Research Article

Light-exposure at night impairs mouse ovary development via cell apoptosis and DNA damage

Yapeng Li¹,², Shunfeng Cheng², Lan Li², Yong Zhao², Wei Shen² and Xiaofeng Sun²

¹College of Animal Science and Technology, Qingdao Agricultural University, Qingdao 266109, China; ²College of Life Sciences, Key Laboratory of Animal Reproduction and Germplasm Enhancement in Universities of Shandong, Qingdao Agricultural University, Qingdao 266109, China

Correspondence: Xiaofeng Sun (xfsun@qau.edu.cn)

The alternation of light and dark rhythm causes a series of physiological, biochemical and metabolic changes in animals, which also alters the growth and development of animals, and feeding, migration, reproduction and other behavioral activities. In recent years, many studies have reported the effects of long-term (more than 6 weeks) illumination on ovarian growth and development. In the present study, we observed the damage, repair and apoptosis of ovarian DNA in a short period of illumination. The results showed that, in short time (less than 2 weeks) illumination conditions, the 24-h light treatment caused the reduction of total ovarian follicle number and down-regulation of circadian clock related genes. Furthermore, the changed levels of serum sex hormones were also detected after 24-h light exposure, of which the concentrations of LH (luteinizing hormone), FSH (follicle-stimulating hormone) and E2 (estradiol) were increased, but the concentration of PROG (progesterone) was decreased. Moreover, 24-h light exposure increased the expression of DNA damage and repair related genes, the number of TUNEL and RAD51 positive cells. These results indicated that 24-h light exposure for 4, 8 and 12 days increased DNA damage and cell apoptosis, thereby affecting the development of ovary.

Introduction

It is well known that photoperiod affects the physiological behavior of animal by adjusting the daily and seasonal changes, and the animals develop specific adaptive periodic activities through rhythm oscillation [1–4]. Many studies have found that the photoperiod regulates animal body, and animals have to adapt to the daily and seasonal changes through the circadian oscillator of hypothalamic suprachiasmatic nucleus. It has been proved that biological rhythms can be determined or regulated by clock genes. And the expression of clock genes in the putative hypothalamus and its suprachiasmatic nucleus varies with the photoperiod or the light condition [5,6]. In mammals, several biological clock genes important for the circadian rhythms have been identified, such as period genes (Per1, Per2), cryptochrome genes (Cry1, Cry2) and RAR-related orphan receptors genes (Rora, Rorb, Rorγ) [7–9]. Per is the first clock gene discovered by Konopka and Benzer in 1971. Different point mutations within Per coding region can lengthen, shorten or abolish circadian rhythms [10]. Moreover, Per gene overexpression could restore the behavior of circadian rhythms caused by Per gene mutated [11], this further showed the important roles of Per gene in generation and maintenance of biological rhythms [12]. In the core feedback loop, transcriptional activator BMAL1 and CLOCK heterodimer initiates the transcription of Per and Cry genes, which are then suppressed by their products of translation and translocation [7,13,14].

Through the rhythm oscillator, photoperiod can adjust downstream genes’ expression of organisms [6,15]. And the daily physiological rhythm disorder can cause many pathophysiological issues, such as cognitive disturbance, mental illness and so on [16–21]. It was hypothesized that the biological clock participated in the daily length measurement [22]. Several groups have investigated the expression of
Table 1 Primers used for qRT-PCR

| Genes       | Sequences of primers                                           | Production (bp) |
|-------------|----------------------------------------------------------------|-----------------|
| β-Actin     | F: TCCTGACCGGCGCTTCTAGG; R: TTGCCCCTTAGGTTCAGGGGG               | 255             |
| Rorα        | F: CCGCTCAGTGGCTTCTCAACA; R: CGGAGGAGTTGGAATGATG                | 95              |
| Rorβ        | F: TACGCTCGGCTATACCAAGG; R: ATGCCCTG GAATGCTTGA                 | 106             |
| Rorγ        | F: TGGCGGGATTTGGGATTCACTCATAGTCG                             | 177             |
| Dbp         | F: CGTGGAGGTGCTTAATGACCTTT; R: ATGGCCTGGAATGCTTGA               | 68              |
| Bmal1       | F: CCGCAAAAGTGAACAGACAGAC; R: GGTCTCTTGATCCTCTCCTTGGT            | 81              |
| Cry1        | F: CTGCGCTGAGATGCATCTG; R: CTGCCGCAATGTAGCTTTA                 | 77              |
| Per1        | F: ACCAGGCGTGCTATGACAGCACATAC; R: CCTCTCGGCTTCTTTTCAG           | 73              |
| Per2        | F: ATGCTCGACTCCCAACAAQA; R: GGGCGAATGGAATGGGAAT                 | 72              |
| Ck1ε        | F: CGCTGCATAGAAGCTGCTGAT; R: AAACCCGTGCGGTGAAGA                 | 71              |
| Rad51       | F: ACCAGACCGGCTTCTTTAC; R: CAAGTGAACAGCATGCTTTC                 | 171             |
| BRCA1       | F: ATGCCGCGGAAAGTGGTCTTCA; R: GGCCTGCAGATGACAAGACTAG            | 171             |
| Rec8        | F: TGATATGGAGAGGCGTACC; R: GCAGCCTCATAAAGGCTTCG                 | 165             |

Photoperiod-induced ovarian circadian clock genes to figure out the effects of photoperiod on biological clock and ovarian development [7,23,24].

In mammals, the development of ovarian follicles consists of serial highly orchestrated events [25–27]. The interaction between oocytes and surrounding somatic cells is particularly important for the development and function of ovaries [28–31]. These interactions regulate the occurrence of germ cells, meiosis and the formation of primordial follicles, and then regulate follicle growth, maturity, ovulation and other processes [32–34]. As a daily and seasonal oscillatory rhythm, photoperiod can alter ovarian development cycle, inhibit or delay the sex maturation of different species [35–37]. At the same time, the endocrine environment and related factors can also be changed by photoperiod, such as changes in the relevant hormone content [38,39]. Light as one of the key external environmental cues can affect the circadian rhythms [40]. Many previous studies have reported that compared with normal light cycle, both increasing the light and decreasing the light exposure can affect ovarian follicular development [41–44]. These investigations suggest that photoperiod and other related signaling pathways affect the development of reproductive system, the secretion of sex hormones.

Even though many studies have presented the effects on ovarian growth and development caused by long-term (more than 6 weeks) illumination [45,46], the effects of the short period of illumination on ovary development have not been thoroughly investigated, and the underlying mechanism remains unclear. Therefore, the purpose of this investigation was to explore the effects of short period of illumination on DNA damage/repairment, and apoptosis in the mouse ovary.

Materials and methods

Animals and experiment design

All procedures of animal handling were approved by the Ethics Committee of Qingdao Agriculture University, Shandong, China [47]. Puberty CD-1 mice (4 weeks old) used in this experiment were purchased from Vital River Laboratory Animal Technology Co. LTD (Beijing, China). They were housed in temperature controlled (21–22°C) and standard diet conditions. 120 mice were randomly divided into two groups. One group was maintained under 24:0 h light/dark cycle (24-h light exposure), and the other was maintained under 16:8 h light/dark cycle (16-h light exposure) [48] as control. The light intensity is about 250 lx. When they were treated for 4, 8 and 12 days, respectively, the mice were euthanized and sampled for further analysis at 4 pm.

RNA extraction, cDNA synthesis and quantitative RT-PCR

Ovaries of mice under different light-dark cycles (exposed for 4, 8 and 12 days) were collected. Total RNA was extracted using RNeasy kit pure MicroKit (Aidlab, RN07, China). Reverse transcription was performed using TUREscript first strand cDNA Synthesis Kit (Aidlab, PC1802). Quantitative real-time PCR (qRT-PCR) was carried out using Light-Cycler® SYBR Green I Master Kit (Roche, Roche 04887352001, Switzerland) with a Roche real time PCR instrument (Roche LC480) according to the manufacturer's instructions. qRT-PCR primers were listed in Table 1. The PCR reaction programs were set as follows: 10 min at 95°C, followed by 45 cycles of 95°C for 10 s, 60°C for 30 s and 72°C for 30 s.
Figure 1. Effects of photoperiod exposure on ovarian development
(A) Ovarian size and morphology, (B) Immunohistochemical staining for VASA, (C) the number of follicles. Data are expressed as mean ± S.D. at least three independent experiments (P < 0.05).

72°C for 20 s. The relative mRNA abundance was normalized to the expression of β-actin according to following formula: $2^{-\Delta \Delta C_T}$ [target gene $C_T$ value - reference gene $C_T$ value]. Data represented were calculated by mean ± standard deviation (S.D.) of at least three independent experiments.
Figure 2. Effects of photoperiod exposure on serum sex hormone levels.
(A) FSH, (B) LH, (C) E2, (D) PROG. Data are expressed as mean ± S.D. at least three independent experiments (*P<0.05, **P<0.01).

Immunofluorescence and immunohistochemistry
For immunofluorescence, ovaries from 24-h light exposed mice and the control group were collected and fixed in 4% paraformaldehyde (Solarbio, P1110, Beijing, China) for 12 h. Next, they were washed with running water, and dehydrated by gradient alcohol and embedded in paraffin. After the ovaries were cut into 5-μm serial sections, they were heated at 60°C for 2 h, dewaxed by xylene and rehydrated by gradient alcohol, Antigen retrieval was performed with trisodium citrate at the condition of 96°C for 10 min. After cooled to room temperature, the ovary slides were blocked with BDT (3% BSA and 10% goat serum dissolved in TBS) for 30 min, then incubated with anti-RAD51 (Abcam, ab88572, U.S.A.) primary antibody at a dilution of 1:1000 overnight at 4°C. The next day, the sections were incubated with Cy3-conjugated goat anti-mouse secondary antibody (Beyotime, A0521, Nantong, China) at a dilution of 1:2000 for 30 min. Finally, Hoechst 33342 was used for nuclear staining. Follicles were amounted and scored as previously described [49]. One out of every four sections was selected from the whole-ovary serial sections for analyzing the percentage of RAD51 positive cells (the number of RAD 51 positive cells/the total number of cells).

For immunohistochemical staining, 3% H2O2 was used to eliminate endogenous peroxidase. Then the sections were incubated with anti-VASA polyclonal antibody at a dilution of 1:1000 overnight at 4°C (Abcam, ab13840). After washing, the sections were incubated with HRP-labeled goat anti-rabbit secondary antibody at a dilution of 1:2000 for 30 min (Beyotime, A0208). After that, the color was developed using DAB peroxidase (Beyotime, P0203) and the sections were counterstained with hematoxylin. All ovaries slices were photographed under Olympus microscope (BX51, Japan). The positive rates were analyzed using Image Plus software, and finally visualized by GraphPad Prism 5. Each immunofluorescence or immunohistochemistry were repeated at least three times.

TUNEL staining
Parallel to procedure stated in immunofluorescence or immunohistochemistry staining, ovary slides were dewaxed, rehydrated. Then TUNEL BrightRed Apoptosis Detection Kit (Vazyme, A1113, Nanjing, China) was used to evaluate ovarian cell apoptosis according to the manufacturer’s instructions. After treated with protease for 20 min at room temperature, ovary sections were incubated with TUNEL reaction mixture (Label Solution and Enzyme Solution; 5:1) for 60 min at 37°C and stained with Hoechst33342. The sections were observed and photographed under BX51 Olympus fluorescence microscope. One out of every four sections was selected from the whole-ovary serial sections for analyzing the percentage of TUNEL positive cells (the number of TUNEL positive cells/the total number of cells).
Figure 3. Effects of photoperiod exposure on cell apoptosis

(A) TUNEL immunostaining of ovaries. (B) Quantification of the percentage of TUNEL positive cells. Data are expressed as mean ± S.D. at least three independent experiments (*P<0.05, **P<0.01).
Figure 4. Effects of photoperiod exposure on mRNA levels of ovarian circadian clock related genes 
(A) Treated for 4 days, (B) Treated for 8 days and (C) Treated for 12 days. Data are expressed as mean ± S.D. at least three independent experiments (\(^*\) \(P<0.05\), \(^{**}\) \(P<0.01\)).

Western blotting
Western blotting analysis of ovary protein lysates was based on procedure represented previously [50,51]. Proteins from ovaries of different treatment were separated and transferred onto PVDF membranes. After blocking with 5% BSA in Tris-buffer, the membranes were incubated with anti-RAD51 antibody (Abcam, ab88572) and anti-ACTIN antibody (Abcam, ab8226) at a concentration of 1.0 \(\mu\)g/ml overnight at 4\(^\circ\)C. Then the membranes were incubated at 37\(^\circ\)C for 2 h with secondary antibodies (Beyotime, A0216) at a dilution of 1:2000. Proteins were detected using the BeyoECL Plus Kit (Beyotime, P0018). The band intensity was qualified with alphaview software.

Levels of luteinizing hormone, follicle-stimulating hormone, estradiol and progesterone
After treatment, the blood samples were collected from the retroorbital sinus immediately. Then the blood samples were centrifuged at 3000 rpm. for 30 min to obtain the serum. The serum samples were quickly removed and kept at \(-80^\circ\)C for subsequent analysis of hormone levels. The serum sex hormone levels were measured using (progesterone) PROG ELISA kit (JingmaBIO, E-20375, China), (follicle-stimulating hormone) FSH ELISA kit (JingmaBIO, E-20418), (luteinizing hormone) LH ELISA kit (JingmaBIO, E-20342) and (estradiol) E2 ELISA kit (JingmaBIO, E-20380) according to the manufacturer’s instructions. Each experiment was carried out in triplicate.

Statistics
All data represent the mean ± S.D. at least three independent experiments. Statistics difference was determined by Student’s \(t\)-test or one-way ANOVA with GraphPad Prism 5. Results were considered statistically significant at \(P<0.05\).
Figure 5. Effects of photoperiod exposure on Rad51 expression

(A) Immunofluorescence of RAD51; (B) Quantification of percentage of RAD51 positive cells. Data are expressed as mean ± S.D. at least three independent experiments (*P<0.05, **P<0.01).
Results

24-h light exposure reduced the number of follicles

Though there were no significant changes in ovarian morphology and size after 24-h light exposure for 4, 8 and 12 days (Figure 1A), the number of oocytes in 24-h light exposure group significantly decreased after 8-day treatment compared with that in control group by staining with oocyte specific marker VASA (*P<0.05; Figure 1B,C).
Photoperiod altered serum sex hormone levels

Based on the methods mentioned above, we speculated that photoperiod might regulate the ovarian development and follicle number by influencing serum sex hormone levels. Therefore, we examined the levels of sex hormones in serum. The results showed that, compared with that in control group, 24-h light exposure significantly increased the levels of FSH (Figure 2A), LH (Figure 2B) after 4- and 8-day treatment, and the level of E2 (Figure 2C) after 12-day treatment, however, significantly decreased PROG concentration (Figure 2D) after 8- and 12-day treatment. Meanwhile, we chose several mice of each group to examine the estrous cycles based on a smear test; however, there was no significant difference between them (data not shown).

24-h light exposure increased the apoptosis of ovarian cells

The positive number of ovarian apoptotic cells in the 24-h light exposure group and control group were analyzed by TUNEL staining (Figure 3A). Compared with the control group, 24-h light exposure for 4, 8 and 12 days significantly increased the number of TUNEL positive somatic cells in ovaries (*P<0.05 or **P<0.01; Figure 3B).

24-h light exposure decreased the expression of murine ovary circadian clock-related genes

After 4 days’ treatment, we found that 24-h light exposure significantly reduced the expression of Rorγ, Bmal1, Cry1 and Per1 (*P<0.05 or **P<0.01) in mRNA level compared with that in control group (Figure 4A). The expression of Rora, Rorβ, Rorγ, Per2 and Ck1ε was significantly decreased after 8 days’ treatment (*P<0.05 or **P<0.01; Figure 4B), and the expression of Rora, Rorβ, Rorγ, Per2 and other genes was significantly decreased after 12 days’ treatment compared with that in control group (*P<0.05 or **P<0.01; Figure 4C).

24-h light exposure increased ovarian DNA damage

To investigate the mechanism of ovarian cell apoptosis, the DNA damage-related protein RAD51 was detected using immunofluorescence and Western blot. The number of RAD51 positive somatic cells in the ovaries was increased significantly after 24-h light exposure for 4, 8 and 12 days compared with that in control group (*P<0.05 or **P<0.01; Figure 5A,B). Furthermore, Western blot results also proved the increased expression of RAD51 (*P<0.05; Figure 6A). Then, the expression of DNA damage and repairment related genes Rad51, BRCA1 and Rec8 were detected significantly up-regulated at mRNA level after 24-h light exposure for 4, 8 and 12 days compared with that in control group (*P<0.05 or **P<0.01; Figure 6B).

Discussion

There have been many studies reported that long-term (more than 6 weeks) exposure to light affected ovarian development in mice, rats, cattle, sheep and other mammals [52–55]. The reproductive cycle is related to the seasonal variation of the photoperiod [56–58], and the prolongation of the artificial photoperiod can affect ovarian development, which is based on the reflect of circadian rhythm to light [8,59,60].

In the present study, we focused on the effects of short term (less than 2 weeks) exposure to 24-h light on ovarian development. In order to avoid the effects of temporally cycling on the level of sexual hormones and clock gene expression, the sampling timing was consistently at 4 pm. Though, there was no significant change in the food intake, and the body weight gain after short term (less than 2 weeks) exposure to light at nights (data not shown), the number of ovarian follicles was decreased. Especially, there was a significant decrease after 8- and 12-day exposure. The relationship between this significant change in the number of follicles and the change in the body weight increasing rate remains to be further studied. We speculate that some of these results may be related to DNA damage repair caused by short-term treatment of 24-h light exposure.

We assume that this periodicity of the photoperiod is closely related to the reproductive cycle of the female. In terms of the levels of serum hormones, our results showed that short-term (less than 2 weeks) exposure to light at night increased the levels of LH, FSH and E2, but decreased the level of PROG. In the present study, large numbers (120 mice) of samples were used and were grouped randomly to exclude the effects of estrus cycle on sex hormones. And the changes of the sex hormones tested were significant, which suggested that the light cycle had a strong interference with reproductive hormones or the reproductive cycle. On the other hand, the changes of hormone level might be one of the reasons for the decreased number of follicles caused by prolonged photoperiodism. In addition, we found that short-term (less than 2 weeks) exposure to light at night increased ovarian apoptosis and DNA damage. It suggested that the short-term (less than 2 weeks) exposure to light at nights might alter the level of sex hormones and induce...
cell apoptosis and DNA damage through neurohormonal regulation, thus affected follicular development; however, the specific regulatory mechanisms remain to be further studied.

It is generally considered that photoperiod exerted its function of neurohumoral regulation via the suprachiasmatic nucleus of mammalian hypothalamus. Based on this, we verified the expression of some genes related to the circadian clock. For a long time, Per was the only circadian gene known, but rapid progress in the circadian field led to the identification of other clock genes [40]. Now, it is known that many clock genes were found to play roles in controlling or regulating biological rhythm, which were also found expressed in animal ovaries [61]. In the present study, we also detected the mRNA expression level of clock genes and found that some of detected biological clock genes significantly decreased after 24-h light exposure for 4, 8 or 12 days. The results showed that the altered circadian rhythm had affected the expression of clock genes, and suggested a potential neuromodulatory effect of the genes associated with peripheral ovarian clocks.

It is well known that environmental toxins, UV and ionizing irradiation usually can induce DNA damage [62]. Light-exposure at night, regard as one of the genotoxic stresses, can disrupt DNA damage response and repair [63]. DNA damage-induced cell apoptosis has been well studied from the year of 2000 [64], in which DNA damage can active DNA repair pathways. If the damage is above the competence of the repair, the cells enter the program of apoptosis and undergo apoptosis. Many reports also confirmed that sex hormones played roles in cell apoptosis [65,66]. Some hormones act as survival factors to inhibit apoptosis and others act as atretogenic factors to induce apoptosis [67–70]. According to our results, 24-hight exposure for less than 2 weeks could induce the reproductive hormones disorder, thereby affecting cell apoptosis. Or 24-hight exposure as one of the genotoxic stresses induced DNA damage then resulted in DNA damage-induced cell apoptosis. The increasing apoptosis was responsible for the number of follicles and the development of ovary.

However, the oscillation phase should be considered in studying the issues of circadian changes. Whether the oscillation phase in sexual hormone secretion or clock gene expression is synchronized or not and whether the oscillation phase in sexual hormone secretion or clock gene expression can be changed by the treatment of the present study will be further explored.

**Author Contribution**

X.S. and W.S.: designed the experiment; Y.L: performed the experiments; and S.C. and L.L.: analyzed the data. Y.Z.: drafted the manuscript. All the authors read and approved the final manuscript.

**Funding**

This work was supported by Shandong Province Natural Science Foundation of China [grant number ZR2017MC033].

**Competing Interests**

The authors declare that there are no competing interests associated with the manuscript.

**Abbreviations**

DAB, 3,3-diaminobenzidine; E2, estradiol; FSH, follicle-stimulating hormone; HRP, horseradish peroxidase; LH, luteinizing hormone; PROG, progesterone; qRT-PCR, quantitative real-time PCR; TBS, tris buffered saline.

**References**

[1] Goldman, B.D. (2001) Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J. Biol. Rhythms 16, 283–301, [https://doi.org/10.1177/074873001129001980](https://doi.org/10.1177/074873001129001980)

[2] Reppert, S.M. and Weaver, D.R. (2002) Coordination of circadian timing in mammals. Nature 418, 935–941, [https://doi.org/10.1038/nature00965](https://doi.org/10.1038/nature00965)

[3] Tomioka, K., Uryu, O., Kamae, Y., Umezaki, Y. and Yoshii, T. (2012) Peripheral circadian rhythms and their regulatory mechanism in insects and some other arthropods: a review. J. Comp. Physiol. B 182, 729–740, [https://doi.org/10.1007/s00360-012-0651-1](https://doi.org/10.1007/s00360-012-0651-1)

[4] Partch, C.L., Green, C.B. and Takahashi, J.S. (2014) Molecular architecture of the mammalian circadian clock. Trends Cell Biol. 24, 90–99, [https://doi.org/10.1016/j.tcb.2013.07.002](https://doi.org/10.1016/j.tcb.2013.07.002)

[5] Dardente, H., Wyse, C.A., Lincoln, G.A., Wagner, G.C. and Hazlerigg, D.G. (2016) Effects of photoperiod extension on clock gene and neuropeptide RNA expression in the SCN of the Soay Sheep. PloS ONE 11, e0159201, [https://doi.org/10.1371/journal.pone.0159201](https://doi.org/10.1371/journal.pone.0159201)

[6] Yasuo, S., Watanabe, M., Okabayashi, N., Ebihara, S. and Yoshimura, T. (2003) Circadian clock genes and photoperiodism: comprehensive analysis of clock gene expression in the mediobasal hypothalamus, the suprachiasmatic nucleus, and the pineal gland of Japanese Quail under various light schedules. Endocrinology 144, 3742–3748, [https://doi.org/10.1210/en.2003-0435](https://doi.org/10.1210/en.2003-0435)

[7] Ko, C.H. and Takahashi, J.S. (2006) Molecular components of the mammalian circadian clock. Hum. Mol. Genet. 15, R271–R277, [https://doi.org/10.1093/hmg/ddt207](https://doi.org/10.1093/hmg/ddt207)

[8] Moore-Ede, M.C. and Moline, M.L. (1985) Circadian rhythms and photoperiodism. Ciba. Found. Symp. 117, 23–37
39 Taranger, G.L., Muncaster, S., Norberg, B., Thorsen, A. and Andersson, E. (2015) Environmental impacts on the gonadotrophic system in female Atlantic salmon (Salmo salar) during vitellogenesis: Photothermal effects on pituitary gonadotropins, ovarian gonadotropin receptor expression, plasma sex steroids and oocyte growth. *Gen. Comp. Endocrinol.* **221**, 86–93, https://doi.org/10.1016/j.ygcen.2015.02.008

40 Dubowy, C. and Sehgal, A. (2017) Circadian Rhythms and Sleep in Drosophila melanogaster. *Genetics* **205**, 1373–1397, https://doi.org/10.1534/genetics.115.185157

41 Shahed, A. and Young, K.A. (2013) Antihuman hormone (AMH), inhibin-alpha, growth differentiation factor 9 (GDF9), and bone morphogenic protein-15 (BMP15) mRNA and protein are influenced by photoperiod-induced ovarian regression and recrudescence in Siberian hamster ovaries. *Mol. Reprod. Dev.* **80**, 895–907, https://doi.org/10.1002/mrd.22215

42 Murphy, B.A., Blake, C.M., Brown, J.A., Martin, A.M., Forde, N., Sweeney, L.M. et al. (2015) Evidence of a molecular clock in the ovine ovary and the influence of photoperiod. *Theriogenology* **84**, 208–216, https://doi.org/10.1016/j.theriogenology.2015.03.008

43 Vasal, S. and Sundararaj, B.I. (1976) Response of the ovary in the catfish, Heteropneustes fossilis (Bloch), to various combinations of photoperiod and temperature. *J. Exp. Zool.* **197**, 247–263, https://doi.org/10.1002/jez.1401970206

44 Gurya, S.S., Sexena, P.K. and Gill, M. (1976) Effect of long photoperiod on the maturation of ovary of the catfish, Mystus tengara (Ham.). *Acta Morphol. Neerl. Scand.* **14**, 331–338

45 Ma, O., Tan, Y., Chen, X., Chen, S., Sun, Y. and Zhou, B. (2018) Regulation of the MAPK signaling pathway by miR-421-5p in rats under light pollution. *Int. J. Mol. Med.* **42**, 3329–3343

46 Lynch, E.W.J., Coyle, C.S. and Stevenson, T.J. (2017) Photoperiodic and ovarian steroid regulation of histone deacetylase 1, 2, and 3 in Siberian hamster (Phodopus sungorus) reproductive tissues. *Gen. Comp. Endocrinol.* **246**, 194–199, https://doi.org/10.1016/j.ygcen.2016.12.008

47 Abdelnabi, M.A., Bakst, M.R., Woods, J.E. and Ottinger, M.A. (2000) Plasma 17beta-estradiol levels and ovarian interstitial cell structure in embryonic Japanese quail. *Poult. Sci.* **79**, 564–567, https://doi.org/10.1093/ps/79.7.564

48 Bedrosian, T.A., Fonken, L.K., Walton, J.C. and Nelson, R.J. (2011) Chronic exposure to dim light at night suppresses immune responses in Siberian hamsters. *Biol. Lett.* **7**, 468–471, https://doi.org/10.1098/rsbl.2010.1108

49 Zhang, X.F., Zhang, L.J., Li, L., Feng, Y.N., Chen, B., Ma, J.M. et al. (2013) Diethylhexyl phthalate exposure impairs follicular development and affects oocyte maturation in the mouse. *Environ. Mol. Mutagen.* **54**, 354–361, https://doi.org/10.1002/em.21776

50 Zhang, P., Chao, H., Sun, X., Li, L., Shi, Q. and Shen, W. (2010) Murine folliculogenesis in vitro is stage-specifically regulated by insulin via the Akt signaling pathway. *Histochem. Cell Biol.* **134**, 75–82, https://doi.org/10.1007/s00418-010-0708-8

51 Chao, H.H., Zhang, X.F., Chen, B., Pan, B., Zhang, L.J., Li, L. et al. (2012) Bisphenol A exposure modifies methylation of imprinted genes in mouse oocytes via the estrogen receptor signaling pathway. *Histochem. Cell Biol.* **137**, 249–259, https://doi.org/10.1007/s00418-011-0894-z

52 Amaral, F.G., Castrucci, A.M., Cicopella-Netto, J., Poletini, M.O., Mendez, N., Richter, H.G. et al. (2014) Environmental control of biological rhythms: effects on development, fertility and metabolism. *J. Neuroendocrinol.* **26**, 603–612, https://doi.org/10.1111/jen.12144

53 Hall, E.S. and Lynch, G.R. (1985) Two daily melatonin injections differentially induce nonsewing thermogenesis and gonadal regression in the mouse (Peromyscus leucopus). *Life Sci.* **37**, 783–788, https://doi.org/10.1016/0024-3205(85)90549-1

54 Schillo, K.K., Hall, J.B. and Hileman, S.M. (1992) Effects of nutrition and season on the onset of puberty in the beef heifer. *J. Anim. Sci.* **70**, 3949–4005, https://doi.org/10.2527/1992.70123994x

55 Platt, T.E., Foster, G.S., Tarnavsky, G.K. and Reeves, J.J. (1979) Influence of ovaries and photoperiod on reproductive function in the mare. *J. Reprod. Fertil. Suppl.* **29**, 564–567, https://doi.org/10.1081/ERC-120026949

56 Dey, R., Bhattacharya, S., Malir, S.K. and Banerji, T.K. (2003) The morpho-anatomy and histology of the pineal complex in a major Indian carp, *Catla catla*: identification of the pineal photoreceptor cells and their responsiveness to constant light and constant darkness during different phases of the annual reproductive cycle. *Endocr. Res.* **29**, 423–443, https://doi.org/10.1081/ERC-120026949

57 Lincoln, G.A. and Davidson, W. (1977) The relationship between sexual and aggressive behaviour, and pituitary and testicular activity during the seasonal sexual cycle of rams, and the influence of photoperiod. *J. Reprod. Fertil.* **49**, 267–276, https://doi.org/10.1530/jrf.0.0490267

58 Freedman, L.J., Garcia, M.C. and Ginther, O.J. (1976) Effect of oocytes and oviposition on reproductive function in the mare. *J. Reprod. Fertil. Suppl.* **39**, 79–86

59 Pickard, G.E. and Turek, F.W. (1988) The hypothalamic paraventricular nucleus mediates the photoperiodic control of reproduction but not the effects of light on the circadian rhythm of activity. *Neurosci. Lett.* **43**, 67–72, https://doi.org/10.1016/0304-3940(88)90130-1

60 Elliott, J.A. (1976) Circadian rhythms and photoperiodic time measurement in mammals. *Fed. Proc.* **35**, 2339–2346

61 Siwicki, K.K., Eastman, C., Petersen, G., Rosbash, M. and Hall, J.C. (1988) Antibodies to the period gene product of Drosophila reveal diverse tissue distribution and rhythmic changes in the visual system. *Neuron* **1**, 141–150, https://doi.org/10.1016/0896-6273(88)90198-5

62 Baille, A. and Gartner, A. (2013) Germ cell apoptosis and DNA damage responses. *Adv. Exp. Med. Biol.* **757**, 249–276, https://doi.org/10.1007/978-1-4614-4015-9

63 Belancio, V.P. (2015) LINE-1 activity as molecular basis for genomic instability associated with light exposure at night. *Mob. Genet. Elements* **5**, 1–5, https://doi.org/10.1080/2159256X.2015.1037416

64 Gartner, A., Milstein, S., Ahmed, S., Hodgkin, J. and Hengartner, M.O. (2000) A conserved checkpoint pathway mediates DNA damage-induced apoptosis and cell cycle arrest in S. cerevisiae. *Mol. Cell.* **5**, 435–443, https://doi.org/10.1016/S1097-2765(00)80438-4

65 Liang, C.C., Lee, T.H. and Chang, S.D. (2013) Effects of sex hormones on cell proliferation and apoptosis in the urinary bladder muscle of ovariectomized rat. *Taiwan J. Obstet. Gynecol.* **52**, 335–340, https://doi.org/10.1016/j.tjog.2012.11.003

66 Yamamoto, Y., Hattori, R.S., Kitahara, A., Kimura, H., Yamashita, M. and Strussmann, C.A. (2013) Thermal and endocrine regulation of gonadal apoptosis during sex differentiation in pejerrey Odontesthes bonariensis. *Sex Dev.* **7**, 316–324, https://doi.org/10.1159/000353506

67 Billig, H., Furuta, I. and Hsuhe, A.J. (1993) Estrogens inhibit and androgens enhance ovarian granulosa cell apoptosis. *Endocrinology* **133**, 2204–2212, https://doi.org/10.1210/endo.133.5.8404672
68 Billig, H., Chun, S.Y., Eisenhauer, K. and Hsueh, A.J. (1996) Gonadal cell apoptosis: hormone-regulated cell demise. Hum. Reprod. Update 2, 103–117, https://doi.org/10.1093/humupd/2.2.103

69 Hsueh, A.J., Billig, H. and Tsafriri, A. (1994) Ovarian follicle atresia: a hormonally controlled apoptotic process. Endocr. Rev. 15, 707–724

70 Hsueh, A.J., Eisenhauer, K., Chun, S.Y., Hsu, S.Y. and Billig, H. (1996) Gonadal cell apoptosis. Recent Prog. Horm. Res. 51, 433–455, discussion 455-436