Oral Presentation (PCS-14)

Potential Reproductive Adaptation of Mice (*Mus musculus*) from Mild Stress using Dark Light Cycle Alteration

D.N. Pristihadi[1], M. Fakhrudin[1], N. M. D. Haq[1], A. Boediono[1*]

[1]Department of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University (IPB) – INDONESIA
* Corresponding author: ab@apps.ipb.ac.id

Keywords: mice, stress, estrus, follicle, ovary.

INTRODUCTION

Stress regarded as a major cause of body destruction. Stress can trigger the gluconeogenesis mechanism that initiates new glucose production from another molecule in the body's storage tissue depot. When the stress occurs, the body gives a 'fight or flight' response. In this condition, the body prioritizes to survive rather than multiplying itself. The reproductive system categorized as the highest rank in the body needs pyramid. Therefore, the reproductive system is on the first line to be sacrificed when the stress occurs.

This research was conducted to observe the potential mice's reproductive adaptation from mild stress using dark light cycle alteration. Mice considered as the small laboratory animal with rapid metabolism rate. This study expected to be able to enrich the information of mice's biology reproductive adaptation.

MATERIALS AND METHODS

This research was conducted using adult female mice DDY albino (6-8 wk). As many as 44 mice were divided into four treatment groups: normal group (without alteration of the dark light cycle/ ADL) and treatments group with ADL for 1, 3, and 7 days. Mice were adapted for a week with normal radiation before treatments. The room illuminated with 12/12 h dark light cycle equal with the solar radiation (light on 06:00-18:00). The lighting source was a LED lamp with ± 135 lux intensity. During the treatment period, the ADL groups illuminated reversed with solar radiation.

Index stress value, estrus cycle length, and dominant follicle formed by stimulation program were analyzed. Submandibular blood collection performed in the evening of the last treatment day. The whole blood sample (± 200 µL volume) tested in the Diagnostic Clinic Laboratory of Faculty Veterinary Medicine, IPB. Index stress value obtained from the ratio of measured neutrophil and lymphocyte.

Estrus cycle observed for seven days after the last treatment day. The vaginal swab performed four times a day (at 06:00 am, 12:00 am, 06:00 pm, and 12:00 pm). The collected samples stained with Giemsa 10% and observed under a light microscope (Olympus CH20) at 100 x magnification.

Stimulation program was conducted using the combination of pregnant mares serum gonadotropin hormone (PMSG; Folligon®, Intervet Netherlands) and human chorionic gonadotropin (hCG; Chorulon®, Intervet Netherlands). PMSG at a dose of 5 IU and hCG 5 IU given intraperitoneally by 47 h interval. Ovaries collected 16 h after hCG injection. Ovarian histology was carried out according to Hamny et al.[1] with minor modifications. Graafian follicle and corpus luteum counted under a light microscope with 40 x magnification.

RESULTS AND DISCUSSION

Alteration of the dark light cycle found to induce mild stress in the mice. This study showed that the ADL treatment for a day can increases 25 % of the index stress value. Index stress value found to be better after three and seven days of ADL treatments. After three days of ADL treatment, the index stress measured was 0.0-4.6 % above the normal value. The result presented in Figure 1.

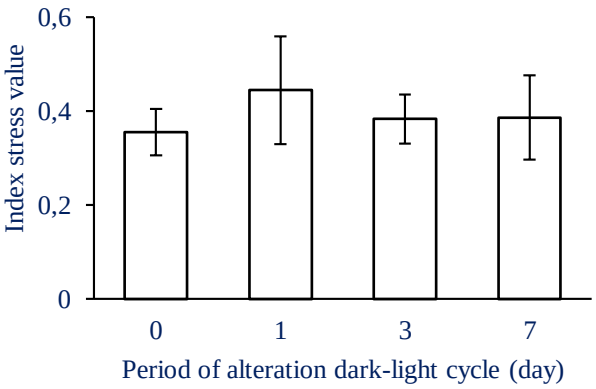

Figure 1 Index stress value of mice with ADL treatments

Alteration of the dark light cycle is a usual stimulus to produce an animal depression model with mild stress[2]. Our result showed that

approximately three days of ADL treatment is sufficient for mice to restore the stress nearly into the normal value. This ability considered fast to happen. Human reported need at least ten days to normalize the stress value after ADL treatment[3].

In this study, ADL statistically insignificant to increase stress in mice. Even with this conditions, ADL causes a significant effect on the reproductive performance. ADL found to be able to decrease the estrous cycle length. The result showed in Figure 2.

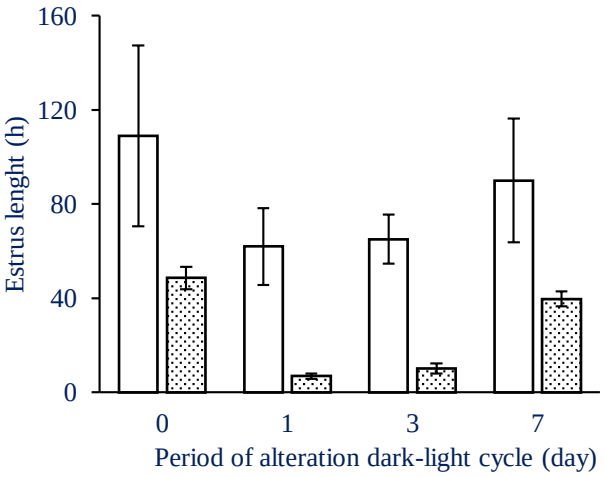

Figure 2 Estrus cycle lenghth (□) and estrus phase lenght (▩) of mice with ADL treatments

Acceleration of the estrus cycle period by ADL treatments was also reported by Yoshinaka *et al.*[4]. Several previous studies reported that the acceleration of mice's estrus cycle after ADL treatments caused by the declining of proestrus phase. Light exposure in the night time known to increase Kisspeptin, a trigger molecule to induce luteinizing hormone (LH) surge secretion and early ovulation[5]. Interestingly, further analysis of this study showed that ADL treatments shortened the estrus phase rather than declining the proestrus phase.

The profile of the estrus cycle and the length of the estrus phase were found to have characteristics inversely proportional to the incidence of stress levels. The peak of stress level was the anticlimactic point of the cycle length and the estrus phase. Pearson correlation value of stress on estrus cycle length and estrus phase was -0.773 and -0.751. This result indicated that stress as the main cause of the reduction of the estrous cycle and estrus phase length.

The ovary is a glucocorticoid organ that responds to stress. Occupation of cortisol in the ovarian glucocorticoid receptors reported being able to trigger granulosa cell's differentiation into lutein cells. Channing *et al.*[6] demonstrated that supplementation of 0.1 µg/mL cortisol with LH, follicle stimulating hormone, and insulin at the same concentration in the porcine granulose culture cell generate this cells to differentiate 4-14 times faster into lutein cells than treatment without cortisol. Another report showed that addition of cortisol in the rat post-ovulatory granulosa cells significantly increases the measured progesterone[7].

This study showed that mice have a fast potency to recover from the mild stress of ADL treatments. On the 3rd day of ADL treatments, mice's estrus cycle observed nearly normal (± 81.69 % of normal estrus cycles length).

The extraordinary recovery potential also discovered on the ovarian dynamics. A day of ADL treatment decreases the total dominant follicle observed. The total dominant follicles observed back to normal after three days of ADL treatments. The results presented in Figure 3.

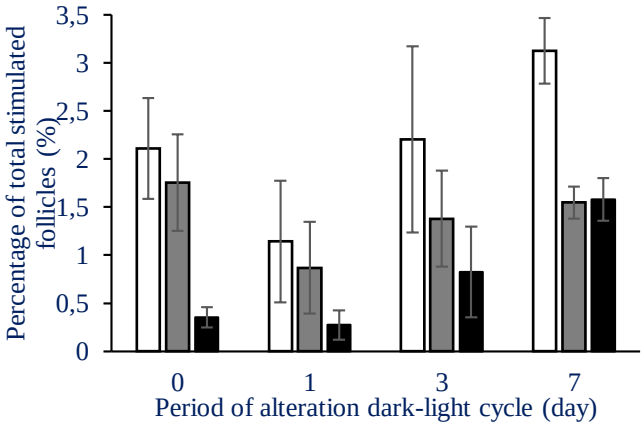

Figure 3 Total dominant follicle (□), corpus luteum (▨), dan Graafian follicel (■) of mice with ADL treatments

Pearson correlation between index stress value and total dominant follicle was -0.631. Stress suspected to able to increase Rfamide-related peptides (RFRPs), an inhibitor of gonadotropin neuron to release gonadotropin-releasing hormone[8]. Stress also suspected to degrade the FSH receptor expressions in the ovarian granulosa cells[9].

ADL treatments found insignificantly change the total corpus luteum formed after stimulation program (Figure 3) and total obtained oocytes (data not shown). Interestingly, ADL treatments for seven days increased the total dominant follicle and total anovulatory Graafian follicle which totally equal with corpus luteum.

CONCLUSION

Mild stress in mice can be emerged by alteration of the dark light cycle for a day. This mild stress affects the reproductive system by decreasing the estrus cycle length, shortened the estrus phase time, and decrease the dominant follicles formed after the stimulation program. Mice found to be able to recover the reproductive

defect after three days of ADL treatments.

ACKNOWLEDGMENT

This research supported by the Ministry of Research, Technology, and Higher Education of Republic Indonesia by *Program Pendidikan Magister Menuju Doktor untuk Sarjana Unggul* (PMDSU) program according to Contract Number 1073/IT3.11/LT/2017.

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