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RESEARCH ARTICLE

Pattern of Estrous Cycle and Ovarian Antiperoxidative Activity in Light Deprived Sprague-Dawley Rats Treated with Sodium Selenate

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Abstract

Studies have enumerated the adverse reproductive outcomes of decremented lighting period in humans and animals. The study aims at determining the effect of selenium supplementations on estrous cycle and ovarian antiperoxidative activity in light deprived rats. 36 cyclical female rats weighing 120-150g were randomly divided into vehicle-treated (VEH), high selenium dose (HSE), low selenium dose (LSE), light deprived (LD), LD+HSE and LD+LSE groups. Rats in photoperiod- control chambers were exposed to 6hr light/ 18 hour dark cycle for two weeks. Selenium supplementations also spanned for two weeks. The result of this study indicates that light deprivation caused a decrease in % estrus phase and estrous cycle ratio and also led to an increase in ovarian glutathione peroxidase when respectively compared with VEH. Selenium administration at high and low doses resulted in higher ovarian glutathione peroxidase. When compared with VEH, LD+HSE and LD+LSE increased ovarian glutathione peroxidase more than LD. LD+HSE also elevated % estrus phase with respect to VEH. We conclude that selenium supplementations improved estrous cycle in light deprived rats dose-dependently and augmented light deprivation induced increase in ovarian antiperoxidative activity in female Sprague dawley rats.

Keywords: Glutathione Peroxidase; Selenium; Photoperiod; Estrous Cycle; Supplementations

Introduction

Seasonal Affective Disorder and other conditions associated with profound shortage of lighting period demonstrate the importance of light/dark cycle for physiological functions [1]. Light, an abiotic factor, plays a major role in the synchronization of natural cycle with biological rhythms, most especially hormonal cycles [2]. In mammals, melatonin rhythm exerts a regulatory influence on physiological activities such as wakefulness, mood and reproduction [2,3].

Studies have established the relationship between seasonal changes in light length and reproductive function. For instance, A study conducted by observed an increased irregular an ovulatory cycles and decreased libido in premenopausal women during winter compared to summer [4]. In animal studies, female hamster rats raised in short day achieved puberty at later age [5]. Short day photoperiod markedly inhibited vaginal patency and reduced ovarian and uterine weights in female deer mice [6].

Human exposure to short lighting period is both natural and human-made. An example of the latter is sensory deprivation. Sensory deprivation techniques such as blindfold restricts and prevents stimulus such as sound and light from entering sense organs [7]. This no doubt may impair reproductive functions.

Another important factor for reproduction is nutrition. Selenium, a micronutrient, is a cofactor for glutathione peroxidase (GPX). GPX, an antiperoxidative enzyme, converts hydrogen peroxide to water [8-10]. Increase in selenium supplementation is widely known to increase the expression of the enzyme thus resulting in increase in antiperoxidative activity and decreased concentration of hydrogen peroxide free radical [9].

On female reproduction, selenium contributed to progesterone production of corpus luteum and maintenance of the function of the corpus luteum and placenta in the latter period of pregnancy [11]. In a study conducted by, patients with unexplained infertility had significantly decreased follicular selenium concentrations [12]. Selenium ameliorated carbendazim induced histological changes in rats' ovaries [13]. In female wistar rats exposed to doxorubicin, nanoselenium improved plasma progesterone [14]. The study is designed to investigate the effect of selenium supplementation on estrous cycle and ovarian glutathione peroxide in light deprived rats.

Materials and Methods

Reagents and Supplies

Sodium selenite (manufactured by Sigma Chemical Co. USA) were obtained from Zayo Sigma Jos. Light Microscope, digital lux meter, glass slides, mercury-in-glass thermometer, weighing balance, dissecting set, needles, syringes and chemicals were obtained commercially.

Animal Care and Management

Thirty six cyclical adult female Sprague-dawley with estrous cycle length of 4-5 days and weighing 120-150g were used for the research work. The diestrus rats were divided into six groups consisting of six animals each. All rats were housed in plastic cages (0.27m x 0.37m) with stainless steel mesh cover.

The rats were kept in six different cages with a wire mesh covering .They were fed pelletized grower's mash adlibitum and provided water through drinking trough. Rats were raised under photoperiod controlled condition and 12hour light/dark cycle respectively.

Photoperiod-control chamber was a well-ventilated room (1.44 m^2) padded with light-proof fabric. The intensity of light in the base of the chamber was measured using digital lux meter.

Ethical Certification

The study was conducted in line with the guidelines of National Institute of Health (NIH) for the use of laboratory rats.

Experimental Procedure

The rats were weighed and randomly grouped into;

Group A: received distilled water in natural 12hour light /12 hour dark cycle for two weeks and was designated as Vehicle-treated group (VEH).

Group B: received 150 µg/kg of selenium supplementation for two weeks in 12hour light/ 12 hour dark cycle and was designated High selenium group (HSe).

Group C: received 100 µg/kg of selenium supplementation in 12hour light/12 hours dark cycle for two weeks and was designated as Low selenium group (LSe).

Group D: were raised in 6hour light/18hour light/dark cycle and was designated as light-deprived group (LD).

Group E: received 150µg/kg of selenium supplementation for two weeks in 6hour light/18 hour dark cycle and was designated as Light deprived and High selenium-treated (LD+ HSe) group.

Group F: received 100 µg/kg of selenium supplementation for two weeks in 8hour light/ 16 hour dark cycle and was designated as Light deprived and Low selenium-treated (LD+ LSe) group.

The experiment lasted for two weeks.

Group	Pattern of light/dark cycle	Light Intensity
VEH	Natural 12hour light/12hour dark cycle	3-10 lux
HSE	Natural 12hour light/12hour dark cycle	3-10 lux
LSE	Natural 12hour light/12hour dark cycle	3-10 lux
LD	6hour light/18hour dark cycle	125 lux
LD+HSE	6hour light/18hour dark cycle	125 lux
LD+LSE	6hour light/18hour dark cycle	125 lux

Table 1: Light/dark regimen

Determination of Estrous Cycle

Phases of estrous cycle were assessed using the method of [15]. 10microliter of distilled water was introduced into the vagina to produce vaginal lavage. With the aid of light microscope (x40 objective lens), epithelial cells, cornified cells and leucocytes were identified.

Diestrus phase: was predominantly characterized by leucocytes with little or no cornified cells.

Proestrus phase: was predominantly characterized by epithelial cells with little or no leucocytes

Estrus phase: was characterized by cornified cells

Metestrus phase: was characterized by leucocytes, cornified cells and few epithelial cells.

Rats cycle from diestrus (D) to proestrus (P) to estrus (E) to metestrus (M). The cycle length was then determined.

The total number of each phase in two weeks was determined and the percentage of each phase was calculated.

Estrous Cycle Ratio (ECR) was calculated as

$$\frac{\text{Percentage of proestrus + Percentage of estrus}}{\text{Percentage of metestrus + Percentage of diestrus}}$$

Determination of Ovarian Glutathione Peroxidase

Glutathione peroxidase (GPx) activity was measured using the procedure of [16].

Supernatant obtained after centrifuging 5% ovary homogenate at 1500×g during 10 min followed by 10000×g for 30 min at 4 °C was used for GPx assay.

1ml of reaction mixture was prepared which contained 0.3 ml of phosphate buffer (0.1M, pH7.4), 0.2ml of GSH (2mM), 0.1 ml of sodium azide (10mM), 0.1ml of H2O2 (1mM) and 0.3ml of tissue supernatant.

Result

Group	Estrous Phases			
	Diestrus (%)	Proestrus(%)	Estrus(%)	Metestrus(%)
VEH	29±0.25	[a]25.04±1.24	17.0±1.75	29±0.25
HSE	*35±2.5	10±2.5	[a]25±0	30±5
LSE	*45±7.5	15±2.5	[a]20±2.5	20±5
LD	44±2.012	22.66±0.67	*6.66±1.67	25.0±6.45
LD+HSE	58±2.22	11.66±3.33	*a27.21±0.55	*0±0
LD+LSE	46.67±11.19	22.22±5.56	[a]15.56±4..46	15.54±3.89

*Significant difference(P<0.05) from VEH. [a]significant difference from LD

Table 2: The effect of light deprivation and selenium supplementations on estrous phase

Compared with VEH, there was a significant decrease in the percentage of estrus phase in LD group but LD +HSE group showed significantly high percentage of estrus. Compared with LD group, HSE, LSE, LD+HSE and LD+LSE exhibited a significantly higher estrus percentage.

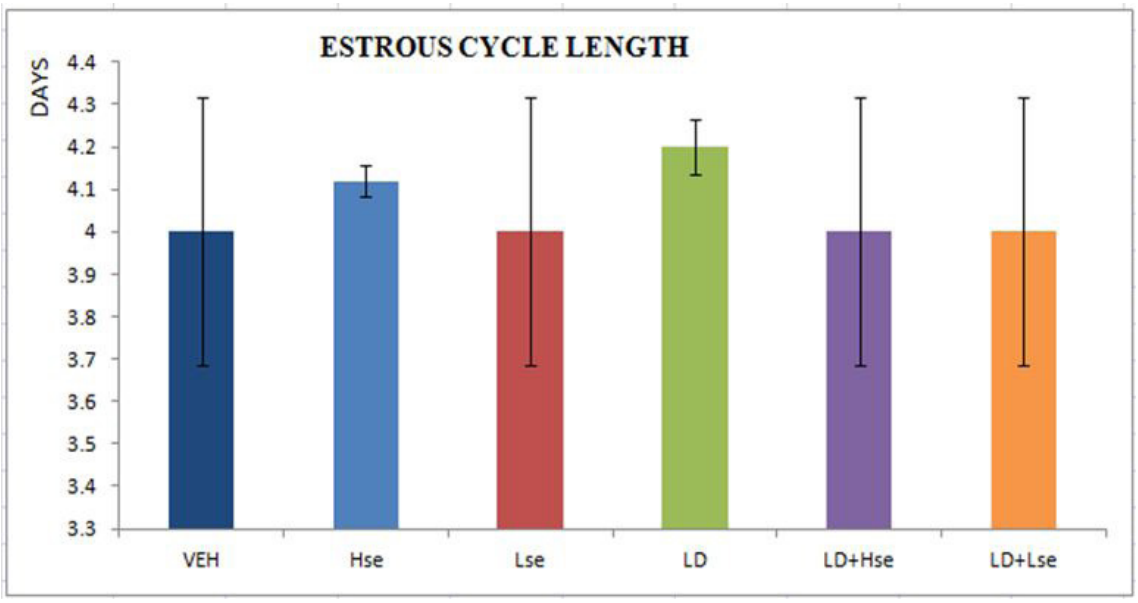
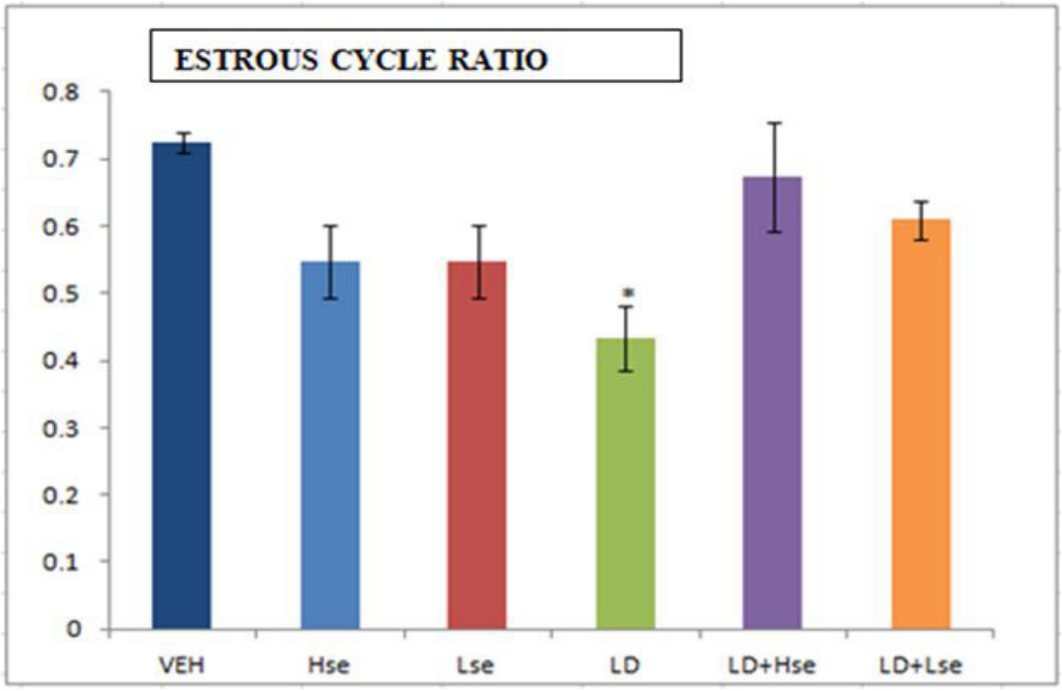
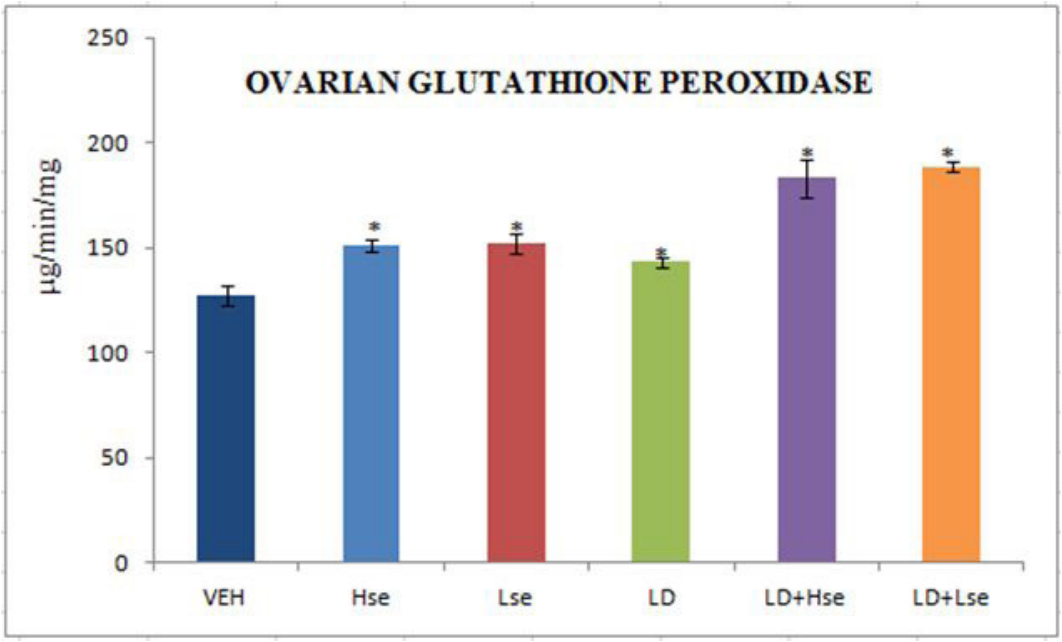


Figure 1: The effect of light deprivation and selenium supplementations on estrous cycle length
Light deprivation and selenium supplementations have no significant effects on estrous cycle length



*Significant difference (P<0.05) from VEH

Figure 2: The effect of light deprivation and selenium supplementations on estrous cycle ratio



*Significant difference (P<0.05) from VEH

Figure 3: The effect of light deprivation and selenium supplementations on ovarian glutathione peroxidase

There was a significant increase in ovarian glutathione peroxidase in HSE, LSE, and LD, LD + HSE and LD + LSE groups.

Discussion

At puberty, female reproduction exhibits a cyclical pattern indicating periodic preparation for fertilization and implantation [17]. Thus, the female reproductive cycles of humans and rodents are susceptible to alteration in environment-borne factors. Such factors may include chemicals and light. The present study investigated the effect of selenium supplementation and light deprivation on estrous cycle and ovarian antiperoxidative activity in sprague dawly rats.

We observed that there was a decrease in percentage of estrus phase in light deprived rats. In rodents, ovulation occurs during estrus phase. Therefore, the decrease in estrus phase in light deprived rats might indicate a blight chance of ovulation. Reports from photoperiod studies show that short day photoperiod markedly reduced ovarian weight, and uterine weight in female deer mice [6]. In male rats, exposure to 6hr light/18hr dark cycle resulted in decreased testicular weight, sperm motility, sperm viability and sperm counts resulted from exposure of adult male Sprague dawley to 6 hr light/ 18 hr dark cycle [18].

Administration of high selenium dose to light deprived rats showed a high percentage of estrus phase. The increase in the percentage of estrus in this group might be due to increased steroidogenic activity of ovaries most especially increased estrogen production. Increased synthesis of estrogen is widely known to increase vaginal cornification and bring about LH surge. In a similar vein, claimed that sodium selenite supplementation increased FSH and estradiol levels in rats exposed to lead [19].

The short length of rodents' estrous makes them suitable as an experimental model. We realized that although, light deprived had a longer estrous cycle but it was insignificant. Similarly, selenium treatments did not affect estrous cycle length.

The status of estrous cycle is often assessed using Diestrus Index (duration of diestrus/duration of estrous cycle) x 100 [20]. In this study, we attempted to include proestrus and estrus in the assessment of estrous cycle status. Therefore, we propose Estrous Cycle Ratio (ECR), a proportion of proestrus and estrus divided by the proportion of metestrus and diestrus. We observed that the ECR of light deprived group is lower when compared with vehicle-treated group. As far as this work is concerned, light deprived group showed the lowest ECR (below 0.5). ECR typifies the ratio of time taken for corpus luteum to develop and time taken for the commencement of development of new corpus luteum. We theorize that the normal ECR is approximately 1. Higher value may imply longer duration of corpus luteum development. Lower value implies delay in commencement of new corpus luteum development.

Many studies have documented the impact of antiperoxidative enzymes most especially glutathione peroxidase on physiological functions [8,9]. The increase in the activities of this enzyme all selenium treated groups is because selenium is the co-factor for glutathione peroxidase. Therefore, increase in the concentration improved the expression of the enzyme.

We also observed that light deprived group have a high glutathione expression but lower than all selenium treated groups. The increase in the enzyme in light deprived group is not surprising. The role of short day photoperiod as a protective and free radical scavenging condition has been documented [21-23]. For instance, reported that short day photoperiod improve cell-mediated immunity [24-26]. As far as this study is concerned combination of light deprivation and selenium supplementations resulted in higher level of glutathione peroxidase.

In conclusion, the findings of the study indicate that selenium supplementation improved estrous cycle in light deprived rats dose-dependently and augmented light deprivation induced increase in ovarian antiperoxidative activity.

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