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Administration of Leptin Increased Concentration of Estrogen and Accelerate the Emergence of Estrus in Post Partum Anestrus of Bali Cattle

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Abstract: Leptin is a hormone synthesized by adipose tissue with a molecular weight of 16 kDa with 146 amino acids that plays a pivotal role in the communication of nutritional status to centers that control reproduction. This study aimed to test the hypothesis that administration of exogenous leptin increased concentration of estrogen and accelerate the emergence of estrus in postpartum anestrus Bali cattle. Twenty seven postpartum anestrus Bali cattle were equally divided into three groups. Control (n = 9), received s.c injections of saline. Treatment I (n = 9) treated with 100 µg/head of recombinant leptin via s.c injection. Treatment II(n = 9) received s.c injection of recombinant leptin 200 µg/head. Exogenous leptinwas administered twice at 12 hours interval. Blood samples were collected via the jugular vein every 12 hours before the start of treatment until the appearance signs of estrus. The results showed that the average concentration of estrogen before treatment for control, treatment1, treatment2 were 54.4 ± 2.3 ng / L; 53.1 ± 2.74 ng / L; 55.8 ± 0.47 ng / L, respectively. After treatment, concentrations of estrogen were 54.1 ± 2.26 ng / L; 80.7 ± 0.58 ng / L and 80.8 ± 1.62 ng / L respectively. The timing of the estrus after treatment 1 and 2 were 57.33 ± 5.29 and 49.33 ± 4.00 hours respectively. While control showed no signs of the emergence of estrus until the end of the study. In conclusion, the administration of exogenous recombinant leptin increased concentration of estrogen and accelerated the emergence of estrus in postpartum anestrus.

Key words: Estrus · Leptin · Concentration of Estrogen · Postpartum Anestrus

INTRODUCTION

Bali cattle are local Indonesian cattle which has meat quality as good as import cattle. Bali cattle has several advantages such as, resistance to unfavorable environment, adaptable to a new environment, high carcass percentage with low fat content, good and efficient as work animals [1]. However, the disadvantage of Bali cattle is having a long calving interval due to the emergence of estrous more than 3 months after delivery [2]. Postpartum anestrus in cattle has been identified as the main causes of low efficiency of reproduction [3]. Postpartum periode is defined as the time between parturition and completion of uterine involution. The postpartum period is a very important stage of the reproduction cycle. Postpartum period can be influenced by prepartum management especially the nutritional management. Peripartumperiod should be taken a good care to improve the fertilization and avoid further economic looses [4].

The main factors affecting the length of calving interval is the nutrients before or after delivery [5]. Nutrient limitation decrease circulating leptin and is associated with decreased secretion of FSH and LH [6]. Energybalanceaffects the lengthofpostpartumanestrous. Lowintakeoffoodeitherbefore and after calving could increase the interval from calvingtonextestrus [7]. The lower of Body Condition Score (BCS) can be related with the increase of anestrus rate, estrus cycle and conception rate. BCS can also be used as tools to manage and select dairy catle in order to improve reproduction performance [8]. Leptin is a hormone that is related to nutrition,

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metabolism and reproduction [7]. Treatment with exogenous leptin increased concentration of FSH hormone and the development of ovarian follicle in postpartum anestrus Bali cattle [9].

The return of estrous cycles after parturition depends on the function of the hypothalamic pituitary -ovarian axis. Increased frequency of GnRH produced by the hypothalamus is a major factor in the increase in circulating FSH and LH from the anterior pituitary. The increase of FSH and LH will be followed by development of ovarian follicle which synthesize and produce estrogen [10]. Leptin is one of the metabolic signal that regulates the hypothalamus-pituitary-ovarian axis. Leptin acts directly on the reproductive organs and through paracrine effects and also regulates synthesis of estrogen [11].

The aim of this study was to investigate the effect of exogenous leptin in inducing estrus via increasing estrogen production.

MATERIALS AND METHODS

Animals and Treatments: This study was a true experimental research which used the "Randomized Pre-Post Group Control Design". The study population has the same BCS which is 3 score with the calving interval more than 14 months. Postpartum anestrus Bali cattle and divided 27 cows divided into three equal groups. Control cattle were treated with s.c injections of saline (n = 9). Treatment I cattle treated withs.c injection of recombinant leptin100 µg/head (n = 9) and treatment II cattle treated with s.c injection recombinant leptin 200 µg/head (n = 9).Treatment with exogenous leptin was done twice with an interval of 12 hours.

Housing and Management of Cow: The study were held at Sobangan Village, District Mengwi, Badung, the Bali Cattle Breeding Cntre. These cattle were housing at conventional/stanchion barn, which is a barn with insulator to avoid prevent the cattle move freely. Theese cattle were positioned in two lines and so the cattle can be face to face each other. In every two lines there is a walk side. The nutrient is greens such as king grass (pennisetum pupureum) and concentrate. The cattle feeds 10 kg/cattle/ day and the concentrate were 2 kg/cattle/ days andwater *ad libitum.*

Blood Sampling an Hormone Assaying: Blood samples were collected at several time, the first was collected via the jugular vein before the first injection and next after 12 hours followed by the second injection. Thereafter, blood samples were collected every 12 hours. Blood was collected in tubes without anticoagulant for serum. Concentration of estrogen was detected Direct Elisa, Double Antibody Sandwich.

Detection of Estrous: Observation of estrous emergence was done twice a day at 06.00-09.00 WITA in the morning and 16.00-18.00 WITA in the afternoon with the signs of estrus observed that their anxiety, vulva swelling and redness covered by mucus transparent.

Statisical Analysis: To determine the significant differences between treatment groups were performed by Kruskal-Wallis test and subsequent test with Mann - Whitney Test. As for knowing the significance difference in each treatment group used Friedman test and subsequent test with Wilcoxon test. Meanwhile, Paired Sample T-test was used to examine differences in the appearance of estrus. The data were analyzed using SPSS 17.0 software for windows

RESULTS AND DISCUSSION

Concentration of Estrogen after Leptin Administration: Serum concentration of estrogen did not differ ($p>0.05$) both on control and treatment group 0 hours of observation. Oon the 12, 24, 36, 48, 60 hours of observation, a significant difference ($p<0.05$) among control and treatment group was clear.

Further test by Mann-Whitney test indicated no difference ($p>0.05$) of serum concentration of estrogen among treatment I and II on 12, 24, 36, 48 hours of observation, while significant difference ($p<0.05$) mean concentration of estrogen on 60 hours of observation (Table 1).

Analysis by Friedman test denoted no significant difference ($p>0.05$) of mean estrogen concentration in the control bali cows on 0, 12, 36, 48, 60 hours of observation while in the treatments group it indicated a significant ($p<0.05$) increase of estrogen concentrations on 12, 24, 36, 48, 60 hours of observation.

Further test by Wilcoxon indicated also a significant ($p<0.05$) increase of estrogen concentrations on 12, 24, 36, 48, 60 hours of observation for treatment I. While for treatment II, estrogen concentrations increasedlinearly and significantly ($p<0.05$) from 12 to 48 hours of observation, with no significant difference between 48 and 60 hours after treatment ($p>0.05$).

Table 1: Average + SE concentration of postpartum anestrus Bali cattle estrogen hormone

	Concentration of estrogen (ng/L)		
Observation (hours) after treatment	Control	Treatment I	Treatment II
0	54.4±0.77^{aA}	53.1±0.91^{aA}	55.8±0.15^{aA}
12	54.1±0.75^{aA}	58.2±0.85^{bB}	60.7±0.60^{bB}
24	54.1±0.75^{aA}	66.6±0.74^{bC}	67.8±0.58^{bC}
36	54.0±0.77^{aA}	74.3±0.69^{bD}	75.9±0.64^{bD}
48	54.1±0.76^{aA}	78.5±0.73^{bE}	80.8±0.54^{bE}
60	54.1±0.75^{aA}	80.7±0, 19^{bF}	79.9±0, 27^{cE}

Means with different superscripts within row (a, b, c) and within column (A, B, C, D, E, F) ARE SIGNIFICANTLY DIFFERENT AT p <0.05

Table 2: Average+ SD time of estrus emergence

	Time of estrus emergence (hours)	
Group	Start of research	End of research
Control	Anestrus	Anestrus
Treatment I	Anestrus	Estrus (57.33±5.29^{a})
Treatment II	Anestrus	Estrus (49.33±4.00^{b})

Annotation: Different letter denote significant difference (p<0.05)

Administration of 100 µg /head and 200 µg/head recombinant leptin in Bali cattle of postpartum anestrous could increase concentration of estrogen gradually respectively reach to 80.7 ng/L and 80.8 ng/L. Leptin has a local influence on the ovaries to stimulate steroidogenesis through the leptinreceptors in granulose cells [12]. Ovary Folikel syntesize steroid hormone such as androgen and estrogen which contribute to the development of folikel by the induction of proliferation and differentiation of granulosa cells respectively through androgen and estrogen receptors [13] Supplement ingleptin *in vitro* in culture media enhanced directly the follicular development by increasing steroidogenesis and insulin With no direct influence on oocytes maturation and preimplantation stage of embryo development in mice [14]. On the other hand, administration of leptin in vivo (dose of 30 ug / ml every 3 hours for 15 hours) or in vitro in mice resulted in the number of oocytes that ovulate less but do not affect the level of steroid. Administration of leptin systemically could inhibit ovulation [15].

Estrogen affected the expression and secretion of leptin, leptin receptor expression and biological activity of leptin. Ovariectomized rats will cause a decline in leptin mRNA in adipose tissue and estrogen can stimulate mRNA expression and secretion of leptin in adipose tissue culture [16]. Estrogen is very sensitive to the leptin signal, low concentration of estrogen increased NPY neurons in hypothalamus and lead to central leptin insensitivity. This suggests that estrogen is important for homeostasis and reproduction [17].

Influence Doses of Leptin on the Emergence of Estrus: Emergence of estrus in treatment group I which injected with 100 µg/head twice with an interval 12 hours was 57.33±5.29 hours while in treatment group II which injected with 200 µg/head twice with an interval 12 hours was 49.33±4.00 hours. Differences time of estrus emergence presented in Table 2.

In this study, subcutaneous injection of 100 ug/head and 200 ug/head recombinant leptin led to the emergence of estrus an average of 57.33 ± 5.29 and 49.33 ± 4.00 hours respectively after administration. This indicated that increasing the dosage of administration accelerated the emergence of estrus.

The dosage of leptin is essential for achieving increased concentrations of LH [18]. Administration of exogenous leptin to accelerate puberty in dairy cattle with dose of 19.2 mg/kg twice daily for 40 days, indicated no elevated levels of LH [19]. Administration of recombinant leptin at a dose of 20 ng/ml in dairy cows are not able to influence the secretion of LH [18]. Chronic administration of leptin is not able to increase the frequency of LH causing the failure of the emergence of early puberty [20]. The high doses of leptin causes an accumulation of the number - suppressor of cytokine signaling 3 (SOCS3) in excess which leptin inhibits JAK / STAT signaling that causes leptin resistance [19].

CONCLUSIONS

The conclusion that leptin increased concentration of estrogen and accelerate the emergence of estrus in postpartum anestrus Balicattle. The difference in dose causes estrus emergence time difference where the appearance of estrus occurs at concentrations of estrogen reaches 80.7 to 80.8 ng / L.

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