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Assessment of reproductive hormones in prepubertal and postpubertal rats (*Rattus norvegicus)* on chronic exposure to various spectra of artificial light at night

Kehinde, F. O.[1*], Dedeke, G. A.[2], Olude, M. A.[3], Ademolu, K. O.[2], Aladesida, A. A.[2] Adewale A. O.[4] and Owagboriaye F. O.[5]
[1]Department of Animal and Environmental Biology, Faculty of Natural Science, Kogi State University Anyigba, Nigeria
[2]Department of Pure and Applied Zoology, College of Biosciences, Federal University of Agriculture Abeokuta, Nigeria
[3]Department of Anatomy, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Nigeria
[4]Department of Biochemistry, College of Biosciences, Federal University of Agriculture Abeokuta, Nigeria
[5]Department of Zoology and Environmental Biology, Faculty of Science, Olabisi Onabanjo University, Nigeria
***Corresponding author:** Kehinde Festus Ola; Kehinde.fo@ksu.edu.ng

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Abstract

Exposures to Artificial Light at Night (ALAN) is on the increase with resultant varied physiological impact related to the light spectrum via the non-visual pathway. This study examined the effects of various spectra of ALAN on Testosterone (Te), Progesterone (Pr) and Estradiol (Es) in rats exposed to Blue (BL), Green (GL), Yellow (YL), Red (RL), White (WL) and Darkness (DD) light wave-lengths while Ambient light (CL) served as the control. Dams with their post-natal day 1 pups were housed in cages exposed to various light wavelengths for 12 hours: (6:00pm–6:00am) daily for 126 days. At d_{63} and d_{126}, five rats per treatments were euthanized; blood serum was collected and the serum hormones: Te, Es and Pr were analysed using ELISA. The results showed that light treatments had significant effect on the level of Te at d_{63} but not at d_{126} in male rats. At d_{63}, onset of puberty, Te was significantly highest ($p < 0.05$) under BL. In females exposed to YL and WL at d_{126}, Te was significant high and Es was significantly low. Estradiol was heightened significantly ($p < 0.05$) in both sexes exposed to RL. In conclusion, BL seemed to stimulate early sexual development in males, WL and GL enhanced post-pubertal male albino rat reproductive system while RL provided the best reproductive environment for females in the long term. Long periods of exposure to YL seemed to pose risks for male and female reproductive function.

Introduction

Artificial Light At Night (ALAN) affects various biological cycles, which invariably and significantly impact animal physiology and behaviour, with the severity and specificity, being determined by the spectral wave-length of light (Falchi *et al* 2011; Dedeke *et al* 2017; Kehinde *et al* 2018; Eran and Noga 2019; Haim *et al* 2019). Likewise, ALAN has reportedly influenced reproduction in animals extensively (Danilenko and Sergeeva 2015) and has become obvious in seasonal breeders that depend on photoperiods (Chang *et al* 2016a; Ali *et al* 2017). In Aves, where extensive ALAN researches have been conducted using various spectra of light at night: red, blue, white and green lights have shown to alter the level of reproductive hormones both in male and female birds, respectively (Diyan *et al* 2014; Wang *et al* 2014; Chang *et al* 2016b). White and red lights were found to stimulate sexual maturity as blue light slowed down sexual maturity in turkeys (Scott and Payne 1937). In ducks, the testicular weight was found to be doubled when exposed to red light (Tilgner 1961). Cao *et al* (2008) also reported that Testosterone (Te) was stimulated when broilers were exposed to blue and green lights.

Studies similar to birds have not been extensively conducted in mammals but earlier studies on mammals revealed that exposure to continuous light (white light) caused persistent ovarian cycle in Sprague-Dawley and Blue Spruce rats (Hoffmann 1970), with more recent reports showing that female rats exposed to white light developed ovarian cysts and infertility (Salvetti *et al* 2003; Ortega *et al* 2004; Miloševi´c *et al* 2005). Gonadotropic hormones were also reported to be stimulated in mammals when exposed to white light by enhancing serum androgen and spermatogenesis (Danilenko and Samoilova 2007; Kripke *et al* 2010). With mammalian studies however

limited to white light, it thus becomes difficult to assume that the physiological response to light spectra in birds will be the same in mammals because non-visual light perception varies between both groups. With little or no empirical data available on the effect of various spectra of light on the reproductive hormones of mammals, this study was therefore aimed to examine the effects of five different light spectra at night on the level of Testosterone, Progesterone and Estradiol in *R. norvegicus.*

Materials and methods

Breeding of experimental animals

Breeder albino rats (at ratio $3_{females}:1_{male}$) were procured from Institute for Medical Research and Training (IMRAT), University College Hospital (UCH), Ibadan, Nigeria for mating and were acclimatized for two weeks with feed and water *ad libtum*. At parturition, pups (without sex discrimination) were distributed with their dams in cages and randomly assigned to different light treatments.

Light treatments

The light spectra used for this study included: Blue (BL), Green (GL), Yellow (YL), Red (RL), White (WL) and Darkness (DD) while Ambient light (CL) served as control. Compact fluorescent bulb of 13 watts (ESTAR, China) was used as the source of light and the light intensity was regulated at 300-350 lux and monitored by the light metre (LX-1010B model). Wave-lengths fell within standard range for each light treatment. The fluorescent bulbs were fixed at the top centre in the cages to ensure uniform distribution of light within the cage. Inverter was used to ensure uninterrupted power supply throughout the study period. Post-natal day one rats were utilized in this research.

At day 28, dams were withdrawn while the juvenile pups were further separated into males and females to receive continuous exposures. Three replicates per treatment per sex were used and each replicate had 4 rats. The rats were exposed to different light colours at night (6pm–6am, 12 hours) for 126 days. Ethical guidelines of animal experimentation (regulation CEE 86/609 EU) were followed during the study.

Collection of Blood

At days 63 and 126, five rats were randomly selected for each treatment, control and euthanized using diethyl ether (SIGMA Aldrich®, England) and sacrificed. Blood was collected by cardiac puncture into plain 5ml bottles, centrifuged at 4000 rps for 15 minutes and the blood serum was collected in Eppendorf tubes and stored at -20ºC till the analysis of hormones.

Analysis of hormones

Serum Testosterone (Te), Progesterone (Pr) and Estradiol (Es) were analysed with a commercial kit (Bio-Inteco, UK) using Enzyme-Linked Immunosorbent Assay (ELISA) according to Danilenko and Sergeeva (2015).

Data analysis

The data collected were subjected to descriptive analysis and analysis of variance (ANOVA) and the means were separated by Duncan's multiple test comparison. Student t-test was used to compare the means of the two periods and the sexes using SPSS version 20.

Results

Male and female hormonal values at 63 days

The mean reproductive hormones of male and female albino rats exposed to the spectral quality of ALAN at d_{63} is as shown in Table 1. The level of Testosterone (Te) was significantly ($p<0.05$) highest in male rats exposed to BL (5.00±0.80ng/ml). Progesterone (Pr) was significantly ($p< 0.05$) higher in male rats exposed to BL, YL, WL and RL (50.98±0.34, 50.87±0.13, 50.66±0.56 and 33.40±4.87 ng/ml, respectively) compared to positive control (21.15± 3.23ng/ml). Estradiol (Es) was significantly ($p<0.05$) highest in rats exposed to RL (16.00±0.00 pg/ml); it was significantly reduced in rats exposed to BL (2.32±0.33pg/ml). The ratio of Te/Es was significantly higher in the male rats exposed to BL ($2.17±0.32×10^{-3}$).

In female rats, Te increased significantly ($p>0.05$) under BL (1.00 ± 0.56ng/ml) and the least value was recorded in rats exposed to RL (0.01±0.00 ng/ml). Progesterone was the highest ($p<0.05$) in rats under DD and those exposed to RL and BL and was significantly ($p<0.05$) low in WL (12.18±2.35ng/ml). Estradiol was significantly ($p<0.05$) highest in rats under WL (25.00±0.50pg/ml).

In comparing the level of reproductive hormones between male and female, Te was significantly higher in male rats exposed to BL (df=4, p=0.002) and YL (df=4, p=0.007) than female rats. Progesterone was significantly higher in the male under WL (df=4, p=0.000) and in the female under RL (df = 4, p = 0.001) and darkness (df=4, p=0.000). Estradiol was significantly higher in male rats exposed to CL (df=4, p=0.002), RL (df=4, p=0.003) and female rats exposed to YL and WL (df=4, p= 0.002 and 0.000, respectively) and DD (df=4, p=0.000).

Male and female mean hormonal values at 126 days

At day 126, no significant difference ($p>0.05$) was observed in the level of Te in male rats but the level was highest in male rat under RL (1.27±1.00 ng/ml; Table 2) and lowest in rats under YL (0.37±0.20ng/ml). Progesterone was significantly high ($p<0.05$) in rats exposed to YL, BL and WL (51.18±0.19, 50.97±0.25, 50.82±0.29ng/ml, respectively) and low ($p<0.05$) in rats under GL and RL (21.54±5.15 and 19.55±2.69ng/ml, respectively) and DD (23.02±7.85ng/ml) when compared to the CL (32.04±1.64 ng/ml). Exposure to RL significantly increased ($p<0.05$) the level of Es (18.75±0.25pg/ml) and Es significantly ($p< 0.05$) reduced under WL (4.03±1.50 pg/ml) when compared to the CL (7.40±2.60pg/ml).

Table 1: Reproductive hormones concentration of male and female *R. norvegicus* exposed to the light of varying wavelength at day 63

Day					63			
light treatments		Darkness	White	Red	Yellow	Green	Blue	Ambient
Testosterone (ng/ml)	Male	0.36±0.14^{a}	0.43±0.12^{a}	1.70±1.40^{ab}	2.05±0.45^{b}	1.40±0.79^{ab}	5.00±0.80^{c}	1.03±0.55^{ab}
	Female	0.45±0.05^{ab}	0.45±0.05^{ab}	0.17±0.28^{a}	0.64±0.15^{bc}	0.53±0.32^{b}	1.00±0.56^{b}	0.30±0.10^{ab}
	P value	0.354	0.830	0.105	0.007	0.154	0.002	0.086
Progesterone (ng/ml)	Male	17.47±5.53^{a}	50.66±0.56^{d}	26.19±4.43^{b}	50.87±0.13^{d}	33.40±4.87^{c}	50.98±0.34^{d}	21.15±3.23^{ab}
	Female	51.15±0.22^{d}	12.18±2.35^{a}	51.06±0.00^{d}	34.07±16.26 ^{bc}	27.39±0.11^{b}	44.73±10.93^{cd}	30.42±5.93^{b}
	P value	0.000	0.000	0.001	0.148	0.099	0.378	0.076
Estradiol (pg/ml)	Male	5.80±0.00^{b}	8.35±0.15^{c}	16.00±0.00^{d}	5.75±2.25^{b}	4.80±0.52^{b}	2.32±0.33^{a}	5.15±0.35^{b}
	Female	9.55±0.45^{c}	25.00±0.50^{f}	11.35±0.15^{d}	14.80±0.20^{e}	5.05±0.35^{b}	4.80±0.00^{b}	2.55±0.55^{a}
	P value	0.000	0.000	0.000	0.002	0.527	0.000	0.002
Testosterone /Estradiol (10^{-3})	Male	0.06±0.03^{a}	0.05±0.01^{a}	0.11±0.09^{ab}	0.42±0.26^{b}	0.30±0.19^{ab}	2.17±0.32^{c}	0.02±0.03^{a}

Means with the same superscript in a column are not significantly different ($p > 0.05$)

Table 2: Reproductive hormones of male and female laboratory rats exposed to the Artificial Light at Night (ALAN) at day 126

Day					126			
light treatments		Darkness	White	Red	Yellow	Green	Blue	Ambient
Testosterone (ng/mL)	Male	1.10±0.00^{a}	0.85±0.25^{a}	1.27±1.00^{a}	0.37±0.20^{a}	1.25±0.75^{a}	1.10±0.46^{a}	0.43±0.21^{a}
	Female	0.21±0.16^{a}	1.95±0.35^{b}	0.05±0.04^{a}	1.85±0.55^{b}	0.30±0.26^{a}	0.16±0.15^{a}	0.35±0.15^{a}
	P value	0.001	0.011	0.103	0.012	0.107	0.027	0.604
Estradiol (pg/mL)	Male	23.02±7.85^{a}	50.82±0.29^{c}	19.55±2.69^{a}	51.18±0.19^{c}	21.54±5.15^{a}	50.97±0.25^{c}	32.04±1.64^{b}
	Female	51.90±0.38^{c}	51.25±0.07^{c}	34.34±1.25^{a}	51.07±0.22^{c}	43.10±0.98^{b}	51.36±0.15^{c}	50.93±0.00^{c}
	P value	0.003	0.065	0.001	0.547	0.002	0.080	0.000
Progesterone (ng/mL)	Male	7.97±2.04^{b}	4.03±1.50^{a}	18.75±0.25^{c}	6.95±1.45^{b}	7.40±0.10^{b}	5.91±1.39^{ab}	7.40±2.60^{b}
	Female	10.33±1.15^{c}	6.00±1.00^{b}	13.05±1.55^{d}	1.50±0.50^{a}	9.35±0.85^{c}	9.40±1.90^{c}	10.60±0.40^{c}
	P value	0.155	0.132	0.003	0.004	0.017	0.062	0.103
Testosterone /Estradiol (10^{-3})	Male	0.15±0.04^{ab}	0.24±0.12^{b}	0.07±0.05^{a}	0.06±0.03^{a}	0.17±0.10^{ab}	0.20±0.09^{ab}	0.07±0.05^{a}

Means with the same superscript in a column are not significantly different ($p > 0.05$)

The value of Te/Es was significantly high ($p<0.05$) in rats exposed to WL ($0.24\pm0.12 \times 10^{-3}$).

In females, Te was significantly ($p<0.05$) high under YL and WL (1.85±0.55 and 1.95±0.35ng/ml respectively). Progesterone was significantly reduced ($p<0.05$) in rats under BL and RL (43.10±0.98 and 34.34±1.25ng/ml respectively). Exposure to RL significantly ($p<0.05$) increased Es (13.05±1.55pg/ml) while YL significantly ($p< 0.05$) reduced it (1.50±0.50pg/ml). In comparing the level of reproductive hormones between male and female, Te was significantly higher in male under BL (df= 4, p=0.027) and DD (df=4, p=0.001). Testosterone was significantly higher in female rats under YL (df=4, p=0.012) and WL (df=4, p=0.011). Progesterone was significantly higher in female rats exposed to CL (df=4, p=0.000), GL (df=4, p=0.002) and RL (df=4, p= 0.001) and DD (df=4, p=0.003). Estradiol was significantly higher in female rats exposed to GL (df=4, p=0.017) and significantly higher in male rats exposed to YL (df=4, p=0.004) and RL (df=4, p=0.003).

Comparison of the level of reproductive hormones between days 63 and 126

Comparison between the levels of reproductive hormones between days 63 and 126 showed that Te was significantly lower ($p<0.05$) in the rats exposed to BL and YL, and significantly higher ($p<0.05$) in the rats under DD at day 126. Testosterone was higher ($p>0.05$) at day 63 in male rats in the CL and under RL and higher at day 126 in rats exposed to WL. The level of progesterone was significantly higher ($p<0.05$) at day 63 in male rats under GL and at day 126 in rats exposed to CL. The level of Es was significantly higher ($p<0.05$) in male rats exposed to BL, GL and RL but lower significantly under WL at day 126 (Figure 1).

The comparison between levels of reproductive hormones in female albino rats on exposure to light of varying wave-length between days 63 and 126 is shown in Figure 2. The level of testosterone (Te) was significantly higher ($p<0.05$) at day 126 in female rats under YL and WL. A lower value was recorded in rats under BL, GL, CL and DD at day 126. The level of progesterone was significantly ($p<0.05$) higher in female rats exposed to GL and WL, DD and CL at day 126. Higher values of progesterone was recorded at day 126 in rats under BL and YL. Estradiol concentration was significantly ($p<0.05$) lower in female rats exposed to YL and WL at day 126. Higher values were recorded at day 126 in rats exposed to RL and DD.

Discussion

This study shows that artificial light spectra at night significantly affected the levels of reproductive hormones in both males and females *R. norvegicus* during the prepubertal age. The spectral-specificity effect of light on invertebrates and some vertebrates has been demonstrated (Falchi *et al* 2011; Kehinde *et al* 2018; Shibuya *et al* 2018). One of the hallmark effects of reproductive hormones in the body is the stimulation of secondary sexual development. The responses to various hormones depend on their levels in the body; below or above a certain threshold, there is always a corresponding consequence on the body physiology and behaviour of the animals. High level of testosterone for instance, in male at puberty is necessary to stimulate body growth and maturation of sexual organs, which are necessary for reproductive fitness.

Significant high levels of testosterone recorded at the onset of puberty (d_{63}) in male and female rats under BL are in agreement with the study of Danilenko and Sergeeva (2015) who reported that exposure to blue-enriched white light increased the production of follicle-stimulating hormones in female human. The same finding was also reported in birds by Change *et al* (2016) who found that blue light stimulated testicular development better than WL and RL at the onset of puberty in gander. Other studies did not investigate blue light, but white light was discovered to significantly affect reproductive hormones. For instance, Hance *et al* (2009) reported elevated reproductive hormones in golden hamster that was exposed to WL from birth till puberty. Biswas *et al* (2013) also reported that rats exposed to 70 days continuous light (WL) had a higher level of testosterone. Danilenko and Samoilova (2007) had earlier demonstrated that human female exposed to early morning white light had higher reproductive hormones.

White light is a combination of several light spectra and from the observation, it is likely that the blue component of white light was actually responsible for the upregulation of the testosterone. To further collaborate this, the ratio of testosterone to estradiol was significantly the highest in male exposed to BL at the onset of puberty and highest in male rats exposed to WL at adult age. This finding also agreed with the observation of Chang *et al* (2016b). Furthermore, the level of estradiol was reported to complement the activity of testosterone.

A direct correlation has been established between the level of testosterone and estradiol in improving spermatogenesis in rat (Rivas *et al* 2002). Akingbemi (2005) remarked that the absolute value of estradiol and testosterone is not what matters in male rats but rather the ratio of testosterone to estradiol. For instance, Liu *et al* (2008) and Chang *et al* (2016b) demonstrated and affirmed that the testosterone-estradiol ratio could be used as a diagnostic tool for evaluating the reproductive potential of individual ganders. Estradiol therefore plays vital role in male reproductive performance (Allan *et al* 2010; Çiftci, 2013; Schulster *et al* 2016).

Moreover, reports have shown that testosterone reduces with age in female human such that its level in older woman is half of what was recorded for younger woman (Copeland *et al* 2004). The present study is in agreement with the above observation except in the female rats exposed to YL

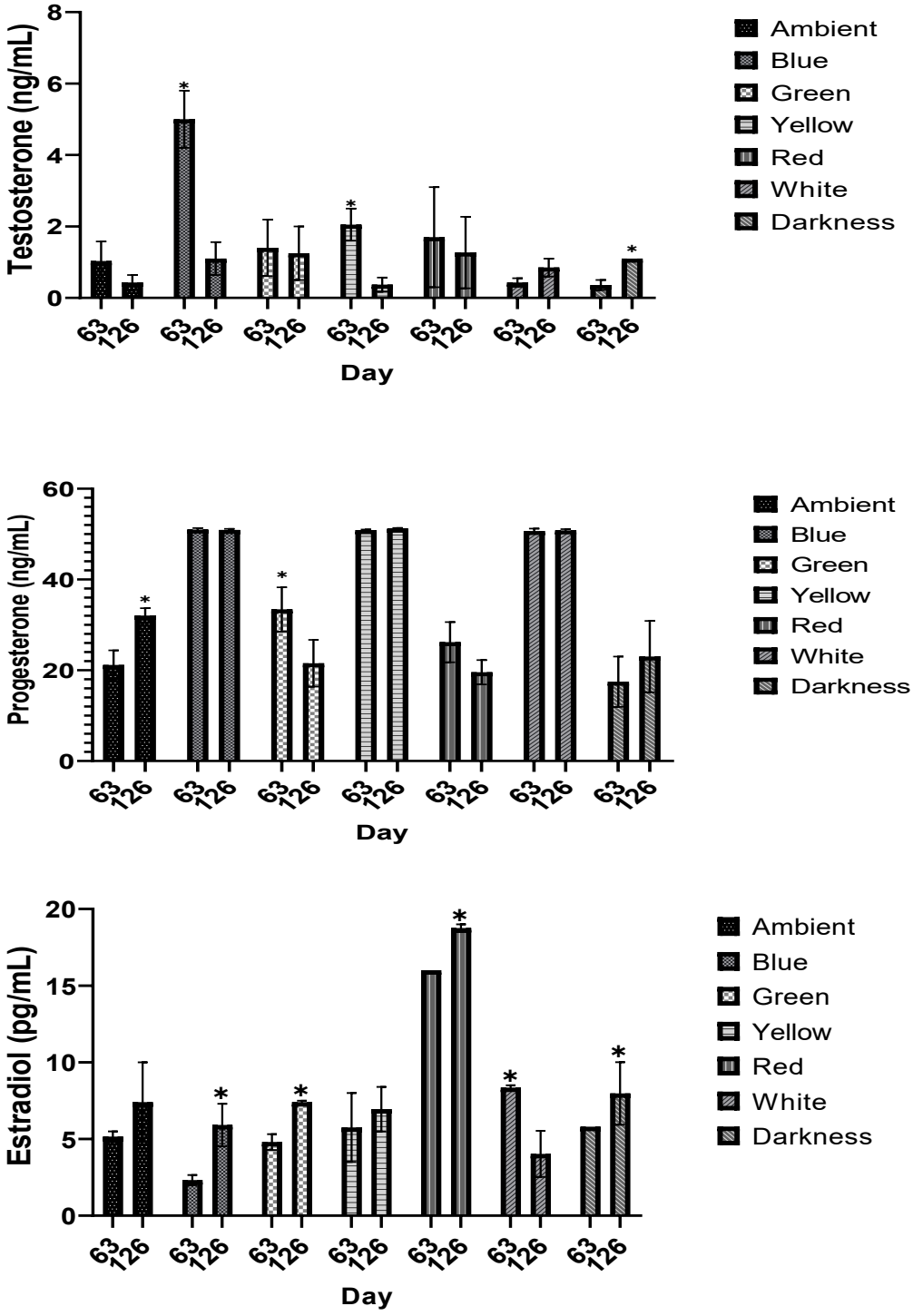

Figure 1. Comparison in the level of reproductive hormones in male albino rats on exposure to light of different colours between days 63 and 126

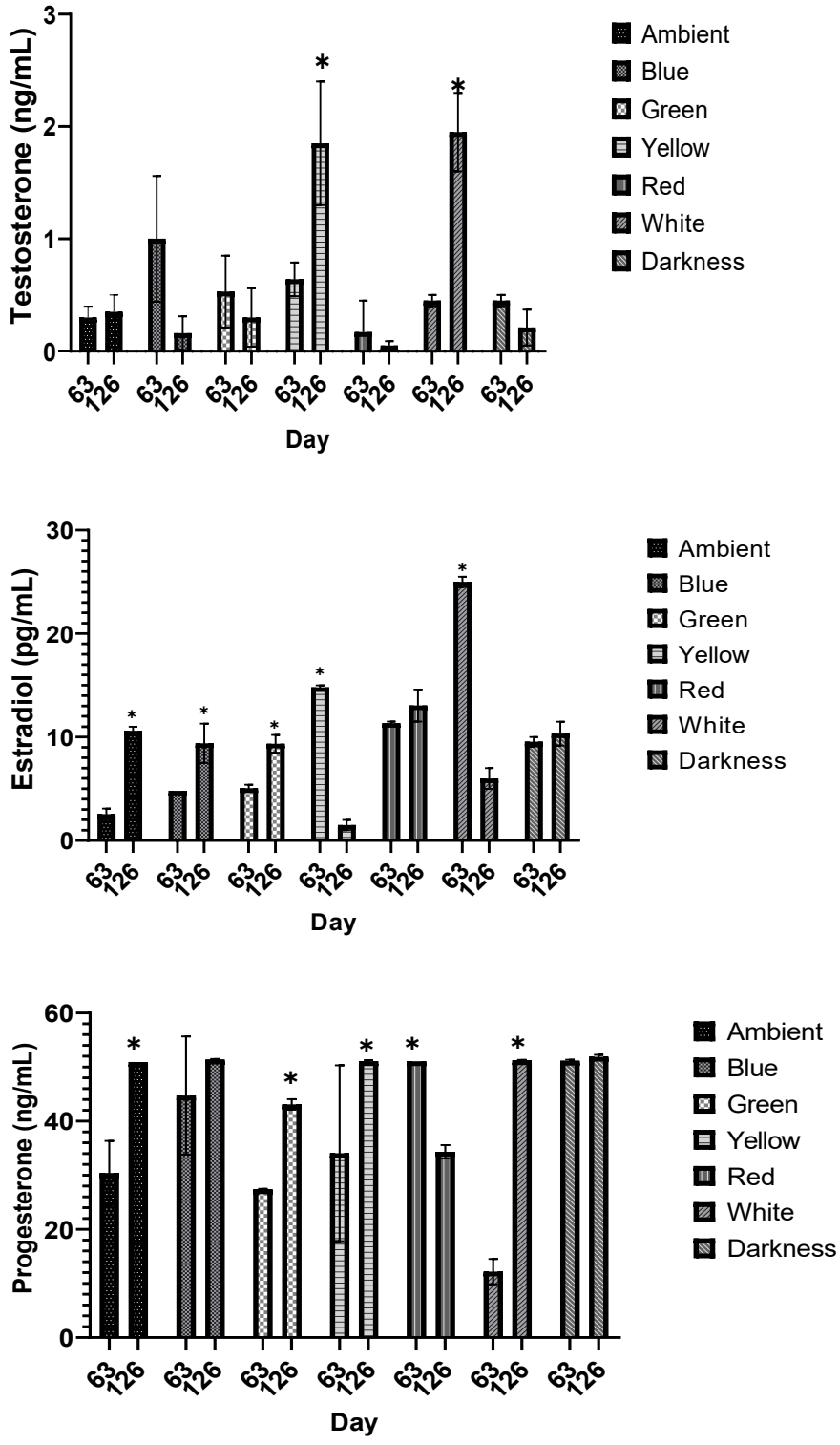

Figure 2. Comparison in the level of reproductive hormones in female albino rats on exposure to light of varying wavelength between days 63 and 126

and WL. Previous studies have shown that female rats exposed to WL developed polycystic ovary and infertility (Ivanisevic-Milovanovic *et al* 1995; Salvetti *et al* 2003; Ortega *et al* 2004). The study of Biyatmo (2014) also showed that exposure to YL as opposed to BL, RL and WL delayed the onset of egg production in the duck. In male rats, YL also significantly reduced the level of testosterone with age. This may result to loss of libido in postpubertal rat and ultimately affect their reproductive performance. The YL and WL could therefore be said to be a stressor; stress has been reported to have negative effect on the activity of testosterone (Swami *et al* 2007) and invariably on the process of spermatogenesis.

In addition, the elevation of testosterone and the subsequence suppression of estradiol by YL and WL in female rats is an indication that these light spectra elicited negative impact on the reproductive performance of the female rats. The YL and WL could be said to act as endocrine disruptor (Russart and Nelson 2018). Endocrine disruptor chemicals such as pyrethroid (Obinna and Kagbo 2017) and gasoline (Ugwoke *et al* 2005) were reported to significantly reduce the serum estradiol in female albino rats. Also, the upregulation of progesterone under YL and WL could have antagonized the activity of estradiol as suggested by Graham and Clarke (1997). Estradiol-progesterone ratio was significantly higher under these lights, the lower the ratio, the better the reproductive performance of the animals. Hence, long period of exposure to YL and WL may pose risk to the female reproductive system.

Worthy of note is the exposure to RL, which significantly reduced the level of testosterone in the female rats throughout the study period. This reduction also coincided with an elevated level of estradiol. The likely explanation for this is that RL probably enhanced the expression of aromatase and thus promoted the subsequent conversion of testosterone to estradiol. There is the need to further probe into this mechanism to ascertain if RL will enhance the expression of aromatase. The RL seems to provide the best reproductive lighten environment for the female rats by eliciting the least testosterone-estradiol ratio, and testosterone-progesterone ratio at day 63. Previous studies in birds revealed that exposure to RL enhanced reproductive performance by stimulating sexual hormonal pathway, ovarian weight, and increased follicular number and thus enhanced higher egg production (Cao *et al* 2008; Kim *et al* 2013; Hassan *et al* 2013). The RL was also reported to stimulate early egg production in chicken (Mobarkey *et al* 2013). Diyan *et al* (2014) reported that hens exposed to RL produced more eggs than those exposed to BL and GL.

The possible mechanism by which artificial light affects reproductive hormone can be linked to the activation of hypothalamic-pituitary-gonad axis as well as the hypothalamic-pituitary-adrenal axis when exposed to light (Ouyang *et al* 2018). These two axes are directly connected and involved in the secretion of the reproductive hormones, though, in different quantities. The stimulation of these axes led to a higher level of testosterone at the onset of puberty under BL.

The activation of hypothalamus results in the release of Gonadotropin-releasing Hormone (GnRH), which invariably excites the adenohypophysis to secrete and release the two gonadotropic hormones, luteinizing hormone and follicle-stimulating hormone. These hormones proportionally activate Leydig cells or ovary to secrete testosterone in male or female respectively. Beside this, pars tuberalis of the pituitary gland, which actually controls the secretion of tropic hormones was discovered to have melatonin receptors (Wood and Loudon 2017; Ouyang *et al* 2018) as well as being very close to Suprachiasmatic Nucleus (SCN) (Child 2009; Ouyang *et al* 2018) and that neuron from SCN innervates pars tuberalis. This is an indication that the activity of pars tuberalis is controlled by light and this may be the major route through which light affects the secretion of reproductive hormones. The BL seems to stimulate pars tuberalis better than other light spectra at the onset of puberty. The reason being that melanopsin, a photoreceptor of the retino-ganglia cells, is more sensitive to BL (LeGates *et al* 2014; Blattner 2018) and also transduces light information to SCN and ultimately affect per tuberalis.

Conclusion

This study reveals that BL stimulated early sexual development as GL accelerates spermatogenesis during post-pubertal age; YL seemed to pose risk to both male and female reproductive performance. The WL also poses risk to female reproductive health. Therefore, BL and GL have the potential to enhance male reproductive performance as RL for female and can be used as alternative therapy.

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