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Administration of Leptin Increases the Level of Follicle Stimulating Hormone (FSH) and Development of Ovarian Follicles in Postpartum Anestrus of Bali Cattle

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Background: Leptin is a metabolic signal that plays a role in reproduction through the Hypothalamic-Pituitary-Ovarian axis. The aim of this study is to determine the effect of leptin on the levels of FSH and development of ovarian follicles of post-partum anestrus in Bali cattle. **Method:** Postpartum anestrus in Bali cattle were divided into three groups of nine; group 1 (control) was injected with physiological saline, group 2 (treatment I) and group 3 (treatment II) were injected with recombinant o-leptin 100 µg and 200 µg/head respectively. Injection was done twice with an interval of 12 hours. Blood sample was collected via the jugular vein at intervals of 12 hours before the start of treatment until signs of estrus had appeared. Follicle diameter was measured using ultrasonography before treatment and during estrus. **Results:** The average levels of FSH before treatment for group 1, group 2, and group 3 were 11.3 + 0.36 mIU/ml, 11.6 + 0.40 mIU/ml, and 11.4 + 0.28 mIU/ml respectively. While at the end of the research, the levels of FSH were 11:02 + 0.39 mIU/ml, 13.0 + 0.48 mIU/ml, and 13.47 + 1.05 mIU/ml respectively. Ovarian follicle diameter for group 1, group 2, and group 3 before treatment were 5.10 + 0.41 mm, 5.21 + 0.36 mm, and 5.00 + 0.37 mm respectively. Whereas at the end of the research, the ovarian follicle diameters were 4.92 + 0.40 mm, 8.37 + 0.43 mm, and 8.45 + 0.38 mm respectively. **Conclusion:** administration of o-leptin in postpartum anestrus of Bali cattle increases the level of FSH and ovarian follicle development.

Keywords: leptin, FSH level, ovarian follicle, postpartum anestrus

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INTRODUCTION

Problems often encountered in raising Bali cattle. In addition to the poor quality of calves[1], they were also caused by less optimal reproductive function. The disorder of reproductive function of cattle will have great impact on the calving rate. This low calving rate of Bali cattle, among others, is due to the high incidence of postpartum anestrus, which were averages of 4.11 months.[2, 3]

Postpartum anestrus is a condition that occurs in cattle where the signs of estrus and ovulation did not appear two months or more after parturient.[4] Postpartum anestrus is related to prenatal nutrition (pre-partum) which caused a decline in the body condition score (BCS) at calving. The low body condition score during calving may prolong the emergence of postpartum estrus.[5]

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Postpartum estrus is important to get one calf every year with the calving interval of 365 days. To achieve this, cattle should be mated no later than 83 days after calving by assuming the cattle gestation period of 276 to 295 days.[6, 7]

The mechanism of postpartum anestrus takes place in the axis of the hypothalamic-pituitary-ovarian, and the interaction of this axis with the center of other central nervous systems that involved in lactation and metabolism. This response involves the metabolic signals that regulates energy and reproductive axis. One of the metabolic signals is leptin. Leptin is required at certain levels to make the hypothalamus sensitive to leptin before the GnRH neuron is activated.[8]

Leptin is a hormone that is secreted by adipose tissue with molecular weight of 16 kDa with 146 amino acids. Leptin also acts directly on reproductive organs and through paracrine effects of leptin, which will regulate the synthesis of estrogen.[9] In the central nervous system, the hypothalamus is the primary target of leptin. Leptin enters through the blood-brain barrier and binds to

the GnRH neurons to stimulate the release of GnRH. GnRH stimulates the production of FSH and LH in the anterior pituitary. Leptin also acts directly on the anterior pituitary to stimulate the release of FSH and LH.[8, 9] Leptin has a local effect on the ovaries by stimulating steroidogenesis through leptin receptors in granulosa cells.[8]

MATERIALS AND METHODS

The research is a true experimental design with Randomized Pre Post Group Control Design. Postpartum anestrus Bali cattle were divided into three groups of nine, group 1 (control) was injected with physiological saline, group 2 (treatment I), injected with recombinant o-leptin 100 µg/head, and group 3 (treatment II) injected with recombinant o-leptin 200 µg/head. Injection was done twice with an interval of 12 hours. Blood sample that used for checking the levels of FSH was collected via the jugular vein at intervals of 12 hours, before the start of treatment until the signs of estrus appeared. Follicle diameter measurements were performed before the treatment and at the appearance of estrus. Blood was collected in tubes without anticoagulant for serum. The level of FSH hormone was observed by using Double Antibody Sandwich ELISA method. Ovarian follicular development was observed by using ultrasonography (USG) specifically for large animals. Signs of estrus were observed from the presence of anxiety, vulva swelling and redness covered by transparent mucus, as well as the accumulated mucous fluid in the vagina. Follicles observed more than 5 mm in diameter. A multivariate test was conducted to test differences in FSH hormone levels in all treatment groups, but if the data were not normally distributed, Kruskal-Wallis with advanced test of Mann-Whitney Test will be used. Meanwhile, Paired Sample T-test was used to examine the diameter differences of the ovarian follicles.

RESULTS

Bali Cattle FSH Levels after Administration of Leptin

The mean of FSH hormone levels in the control group, the treatment I, and the treatment II on the observations of 0, 12, 24, 36, 48 and 60 hours after administration of leptin are shown in Table 1.

Treatment by leptin administration showed significant differences ($P < 0.05$) between the control and the treatment groups on 12, 24, 36, 48, and 60 hours after administration of leptin. While between treatment I and II, significant differences ($P < 0.05$) between treatment I and II occurred after 24, 36, 48, and 60 hours. Meanwhile, the increase in FSH levels occurred significantly ($P < 0.05$) 48 hours after administration of leptin for the treatment I and the observation of the 24, 36, 48 hours after administration of leptin for treatment II. However, after 60 hours there was a decrease of FSH levels both in treatment I and II.

Table 1. Mean ± SD levels of Postpartum Anestrus Bali Cattle FSH Hormones

Observation (Amount of Hours After Treatment)	Group		
	Control	Treatment I	Treatment II
0	11.3 ± 0.36^{aA}	11.6 ± 0.40^{aA}	11.4 ± 0.28^{aA}
12	11.2 ± 0.36^{aA}	12.3 ± 0.39^{bB}	12.4 ± 0.35^{bB}
24	11.3 ± 0.33^{aA}	13.0 ± 0.40^{bC}	13.4 ± 0.39^{cC}
36	11.3 ± 0.36^{aA}	13.5 ± 0.42^{bC}	14.1 ± 0.43^{cC}
48	11.3 ± 0.37^{aA}	13.70 ± 0.53^{bD}	13.47 ± 1.05^{cC}
60	11.2 ± 0.39^{aA}	13.0 ± 0.48^{bC}	12.2 ± 1.22^{cB}

Notes:
- Different letters (uppercase) towards rows showed significant differences ($P<0.05$) between observations.
- Different letters (lowercase) towards the column showed significant differences ($P<0.05$) between treatments.

The Development of Ovarian Follicles in Postpartum Anestrus Bali Cattle after the Administration of Leptin

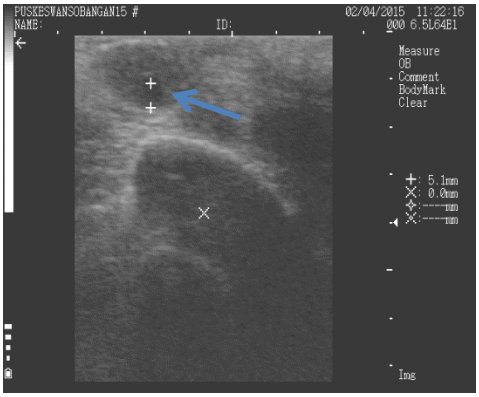
The average diameter of the ovarian follicles before and after the administration of leptin were measured using ultrasonography, as shown in Table 2.

Table 2. Mean ± SD of Ovarian Follicles Diameter (mm) of Bali Cattle after the Administration of Leptin

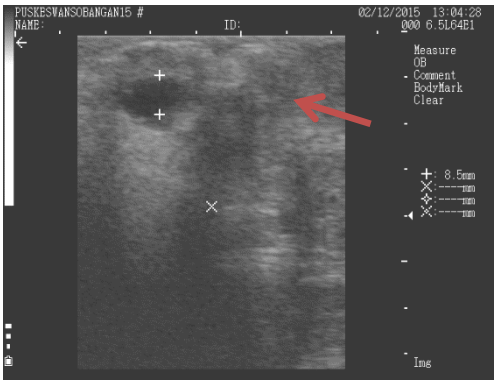
Group	Condition	
	Start	End
Control	5.10 ± 0.41^{aA}	4.92 ± 0.40^{aA}
Treatment I	5.21 ± 0.36^{aA}	8.37 ± 0.43^{bB}
Treatment II	5.00 ± 0.37^{aA}	8.45 ± 0.38^{bB}

Notes:
- Different letters (uppercase) towards rows showed significant differences ($P < 0.05$) between observations.
- Different letters (lowercase) towards the column showed significant differences ($P < 0.05$) between treatments.

The average diameter of ovarian follicles before and after administration of leptin were significantly different ($P < 0.05$) both in the treatment I and treatment II, while there were no significantly different ($P > 0.05$) in the control group. Between control group and treatment group there were significantly different ($p < 0.05$) in the average of ovarian follicles diameter.



Group A



Group B

Legend:

= Ovarian Follicles Bali Cattle on Non Estrus Group

= Ovarian Follicles Bali Cattle on Estrus Group

Figure 1. Ovarian Follicles of Bali Cattle on Non-Estrus (Group A) and Estrus (Group B)

DISCUSSION

Bali Cattle FSH Levels after the Administration of Leptin

The FSH levels of postpartum anestrus Bali cattle was 10.94 - 12 mIU/ml initially. Administration of 100 µg/head leptin caused FSH levels to rise gradually up to 13.70 ng/ml on the 48-hour observation, but at the time of estrus, they began to drop down to 13 ng/ml (on the 60-hour observation). Meanwhile the administration of 200 µg/ml leptin caused FSH levels to rise gradually until they achieve the highest levels of 14.1 ng/ml on the 36-hour observation. However, on the 48-hour observation (at the time of estrus), the FSH levels began to drop down to 13.47 ng/ml.

After the peak concentration of FSH was reached, then it will decrease gradually for several days, but the growth of the follicles will still occur, with observed growth of 4.0 to 8.5 mm. Increase of the concentration of FSH leads to one of the selected follicles to grow into the dominant follicle. This dominant follicle will be maintained by FSH in the basal concentration.[10]

This dominant follicle will suppress the FSH secretion and the emergence of new follicles wave due to the production of inhibin and estradiol from the follicles. Productions of inhibin, activin and follistatin are only taking place in the ovary, which is an endocrine factor to regulate the pituitary in FSH secretion.[11]

Leptin and FSH have a synergistic effect in stimulating the activity of aromatase in human granulosa cells of pre-ovulatory follicles. Leptin increases mRNA P450arom, P450arom protein expression, and aromatase activity by directly working on granulosa cells. Leptin also further increases the production of estrogen stimulated by FSH and/or IGF-1, which is a stimulator of aromatase.[12] FSH receptors are on the granulosa cells from the small, medium and dominant follicles. FSH induces the aromatase enzyme activity of the follicle, resulting in the conversion of androgens to estrogens. But the granulosa cells of small follicles are more sensitive to FSH so that the cAMP response to FSH was higher than the granulosa cells of large follicles.[13]

The Development of Ovarian Follicles of Bali Cattle after the Administration of Leptin

The average diameter size of ovarian follicles of postpartum anestrus Bali cattle observed in this study were 4.52 to 5.57 mm. Administration of leptin 100 µg/head and 200 µg/head, increases the average size of the ovarian follicle diameter was 8.37 mm and 8.45 mm, respectively.

Administration of leptin (300ng - 10µg per animal) every day for 21 days in mice showed that an increasing number of ovulation of immature oocytes and reduce the incidence of apoptosis in the follicles.[14] Administration of leptin by in vitro of culture media showed that leptin directly enhances the follicular development by increasing steroidogenesis and insulin in mice.[15]

The process of follicular growth, ovulation, and the formation of CL is strongly influenced by the circulation of reproductive hormones in the body. Hypothalamus produces GnRH that serves to stimulate the release of FSH and LH by the anterior pituitary in response to estrogen. FSH and LH respectively binding to specific receptors on the surface of granulosa and theca cells. Activation of follicle stimulating hormone receptor (FSHR) and LH stimulates mitosis and differentiation of granulosa and theca cells.[16]

After parturient, there is an increase of FSH followed by the emergence of the first follicular wave (3-4 follicles with a diameter of 4-6 mm) within 10-14 days of postpartum.[17] Each wave progresses through different stages of development, namely the emergence, selection, dominance and atresia or ovulation.[5, 16] The wave of follicular

growth occurs not only during the estrus cycle, but also had occurred before puberty, during pregnancy and during the postpartum period.[16]

Factors affecting the dominant follicle of the postpartum are strongly related to the metabolic status of animals, for example the prenatal feed or nutrition, the postpartum energy balance, as well as the parity.[18, 19] Dominant follicles in cattle occur after the selected follicles reach a size of 8 to 8.5 mm and produce high concentrations of estrogen and inhibin which cause a decrease in the concentration of FSH. In cattle species of *Bos Taurus*, the dominant follicle occurs when the follicle reached a diameter of 8 mm and 7 mm on the type of *Bos Indicus* cattle.[20] While ovulation occurs when the follicle reaches a maximum diameter of 10.9 mm in two waves of period and 11.0 mm in three waves of period.[21]

CONCLUSION

Based on the results of the study, it can be concluded that leptin increased the levels of the FSH hormone and aided in the development of ovarian follicle of the postpartum anestrus Bali cattle. There was a positive correlation between the size of follicle and the level of FSH during the treatment with leptin. This result suggested that leptin at the dose of 100 or 200 µg/head could be used to treat postpartum anestrus in Bali cattle.

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