic degeneration of seminiferous tubules was observed under darkness with age. Evidence of displacement and degeneration of the spermatogonia was observed under YL. Exposure to YL and darkness appeared to cause a threat to reproduction as BL and GL to enhance male reproductive function in the albino rats.

Keywords: Albino rats, ALAN spectral, Testicular weight, Histopathology

Introduction

ALAN is the light that animals are exposed to at night which is considered hazardous or pollutant, it was first described by the astrologist and secondly by a chronobiologist and presently of a concerns to oncologist. Exposure to artificial light at night is currently a global phenomenon (Haim *et al.*, 2019) and it has been implicated in several physiological alterations including: polycystic ovarian dysfunction in female rodents (Salvetti *et al*., 2003; Miloševi´c *et al*., 2005); alteration of circadian rhythm and metabolic processes (Fonken *et al.*, 2013; Challet and Kalsbeek, 2017;Ali *et al.*, 2017); melatonin suppression and ultimately downregulation of physiological processes (Falchi *et al.*, 2011; Haim and Portnov, 2014; Zubidat *et al*., 2018). It also disrupts endocrine activity resulting in hormonal irregularities. Vital glands in the body that either directly or indirectly have an impact on reproduction are also discovered to be affected by light (Olatunji-Bello and Sofola, 2001; Gawad *et al.*, 2019).

A photoperiodic animal like the albino rat depends largely on light signals for an array of events that position and prepare them for reproductive success (Hoffmann, 1970). Since artificial light is also presently unavoidable because it enhances working capacity, productivity, etc. For instance, most of the gadgets used today radiate one

type of light color or the other. The impact of light at night on various physiological processes in the body therefore cannot be underrated.

Majority of studies on photoperiodism have focused on white light (Freeman *et al.,* 2000; Biswas *et al.,* 2013; Dominoni *et al.,* 2013; Robert *et al.,* 2015). However, studies have shown that various light spectra have different physiological responses on various organs in the body (Ashkenazi and Haim, 2012; Haim *et al.,* 2019; Zubidat *et al.,* 2011) with light colors affecting melatonin secretion, glucocorticoid concentration and DNA methylation in social voles (*Microtus socialis*) and 'blind' mole rats (*Spalax ehrenbergi*).

Artificial light has a significant effect on the gonads of avians (Chang *et al*. 2016), and increase testicular weight in rodents after long exposure to white light (Hasting *et al.,* 1989, Olatunji-Bello and Sofola, 2001; Hance *et al*., 2009; Bisway et al., 2013). However, Ali *et al*. (2017) revealed the opposite in giant rats with a decrease in testicular weight when exposed to white light and the reason for this variance was because giant rats are nocturnal animals. Recently, infertility is on the increase, therefore this study was undertaken to understand the effect of different light spectra on the gonadal weight and testicular histomorphology of laboratory rat, which will also serve as a model to evaluate such consequences on other animals and human.

Materials and methods

Study area: The study was carried out at the Zoo Park, Federal University of Agriculture Abeokuta, Ogun State, Nigeria.

Experimental rats: The rats used for the study were obtained from the Institute for Medical Research and Training (IMRAT), University College Hospital (UCH) Ibadan, Nigeria.

Light treatments: The light treatment is as described in Kehinde *et al*. (2023). Briefly, rats were exposed to red, yellow, green, blue and white lights. The wavelength of the light colors used was not determined. The light bulb used was of compact florescent and 11 watts power rating. Rats were also subjected to ambient light and total darkness as the controls.

Study design: The experimental design was in accordance with the work of Kehinde *et al*. (2023). Briefly, 105 day-old rats were distributed in triplicate of 5 rats into various treatments and the controls. Light exposure was done between 6 pm and 6 am and lasted for 126 days.

Collection and measurement of testes weight: Four rats from each treatment were randomly selected and anaesthetized using diethyl ether and sacrificed at days 63, 91, and 126. The paired testes were dissected out quickly and washed in 0.9 % (w/v) cold normal saline, pat, dry, and weighed using the top loading mass meter and the values recorded on the nearest 0.01g. The testes were further fixed in 10 % formalin for histomorphological studies.

Histomorphological analysis: The testes were randomly selected and processed for histological study. According to (Shobikhuliatul, 2013), the testicular tissues were sectioned at 5 µm and stained with Haematoxylin and Eosin and mounted in Canada balsam. The slides were then read under the binocular microscope, OLYMPUS CX 21, New York, using x 100 magnification.

Statistical analysis: The data collected were subjected to the analysis of variance (ANOVA) and the means were separated using Duncan multiple comparison. SPSS version 20 was used for the analysis.

Results

Changes in testicular weight of the rats exposed to light spectra at night: The mean testicular weights of laboratory rats exposed to Artificial Light at Night (ALAN) at days 63, 91, and 126 are shown in Table 1. The testicular weights of the rats under light color were significantly different ($p < 0.05$). At day 63, rats exposed to BL had the highest ($p < 0.05$) testicular weight (2.32 ± 0.13 g), followed by those under YL (2.25 ± 0.24 g) and significant ($p < 0.05$) weight was recorded under GL (1.00 ± 0.12 g). At day 91, testicular weight was significantly higher in the rats exposed to BL (2.42 ± 0.14 g), GL (2.36 ± 0.17 g), RL (2.34±0.19 g) and darkness (2.36 ± 0.48 g) and the least value recorded under ambient light (1.85±0.68 g). At day 126, the testicular weight of rats under BL was significantly ($p < 0.05$) highest (3.18±0.14) followed by those under GL (2.71 ± 0.04 g), darkness (2.69 ± 0.22 g) and WL (2.58±0.19 g) while rats exposed to YL (2.28 ± 0.11 g) had the least significant ($p < 0.05$) weights.

Table 1: Testicular weight of rats exposed to various light spectra at night at days 63, 91, and 126

Days / Light treatments	Testicular Weight (g) at Day		
	63	**91**	**126**
Control	1.71±0.46^{bc}	1.85±0.68^{a}	2.35±0.30^{ab}
Blue	2.32±0.41^{c}	2.42±0.14^{b}	3.18±0.14^{d}
Green	1.00±0.45^{a}	2.36±0.17^{b}	2.72±0.13^{c}
Yellow	2.09±0.39^{bc}	1.90±0.20^{a}	2.32±0.15^{a}
Red	2.20±0.45^{c}	2.34±0.19^{b}	2.55±0.09^{bc}
White	1.89±1.03^{bc}	2.27±0.23^{ab}	2.58±0.19^{c}
Darkness	1.43±0.39^{ab}	2.36±0.48^{b}	2.69±0.22^{c}

Means with different superscript in a column are significantly different ($P < 0.05$).

The increases in the testicular weight of albino rats between the various days were evaluated and shown in Figure 1. The testicular weight gain between days 91 and 63 showed a significant difference ($p < 0.05$) and was higher in rats exposed to GL (1.36 ± 0.30 g) and least in the rats exposed to YL (-0.15 ± 0.20 g). The testicular weight gain between days 126 and 91 showed a significant difference ($p < 0.05$). The weight gain was significantly higher in rats exposed to BL (0.76 ± 0.07 g) and least in rats exposed to RL (0.21 ± 0.11 g). The testicular difference between days 126 and 63 showed significant difference ($p < 0.05$). Rats exposed to GL (1.72±0.33 g) had the highest significant ($p < 0.05$) value followed by DD (1.27 ± 0.21 g), BL (0.86±0.28 g) and the least significant value was recorded under YL (0.23±0.25 g).

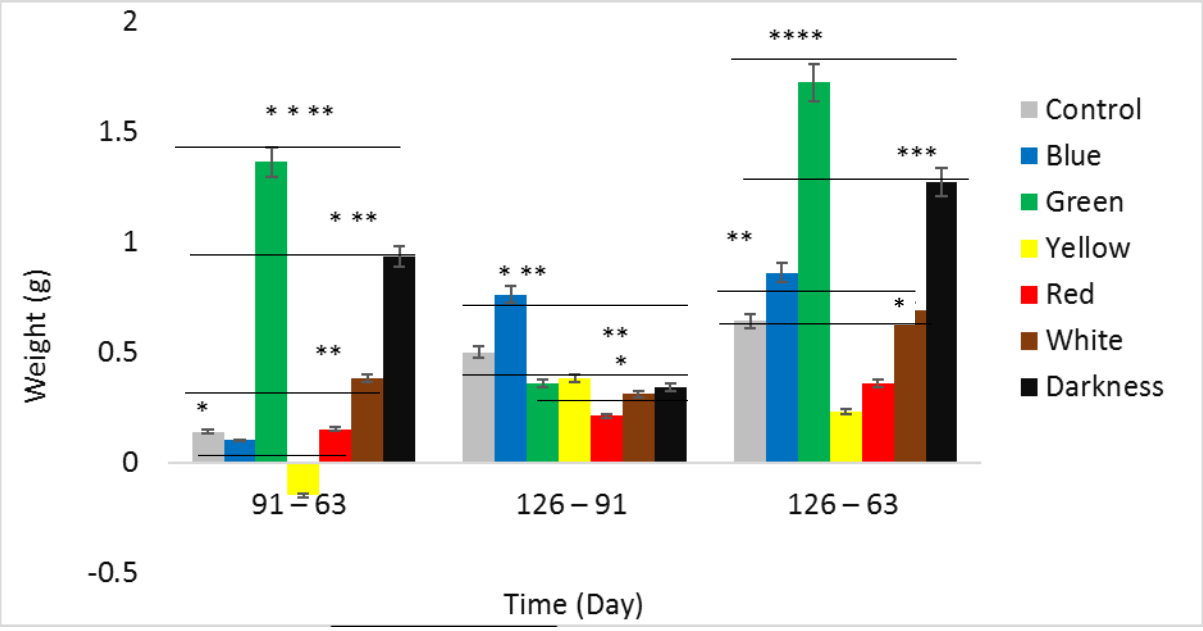

Figure 1: Testicular weight gain of albino rats between various days (63, 91, and 126 days) on exposure to various light colours at night

Histomorphology of testes of albino rats exposed to different light spectra for 126 days: The photomicrograph of the testes of albino rats under CL and DD at days 63, 91, and 126 are shown in Plate 1. Transverse section of the seminiferous tubules of animals under CL revealed normal testicular cytoarchitecture. Plates predominantly show basal spermatogonia as round, oval, darkly stained with dark or condensed chromatin (arrowheads) along the basement membrane with intact myofibroblasts (M). Other spermatogonia types and spermatids (SP) are unclearly differentiated with minimal spermatozoa (SZ) in the lumen. The Leydig cells (L) appear within the interstitium as normal cells with minimal lipid vacuoles indicative of reduced or yet to be fully activated testosteronergic activity. Spermatogenic activities increased on days 91 and 126.

The rat under DD had disoriented seminiferous tubules and showed a moderate degree of oedema, and the interstitial spaces appeared mildly oedematous. There was partial depletion of round spermatids accompanied with sloughing off into the lumen at day 63. At day 91, most of the spermatogonia type A was not resting on the basement membrane indicating depletion and degeneration, and other spermatogonia types and spermatids were disoriented. At day 126, there was nonspecific tubular degeneration, atrophy, and depletion of germ cells from tubules with no cell or stage specificity and seminiferous tubules appeared shapeless and disoriented.

At day 63, production of spermatozoa was observed in the lumen of the seminiferous tubules of rats exposed to BL. Spermatogenesis seemed to commence early in this group with histological pictures showing matured architecture. At day 91, tubules had the greatest diameter and spermatozoa production was comparable to control. At day 126, seminiferous tubules were elongated, typical as control animals.

On exposure to GL, no spermatozoa were however observed within the lumen, but there were hypertrophic germinal cells. The proliferation of myofibrils was observed although. On day 91, spermatozoa were observed in the lumen of the tubules, but there was partial depletion of elongated spermatids and depletion of germinal layer cells. There were spermatozoa, tubular atrophy was seen in one tubule, but at day 126, spermatozoa production appeared normal comparable to control.

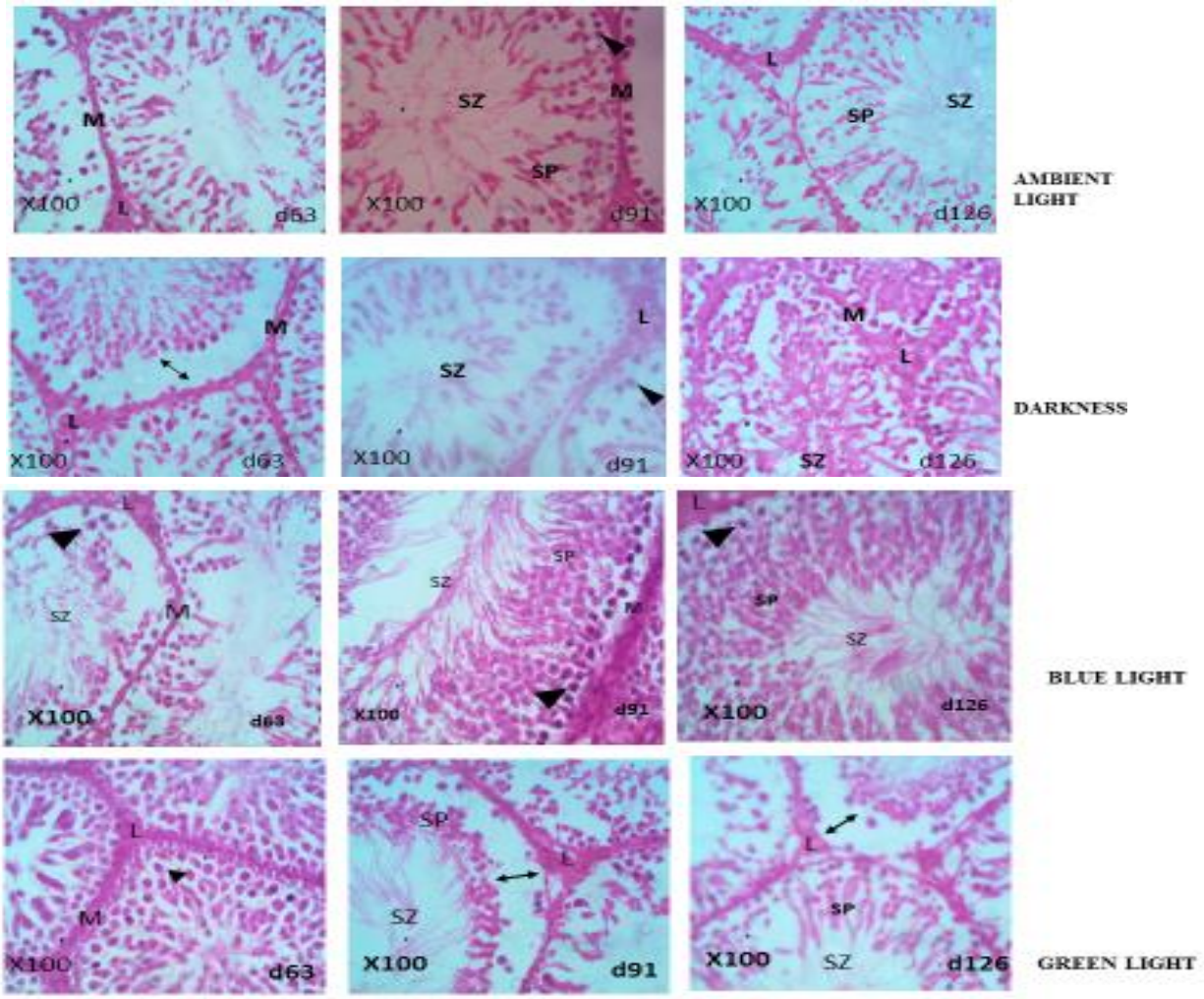

Plate 1: Representative photomicrographs of testes of rats under ambient and darkness at days 63, 91, and 126. Plates show basal type spermatogonia (arrowheads), basement membrane with myofibroblasts (M), Spermatogonia type; spermatids (SP) and Spermatozoa (SZ) in the lumen. Leydig cells (L) appear within the interstitium. Control (Ambient light) showing normal; seminiferous tubules, SP, L, no SZ at day 63. DD showing thinning of myofibres with evidence of hydropic changes, nonspecific atrophic degeneration of seminiferous tubules, there is evidence of SZ at days 91 and 126.

The photomicrograph of the testes of albino rats exposed to YL, RL, and WL at days 63, 91, and 126 are shown in Plate 2. For rats under YL, displacement of germinal cells was observed along layer VII with karyolysis and degenerative changes of clumped chromatin occurring in displaced cells at day 63. Pyknotic basal cells were also observed close to the lumen. The histology showed evidence of early (day 63) spermatozoa production in the lumen just as observed under blue light. At days 91 and 126, the histology is comparable to control but evidence of displacement and degeneration of the spermatogonia.

For the rats exposed to RL, there was a proliferation of myofibrils, although less than in green light. There is slight depletion of germinal cells but was normal as the control. There was no evidence of spermatozoa at day 63 but became obvious at day 91 and more conspicuous at day 126.

For the rats exposed to WL, there was a variety of changes; a slight depletion of the germinal epithelium but was comparable to normal. There was no evidence of spermatozoa at day 63 but was obvious at days 91 and 126.

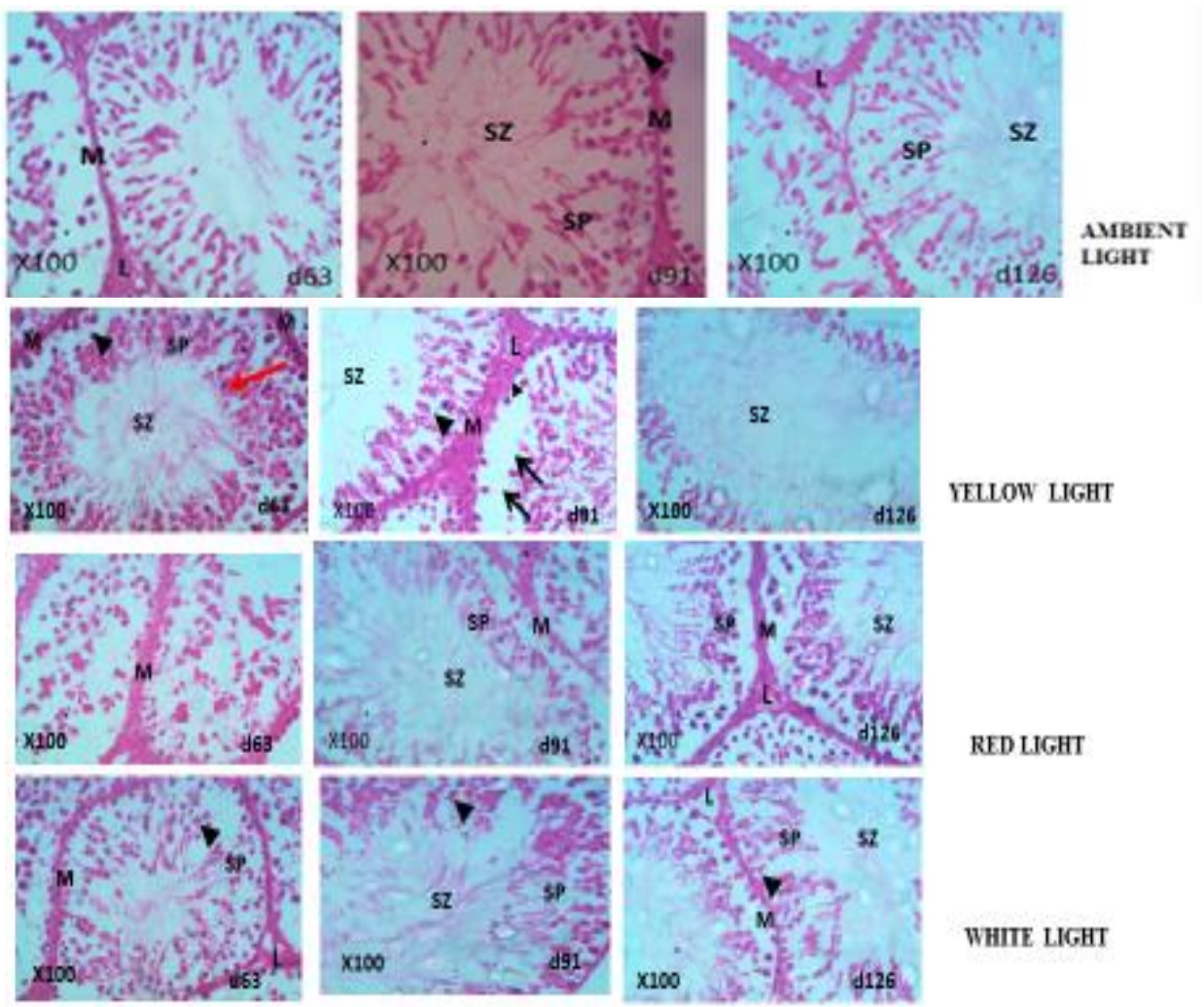

Plate 2: Photomicrographs of testes of albino rats exposed to yellow, red, and white light at days 63, 91, and 126. Plates show basal type spermatogonia (arrowheads), basement membrane with myofibroblasts (M), Spermatogonia type and spermatids (SP), and Spermatozoa (SZ) in the lumen. Leydig cells (L) appear within the interstitium. YL showed evidence of displacement and degeneration of the spermatogonia ( ), pyknotic basal cells were also observed close to the lumen. There was, however, evidence of SZ at day 63. RL showed normal cytoachitecture comparable to green light but progressively as control. WL showing a variety of changes; slight depletion of germinal epithelium but was comparable to the control

Discussion

Light as an environmental factor has been reported to affect reproductive function in animals, but not much is known as regards the effect of spectral nature of light on reproduction in mammals (Danilenko and Sergeeva, 2015). Exposure to various light colors has become part of life these days Kehinde *et al*. (2022). This study demonstrated that the spectral power distribution of light has a significant role in male reproductive performance, with blue and green light having a profound effect on the testis of the rats in that the highest weight of the testes was always the highest under blue light. Rats under blue light had spermatozoa in the lumen at day 63 and elongated seminiferous tubules at days 91 and 126, which was not typically present in other treatments and control as well. This also tallied with the highest testicular weight seen in rats exposed to blue light, which suggests the earliest attainment of puberty and sexual maturity in this group. Previous studies have established a correlation between testicular weight and spermatogenesis (Berndtson and Thompson, 1990; Johnson *et al.*, 1994). Literature reports also show that blue light increases the maturation of reproductive organs and shortens the onset of puberty in gender (Chang et al., 2016). Danilenko and Sergeeva (2015) reported that female human exposure to blue-enriched white light increased the production of follicle-stimulating hormone. This suggests the reason while blue lights may have more impact on the spermatogenesis of the rats. Olatunji-Bello and Sofola (2001) had earlier reported that rats exposed to light contained more spermatozoa when compared with their controls. The report of Chang *et al*. (2016) showed that the testicular weight of gander was highest in those exposed to blue light at the onset of puberty and white light at sexual maturity.

Furthermore, the thick myofibres, hypertrophic germinal cells of the seminiferous tubules in rats exposed to green light at day 63 indicate the influence of this light spectrum on the growth of myofibres (Cao *et al*., 2008). Hypertrophic germinal cells observed at day 63 were followed by a progressive change in testicular weight at day 91. The gonadal growth under green light at day 91 was 13 times greater than that of blue light. This is an indication of late sexual development on exposure to green light as compared to blue light, although the two colors enhanced sexual development than any other color. This study agreed with the finding of Cao *et al*. (2007) who reported that blue and green light increased testicular development in broilers.

Exposure to yellow light on the other hand, just as blue light had spermatozoa at day 63 but presented progressive displacement of germinal cells with karyolysis and degenerative changes with chromatin clumping and pyknotic basal cells. This indefinitely led to a decrease in testicular weight at day 91 and the insignificant weight gain at day 126. Kehinde *et al*. (2023) demonstrated that exposure to yellow light significantly reduced the testosterone in male rats. Report also revealed that Duck exposed to yellow light had delayed the onset of egg production and terminated egg production earlier when compared to as opposed to blue, red, and white light (Biyatmoko, 2014). Kehinde (2019) also revealed that rats exposed to yellow light had elevated serum corticosterone. The degeneration observed in the testes of rats exposed to yellow light could be as a result of stress since stress has been correlated with low testosterone (Swami *et al*., 2007), hence retarded gonadal growth. Therefore, the retarded testicular weight in rats exposed to yellow light could be as a result of upregulation of corticosterone via receptors in Leydig cells (Pellegrini *et al*., 1998).

Exposure to darkness also resulted in some histopathological disorders with thinning of myofibres, hydropic and nonspecific atrophic degeneration of the seminiferous tubules at day 126. The high testicular weight recorded can be attributed to accumulation of water with evidence of a moderate degree of oedema in seminiferous tubules and interstitial spaces which appeared mildly oedematous. Therefore, the heavy testicular weight recorded was not as a result of cellular growth but rather an accumulation of water. Reiter and Hester (1966) and Wade and Bartness (1984) earlier reported that hamsters kept in darkness develop marked atrophy in the gonads and delayed growth of accessory sex organs. Olatunji-Bello and Sofola (2001) also reported that rats exposed to darkness had reduced gonad activity.

In conclusion, this study demonstrates the spectral effect of light on testicular weight and histomorphology. Blue and green lights appear to favor myofibril growth, testosterone stimulation, and spermatogenesis. Exposure to yellow spectrum and darkness showed deleterious effects on testicular tissue and overall cytoarchitecture of the testes with age.

Acknowledgement

We would like to thank Dr. Moses Oyatogun, the former director of FUNAAB Zoo Park, for providing us with a befitting space as well as an office while we were doing the research.

References

Ali MN, Onyeanusi BI, Ayo JO, Salami SA, Hambolu JO: Effects of continuous light exposure on testicular structure and function of the African giant rat (*Cricetomys gambianus*). Niger Vet J, 38(2): 159- 166. 2017.

Ashkenazi L, Haim A: Effect of light at night on oxidative stress markers in golden spiny mice (*Acomys russatus*) liver. Comp Biochem Physiol Mol Part A, 165: 353–357. 2013.

Berndtson WE, Thompson TL: Changing relationships between testis size, sertoli cell number and spermatogenesis in Sprague-Dawley rats. J Androl, 11:429-435. 1990.

Biswas NM, Biswas R, Biswas NM, Mandal LH: Effect of continuous light on spermatogenesis and testicular steroidogenesis in rats: Possible involvement of alpha 2u-globulin. Nepal Med Coll J, 15(1): 62-64. 2013.

Biswas R, Sarkar M, Biswas NM: Protection of testicular activity by continuous light in rats treated with lithium. Med Sci Res, 24: 297-298. 1994.

Biyatmoko D: Effects the combinations of light color and intensity of light to age at first laying and production egg of alabio laying ducks. Int J Biol Sci, 5(5): 80-85. 2014.

Cao J, Liu W, Wang Z, Xie D, Jia L, Chen Y: Green and blue monochromatic lights promote growth and development of broilers via stimulating testosterone secretion and myofiber growth. J Appl Poult Res, 17: 211–218. 2008.

Chang SC, Zhuang ZX, Lin MJ, Cheng CY, Lin TY, Jea YS Huang SY: Effects of monochromatic light sources on sex hormone levels in serum and on semen quality of ganders. Anim Reprod Sci, 167: 96-102. 2016.

Danilenko KV, Sergeeva OY: Immediate effect of blue-enhanced light on reproductive hormones in women. Neuroendocrinol Lett, 36(1):84–90. 2015.

Dominoni D, Quetting M, Partecke J: Artificial light at night advances avian reproductive physiology. Proc R Soc B Biol Sci, 280: 20123017. 2013.

Falchi F, Pierantonio C, Christopher DE, David MK, Haim A: Limiting the impact of light pollution on human health. Environment and stellar visibility. J Environ Manage, 92(10): 2714-2722. 2011.

Fonken LK, Lieberman RA, Weil ZM, Nelson RJ: Dim light at night exaggerates weight gain and inflammation associated with a high-fat diet in male mice. Endocrinology, 154(10):3817-25. 2013.

Gawad FAA, El-Shaarawy EAA, Arsanyos SF, El-Galil TIA, Awes GN: Can constant light exposure affect the thyroid gland in prepubertal male albino rats? Histological and ultrastructural study. Folia Morphol, 78(2): 297–306. 2019.

Haim A, Portnov BA: *Light Pollution as a New Risk Factor for Human Breast and Prostate Cancers*. Springer, Dordrecht Heidelberg. New York. London. pp.165. 2014.

Haim A, Boynao S, Zubidat AE: Consequences of artificial light at night: The linkage between chasing darkness away and epigenetic modifications: *In*: Epigenetic. R Meccariello (Eds). IntecOpen pp. 21. 2019.

Hastings MH, Walker, AP, Power JB, Hutchison J, Steel EA, Herbert J: Differential effects of photoperiodic history on the responses of gonadotrophins and prolactin to intermediate day-lengths in male Syrian hamster. J Biol Rhythms, 4: 334 – 35. 1989.

Johnson L, Carter GK, Varner DD, Taylor TS, Blanchard TL, Rembert MS: The relationship of daily sperm production with number of Sertoli cells and testicular size in adult horses: role of primitive spermatogonia. J Reprod Fertil, 100: 315 – 321. 1994.

Kehinde FO, Dedeke GA, Olude MA, Ademolu KO, Aladesida AA, Adewale AO, Owagboriaye FO: Assessment of reproductive hormones in prepubertal and postpubertal rats (*Rattus norvegicus)* on chronic exposure to various spectra of artificial light at night. Zoologist, 22: 31-39. 2023.

Kehinde FO, Dedeke GA, Olude MA, Aladesida AA, Ademolu KO: Assessment of haematological variation in rat bred under various artificial light spectra at night (ALAN) Green Report, 3(8): 1 – 6. 2022.

Kehinde FO: Non-visual effect of spectral quality of artificial light at night (SQALAN) on the physiology of laboratory rat (*Rattus norvegicus* var. *albinus*). Ph.D. Thesis, Federal University of Agriculture Abeokuta, pp. 258. 2019.

Miloševi´c V, Trifunovi´c S, Sekuli´c M, Šoši´c-Jurjevi´c B, Filipovi´c B, Negi´c N, Nestorovi´c N, Stojanoski MM, Starčevi´c V: Chronic exposure to constant light affects morphology and secretion of adrenal zona fasciculata cells in female rats. Gen Physiol Biophys, 24: 299-309. 2005.

Olatunji-Bello II, Sofola OA: Effect of continous light and darkness exposures on the pituitary-gonadal axis and thyroid activity in male rats. Afr J Biomed Res, l 4: 119 – 122. 2001.

Pellegrini A, Soldani P, Gesi M, Lenzi P, Martin F: Diazepam reduced ultra-structural changes induced by noise stress in rats adrenal gland. J Submicrosc Cytol Pathol, 30: 385- 391. 1998.

Reiter RJ, Hester RJ: Interrelationships of the pineal gland, the superior cervical ganglia and the photoperiod of the endocrine systems of hamsters. Endocrinology, 79: 1168-1170. 1966.

Robert KA, Lesku JA, Partecke J, Chambers B: Artificial light at night desynchronizes strictly seasonal reproduction in a wild mammal. Proc Biol Sci, 282(1816): 20151745. 2015.

Salvetti N, Gimeno EJ, Canal AM, Lorente JA, Ortega HH: Histochemical study of the extracellular matrix components in the follicular wall of induced polycystic ovaries. Brazil J Morph Sci, 20: 93–100. 2003.

Swami CG, Ramanathan J, Jeganath C: Noise exposure effect in testicular histology, morphology and on male steroidogenic hormone. Malays J Med Sci, 14(1): 28-35. 2007.

Wade EP, Bartness JT: Seasonal obesity in Syrian hamsters: effect of age, diet, photoperiod and melatonin. Am J Physiol, 247: R328 – R334. 1984.

Zubidat A, Nelson RJ, Haim A: Spectral and duration sensitivity to light-at-night in 'blind' and sighted rodent species. J Exp Biol, 214:3206–3217. 2011.

Zubidat AE, Fares B, Fares F Haim A: Artificial light at night of different spectral compositions differentially affects tumor growth in mice: Interaction with melatonin and epigenetic pathways. Cancer Control, 25: 1-15. 2018.