Research Article

Constant light exposure terminates pregnancy in rats with pineal gland dysfunction, low melatonin level and pro-inflammatory response

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ABSTRACT

In the current study, the prolonged light exposure at night on the function of pineal gland, melatonin production, pro-inflammatory response and progress of pregnancy in pregnant rats were investigated. A long term (entire gestation stage) of 24 h light exposure not only modify the morphologies of pinealocytes by decreasing their nucleus/cytoplasm ratio and mitochondrial numbers but also reduce the level of circulating melatonin. The biological consequences for this constant light exposure in pregnant rats were the elevated pro-inflammatory response indicated by the increased production of IL-6 and finally, the termination of pregnancy compared to their controlled counterparts under the normal light/dark cycle. The result showed that the pregnancy was terminated at the early stage of embryo development. The report, for the first time, established a potential association among the pineal function, pro-inflammatory reaction and pregnant progress under the influence of light exposure. This observation has a high relevant to the rise in human infertility since humans have overexposed to the light at night with the increased light pollution globally.

Key words: Melatonin, pineal gland, pregnancy, rat, reproduction, pro-inflammatory response, mitochondria, light pollution.

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1. INTRODUCTION

The pineal gland (epiphysis) in vertebrates has attracted a great attention of researchers. It is considered as neuro-endocrine "converter" which responses to neural impulses by mainly secretion of a chemical named melatonin (1-3). The roles of melatonin have been widely studied in organisms from bacteria to humans (4-6). Especially, its activities on reproductive system have been well documented. For example, melatonin promotes embryo implantation (7, 8) regulates labor activity (9), improves placental efficiency and birth weight (10), decreases oxidative stress in case of preeclampsia (11) and increases the pregnancy rates in animals and also in humans (7, 12, 13).

In addition, both oocytes (14, 15) and the trophoblast of placenta (16) synthesize melatonin. It is believed that the locally produced melatonin is used to promote and protect the embryo development (13). In other hand, melatonin is also involved in regulation of the immune system by impacting the production of cytokines. Esroy *et al.* (17) showed that this molecule moderated inflammatory immune response by decreasing the level of interleukin-6 (IL-6) and increasing of level of interleukin-10 (IL-10). It also reduced the inflammation induced by stress in mice (18).

Based on these evidences, we speculate that the natural melatonin levels as well as its circadian rhythm may influence the progress of pregnancy via the regulation of inflammatory response in pregnant mothers. This influence may start from implantation and also during the stage of trophoblast/placenta formation. To test this hypothesis, the different light exposure processes are selected to modify the melatonin circadian rhythm in pregnant rats. It is well known that the light exposure is the principle factor to suppress melatonin production in vertebrates (19). This suppression is involved in the retino-hypothalamic tract to reduce the pineal activity (20). Many previous studies have confirmed that the prolonged light exposure during night significantly reduces melatonin production and diminishes melatonin circadian rhythm in rats (21, 22). As a result, the altered melatonin levels will promote the pro-inflammatory response and finally jeopardize the progress of pregnancy in animals. The results of the current study will clarify the potential associations among pineal function, melatonin production, pro-inflammatory response and reproductive consequence in tested animals.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents.

All chemicals were purchased from Novamedline Company (Kyiv, Ukraine); otherwise they will be mentioned. Melatonin assay kit was obtained from IBL (City, Germany). The assay agents for factor-α (TNF-α), interleukin-1-β (IL-1-β), interleukin-6 (IL-6), interleukin-4 (IL-4) and interleukin-10 (IL-10) were purchased from Vector-Best, (Kyiv, Ukraine). The samples were analyzed by automatic immunoenzyme analyzer & thermoshaker StatFax 2200 (manufactured by Awareness Technology, Inc., Palm City, FL, USA).

2.2. Animals.

The nonlinear female white rats (*Rattus Norvegicus* Wistar) with body weight 200 – 280 g, aged 17-24 weeks were obtained from the bank of experimental animals of Bukovinian State Medical University (Chernivtsi, Ukraine). All the animals were primigravida. The rats were kept in vivarium, 4 rats/ cage, with the room temperature at 20 ± 24 ^{o}C and relevant humidity of 60-75%. The rats were allowed to access the food and water *ad libitum*. The animals were treated according to the Recommendations of UNESCO Bioethics International Committee.

2.3. Experimental procedure.

This animal study was approved by Commission of Biological and Medical Ethics of Bukovinian State Medical University with the protocol №3 dated March 30th, 2017. After 3 days of acclimation the females were allowed to pair with males of a reproductive age (20-28 weeks). The pregnancy occurrence was confirmed by microscopic examination of vaginal smears and by finding the spermatozoons in them (23). In the day 3 (D3) after pregnancy confirmation, a group of animals (14 rats) were exposed to the constant light (24 h light exposure per day, light/dark cycle of 24 h/0 h) as referred as the experimental group. Another 10 animals were exposed to light in a regimen of 12 h light/ 12 h of darkness (light/dark cycle of 12 h/12 h, light on at 8:00 am and off at 8:00 pm) as the control group. The 20 W "Philips" halogen light bulbs of "white" light were used (color temperature 6500 K, light output 1200 lm). The distance between a cage and a light source was 50 cm. In the day 24 after confirmation of pregnancy (after 21 days of lighting exposure) the animals of the constant light exposed group failed to give any birth and they were sacrificed by decapitation with usage of thiopentalum natrium anesthesia. The animals in the 12 h/12 h light exposed group naturally gave the birth of 4-6 fetuses per mother. The average duration of pregnancy in this group was 21 days. Immediately after delivery the animals in control group were sacrificed. The animals in the constant light exposed group were sacrificed on the same day as the control group; all the animals from both groups were sacrificed between 8 am and 10 am. The blood was collected for biochemical analyses and the pineal glands were collected for the morphological examinations.

Additionally, the pineal glands of 14 non-pregnant female rats with the same age and body mass, exposed to the constant light and another 14 non-pregnant female rats exposed to 12 h / 12 h light/ dark cycle, were collected as the controls for optical and electronic microscopic analyses. The treatments on the non-pregnant rats were same as the pregnant rats and also were sacrificed at the day 21 after different light exposure regimens.

2.4. Methods.

2.4.1. Melatonin assay.

Concentrations of melatonin in the blood of rats were measured using the kit Melatonin ELISA. The assay procedure is based on the basic principle of competitive ELISA, whereby there is competition between a biotinylated and a non-biotinylated antigen for a fixed number of melatonin antibody binding sites. The amount of biotinylated antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When the system is in equilibrium, the free biotinylated antigen is removed by a washing step and the antibody bound biotinylated antigen is determined by use of streptavidine alkaline phosphatase as marker and p-nitrophenyl phosphate as substrate. Quantification of unknowns is achieved by comparing the enzymatic activity of unknowns with a response curve prepared by using known standards of melatonin.

2.4.2. TNF-α assay.

The blood levels of TNF-α were measured using the reagent kit manufactured by "Vector-Best", Kyiv, Ukraine. A highly sensitive sandwich enzyme immunoassay for human TNF-α was used (description see below).

2.4.3. Interleukins assay.

The blood levels of the cytokines were measured using the reagent kits manufactured by "Vector-Best" (Kyiv, Ukraine). A highly sensitive sandwich enzyme immunoassays for human interleukins (IL-1-β, IL-6, IL-4, IL-10) were used. Each interleukin was measured separately. At first stage, the tested samples are incubated in wells with immobilized antibodies, specific for a certain interleukin; the antibodies bind with interleukins. At the second stage, bound interleukins react with the conjugate #1 (the biotin-conjugated antibody specific to the certain interleukin). At the third stage, the bound conjugate #1 reacts with conjugate #2 (avidin-horseradish peroxidase). Quantity of the conjugate #2 is established by a color reaction with usage of substrate of horseradish peroxidase – hydrogen peroxide, and chromogen tetramethylbenzidine. The intensity of yellow color (wavelength 575 nm) is proportional to quantity of the investigated interleukin in the sample. The optical density of the solution is estimated using calibration graph.

2.4.4. Morphological assay of pineal glands.

The optical and electronic microscopy were used to examine the pineal morphology. The pineal gland samples were prepared as: paraffin blocks obtained and fixated in neutral 10% solution of formalin, buffered according to Lillie (24) for 24 h; after this, they were dehydrated through an ethanol series and put in paraffin at temperature 58 ^{o}C. Histological sections 5 mcm in thickness were stained with methylene blue and examined under light microscope (MICROmed SEO SCAN) and images were taken by Vision CCD Camera (microscope and camera manufactured by Ningbo Shengheng Optics & Electronics Co, Ltd, Zhejiang, China). To estimate the optical density, digital transformation of the obtained images of light microscopy of the pineal glands was performed using free-licensed software ImageJ (version 1.50 b). Target areas of the images (without blood vessels) were converted into 8-bit format; after that, black-and-white images were created using

instrument "Binary". Then the instruments "Analyze Particles" and "Measurements" of ImageJ were used to calculate the quantity of the cells and nuclei and to determine the area of nuclei and cytoplasm (for optical microscopy). The optical densities of cytoplasm and nuclei of pinealocytes were estimated using 0-255 RGB gradation (0 – black color, 255 – white color). Each pineal gland was estimated twice, with random selection of area of estimation; therefore, in the constant light exposed group n = 28, in 12 h/12 h light/dark cycle exposed group n=20, in both groups of non-pregnant rats (24 h/0 h and 12 h/12 h lighting/dark cycle) n = 28. The electronic microscopic samples were prepared as mentioned by Kus (25). The sample was analyzed by ПЕМ-125К electronic microscope (manufactured by JSC "SELMI", Sumy, Ukraine) using lead citrate (26). The instrument "Measurements" of ImageJ was used to calculate the total area of the mitochondria and length of their cristae in pinealocytes (for electronic microscopy).

2. 5. Statistical analyses.

The data of optical analysis of pineal glands were expressed as Welch-test for unequal samples. The results of melatonin, TNF-α and cytokines assays were statistically processed by Mann-Whitney U-test for small unequal samples with calculation of 95% confidence interval for median. The data were expressed as mean ± SD and were statistically proceeded using MedCalc statistical software, developed by "MedCalc Software", Ostend, Belgium. $P < 0.05$ was considered as statistically significant.

3. RESULTS

3.1. Effects of different light exposure schedules on the pregnant progress in rats.

The results showed that under the normal light exposed (12 h/12 h, light/dark), all females (10 rats) had normally delivered 4-6 pups/mother at the end of the gestation. Surprisingly, none of the pregnant rats (12 rats) under the constant light exposure (24 h/0 h light/dark) had given birth at the end of the gestation period. The data are listed in Table 1 and also illustrated in Figure 1.

Table 1. Effects of the different light exposure schedules on the pregnancy in each of the rats.

Pregnant rats under 12 h/12h light/dark		**Pregnant rats under 24 h/0 h light/dark**	
The orders of rats	Pups/litter	The orders of rats	Pups/litter
1	4	1	0
2	5	2	0
3	4	3	0
4	6	4	0
5	6	5	0
6	5	6	0
7	4	7	0
8	6	8	0
9	5	9	0
10	4	10	0
		11	0
		12	0
		13	0
		14	0
Average pups/litter	4.9 ± 0.88 (mean ± SD)	Average pups/litter	0 ± 0 (mean ± SD)

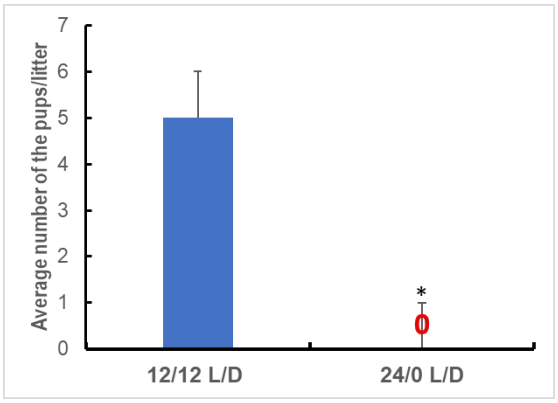

Fig. 1. Effects of the different light exposure schedules on the pregnant outcome in rats.

*The data were expressed as mean ± SD. For the 12 h/12 h LD (n = 10), for the 24 h/0 h LD (n = 14). 0: no pups, * p < 0.05 vs 12 h/12 h L/D rats.*

Further analysis of the uteruses for the pregnancy terminated rats under the constant light showed that the macroscopic sites of implantation were marked by implanted traces in uterine horns (4-6 in each horn) (Figure 2). No significant hyperemia was observed, probably, due to early pregnant termination provoked by exposing to the constant light.

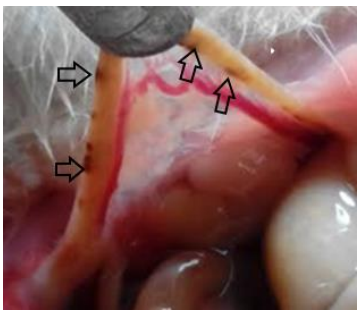

Fig. 2. A representing image of macroscopy of uterine horn of a rat under constant light exposure.

The implantation trace sites in uterine horn (4-6 in each horn) of constant light exposed pregnant rat were obvious. This indicated the early termination of pregnancy. The arrows indicate the implantation trace sites.

Histological examination of the uteruses has confirmed the aborted pregnancy. The decidualization of endometrium (Figure 3, left panel) and reaction of Arias-Stella (Figure 3, right panel) were found. The histologic analyses unambiguously indicated that pregnancy had occurred previously but terminated at its early stage.

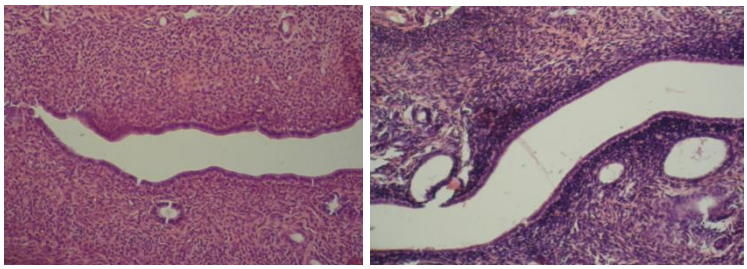

Fig. 3. Histologic evidence regarding the previous pregnancy in rats under the constant light exposure.

Left panel indicated the decidualization of endometrium. Stained by hematoxylin and eosin. x200. There are many polygonal cells of stroma with well-expressed and size increased nuclei.

The active growth of new blood vessels is seen. Right panel: Arias-Stella reaction of endometrium. Stained by hematoxylin and eosin. x200. The nuclei of the endometrial cells are fragmented and hypertrophic, the remarkable vacuolization of cytoplasm is present. This phenomenon is typical only for mammals during pregnancy.

3.2. Effects of the different light exposure schedules on the morphology of pineal gland in pregnant and non-pregnant rats.

To investigate whether the light exposure had impacts on the pineal gland, its morpologies were examined under the optic micropscopy. The results have showed that the pineal gland of pregnant rat which is under 12 h/12 h L/D cycle has widely spreaded dark pinealocytes with basophile nuclei. This is due to, as supposed, the presence of more granules of melatonin secretion (Figure 3, A). In contrast, for the pineal gland of pregnant rats in constant light exposure, its pinealocytes were mostly bright with roundish nuclei and well-expressed chromatin (Figure 3 B). As a control, the pineal gland of non-pregnant rat under 12 h/12 h L/D cycle is more or less similar to its pregnant counterparts represented with prevalence of dark pinealocytes with significant amount of inclusions of melatonin in the cytoplasm (Figure 4, C). The structure of the pineal glands of non-pregnant rats under 24 h/0 h L/D cycle is represented with prevalence of bright pinealocytes due to a weak secretory activity (few granules of melatonin are present in cytoplasm) and these are the features of its pregnant counterpart (Figure 4, D). However, the well expressed growth of gliocytes in these rats are much less in their pregnant counterparts under the constant light

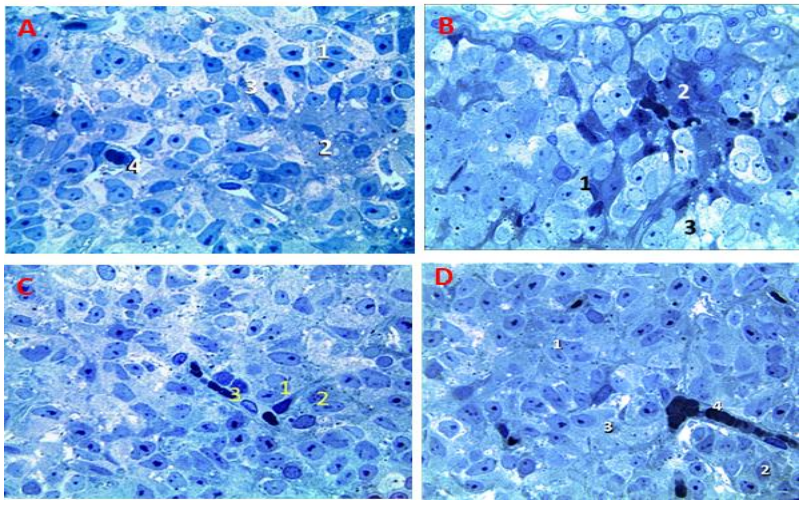

Fig. 4. Effects of the different light exposure schedules on the morphology of pineal gland in pregnant and non-pregnant rats: a light microscopic view.

A. An image of pineal gland of a pregnant rat under 12 h/12 h L/D. 1: bright pinealocytes, 2: cluster of dark pinealocytes, 3: gliocyte, 4: blood capillary. B. Pineal gland of a pregnant rat under 24 h/0 h L/D cycle. 1: large area of bright pinealocytes, 2: small portion of dark pinealocytes, 3: gliocyte. C. Pineal gland of a non-pregnant rat under 12 h/12 h L/D cycle. 1: bright pinealocytes, 2: dark pinealocytes, 3: gliocyte. D. Pineal gland of a non-pregnant rat under 24 h/0 h L/D cycle. 1: much more of bright pinealocytes than (B), 2: dark pinealocytes, 3: gliocyte, 4: blood capillary. All samples were stained with methylene blue. x400.

To quanitity the observations from the images of light microscopy the method of digital estimation was used. the areas of the nuclei from pinealocytes in pregnant and non-pregnant rats under 12 h/12 h L/D cycle were established to be maximum. The results showed that in pregnant rats who were exposed to constant light, the area of the nuclei was certainly the smallest, and the area of cytoplasm (without nuclei) was the biggest among the groups. These alterations are the

evidence of nuclei pyknosis. In addition, their nucleus/cytoplasm ratio was significantly decreased and this was more significant in pregnant rats comparing to non-pregnant rats. All these suggested that the secretory and metabolic activity of pineal glands of these rats, particularly in the pregnant rats were decreased. It appeared that the pregnancy is an additional burden for the pineal gland in mammals under constant light exposure. This is supported by the further observation that the optical density of nuclei and cytoplasm were weak. In other words, these pinealocytes contained the lowest amount of secretory granules of melatonin compared to the rats under 12 h/12 h L.D cycle. The statistical analyses on these parameters were illustrated in the Figure 5 and 6.

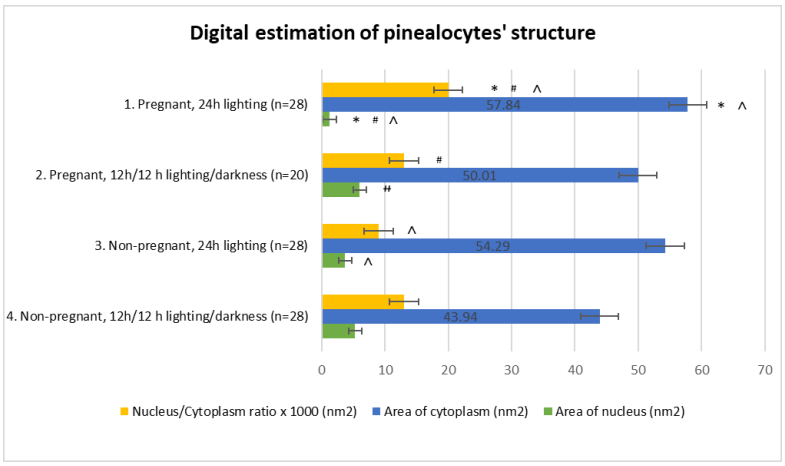

Fig. 5. Effects of different light exposure schedules and prognancy on the structure of pinealocytes.

*The data were expressed as means ± SD (n = 20 – 28). Data were from the imanges of light microscopy and analysized by digital estimation using ImageJ software, * p < 0.05 vs line 2, # p < 0.05 vs line 3, ^ p < 0.05 vs line 4.*

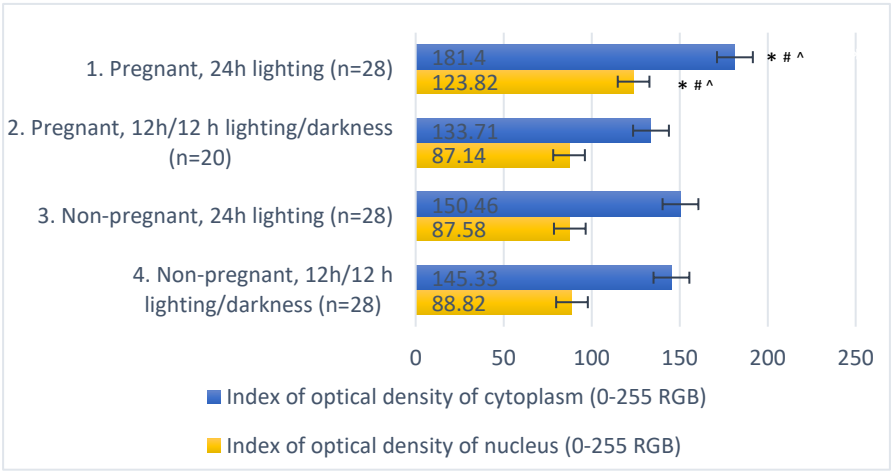

Fig. 6. Effects of different light exposure schedules and pregnancy on the the estimated activities of pinealocytes.

*The estimated activities of pinealocytes was indicated by the optical density of pinealocytes. The closer to "black" cytoplasm (the optical density index closer to 0 RGB) suggests the higher activity of the pinealocytes. The data were expressed as means ± SD (n = 20 – 28). Data were from the imanges of light microscopy and analysized by digital estimation using ImageJ software. * p < 0.05 vs line 2, # p < 0.05 vs line 3, p < 0.05 vs line 4.*

To analysize the utrastructures of the pinealocytes, the electronic microscopic evaluation was performed. The results showed that the pinealocytes from the pregnant rats under 24 h/0 h L/D cycle had significantly less total area of mitochondria and length of mitochondrial cristae than that

of their counterparts who were under the 12 h/12 h L/D cycle, and even less than all non-pregnant animals, including ones under 24 h/0 h L/D cycle. These confirmed the suggestion based on the results of light microscopic analysis. Pregnant rats under 12 h/12 h L/D cycle had the highest total area of mitochondria and length of mitochondrial cristae among all groups. In addition, the nuclei of pinealocytes of constant light exposed pregnant rats were mostly pyknotic and had invaginations of caryolemma compared to their 12 h/12 h L/D exposed controls. These indicated the initial signs of nuclei degeneration in the pinealocytes under the constant light exposure (Figure 7) and the statistical analysis on mitochodrial alterations were illustrated in Figure 8.

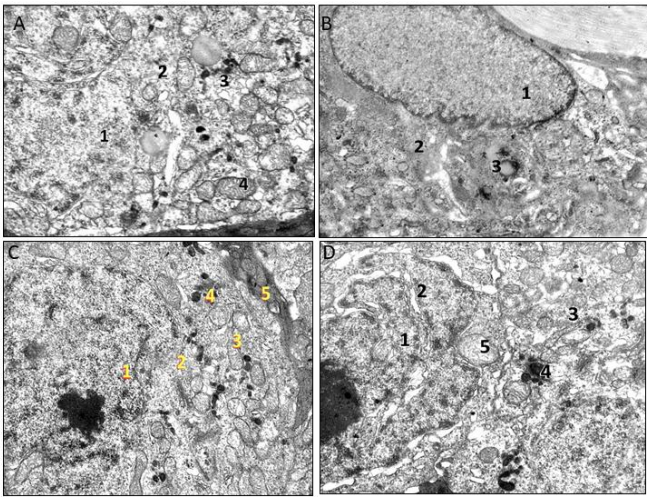

Fig. 7. Effects of the different light exposure schedules on the morphology of pineal gland in pregnant and non-pregnant rats: a electronic microscopic view.

A. An image of the pineal gland of a pregnant rat under 12 h/12 h L/D cycle 1: nucleus, 2: cytoplasm, 3: inclusion of melatonin, 4: mitochondrion. x9000. B. The pineal gland of a pregnant rat under 24 h/ 0 h, L/D cycle. 1: nucleus, 2: cytoplasm, 3: secretory inclusion of melatonin (few mitochondria were identified). x9000. C. the pineal gland of a non-pregnant rat under 12 h/12 h, L/D cycle. 1: nucleus, 2: cytoplasm of pinealocyte, 3: mitochondria, 4: secretory inclusions of melatonin, 5: tail of dark pinealocyte. x 9 000. D. The pineal gland of a non-pregnant rat under 24 h/0 h L/D cycle. 1: nucleus, 2: caryolemma invagination, 3: cytoplasm, 4: secretory inclusion of melatonin, 5: mitochondrion. X14000.

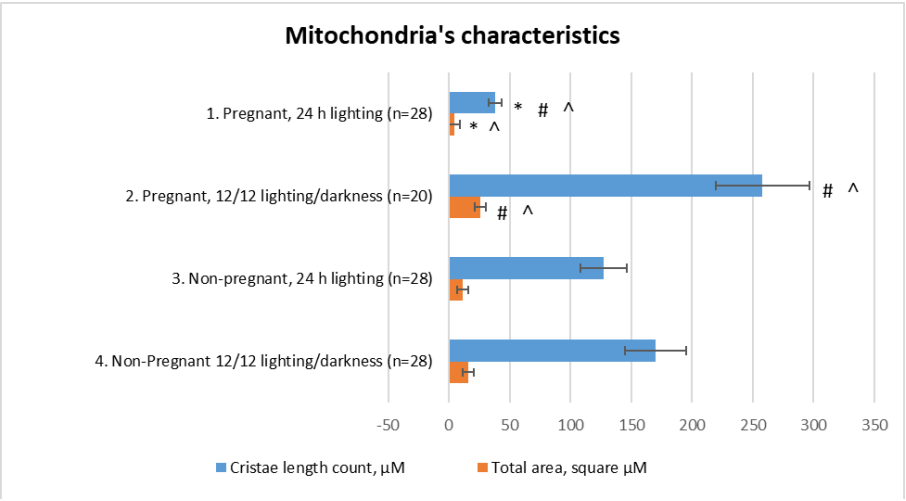

Fig. 8. Statistical analyses of the effects of different light exposure schedules and prognancy on the utrastructures of mitochondria in pinealocytes.

*The data were expressed as means ± SD (n = 20 – 28). Data were from the imanges of electron microscopy. * $p < 0.05$ vs line 2; # $p < 0.05$ vs line 3; ^ $p < 0.05$ vs line 4.*

3.3. Effects of the different light exposure schedules on the levels of blood melatonin in pregnant rats.

Whether the morphological alterations of the pineal gland caused by the different light exposure schedules will modify the melatonin production in the animals, the blood melatonin levels in pregnant rats were measured. The result showed that the melatonin level in constantly light exposed rats were significantly lower than that in their control group (Figure 9).

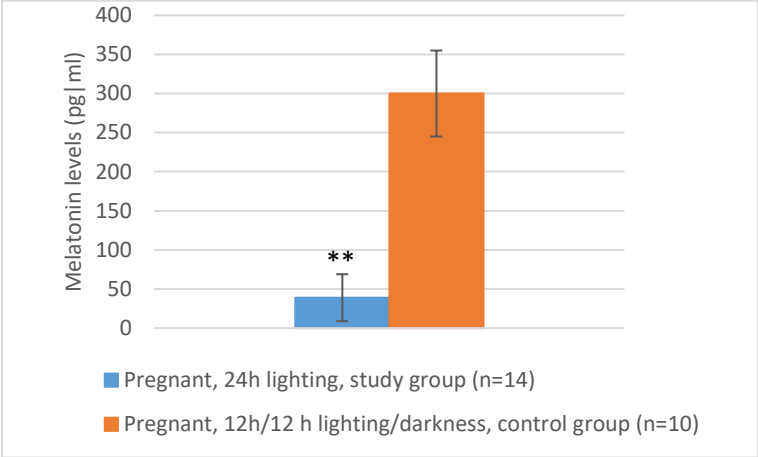

Fig. 9. Blood melatonin levels (pg/ml) of the pregnant rats under different light exposure schedules.

*Data were expressed as mean ± SD (n = 10-14). ** p<0.01 vs control.*

3.4. Effects of the different light exposure schedules on the levels of cytokines in pregnant rats.

To determine whether the light exposure has influence on the pro-inflammatory response several important cytokines including IL-6, IL-10, IL-1-beta, IL-4 and TNF-alpha were also measured. It was found that among other cytokines, only IL-6 was significantly elevated in the pregnant rats who were under constant light exposure compared to their 12/12 L/D exposure controls ($p < 0.05$, Figure 10).

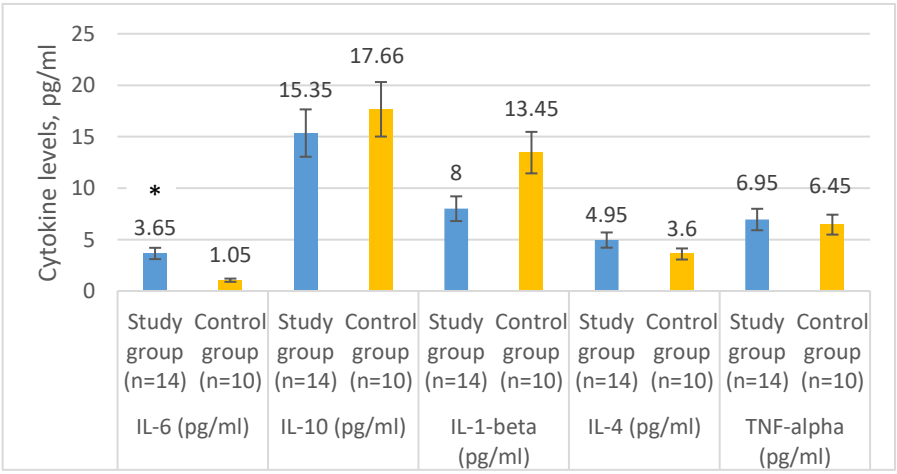

Fig. 10. Effects of the different light exposure schedules on the levels of cytokines in pregnant rats.

*Data were expressed as mean ± SD (n = 10-14 as indicated in the figure.). *p < 0.05 vs its respective control.*

4. DISCUSSION

The prevalence or incidence of the infertility or miscarriage is in rise globally for the modern populations. Many risk factors are attributed to this tendency. Among them, the light pollution is a substantially underestimated factor for this aspect. Light pollution, particularly prolonged light exposure during night, for example, shift workers, night club social activities, has adverse effects on human health. It may associate with insomnia, depression, heart disease, metabolic disorders, obesity and cancers (27-34). Based on solid evidence, the light at night has been classified as a Group 2A carcinogen by the International Agency for Cancer Research (IACR), i.e. a probable carcinogen (35). The influence of light pollution on animal reproductive activity has also be reported (36). For example, the elevated artificial light intensities at night would increase corticosterone and reduced estrone levels in female birds (37). For mammals, the light at night also desynchronized their seasonal reproduction and delayed birth (38). All these reproductive alterations caused by prolonged light exposure at night were attributed to the suppressed melatonin levels in these animals. In the current study, for the first time, we have observed that the pregnant rats under normal light (12 h/12 h L/D) exposure had natural pregnant process and delivered healthy pups, in contrast, the pregnant rats under constant light exposure completely terminated their pregnancy and none of them had given birth. The result is surprising and such a profound effect of light at night on mammalian reproduction, particularly in rats, is not expected. In one hand, light at night is referred as the chemical pinealectomy regarding its suppressive effect on melatonin production and thus, it can impact the mammalian reproduction as we mentioned above. In other hand, we also realized that pinealectomized hamsters, in which their melatonin circadian rhythm was diminished, still could have a successful pregnancy and give birth but at the wrong season (39). This inconsistence may be the species specific. Most importantly, the light pollution may have more profound adverse effects than that of pinealectomy on mammalian reproduction. This issue is warranted for further study. Evidence shows that the pregnant termination caused by constant light occur at an early stage of the pregnancy since no significant hyperemia was observed in uterus of the rats. This indicates that the early stage of embryo development is vulnerable to the environmental challenge caused by constant light. Light at night suppresses melatonin production and this was confirmed in our study. As seeing in Figure 9, the melatonin level in females under constant light exposure is only 1/5 of that in their 12 h/12 h L/D exposed controls. In our study, the melatonin levels appeared higher than those in some reports but were comparable to other previous reports in rats (40, 41). The differences might relate to the different assay methodologies. Nevertheless, the ratio of the melatonin levels between the groups is a reliable index to indicate this difference at the same set of analysis. Melatonin is an important molecule to target the mammalian reproductive activities. It is not only a chemical signal for the reproduction in photoperiodic breeders (42-45), but it is also a local regulator in reproductive organs (46). Melatonin promotes the embryo development in the *in vitro* and *in vivo* conditions (47-51) and therefore, increases the pregnancy as well as birth rates in mice, cows and goats (52-55). The low levels of melatonin lead to reduced reproductive success in animals and also in humans (56-60). We speculate that the early termination of the pregnancy in our study is related to the extremely low melatonin level compared to the control. The exact mechanisms for this are unknown. The antioxidant capacity of melatonin on protection of embryo development has been well documented (61). In addition to this, our data indicated the increased pro-inflammatory reaction might be another mechanism. Melatonin is an excellent anti-inflammatory molecule and it can reduce the pro-inflammatory cytokines secretion (62-66). We also observed that the low melatonin caused by the constant light exposure in the pregnant rats resulted in the pro-inflammatory response indicated by the increased level IL6. The local inflammatory reaction in the implantation place would jeopardize the embryo development and if this event were not counteracted properly, the gestation would be terminated as observed in our study. The morphological study of pineal gland has confirmed that the low level of melatonin is due to the dysfunction of this gland. Under the constant light exposure, the pinealeocytes tend to degeneration with reduced nucleus/cytoplasm ratio, less

area of mitochondria and the length of their cristae, which were classified as bright pinealocytes compared to the dark ones observed in the control. Currently, the mitochondria have proved to be the major sites to synthesize melatonin in all cells and particularly in the pinealocytes (67). The reduced mitochondrial numbers and damaged mitochondrial cristae inevitably will render the low level of secretory melatonin granulas and the low blood melatonin production as well as the increased inflammatory reaction in the implanted sites of uterine observed in our studies. Whether melatonin supplementation will correct these disorders is the goal of our future studies.

In conclusion, it is for the first time that we reported that constant light exposure completely terminated the pregnancy in already pregnant rats. This observation may also apply to other mammals; however, it requires further confirmation. The potential mechanisms involve pineal dysfunction caused by the light exposure at night, reduced blood melatonin level and elevated proinflammatory reactions. All these factors are attributed to the early termination of the pregnancy of rats (Figure 11). These observations have direct relevant to the human reproduction under the increased light pollution globally. The adverse effects of light pollution on increased human infertility and miscarriage cannot be underestimated based on our observations in the current study.

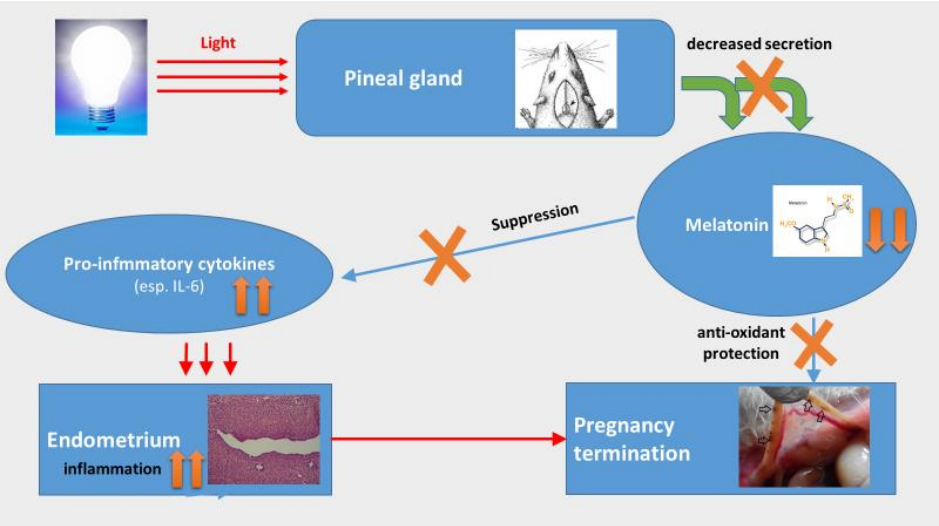

Fig. 11. The potential mechanisms of constant light exposure on pregnancy in rats.

Red and green arrows indicate the orders of the processes; orange arrows indicate the directions of the alterations; X indicates the block of the processes, IL-6: interleukin-6 .

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AUTHORSHIP

Andrii Berbets was responsible for conducting the experiment, sample processing, searching in databases, statistical analysis and drafting the manuscript. Adrian Barbe was responsible for conducting the experiment, sample processing, statistical analysis and drafting the manuscript. Oleksandr Yuzko was the project leader and was responsible for finalizing the manuscript.

CONFLICT OF INTEREST

The authors declared no conflict interest.

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