LEVEL OF ESTRADIOL 17-β SERUM AND OVARIAN FOLLICULARE DYNAMICS IN SHORT ESTROUS CYCLE OF BALI CATTLE

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui kadar estradiol 17-β dan gambaran dinamika folikel yang menyertai kejadian siklus estrus yang pendek Penelitian ini menggunakan 7 ekor sapi Bali yang ada di Kebun Pengembangan Penelitian Pertanian, dan Peternakan (KP4), betina, umur 2 tahun, sehat dan bersiklus estrus normal. Pengukuran diameter folikel menggunakan ultrasonografi (USG) dan darah diambil dari vena jugularus dimulai hari pertama setiap hari dalam waktu yang bersamaan selama 3 siklus. Kadar estradiol 17-β dianalisis menggunakan metode Enzyme Immuno Assay (EIA) Hasil penelitian menunjukkan 4 ekor sapi Bali mempunyai siklus estrus pendek (n=7) diantara siklus estrus normal. Sapi Bali tersebut mempunyai 1 gelombang perkembangan folikel dengan panjang siklus 7-10 hari, diameter folikel ovulasi maksimal dan kadar estradiol 17-β menyerupai siklus normal. Kadar tertinggi estradiol 17-β pada siklus tersebut 107,77 ± 55.94 pg/ml pada hari ke 7-10 saat ukuran folikel ovulasi mencapai 10.5 ± 0,38 mm. Kesimpulan penelitian ini adalah kejadian siklus estrus pendek dapat terjadi diantara siklus normal pada sapi Bali.

Kata kunci : siklus estrus pendek, sapi Bali, estradiol 17-β, serum, folikel ovulasi

ABSTRACT

The aims of the research were to confirm the short estrous cycles and determine the blood level of estradiol 17-β and ovarian follicukar dynamics in these cases. The research was conducted using seven Bali cattle, approximately 2 years of age, kept in healthy condition with normal estrous cycles. Observation of estrus symptoms was performed daily. Ovarian follicles was examined and measured using ultrasonography started at the estrus day. Blood samples were collected from jugular vein, blood sample collection and ultrasonographical examination of the ovaries were performed daily in the same time. Serum level of estradiol 17-β was performed using EIA. The short cycle estrus were observed in 4 Bali cattle (n=7) among natural estrous cycle. They have only one wave ovarian follicular development whereas the maximal size of ovarian follicles ovulation likes the normal cycle The duration of short estrous cycle was 7-10 days with normal usual estrus behavior. The peak of blood serum level was 107.77 ± 55.94 pg/ml when the diameter dominant follicle of short estrous cycle was reached 10.5 ± 0.38 mm. It can be concluded that the short estrous cycles may occur in Bali cattle after puberty among normal cycles.

Keywords : Short estrous, Bali Cattle, Estradiol 17-β, Serum, Ovarian Follicle

INTRODUCTION

Bali cattle can be found spreading all over Indonesia outside Java island. They are true tropical, have a high tolerance under poor environment, and late maturing animals as well

(Toilehere *et al*., 2003) The length of estrous cycle is not differ from the other cattle breeds. It consits luteal and follicular phase, the average length of the oestrus cycle is 21 days, with some evidence that it is shorter when nutrition is poorer (Geoffry *et al*., 2003)

Short estrus is an estrus cycle that occurs in short duration with normal estrus behavior. The duration of short estrous cycle is 7-10 days but an 8-day was most frequent (Odde *et al*., 1980). Short estrous cycle are connected to the attainment of puberty or the resumption of cyclicity postpartum (Taponen, 2002). The results of some researches showed that a short estrous cycle a normal phenomenon (Edq *cit in* Mukasa, 1991). Although the ova of short cycle can be fertilized but it so difficult to predict estrus time normally. The short estrsu cycle signifcance initially lies in difficulty to predict subsequent estrus particulary for cow not interacting with bulls (Mukasa, 1991). The short cycle are well documented in cattle during puberty with spontaneous or induced ovulation postpartum.

In the short estrous cycle, the corpus luteum can demise early because prostaglandin excreted from uterus quickly. In day 6 after estrus, the uterus more sensitive to the luteolytic effect of prostaglandin. The follicle plays a fundamentally important role in reproduction which development and ovulation are important to improve and control reproductive function in farm and companion animals (Roche, 2004). Estradiol is produced by granulosa cells and theca sel of follicle ovaria, the increase in follicular size is associated with an increase in estradiol concentration. It means that the fluctuative estradiol level can determine stage of development ovarian follicle.

The behaviour of short estrus as same as with the normal cycle but in this period the ova can not be fertilized. If the short estrus occured, it can disrupt a recording of estrus cycle and difficult to predict the true time estrus with fertile ova. Information about blood level of estradiol, ovarian follicular dynamics and length of short estrous cycles in Bali cattle is very limited. In the early studies, the authors had seen short estrous cycles in normally cycling of Bali cattle (Airin, Unpublished data).

The aims of the present research were to detect blood level of estradiol 17-β and ovarian follicular dynamics in short estrous cycles of Bali cattle.

MATERIALS AND METHODS

Animals and Blood Sampling

The research was used seven Bali cattle kept in Agricultural Training, Research and Development Station (KP 4), Gadjah Mada University-Yogyakarta. The Bali cattle had the same age (average 2 years) and healthy condition. Blood sample collection and ovarian examination were initiated when the animals showing estrus symptoms, i.e. standing heat (standing still while being mounted by other cattle), transparent vaginal discharge, changes in vulva (it becomes warm, oedematus and reddish in colour) (Toelihere, 2003).

Ultrasound Examinations

Transrectal ultrasound examinations were performed by a single operator using a real-time, B-mode scanner with an 8.0-MHz linear-array transducer (Honda-Japan). Ovarian maps were drawn at each examination and the relative positions and sizes of ovarian follicles greater than 3.0 mm in diameter and CL were recorded daily. The dominant follicle was defined as the follicle reach 5 mm of diameter (Evan, 2004; Umut *et al*., 2008).

Blood Sampling and Determination of Estradiol 17-β from Serum Samples

Blood samples were collected by vacuum puncture of a jugulair vessel into silicone plain tubes (venoject) by 20 G needles (vacutainer) in all bali cattle daily, serum was seperated and stored at −20°C until the time of analysis. Analysis of blood serum estradiol 17-β was only performed from short estrous cycle animals. The first step was adding 25 µl of standard and samples into appropriate wells and 200 µl of enzyme conjugate into each wells. The plate was incubate at room temperature for 120 minutes then remove the incubation mixture by flicking plate contents into a waste container. The each wells was rinsed for 3 times with distilled or deionized water than dispense 100 µl substrate solution into each well. The reaction was stopped by add 100 µL of stop solution to each well. The last step, plate was read used OD at 450 nm within 10 minutes.

Statistical Analysis

The data analysis was performed descriptively with individual data. The individual data would be compared between dynamics of

follicles and level estradiol 17-β in short and normal estrous cycle.

RESULTS AND DISCUSSION

Ovarian Follicular Dynamics in Short Estrous Cyle

Folliculogenesis is the process in which a recruited primordial follicle grow and develops into a specialized graffian follicle with the potential to either ovulate its egg into the oviduct at mid-cycle to be fertilized or to die by atresia (Gregory, 2008**)**. In a number spesies, follicle growth is characterized by follicle wave which follicular wave occurring during the normal estrous cycle (Evan, 2004). The development of dominant anovulatory follicles comprised three phases: growing, static and regressing phase (Noseir, 2003).

Cattle are polyestrous animals and displays estrous behaviour every 21 days but in the short estrous cycle this phase can occurred in 7-10 days. In the present study indicated that short estrous cycle found in 4 Bali cattle number 1,2,3 and 4 (n=7), its mean that 60% popullation Bali cattle of KP4 have short estrus cycle. Based on follicular dynamics, the length of short estrous cycle was 7-9 days with only one follicular development wave (Figure 1a, Figure 1b and Table 1). The follicular dynamics of Bali cattle no. 5, 6 and 7 were normal, they did not have short estrous cycle. It means that they have a normal cycle. The majority of bovine estrus cycle are composed of two or three folliculare waves, whereas emergence of the first folliculare wave occurs on the day of ovulation (day 0), the second wave occurs on day 9 or 10 in two-wave cycles. In the three waves, emergence second waves on Day 8 or 9, and the third wave emerges on day 15 or 16 (Mitesh and Govin, 2007; Adam *et al.*, 2008).

The short estrous cycle can occur after parturition but in some studies reported that the short estrus cycle a normal phenomenon (Edqvist *et al*., 1984). In the short estrous cycle ova can be fertilized and corpora lutea have short life-spans, they have only one development follicle wave. Production of progesterone to be sub-optimal, it means that rate of pregnancy tend in the short cycle (Galina *et al.,*1982). Pregnancy rates in cattle with two- versus three-wave patterns were compared based on the notion that the preovulatory follicle in the two-wave pattern grows for a relatively longer period and may contain a relatively aged oocyte. In contradictory results reprorted by Blech *et al*. (2004), the wave of development follicle cannot influence rate of pregnancy.

The most frequent length of short cycle was 11 days which all the short cycle was either first or second postpartum estrus in buffalo (Chohan, 1992). Yavas and Walton (2000) reported that short estrous cycles occur in approximately 80% of all cows following the first ovulation after calving. The time of estrus onset depend on stage of follicular wave when corpus luteum regression is induced (Taponen, 2002). The interval can be shortened with administration of PGF2a when a dominant follicle mature is present, this phenomenon can prolong if the follicle wave is emerging or undergoing selection process (Roche *et al*., 1996).

Table 1. The Concentration of Blood Estradiol 17-β (pg/ml) and Diameter of Follicle Ovulatory of Bali Cattle in the Short and Normal estrus Cycle.

	Cattle	Estradiol 17-β (pg/ml)	Diameter of Follicle Ovulatory (mm)	Lenght of Cycle (mm)
Short cycle	1	92.74	10.55	7
	2	190.62	10.60	7
	3	74.36	10.90	10
	4	73.37	9.98	8
Normal cycle	5	70.20	10.40	18
	6	72.40	10.02	21
	7	71.40	10.05	18

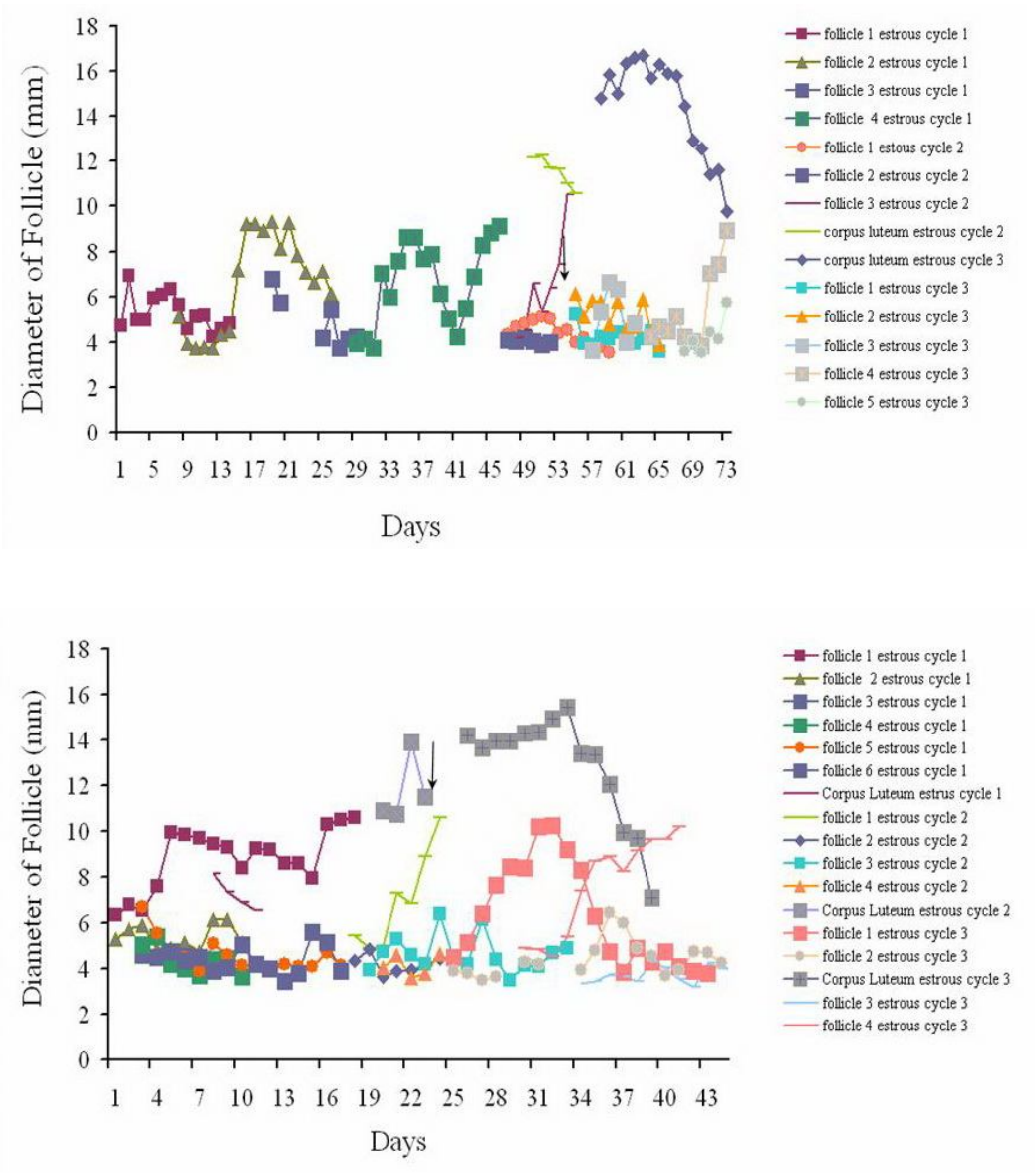

Figure 1a. Diameter of ovarian follicular dynamics Bali cattle (no 1-2) during estrus cycle. In the grafik shown that short estrous cycle can emerge among normal estrus cycle. ↓ = the short estrus cycle

In the present research show that the short estrous cycles occurred in Bali cattle heifers. It meant that phenomena short estrous cycle not usually occurring following the first ovulation after calving. The short estrous cycle can occur in cattle during puberty (Taponen *et al.*, 2002).

Level of Estradiol 17-β during Short Cycle

The estrous cycle is regulated by hormones of the hypothalamus, the pituitary, the ovaries and the uterus (Forde *et al.*, 2011). Estradiol 17-β concentration increased during folicular phase of estrous cycle. Noseir (2003) reported that the increase in follicular size was associated with an increase in estradiol 17-β concentration, it means that the level of blood estradiol can use to determine stage of follicle development. Putro *et al* (2014, in press), the level of hormone in the folliculare fluid as same as with blood such as tiroid hormone. Follicular fluid (FF) is an avascular compartment separated from the perifollicular stroma by the follicular wall within the mammalian ovary (Abd Ellah *et al.*, 2010; Albomohsen *et al.*, 2011; Nasroallah *et al.*, 2012).

A progresive increase of estradiol 17-β concentration was observed during growth phase

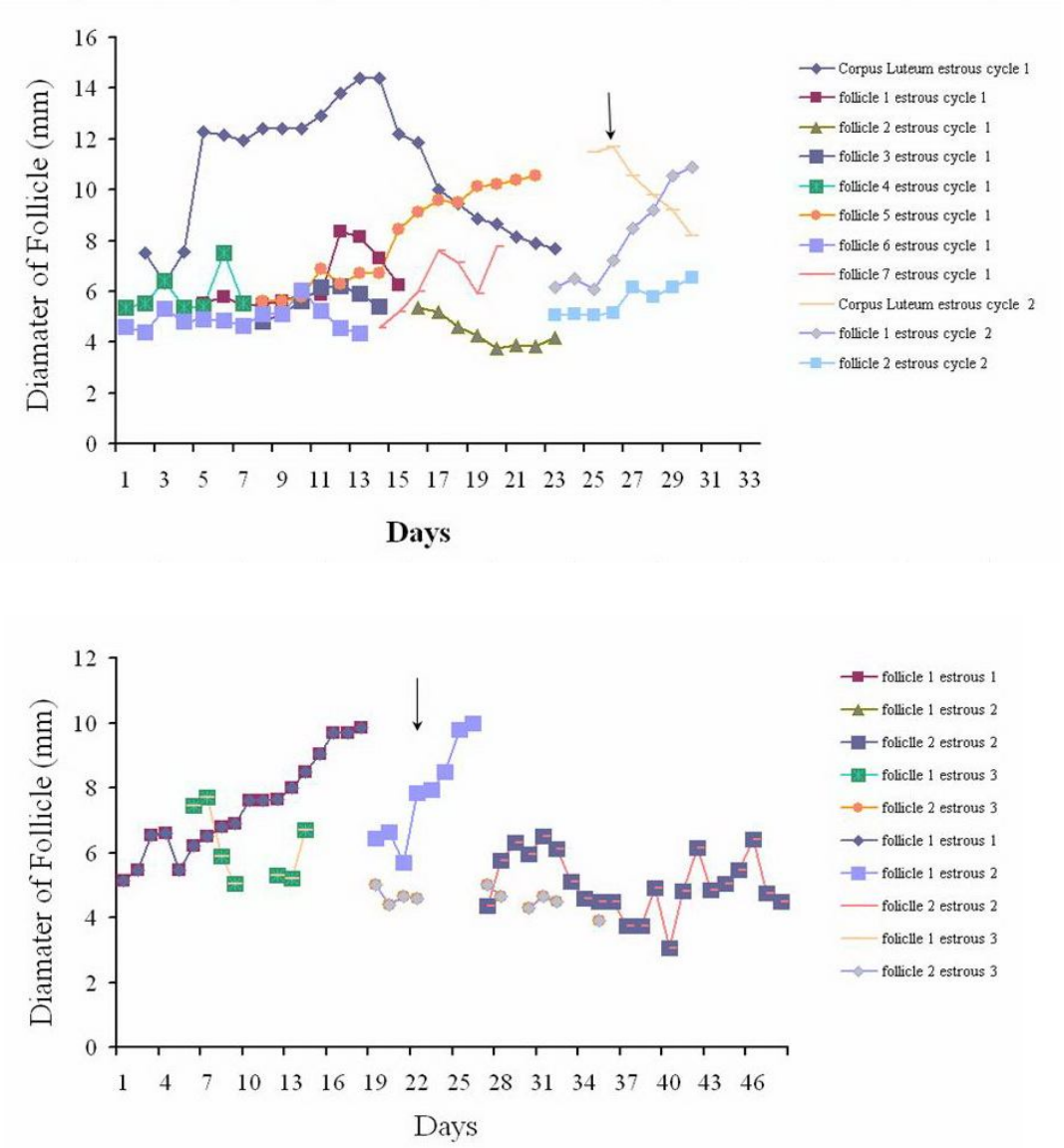

Figure 1b. Diameter of ovarian follicular dynamics Bali cattle (no 3-4) during estrus cycle. In the grafik shown that short estrous cycle can emerge among normal estrus cycle. ↓ = the short estrus cycle

but progresive decrease in estradiol 17-β concentration during static phase with more and less constant size of follicles. In the regresing phase, there was a constant decrease in follicular size and increase in estradiol production (Noesir, 2003). Vascularization is the important in determining the fate of follicle and necessery for folliculare health (Young and Mc Nelly, 2010). In the present research, the progresive increase of blood estradiol 17-β concentration was following ovulation without static phase. In the short estrous cycle, the peak of blood estradiol 17-β was 107.77±55.94 ng/ml in 7-10 days and in the normal cycle was reached in 18-21 days (Table 1). This fact was supported by the follicular dynamics which the size of ovarian dominant follicle was 10.5 ± 0.38 mm in 7-10 days before ovulation.

In the normal estrous cycle, blood estradiol 17-β from preovulatory follicle may induce uterine progesterone receptor which are required to establish progesterone dominance of subsequent ovulation. The dominance of progesterone result inadeguate uterine progesterone receptor synthesis and the estradiol 17-β concentration will decrease. The uterus may lose progesterone dominance earlier which this would be initiated the positive feedback loop between oxytocin and $PGF_{2\alpha}$ earlier in the estrous cycle (Ottobre *cit in* Zoller 1993). Short *et al.*

(1990) reported that the corpus luteum formed is smaller in the short estrous, secretes less progesterone and is less responsive to stimulation. In the normal luteolysis, the timing of luteolysis was influenced by the concentration of severe m-RNA such 3β-HSD and sTAR (Gordon *et al.*, 2000). Branden *et al.* (1988) reported that during luteal regression, initial decreased in concentration of progesterone do not appear to be due to loss of steroidogenic luteal cells. Vascularition can influence development of follicle and life span of corpus luteum ovarium. Hamish (2006) reported that the grow of follicle ovarium assosiate with development individual cappilary network, while it can receive nutrients and oxygen by passive diffusion from stroma blood vessel. The decreased secretion of progesterone caused decreased luteal blood flow by $PGF_{2\alpha}$ and, thus may reduce deliver of nutrients and substrate of steriodogenesis (Gordon *et al.*, 2000).

CONCLUSION

It can be concluded that the short estrous cycles may occur in Bali cattle after puberty among normal cycles with length of cycle was 7-10 days

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