ORIGINAL ARTICLE

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Effects of Blue Light on Puberty and Ovary in Female Rats

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What is already known on this topic?

Blue light is a natural light source. However, in recent years, exposure to blue light has increased with the use of mobile devices and tablets. The severity of the impact of blue light exposure increases as the eye-screen distance decreases. The use of these devices in children has increased, and melatonin suppression by blue light is known to cause disruption in circadian rhythm, increased appetite, and obesity. However, its effects on puberty are unknown.

What this study adds?

Early puberty was observed due to exposure to blue light in the prepubertal period. When exposure to blue light increased, apoptosis and the appearance of polycystic ovary were detected in the ovaries.

Abstract

Objective: This study was designed to examine the effect of blue light exposure and exposure time on puberty in an animal model.
Methods: Eighteen 21-day-old female Sprague Dawley rats were divided into three equal groups which were: control group (CG); blue light-6 hours (BL-6); and blue light-12 hours (BL-12). CG rats were maintained with 12/12-hour light-dark cycles. The animals in BL-6 and BL-12 were exposed to blue light of wavelength 450-470 nm and intensity of 0.03 uW/cm^2 for 6 and 12 hours, respectively. Exposure to blue light continued until the first signs of puberty. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, testosterone, dehydroepiandrosterone sulfate (DHEA-S), leptin and melatonin were measured. Subsequently the ovaries and uterus were examined histomorphologically.
Results: The median day of puberty start was 38, 32 and 30 for the CG, BL-6, and BL-12 groups, respectively (p = 0.001). FSH, testosterone, DHEA-S, and leptin concentrations of all groups were similar. However, LH and estradiol concentrations in BL-6 were higher compared to CG (p = 0.02). There was a negative correlation between blue light exposure, exposure time, and melatonin concentrations (r = -0.537, p = 0.048). Ovarian tissue was compatible with puberty in all groups. As blue light exposure time increased, capillary dilatation and edema in the ovarian tissue increased. Prolonged exposure was associated with polycystic ovary-like (PCO) morphological changes and apoptosis in granulosa cells.
Conclusion: These results suggest that exposure to blue light and the duration of exposure induced earlier puberty in female rats. As the duration of blue light exposure increased, PCO-like inflammation, and apoptosis were detected in the ovaries.
Keywords: Blue light (470 nm), early puberty onset, rat, apoptosis, melatonin

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Introduction

Sunlight contains red, orange, yellow, green, and blue light. Light entering the retina produces a stimulus that is transmitted to the suprachiasmatic region of the hypothalamus and regulates circadian rhythm by controlling the body's biological clock. This mediates the timing of functions, such as cortex activity, body temperature, and the sleep-wake cycle. Evening light exposure causes a decrease in the release of the hormone melatonin, leading to disruption of the circadian rhythm and reduction in the antioxidative effects of melatonin (1,2,3). Blue light exposure in daylight during daytime increases alertness and promotes memory and cognitive functions (4,5). However, it is known that exposure to blue light at night has a significant melatonin-suppressing effect (6).

Electronic mobile devices emit high-energy, short-wavelength blue light (7). In the last century, blue light sources such as fluorescent and LED lighting and television became common in daily life. However, over the past ten years, the use of touch-screen devices, such as tablets and smartphones, has increased in all age groups (8). Blue light exposure is more intense with these devices because of the shorter eye-screen distance. In recent years, the age of children using these devices has rapidly decreased (9). Since the Coronavirus disease-2019 (COVID-19) pandemic, screen exposure in children and adolescents has increased substantially due to remote education and more screen time at home during lockdowns (10,11,12). Of note, an increase in the incidence of precocious puberty was observed during the pandemic period compared to the pre-pandemic period (13,14,15).

One of the factors that initiate puberty is a decrease in melatonin. The melatonin hormone has a suppressive effect on gonadotropin-releasing hormone (GnRH) released from the hypothalamus at the onset of puberty. And as melatonin level decreases, GnRH synthesis increase and puberty begins (16). In children living near the equator with lower melatonin concentrations because of the long daylight hours, puberty occurs earlier than in those at higher latitudes (17). It is known that light-exposure at night has a suppressive effect on melatonin and it has been shown that blue light suppressed melatonin production more than any other color such as green, red (7).

However, the impact of this type of blue light exposure on the pubertal process is unclear. The aim of this study was to examine the effects of blue light exposure including duration of exposure in an animal model of puberty.

Methods

Animals

Eighteen prepubertal 21-day-old female Sprague Dawley rats weighing 35-50 g were procured from the Experimental Animal Center of Gazi University (Ankara, Turkey). The study groups were isolated from male rats after postpartum 21 days. The rats were housed in polysulfone cages (42.5 × 26.6 × 18.5 cm in size; three rats per cage) at 21-24 °C and 40-45% humidity at the Laboratory Animals Breeding and Experimental Research Center of the Faculty of Pharmacy, Gazi University (Ankara, Turkey). The animals were fed a standard pellet diet and water *ad libitum* during the experimental period. All the animals were maintained by the Guide for the Care and Use of Laboratory Animals (18), and the experimental procedures were approved by the Experimental Animal Ethics Committee of Gazi University (project no: G.Ü.ET-21.052, date: 09.07.2021).

Light Exposure Protocol

A blue LED strip (FSHI. 1048.B020.6012, HI-LED, FLEX honor-, ILED- İstanbul, Turkey) was the source of the blue light at a wavelength of 450-470 nm was placed approximately 20 cm above the center of each cage in the experimental groups (Figure 1).

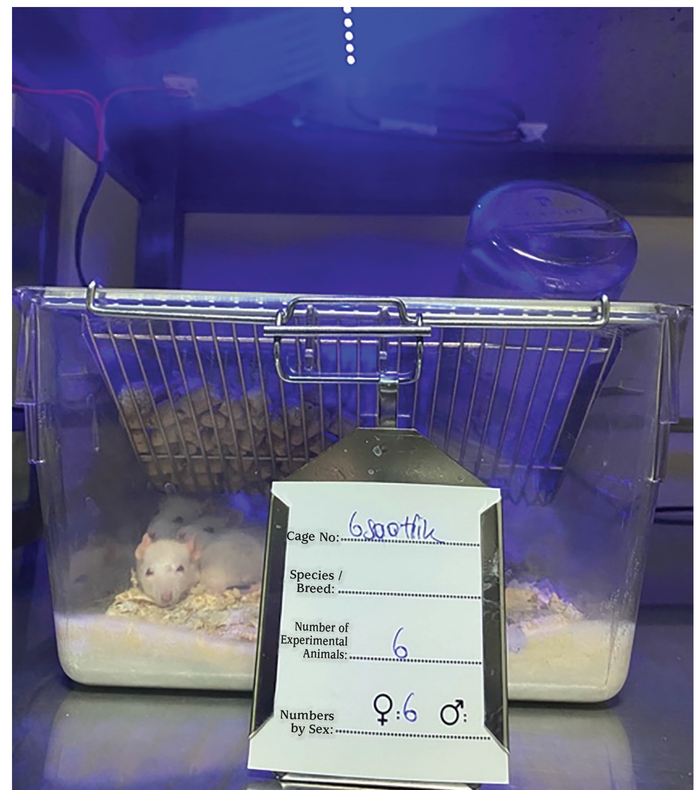

Figure 1. Experimental set-up of the room

In the experimental setup, the blue light source was used at an intensity that lowered rat melatonin concentrations but would not cause retinal damage (19,20,21). This was determined to be an irradiance level of 0.03 uW/cm^2 at the eye level of the animals. The irradiance in the entire area of the cage where the rats were housed was measured with a spectroradiometer and adjusted to the same level.

Experimental Design

The rats were randomly divided into three groups of six rats: the control group (CG), blue light-6 hours (BL-6), and blue light-12 hours (BL-12). CG rats were maintained under standard conditions with 12/12-hour light-dark cycles. The light/dark cycle condition for the BL-6 and BL-12 groups were exposed to blue light (450-470 nm) for 6 hours (light time 6:00 a.m.-6:00 p.m.; blue light time 6:00 p.m.-12:00 p.m.; dark time 12 p.m.-6:00 a.m.); for 12 hours (light time 6:00 a.m.-6:00 p.m.; blue light time 6:00 p.m.-06.00 a.m.), respectively.

The rats were weighed at the beginning and end of the experimental procedure, and the percentage weight gain was calculated with the formula Weight gain (%) = (Last day-First day)/First day x 100.

Vaginal Examination and Cytology

Vaginal opening is one of the external signs of puberty in rodents (22). The rats were examined daily, starting at 22 days of age, to detect vaginal opening. After vaginal opening, vaginal smear samples were collected to determine the estrus stage. To do this, a moistened cotton swab was inserted into the vagina. Cells from the vaginal lumen and walls were gently taken and transferred to a glass slide. After the samples were allowed to air-dry, they were stained with Giemsa stain and examined under a light microscope.

The stages of the estrous cycle were classified as *proestrus* (oval nucleated epithelial cells), *estrus* (irregular-shaped, cornified squamous epithelial cells), *metestrus* (fragmented, cornified epithelial cells and smaller, darker stained leukocytes), and *diestrus* (nucleated epithelial, predominantly leukocytes) (23). Rats were exposed to blue light until the first estrus stage after vaginal opening.

Termination of the Experimental Procedure

At the first estrus stage, all the rats were sacrificed by taking blood from the heart at 8:00 p.m. to determine the peak melatonin rhythm of the rats (24) under general anesthesia (10 mg/kg xylazine hydrochloride and 50 mg/kg ketamine hydrochloride). After the anesthesia procedure, blood samples were obtained by the intracardiac puncture. The blood samples were centrifuged at 3000 rpm (906 x g) for 15 minutes and the serum was separated. The serum samples were stored at -80 °C until analysis. The height of the ovarian tissues was measured by a micrometer, and the uterine and ovarian tissues were dissected and weighed.

Determination of Biochemical Parameters

The collected blood was centrifuged at 3000 rpm for 10 minutes at +4 °C and stored at -80 °C. The serum concentration of the follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, testosterone, dehydroepiandrosterone sulfate (DHEA-S), leptin, and melatonin were evaluated by enzyme-linked immunosorbent assay (ELISA) (Rat-specific ELISA, Bioassay Technology Laboratory, China).

Histopathological Methods

The right and left ovaries and the uterus were weighed after dissection, then the ovaries and the uterus were fixed in Bouin's fixative and embedded in paraffin blocks using standard procedures. Sections of 4-5 micron thickness were taken from the prepared paraffin blocks and stained with hematoxylin and eosin. The samples were examined for histomorphological changes by light microscopy using the Leica DM4000 (Leica, Germany) computer-assisted imaging system, and images were obtained using the Leica-Qwin program.

Statistical Analysis

Statistical Package for the Social Sciences, version 26 was used for statistical analysis (IBM Inc., Armonk, NY, USA). The Kruskal-Wallis test was used when comparing the medians of three independent groups for data that did not fit the normal distribution, and the Mann-Whitney U test were used when comparing the medians of two independent groups. While investigating the associations between non-normally distributed and/ordinal variables, the correlation coefficients and their significances were calculated using the Spearman's test. All data are given as median (minimum-maximum). Bonferroni correction was used in *post hoc* tests. Statistically, $p < 0.05$ was considered significant.

A power analysis was performed using G*Power version 3.1.9.7 to determine the minimum sample size required to test the study hypothesis. Results indicated that a sample size of n = 18 is required to achieve 80% power for detecting a large effect at a significance of $\alpha = 0.05$.

Results

The mean±stardard deviation (SD) initial weight of the female rats in CG, BL-6, and BL-12 were 42.5±4.7, 42±3.4,

and 42.3±2.7 g, respectively (p = 0.91). The median day of puberty onset was 38th, 32nd, and 30th days in the CG, BL-6 and BL-12 groups, respectively. Puberty onset was significantly earlier in BL-12 compared to the CG (p = 0.001) (Table 1). The age of onset of puberty decreased as the duration of blue light exposure increased (r = -0.910, p < 0.001). The mean±SD weight at onset of puberty were 85.1 ± 9.7 g, 91.6 ± 5.5 g, 80 ± 5 g in CG, BL-6, and BL-12 groups, respectively. The weight at onset of puberty in BL-6 was significantly greater than in BL-12 (p = 0.04).

Median percentage weight gain in CG, BL-6, and BL-12 was 110%, 117%, and 93%, respectively. Percentage weight gain was higher in BL-6 compared to CG, while rats in BL-12 had the least weight gain (Table 1).

Serum concentrations of FSH, estradiol, testosterone and DHEA-S in the blue light-exposed groups were similar to those of controls (p > 0.05) (Table 2). LH concentrations were higher in BL-6 than in CG (p = 0.027). The high concentrations of LH and estradiol in BL-6 can be attributed to the hormonal peak during estrus. The estrus stage of the rats in the BL-12 group was observed in the earlier hours of the day and the time between the estrus stage and time at sacrifice was longer. Therefore, LH and estradiol surges were not detected. On the contrary, we detected LH and estradiol surges in the BL-6 group due to the shorter estrus stage and earlier time of sacrifice.

Serum concentrations of leptin showed no significant difference among the groups (p > 0.05) (Table 1). There was no correlation between percentage weight gain, leptin, and the day of puberty onset (p > 0.05).

Median (minimum-maximum) melatonin levels were 144 (126-197) ng/L in CG, 143.7 (132-152) ng/L in BL-6, and 121 (116-151) ng/L in BL-12 (p > 0.05). Melatonin levels decreased as the duration of exposure to blue light increased (r = -0.537, p = 0.048).

The ovarian size, ovarian weight, or uterine weight of groups were similar (p > 0.05) (Table 3). On histologic examination, ovarian tissue was compatible with puberty in all groups. Primary, antral, and tertiary follicles and corpus luteum were observed in CG (Figure 2) and BL-groups. Edema and congestion in the medulla increased as the exposure time to blue light increased.

When the ovarian tissue of BL-6 was examined, a lower antral and Graafian follicle density and higher preantral follicle density were noted in BL-6 compared to the CG group.

Table 1. Timing of puberty onset, percentage weight gain, and leptin concentrations of the groups

	Control	BL-6	BL-12	p value
Puberty onset (day)	39 (38-40)	33 (30-34)	30 (30-32)	***0.001**
Weight gain (%)	110 (67-133)	117 (98-152)	93 (56-110)	0.09
Leptin (ng/mL)	3.1 (2.6-4.1)	3.2 (2.5-4.4)	3.4 (2.7-11.3)	0.51

Values represent median (minimum-maximum). *Control vs. BL-12.
BL-6: blue light-6 hours, BL-12: blue light-12 hours

Table 2. The hormone and melatonin concentrations of the groups

	Control	BL-6	BL-12	p value
FSH (IU/mL)	16.6 (9.6 -20.2)	19.4 (6.6-24.2)	9.2 (6.3-17.8)	0.07
LH (IU/mL)	67.5 (38.4-99.8)	106.9 (79-153.2)	82 (42.1-101.7)	***0.02**
Estradiol (pmol/L)	92.5 (57.5-166.2)	110.9 (89.6-150.5)	99.6 (46.7-123.1)	0.60
DHEA-S (µmol/L)	1.13 (1.01-1.13)	1.26 (0.90-1.57)	0.99 (0.64-1.54)	0.15
Testosterone (nmol/L)	335 (229-439)	243 (239-534)	261 (134-290)	0.30
Melatonin (ng/mL)	144 (126-197)	143 (132-152)	121 (116-151)	0.11

Values represent median (minimum-maximum). *Control vs. BL-6 p = 0.03.
BL-6: blue light-6 hours, BL-12: blue light-12 hours, FSH: follicle stimulating hormone, LH: luteinizing hormone, DHEA-S: dehydroepiandrosterone sulfate

Table 3. Ovary length and ovary and uterus weights of the groups

	Control	BL-6	BL-12	p value
Right ovary (mm)	4.5 (3.7-5.5)	3.8 (2.3-4.9)	4.9 (4.5-5.5)	0.06
Left ovary (mm)	4.5 (4.4-6.2)	4.3 (2.3-5.6)	4.5 (4.4-6.4)	0.43
Ovary weight (mg)	120 (40-130)	120 (20-140)	110 (60-150)	0.31
Uterus weight (mg)	410 (210-820)	510 (300-650)	640 (250-900)	0.21

Values represent median (minimum-maximum)

Perivascular edema and capillary dilation were prominent in the medulla of the BL-6 group (Figure 3a). At high magnification, extracellular edema in the granulosa cells in the preantral follicles and the presence of apoptotic cells with pyknotic nuclei in the granulosa layer of antral follicles were noted (Figure 3b). The most remarkable finding in the examination of BL-12 ovarian tissue was the presence of the corpus luteum covering most of the organ. In contrast to CG and BL-6, numerous Graafian follicles were noted in BL-12 group (Figure 4a). At high magnification, it was evident that the Graafian follicles had a thinner granulosa layer than in the CG. This appearance was consistent with an appearance of polycystic ovary (PCO). In addition, the extracellular edema in the granulosa cells and the presence of apoptotic cells with pyknotic nuclei in the granulosa layer of the antral follicles were more pronounced in BL-12 than in BL-6 (Figure 4b). Blue light exposure appeared to induced granulosa cell apoptosis, and the number of apoptotic cells increased with longer exposure time.

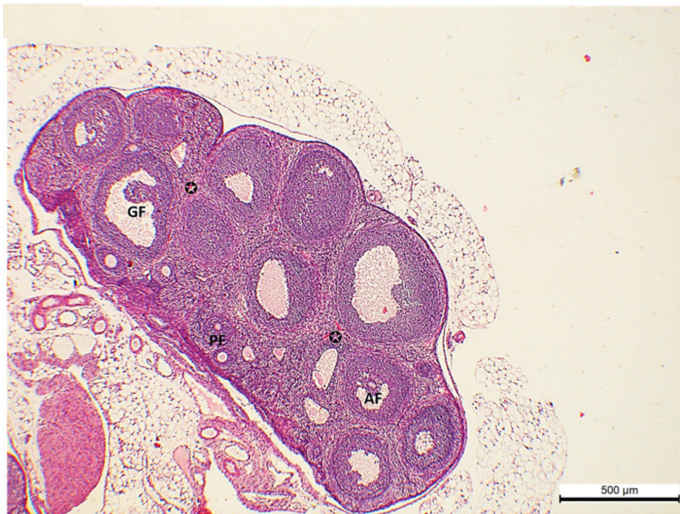

Figure 2. Histological findings of ovary-control group (H&E x40). All developing preantral, antral, and Graafian follicle structures and stroma appeared normal. Normal structure and distribution of blood vessels (✪)

PF: preantral follicle, GF: Graafian follicle, AF: antral follicle

Histological examination of uterine tissue from control rats was normal (Figure 5). In BL-6, the most remarkable histomorphological finding in the proliferative phase uterus was that the uterine epithelium thickened and became high prismatic epithelium, compared to CG (Figure 6). In BL-12, the uterine and gland epithelium in the proliferative phase uterus had a similar histological appearance to the samples from BL-6. The most prominent change in BL-12 was vessel dilation in the endometrial lamina propria and the capillaries reaching the surface (Figure 7). This finding was consistent with the endometrium entering the secretory phase with changes consistent with ovulation.

Discussion

To the best of our knowledge, the present study is the first to demonstrate the effects of blue light exposure on puberty in experimental animals. Blue light is the dominant light used for increasing screen brightness of mobile devices. The wavelength of blue light is between 450 and 490 nm and exposure is exacerbated with the reduction of eye-screen distances (7,25). In the last decade, several studies have suggested that night-time exposure to blue light emitted by devices, such as phones and tablets, can impair the normal

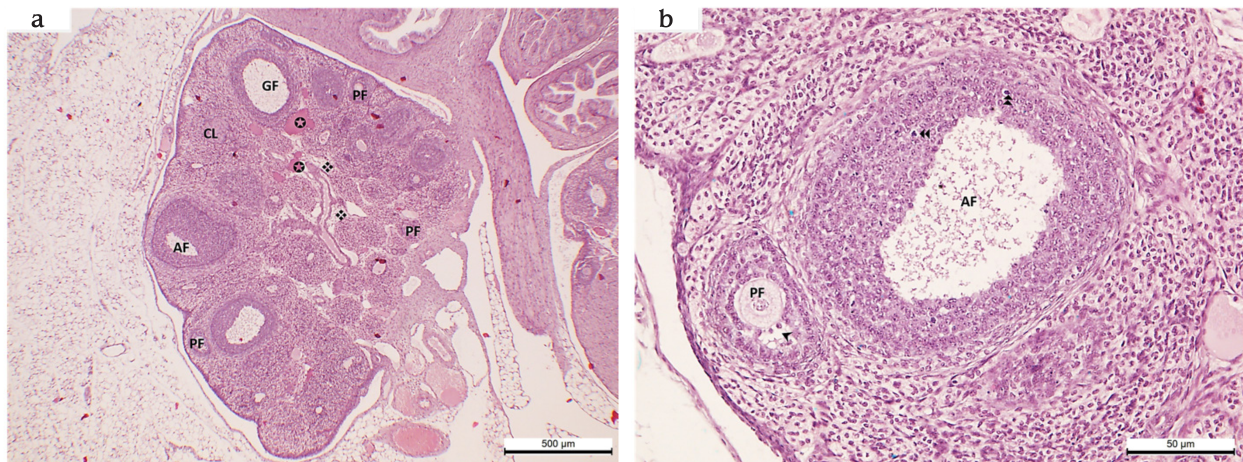

Figure 3. a) Histological findings of ovary-BL-6 group (H&E x40). At low magnification, the lower AF and GF density and higher PF density were noted in the cortex. Small CL structures were observed. There was prominent capillary dilation (✪) and especially perivascular edema (❖). **b)** Histological findings of ovary-BL-6 group (H&E x200). Extracellular edema (⏴) in the granulosa cells of the PF and apoptotic cells (⏪) in the granulosa cells of the AF were prominent

PF: preantral follicle, AF: antral follicle, GF: Graafian follicle, CL: corpus luteum, BL-6: blue light-6 hours

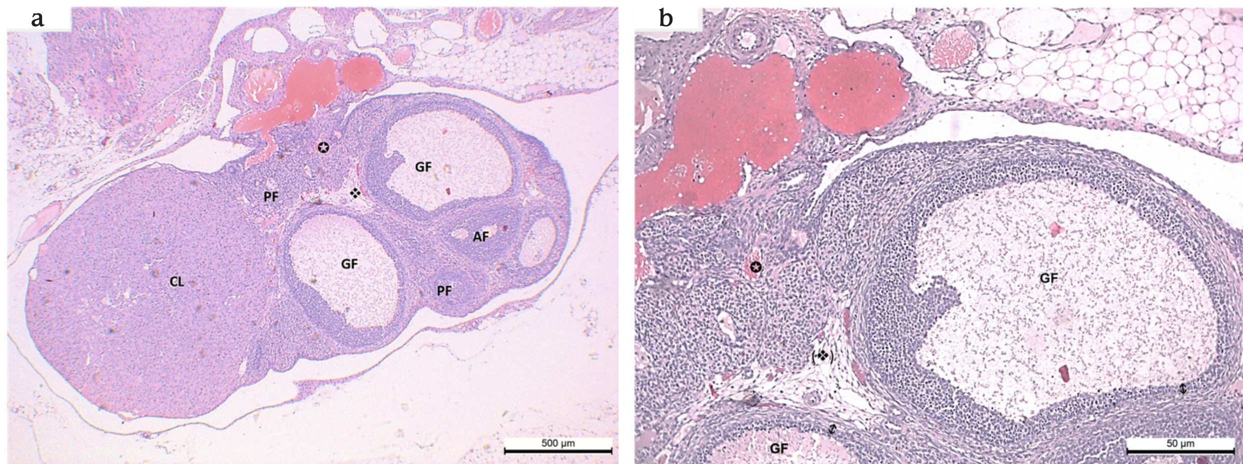

Figure 4. a) Histological findings of ovary-BL-12 group (H&E x40). Pronounced thinning of the granulosa layer of the follicles (⇕) was noted. There was increased edema (❖) in the medulla and pronounced capillary dilation (✪). **b)** Histological findings of ovary-BL-12 group (H&E x200). Pronounced thinning of the granulosa layer of the follicles (⇕) was noted

✪: capillary dilation, ❖: perivascular edema, PF: preantral follicle, AF: antral follicle, GF: Graafian follicle, CL: corpus luteum, BL-12: blue light-12 hours

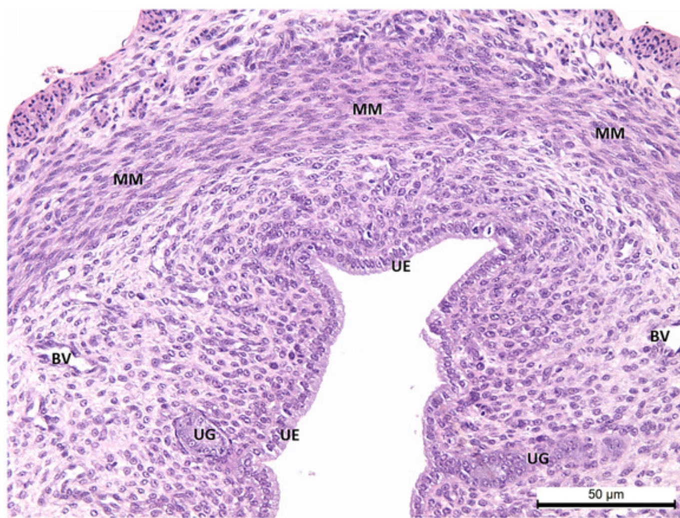

Figure 5. Histological findings of uterus-CG (H&E x40). Uterine tissue from control rats revealed normal UE of proliferative phase endometrium, a small number of UG in the lamina propria, and spiral arterioles that had not yet reached the surface. The myometrial structure was typical

UE: uterine epithelium, UG: uterine glands, BV: blood vessels, MM: myometrium, BL-12: blue light-12 hours

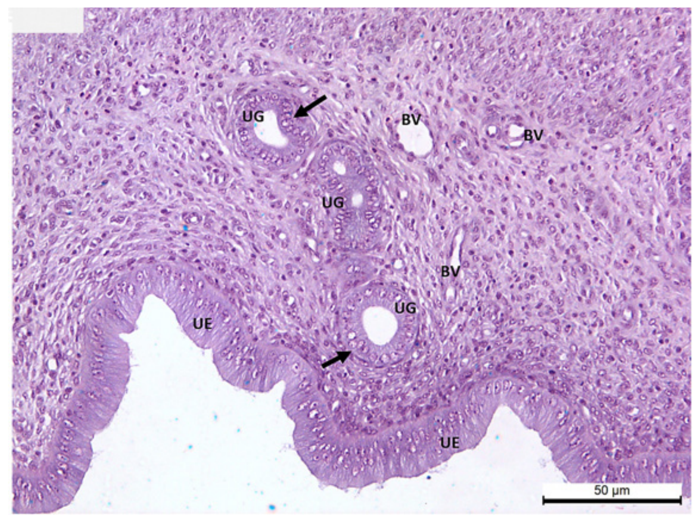

Figure 6. Histological findings of uterus-BL-6 (H&E x40). UE height was relatively increased. UG were more numerous and epithelial height increased. BV advanced towards the surface, the number of UG was also relatively increased in BL-6 group, and UG epithelial cells were larger than in CG. The endometrial vessels spread superficially. All these findings indicated that the uterus was entering the secretory phase

UE: uterine epithelium, UG: uterine glands, BV: blood vessels, BL-6: blue light-6 hours

function of the biological clock, alter sleep-wake cycles, and induce metabolic changes (26,27). The effect of prepubertal exposure to blue light on puberty, however, has not been previously investigated.

The results of the present study suggest that blue light was associated with early puberty in female rats, with blue light exposure and exposure time accelerating the onset of puberty. The levels of FSH, LH, and estradiol in the CG demonstrated that puberty initiated in the hypothalamic-pituitary-gonadal axis. The lack of a difference in hormone levels between the control and both BL groups suggests that puberty also had a central onset in the BL groups. The high concentrations of LH in BL-6 may be attributed to the hormonal peak during estrus. Furthermore, we did not

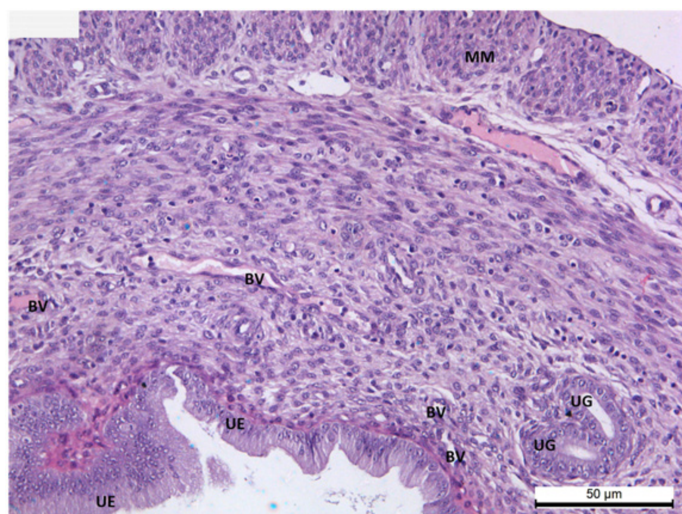

Figure 7. Histological findings of uterus-BL-12 (H&E x40). UE height was relatively increased, UG were more numerous and epithelial height increased. BV extend to the surface and appear dilated

UE: uterine epithelium, UG: uterine glands, BV: blood vessels, MM: myometrium, BL-12: blue light-12 hours

detect LH and estradiol surges in the BL-12 group. On the contrary, we may have detected LH surges in the BL-6 group due to the shorter estrus stage and earlier sacrifice time. The histological findings of the ovarian tissue in all groups were consistent with puberty, providing additional evidence that all the rats had entered puberty.

Through its effects on the hypothalamic center, blue light exposure increases food intake by reducing the release of leptin, which regulates hunger signals (28). Light exposure at night was also shown to increase body weight and body fat in mutant mouse experiments (29), and there are reports that weight gain accelerates puberty onset in rats (29,30). In our study, the group with the longest exposure to blue light had the lowest percentage of weight gain. There was no correlation between percentage weight gain, leptin, and the day of puberty onset. Therefore, this study may rule out weight gain and leptin as factors that accelerate puberty due to blue light.

Another factor contributing to puberty with exposure to blue light could be decreased melatonin secretion. We observed that melatonin levels decreased with increased blue light exposure time and puberty occurred earlier. The melatonin release pattern during the human lifespan involves an increase in melatonin concentrations from the neonatal period to the pubertal period, followed by a decrease at the onset of puberty (31). Neuroendocrine control of the sexual maturation process is influenced by the pattern of melatonin secretion resulting from the light-dark cycle. High melatonin concentrations are thought to have an inhibitory effect on the GnRH (32). *In vitro* studies of cultured prepubertal rat pituitary glands demonstrated that melatonin plays a role in the timing of developmental stages by inhibiting the release of GnRH and, therefore LH (33). A study comparing girls with precocious puberty and age-matched controls found that lower melatonin concentrations were associated with early puberty (32). Lee et al. (34) found that blue light exposure in the evening suppressed melatonin more in children than adults, even if the exposure was shorter. A study examining the effect of light on the circadian system of children in early puberty and mid-puberty showed that children in early puberty were more sensitive to evening light and their melatonin concentrations were more suppressed (35). During the COVID-19 pandemic, online education via electronic mobile devices and an increased time at home resulted in longer screen exposure in the younger age group. Studies have shown an increase in precocious puberty and accelerated puberty during pandemic-related lockdown compared to the pre-pandemic period (13,14). Among these studies, Stagi et al. (13) compared data from the pandemic period and the five years before the pandemic and reported that the incidence of newly diagnosed precocious puberty cases increased, and the rate of puberty was accelerated. They found a significant increase in body mass index (BMI) and pre-sleep screen device usage time among patients diagnosed and followed up during the pandemic.

Similarly, Chioma et al. (14) reported an increase in precocious puberty cases during the pandemic compared to the corresponding months of the previous year. Although there was no difference in BMI between the groups, the authors observed an increase in the duration of electronic device use and a decrease in physical activity. Our study demonstrated the effects of blue light exposure on puberty and the relationship with increased exposure time.

Early-life stress exposure is one of the common risk factors for psychopathology and deviations in pubertal timing. Several studies have demonstrated that stress promotes puberty in girls and female rats (36,37). Exposure to blue light may have induced stress in the rats. Stress may have also contributed to early puberty.

The histological findings in the ovarian tissue samples were striking. There was increased edema and capillary dilation in the ovarian medulla, accompanied by increased granulosa cell apoptosis, and histomorphological changes consistent with PCO in the BL-12 group. PCO syndrome (PCOS) is a common endocrine disorder in adolescence. Genetic abnormalities, lifestyle, prenatal hormonal imbalances, and environmental factors have been implicated in the

etiology of PCOS (38). Simon et al. (39) demonstrated that the effects of circadian rhythm disruption may contribute to PCOS in humans. In a PCOS study in which 6-week-old rats were exposed to continuous light for four weeks, PCOS-like results were discovered in the ovarian tissue of the rats. The authors suggested that constant light exposure appears to induce PCOS-like changes although they detected no differences in serum concentrations of FSH, LH, estradiol, or testosterone (40). In another rat study, 6-week-old rats were exposed to 600 lux light for 16 weeks. The study was conducted to model PCOS in rats and revealed PCOS-like histological findings in the ovaries and increased testosterone concentrations (41). In both studies, it was observed that prolonged light exposure in mature rats appeared to induce PCOS. In our study, BL-12 showed a thin granulosa layer in the Graafian follicles, which was a PCO-like finding (42,43). The absence of other signs of PCO and the lack of difference in androgen concentrations may be related to the duration of blue light exposure, irradiance level, and early sacrifice of the rats. In the present study, increased edema and capillary dilation in the ovarian medulla and apoptosis of the granulosa cells was observed in the rats exposed to blue light. There are no previous publications associating blue light exposure with apoptosis in the granulosa cells of ovarian tissue. However, increased edema and capillary dilation in the ovarian medulla may have been triggered in the experimental groups due to reduced melatonin and the subsequent increase in the pro-inflammatory process (44).

Study Limitations

As one of the limitations of our study, groups exposed to daylight should have been included. In the female rat study in which light-dark, continuous light and continuous darkness were applied, no difference was found in the days of puberty-onset (45). Furthermore, the reason for excluding other wavelengths of light from this investigation arises from the fact that the inhibitory impact of these wavelengths on melatonin was comparatively weaker as compared to blue light. Consequently, we excluded these groups from our study to comply with the 3R rule (46), which pertains to using a minimum number of animals in our research. The high concentrations of LH in BL-6 may be attributed to the hormonal peak during estrus. Furthermore, we did not detect LH and estradiol surges in the BL-12 group. Rats were sacrificed at the time interval when melatonin levels were highest (23). However, at this time, the gonadotropin levels were not at peak in the cycle due to the difference between puberty onset time and the time of sacrifice. We could not show a direct effect of melatonin on Kisspeptin and GnRH. A further limitation was that hormonal measurements were performed with ELISA. Liquid chromatography-mass spectrometry/mass spectrometry or high-performance liquid chromatography methods may have provided a greater degree of accuracy and sensitivity for hormonal measurement.

Conclusion

The blue light exposure and duration of exposure caused earlier onset of puberty. With increased blue light exposure duration, signs of PCO, inflammation, and apoptosis were detected in the ovaries. In the future, human studies are needed to demonstrate that blue light accelerates puberty onset and determine its short- and long-term effects on ovaries.

Ethics

Ethics Committee Approval: The study was approved by the Experimental Animal Ethics Committee of Gazi University (project no: G.Ü.ET-21.052, date: 09.07.2021).

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Aylin Kılınç Uğurlu, Aysun Bideci, Design: Aylin Kılınç Uğurlu, Aysun Bideci, Mürşide Ayşe Demirel, Gülnur Take Kaplanoğlu, Duygu Dayanır, Özlem Gülbahar, Tuba Saadet Deveci Bulut, Esra Döğer, Mahmut Orhun Çamurdan, Data Collection or Processing: Aylin Kılınç Uğurlu, Mürşide Ayşe Demirel, Duygu Dayanır, Tuba Saadet Deveci Bulut, Esra Döğer, Analysis or Interpretation: Aylin Kılınç Uğurlu, Aysun Bideci, Mürşide Ayşe Demirel, Gülnur Take Kaplanoğlu, Duygu Dayanır, Özlem Gülbahar, Esra Döğer, Literature Search: Aylin Kılınç Uğurlu, Aysun Bideci, Mürşide Ayşe Demirel, Gülnur Take Kaplanoğlu, Özlem Gülbahar, Esra Döğer, Mahmut Orhun Çamurdan, Writing: Aylin Kılınç Uğurlu, Aysun Bideci, Mürşide Ayşe Demirel, Gülnur Take Kaplanoğlu, Özlem Gülbahar, Esra Döğer.

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