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Induction of abnormal oocyte division under the constant light in the young adult rat

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ABSTRACT

In order to study the effect of constant light on the ovary, 12 young adult female rats were exposed to constant light for 37 days and their estrous cycles were recorded by daily examination and the ovaries were examined histologically. The results showed that constant light induced in an abnormal and uncontrolled division of oocytes. In these divided–oocyte follicles (DOFs), two or more divided–oocytes shared one zona pellucida and usually floated freely in the follicular antrum. This fantastic phenomenon was discovered for the first time, and it was different from multioocyte follicles (MOFs) and polyovular follicles (POFs) founded in rats, humans, rabbits, mice and dogs.

1. Introduction

Photoperiod is one of the most important regulators of a wide variety of physiological rhythms in animals[1–3]. However, with the invention of electricity and artificial light, the photoperiod was dramatically changed by the ecological light pollution nowadays[4], and caused a series of problems. For example, exposure to light at night not only affected circadian rhythms, behavior[5], hormonal function[6], follicular kinetics[7] and reproductive processes [8], but also possibly increased the risk of many diseases [9]. Moreover, some evidences had revealed that there was correlation between cancer and photoperiod, especially for breast, colon and endometrial cancers[3,10–13]. Up to now, however, there was a lack of an appropriate explanation and morphological evidence *in vivo*.

In the current study, we accidentally found that the constant light caused abnormal and uncontrolled division of oocytes. We described the follicles with divided–oocyte as "divided–oocyte follicles (DOFs)". Unlike multioocyte follicles (MOFs) and polyovular follicles (POFs) founded in rats[14], mice[15] and dogs[16], these divided oocytes in DOFs seemed from one original oocyte by uncontrolled and continual division, and shared one zona pellucida.

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2. Materials and methods

2.1. Animals and experimental design

Sprague–Dawley rats (Qinglongshan Experimental Animal Supply Co. Ltd, Nanjing, China) were used. Twenty–four female rats at 65 days old were kept in a standard 12:12 light/dark (LD) photoperiod for 10 days as pilot experiment. Subsequently, two groups were subdivided randomly, and kept at different light/dark regimens: the LD (n=12) and constant light (LL, n=12) groups. With food and water *ad libtum*, every four animals were kept in a plastic cage (70 cm×40 cm×40 cm) and provided with wood shavings as nesting material. For LD group, fluorescent lamp was used, and avoid illuminating directly. While, the mixed light of incandescent lamp and fluorescent lamp were used and illuminated directly for the LL group. The light intensities were ~600 lux and ~1800 lux in LD and LL groups, respectively. The stages of estrous cycle were assessed by vaginal smears. After 37 days of constant light, rats were sacrificed with an overdose of anesthetic ether and the ovaries were taken for histological observation.

2.2. Histological observation of ovaries

The ovaries were collected, fixed and embedded for histological study. Paraffin sections of the ovaries were serially cut at 10 μm thickness and stained with hematoxylin and eosin (HE). The images were captured in a Nikon 80i (Nikon, Tokyo, Japan). The experimental protocols involving animals were approved in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

3. Results

3.1. Effects of constant light on estrous cycles

At four estrous cycles in length (17 days) after lighting condition, the estrous cycle began disordered in the LL group rats, and two rats had a prolonged vaginal estrus for more than four days (Figure 1). In addition, taken the 17–37 days records into account, all the LL group rats exhibited persistent vaginal estrus for 5–14 d and the estrous cycles were in disorder. However, the ovulation was not blocked. It was suggested that the constant light could not abolish the estrous behavior completely. In contrast, the LD group rats were all normal at the same period.

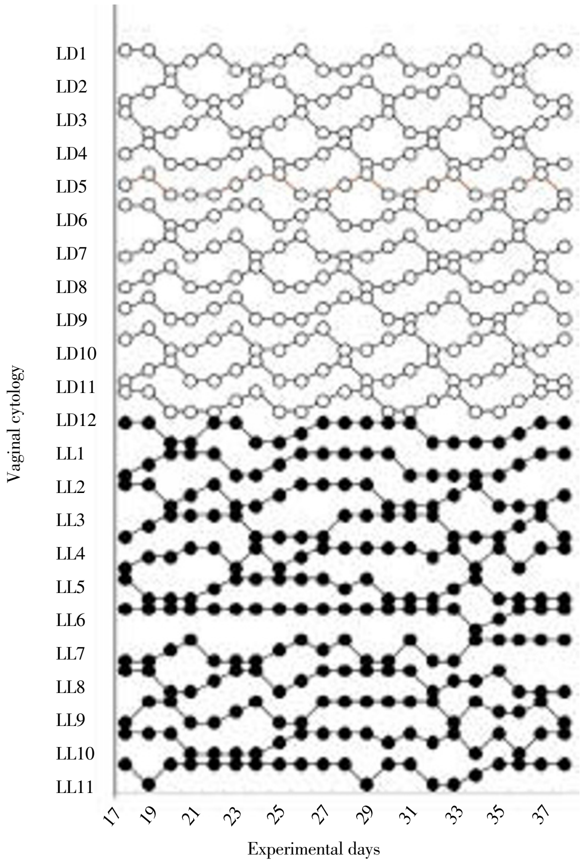

Figure 1. Stages of the estrous cycle after 16 days of constant light. A–C, LD group; D–F, LL group. Vaginal cytology was monitored for every day after 16 days. Open circles and dark circles denote LD (control) *vs.* LL (constant light) group rats, respectively. The plot for each animal is a three-level depiction of the estrous cycle; data points on the lower, middle, and top level represent a day of metestrous or diestrus, proestrus, and estrus, respectively.

3.2. Effects of constant light on the oocyte division

The most obvious change in the ovaries of LL group was DOFs appeared (Figure 2). In the LD group, each ovarian follicle contains only one oocyte which was surrounded by lots of granulosa cells (Figure 2A and B). However, in DOFs, two or several oocytes shared one zona pellucida, and floated in the follicular antrum freely. Generally speaking, the major characteristics of DOFs can be summarized as follows:

Two daughter divided–oocytes derived from oocyte had similar spherical shape and volume, and stained consistent, showed prominent nucleoli (Figure 2C). In addition, these divided–oocytes might divide once again, and formed smaller cells with different sizes (Figure 2D). It was evidence that the division manner of daughter cells might be asynchronous and continuous (Figure 2E). It was indicated that the division of divided–oocyte was uncontrolled. When the daughter cells degenerated, surprisingly, the nuclei were affected in the final stage (Figure 2F).

Another type of DOFs was more interesting. The antral follicle seemed normal, but some divided–oocytes floated in the antrum (Figure 2G). In such type of DOFs, the oocyte surrounded by cumulus cells was in non–divided state. However, these oocytes floated in the antrum kept continued division (Figure 2H). Furthermore, the non–divided–oocyte and the divided–oocyte were separated by cumulus cells, and enveloped by zona pellucida, respectively. Therefore, it was obvious that the non–divided–oocyte was not derived from the divided–oocyte. However, the origin of this type of DOFs was unclear.

In DOFs, the divided–oocyte always appeared after the elimination of cumulus cells. It was seemed that cumulus cells inhibited oocyte division in DOFs (Figure 2G, 2H). Although these oocytes divided continually in DOFs, they degenerated finally (Figure 2F). In addition, there were no blood vessels or blood cells appeared in the antrum of DOFs, but some debris of dead granulosa cells occasionally (Figure 2D). It was suggested that the degeneration of oocytes in DOFs was induced by poor nutrient supply.

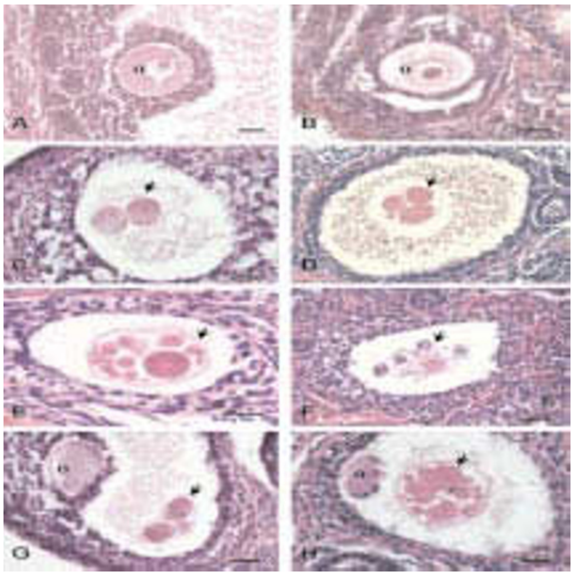

Figure 2. Induction of abnormal oocyte division by constant light. Short arrows, divided–oocytes; O, oocyte. Bar=25 μm.

4. Discussion

Photoperiod was known to exert a strong influence on reproductive processes in the female rat[8]. In this study, constant light resulted in persistent vaginal estrus[17], and the abnormal oocyte division in the LL group.

It was widely accepted the photoperiod played a major role in controlling the circadian rhythms[1], and circadian rhythms might affect cell division in many organisms [18]. In DOFs the divided–oocyte sustained the ability of continued division, and didn't show the typical characteristics of cell death[19]. On the contrary, the granulosa cells were eliminated firstly. It was obvious that the roles of constant light were different between divided–oocyte and granulosa cells, but the reasons need to be further study.

Furthermore, these divided–oocytes were of different cell cycles and volumes. It was suggested that the cell division of divided–oocyte were uncontrolled. Although there was no evidence revealed that the divided–oocyte would become tumor or cancer cells, the potential possibilities were existed in case of adequate nutrition supply.

Conflict of interest statement

We declare that we have no conflict of interest.

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