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Profile of estrogen and progesteron hormones in bali cows that are exposed in the natural grazing filed

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Abstract
The reproductive process in productive cows, controlled by hormones, and physiological events is important to understand in improving reproductive efficiency. The study aims to determine the hormone profile of estrogen and progesterone in bali cow during one estrous cycle. The study used 10 bali cattle aged 3-4 years, Body Condition Scor (2-3), not breastfeeding, and in the luteal phase. Cows were synchronized with estrus by injecting 25 mg PGF2α (Dinoprost) intramuscularly. Detection of the emergence of estrus is done twice a day, namely: morning and evening after being treated. Blood collection is carried out on day 0 (during estrus), 3rd, 9th, 15th, and 17-23th to determine the levels of the hormones estrogen and progesterone. Measurement of estrogen (pg/ml) and progenteron (ng/ml) levels by the method of Enzyme Linked Immunoabsorbant Assay (ELISA). The results showed that the appearance of estrus in cows was 3.40±0.52 days after PGF2α injection, with an estrous cycle length of 22 days. Estrogen hormone levels on days 0, 3, 9, 15, and 17-23 were: 56.97-1.19; 16.40±0.40; 21.89±1.02; 15,37±0,37; 17.87±0.52; 26,32±0.43; 31.31±0.82; 44.01±0.83; 53.74±0.96; 55.71±0.65; 53.94±0.77 pg/ml, respectively and levels of the hormone progesterone on days 0, 3rd, 9th, 15th, and 17-23 were: 0.35±0.10; 1.35±0.72; 5.07±0.15; 7.15±0.08; 6.24±0.13; 5.69±0.04; 4.67±0.05; 3.20±0.03; 1.18±0.05; 0.43±0.02; 0.43±0.03 ng/ml, respectively. It can be concluded that the levels of estogenic hormones at the time of estrus were highest (56.97±1.19 pg/ml and 55.71±0.65) with basal progesterone levels (0.35±0.10 and 0.43±0, 03 ng/ml).

Keywords: Synchronization of estrus, progesterone, estrogen, estrus cycle of Bali cow

Introduction
Bali cattle is one of the local beef cattle germplasm in Indonesia. Data on the appearance of production and reproduction in bali cow has been widely reported, but there are no reports available on the profile of the hormones progesterone and estrogen in bali cows that are grazed in grazing areas.One of the technical implementation units (UPT) under the Indonesian Ministry of Agriculture that is tasked with increasing the population of native bali cattle in Indonesia is the Center for Livestock Feed-Animal Forage (BPTU-HPT) Denpasar-Bali. In carrying out their duties, BPTU-HPT Denpasar is faced with several obstacles, one of which is the ineffective management of effective mating to improve the reproductive performance of Bali cattle, especially in predicting estrus and ovulation time. This is seen in the report Siswanto *et al.* (2013) [47] that some of the original Balinese cows raised in BPTU-HPT Denpasar conception rates of artificial insemination are high enough to reach 4. Failure to detect and interpret signs of estrus correctly can contribute to the loss of livestock business (Mekonnin *et al.* 2017) [29]. Visually observing the symptoms of estrus in livestock is a common method used to detect estrus, this traditional method has not been able to provide a complete picture when done alone (Selvam and Archunan, 2017) [45]. In developed countries, several techniques have been used to confirm estrus, including measuring estrogen and progesterone levels in serum/plasma (Mondal *et al.* 2006; Rao *et al.* 2013 and Naik *et al.* 2013) [30, 41, 32] used to predict ovulation success.The hormones estrogen and progesterone play an important role in various reproductive functions. One of the functions of the two hormones during one estrous cycle is to regulate the length of the estrogen cycle and determine the time of ovulation. Measurement of hormone levels can use the enzyme linked immune sorbent assay (ELISA) method (Lequin, 2005) [25]. Accurate information about reproductive hormones during the estrous cycle needs to be investigated as a basic concept in the development of native Balinese cattle in Bali.

This study aims to determine the hormone profile of estrogen and progesterone in bali cattle during one estrous cycle.

Material and Methods

Experimental design, animals, housing and diets

This research was conducted at BPTU-HPT Denpasar, for one month in July 2018. The study used ten clinically healthy Bali cattle, having a Body Condition Scor (2-3) (Soares Dryden, 2011) [15, 56]. having 1 or 2 breeds (not breastfeeding)), age 3-4 years, and 90 days post parturition. In addition, the cattle used are having corpus luteum (CL) in the ovary and are not pregnant by palpation per rectal. Cows are kept in cattle yards with an area of about 902.8 m^2, fed with king grass with crude protein content (CP 8.47%), 10% of body weight; and commercial concentrate (CP 17%), 1% of the average body weight. Drinking water is given ad libitum.Estrus synchronization in cattle was carried out with a single injection of PGF2α (Dinoprost) (Enzaprost®-T, Ceva Santé Animale, France), a dose of 25 mg intra-muscularly. Visually estrus detection is done twice a day, namely: morning (06: 0-08: 00) and afternoon (16: 00-18: 00) after treatment. The speed of the emergence of estrus is measured by observing the time interval between the end of administration of Dinoprost and the beginning of the time of emergence of estrus. The percentage of estrus is determined by counting the number of animals that are lust divided by the number of animals that are treated multiplied by 100 percent. Cows are said to have estrus when visually showing symptoms of anxiety, revealing, swollen vulva, red and full of transparent mucous secretions that hang from the vulva or appear around the base of the tail (Toelihere, 1979) [54]. In addition, female cows appear to be active and when collected with fellow females show a standing behavior.A total of 3 ml of blood samples were taken through the jugular vein using the vacutainer vacuum (red top 5 ml) at the time of the appearance of estrus day 0 (during estrus). Furthermore, it is carried out on days 3, 9, 15, and days 17-23. The vacumvacutainer tube containing blood was left at room temperature for 2 hours with the tube tilted. The serum is separated from the blood by centrifuge at a speed of 3000 rpm for 15 minutes. The serum obtained was put into a 1.5 ml eppendrof that was labeled and stored in a freezer at a temperature of minus 200C until the test was carried out. Estrogen (pg/ml) and progesterone (ng/ml) levels in serum were tested by the Enzyme Linked Immunoabsorbant Assay (ELISA) method.The examination was carried out in duplicate according to the protocol on the Bovine Es (Estrogen) ELISA Kit, and Bovine Pg (Progesterone) ELISA Kit (Wuhan Fine Biological Technology, China). ELISA micro plate reading uses Elisa Reader (EpochTM BioTek) at a wavelength of 450 nm at theDenpasar Veterinary Center (BBVet).

Statistical analysis

Data regarding the time of emergence of estrus, percentage of estrus and estrous cycle length are presented descriptively. For the Optical Density (OD) absorption data obtained, it is converted using Curve Expert 1.4 for windows. The average value of the hormone is analyzed statistically using the General Linear Model (GLM) in the IBM SPSS Statistics 20 for windows program.

Results

All study cows injected by Dinopros experienced estrus (100%). The emergence of estrus after treatment occurred on the third day of six tails (60%) and the fourth day of four tails (40%). At the time of estrus, clinical symptoms that appear to meet the established estrous criteria. The time of emergence of estrus after treatment, ranged from 3-4 days with an average of 3.40 ± 0.52 days. The length of the estrous cycle is between 22-23 days with an average of 22.50±0.50 days.Average estrogen levels in bali cow serum during one estrous cycle on days 0, 3rd, 9th, 15th, 17th, 18th, 19th, 19th, 20th, 21st, 22nd and 23rd respectively: 56.97±1.19; 16.40 ± 0.40; 21.89±1.02; 15.376±0,38; 17.87±0.52; 26.32±0.43; 31.31±0.82; 44.01±0.83; 53.74±0.96; 55.71±0.65; 53.94±0.77 pg/ml. In this study, two estrogen peak points were found, namely day 0 and day 22 of the estrous cycle. The time span between the two peak points is three waves of decline and three surges in estrogen levels. A wave of decreasing estrogen levels sequentially occurs on the 3rd day, then the 15th day and 19th day of the estrous cycle. Conversely, surges below the maximum level occur on the 9th, 18th and 20th days of the estrous cycle. The dynamics of the development of estrogen levels can be seen in Figure 1

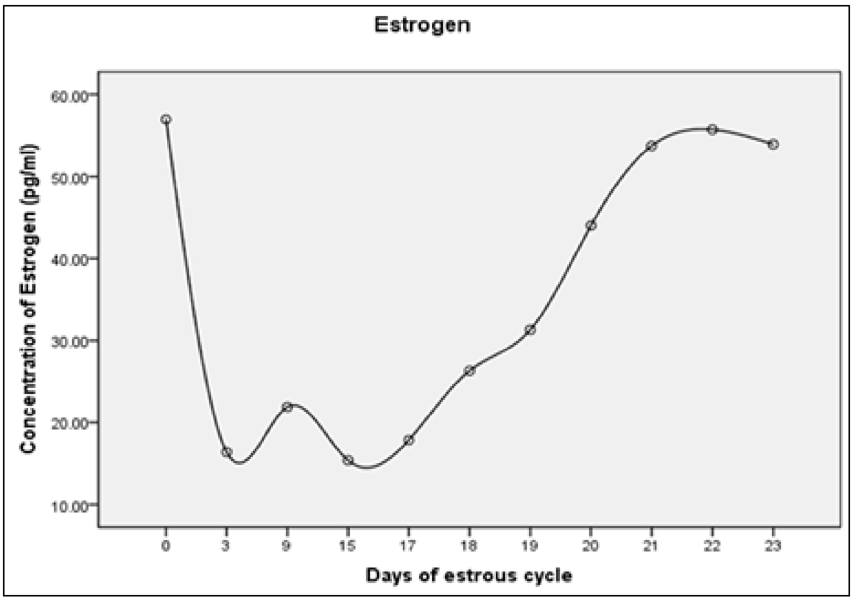

Fig 1: Average pattern of estrogen hormone levels in bali cow serum (n=10) during one estrous cycle (day 0 = estrus)

Average levels of the hormone progesterone in bali cow serum (n=10) during one estrous cycle on days 0, 3rd, 9th, 15th, 17th, 18th, 19th, 19th, 21st, 22nd and 23rd respectively: 0.35±0.10; 1,35±0,72; 5,07±0,15; 7.15±0.08; 6.24±0.13; 5.69±0.04; 4.67±0.05; 3.20±0.03; 1.18±0.05; 0.43±0.02; 0.43±0.03 ng/ml. The dynamics of the development of progesterone hormone levels during one estrous cycle can be seen in Figure 2.

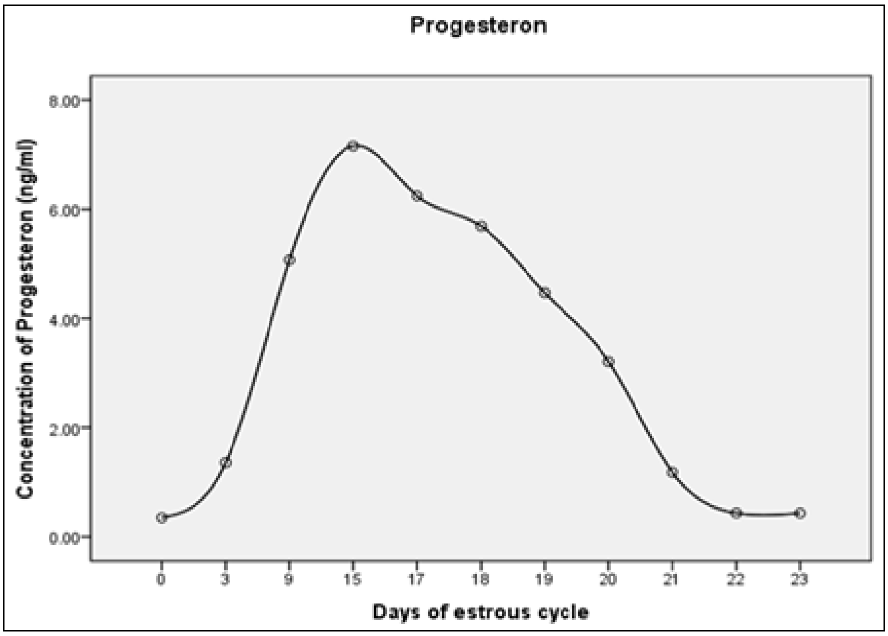

Fig 2: Even pattern of progesterone hormone levels in bali cow blood serum (n=10) during one estrous cycle (day 0=estrus)

Figure 2. illustrates that the average level of the hormone progesterone is low during estrus day 0 and day 22 respectively: 0.35±0.10 and 0.43±0.02 ng/ml. The increase in progesterone levels starts on the 0th day after estrus and sharply occurs from the 3rd day to reach a peak of about 7.16/0.08 ng/ml on the 15th day of the estrous cycle (luteal phase). Then it gradually decreases after the 15th day until the 21st day. Progesterone levels again decline to basal levels 22nd and 23rd day of the estrous cycle. A decrease in progesterone levels <1 ng/ml on that day, indicates the time of estrus.

Discussion

The time of emergence of estrus after treatment in this study ranged from 3-4 days with an average of 3.40 ± 0.52 days. The time of emergence of estrus above is similar to reports of the use of Dinoprost for synchronization in cattle (Pursley *et al*. 2012 and Baryczka *et al.* 2018) [27, 40, 8]. The results of this study indicate that the administration of luteolytic Dionprost in cattle that have CL causes regresicorp luteum (Stevenson *et al.* 2010; Esterman *et al*. 2016; Montaser *et al*. 2016) [31, 51, 52]. CL regression occurs through a counter current mechanism (Senger, 2003) [44], which results in a sudden decrease in the level of the hormone progesterone in the blood (Martins *et al.* 2011) [27]. Low levels of the hormone progesterone and high levels of estrogen in the blood cause negative and positive feed-back in the hypothalamus which results in the release of FSH and LH from anterior pituitary (Senger, 2003; Ball and Peters, 2004 and Forde *et al*. 2011) [15, 44, 51]. The two hormones mentioned above, work together to stimulate the development and maturation of follicles in the ovary. As follicles mature, estrogen levels are generated more and more, and this condition causes estrus (Stötzel *et al*. 2012) [53]. All research cows showed estrous symptoms. The effectiveness of estrus synchronization using Dinoprost in this study is the same as that reported by Montaser *et al.* (2016) [31] which reached 100%. Siregar *et al.* (2015) [46] also reported a similar finding occurring in cattle in the Aceh region, Indonesia. With the same method, the percentage of estrus in this study was higher than that of Angus beef cattle which was 58% and Brahman cattle by 52% (Esterma *et al.* 2016) [14]. Whereas in dairy cows of 61.7% (Ribeiro *et al.* 2012) [43]; 45.6% (Pursley *et al*. 2012) [27,40]; 57.6% (Brayzaka *et al.* 2018); and by 70% (Balumbi *et al*. 2019) [7]. The high percentage of estrus in this study is thought to be caused by the uniformity of functional CL age in the ovary at synchronization. Gordon (1996) [18] states that the use of a single dose PGF2α in the estrus synchronization program should be carried out in cows that have mature CL in the ovaries. To increase the effectiveness of PGF2α work in lyses CL requires an appropriate estrus synchronization protocol (Islam, 2011) [21] and a complete understanding of the physiological status of CL (Paul *et al.* 2015) [37]. According to Nalley*et al*. (2011), the determination of one estrous cycle is based on the interval between the two lowest points of the hormone progesterone level or the two highest points of the estrogen hormone level. Based on the analysis of hormone levels, it was found that the length of the estrous cycle after treatment (Figure 1 and Figure 2) was on the 22nd day of the estrous cycle. The long duration of the estrous cycle after this treatment, is not much different from the previous findings in the same type of cow, which is on the 21st day of the estrous cycle (Arimbawa *et al*. 2012) [3] or in different breeds, such as ongol crossbred cattle (PO) (Priyoatmojo *et al.* 2012) [39]. However, some researchers report that the long duration of the estrous cycle after treatment is on the 19th day of the estrous cycle (range 18.3-22.3 days) in Holstein cattle (Beal *et al.* 1980) [9] and on the 20th day, 0±2.3 of the estrous cycle in Zebu cattle are present (Oyedipe *et al.* 1986) [36]. The long duration of the estrous cycle after treatment in the cattle mentioned above is still in the normal range, which is between 18-24 days with an average of 21 days (Toelihere, 1979) [54]. The developmental dynamics of estrogen in bali cattle during estrus (Figure 1) are similar to the estrogen levels in Punganur cattle reported by Naik *et al.* (2013) [32]. Estrogen hormone levels during estrus in this study were higher than estrogen levels of ongol crossbreed (PO), F1 Simental and F2 Simental (Yanhendri, 2007) [57], mithun cattle (Mondal *et al*. (2006) [30], Holstein cattle (Lopez *et al.*, 2002) [26] In the same type of cow, the results of this study were lower compared to the findings of Airin *et al*. (2014) [1] and Pemayun *et al.* (2016) [38] The differences in observed estrogen hormone levels might be caused by several factors, one of which is the treatment The results of this study are in accordance with what reported by Dezaza *et al.* (2013) that an increase in estrogen hormone levels can be associated with a synchronizing effect, but does not occur in subsequent estrus Trwary (2006) [55] also observed high estrogen levels (17.69±4.49 up to 55.89±4.45 pg/ml) on estrous induced and 7.27±0.64 to 7.38±0.58 pg/ml before treatment. Jena *et al.* (2016) [22] also reported the same thing. namely a significant increase in estrogen levels fish after treatment from 41.80±5.00 to 43.06±2.34 pg/ml. Estrogen hormone concentrations show a sharp decrease from day 0 (56.97±1.19 ng/ml) towards day 3 (16,40±0.40 ng/ml) of the cycle caused by ovulation and changes from follicular phase to the luteal phase under the influence of the hormones FSH and LH (Gordon, 1996) [18]. Immediately after the 3rd day, estrogen levels show a temporary increase, until the 9th day and estrogen concentrations do not reach peak levels indicating the development of the first dominant follicle (Alvarez *et al.* 2000) [2]. Added Martinez *et al.* (1999) [28] that the emergence of new follicular waves spontaneously occurred after being induced. After the 9th day the concentration of the estrogen hormone sharply decreases on the 15th day indicating atresia of the dominant follicle that grows during the first wave of follicles (Hafez, 2008 and Noakes *et al.* 2001) [19, 35]. During this period, ovarian activity in growing follicles is relatively reduced. Towards the end of the luteal phase, on the 17th day through the 18th day the estrogen concentration showed an upward trend of half of the optimum level indicating the development of the 2nd single dominant follicle. However, follicles do not seem to have the ability to develop as a result of changes in hormonal levels (Woodruff and Shea, 2010) [56]. So that the development of a single dominant follicle is suppressed and the 2nd dominant follicular atresia occurs on the 19th day of the cycle (Fortune, 1994) [16]. Furthermore, after the 19th day there was a sharp increase in estrogen levels until the next estrus day 22, where estrogen concentrations peak and decrease after estrus. High estrogen levels during estrus are caused by the development of mature pre-ovulatory follicles (Hirshfield, 1991 and Sunderland *et al.* 1994) [20, 50] in one of two ovaries (Forde *et al.* 2011) [15]. At this stage, the anterior hypothalamus releases GnRH before ovulation (Senger, 2003; Ball and Peters, 2004 and Forde *et al*. 2011) [15, 44]. It was further said that, the GnRH hormone causes the pituitary gland in the hypotalamus to release FSH and LH in the blood to the ovaries to control follicular growth and development. Surges of FSH and LH hormones secreted during and immediately after estrus in this study allowed spontaneous 3rd wave follicles on the 22nd day spontaneously based on measurements of estrogen and progesterone hormone levels (Figures 1 and 2). The pattern of estrogen levels during proestrus increases when the cow approaches estrus and matches the expected pattern.In Bali cow, the pattern of increase and decrease in progesterone levels during one estrous cycle is similar to that found in other types of cattle. The average level of progesterone in serum at estrus is low (<1 ng/ml) (Figure 2) in accordance with previous findings in the same type of cow (Arimbawa *et al*. 2012; Astiti and, Panjaitan, 2013) [3, 4] and in the breed different as in dairy cows (Beal *et al*. 1980) [9], Punganur cows (Naik *et al.* 2013) [32] and crossing of Zebu cows with Friesian holstein (Mekonnin *et al.* 2017) [29]. Low levels of progesterone in this study indicate that CL is not functioning and estrus is ongoing. Progesterone levels reach the lowest (basal) level when estrus in this study lasts up to 3 days. Knowing the level of the hormone progesterone, of course ovulation time can be predicted. Toelihere (1979) [54] states that ovulation will occur around 10-15 hours after the onset of estrus with estrous duration of about 18 hours. Thus, the optimum time for mating is around mid to late estrus, because the formation of CL occurs just after ovulation (about 48 hours after ovulation), where the hormone progesterone begins to be produced (Quintal-Franco *et al.* 1999).Larson and Randle (2014) [24] report that in general functional CL begins on day 5 and reaches its peak point on day 17 of the cycle. The research data obtained gives a strong indication that, the gradual increase in the level of the hormone progesterone begins on the 3rd day after estrus follows CL activity in the ovary and reaches its peak on the 15th day of the estrous cycle (in the luteal phase). The results of this study are in line with those reported by Balakrishnan *et al.* (1998) [5], Mondal *et al.* (2006) [30], Ginther *et al*. (2010) [17] and Naik *et al*. (2013) [32], that the level of the hormone progesterone remains low during estrus and increases gradually from day 3 to peak at day 15 of the cycle. However, some researchers (Diaz *et al*. 1986; Khanum *et al*. 2008 and Arimbawa *et al.* 2012) [3, 13, 23] reported the time when peak levels of progesterone occurred between days 12-14 of the estrous cycle.The prolongation of peak progesterone levels found during the estrous cycle in this study, can be caused by one of them being a long luteal period of up to 17 days (Larson and Randle, 2014) [24]. Toelihere (1979) [54] said that in the luteal phase, CL achieves its maximum growth and function, and the levels of the blood progesterone hormone produced are very high. During this period, the activity of the ovaries in growing follicles decreases and as a result the hormone estrogen becomes lower.The hormone progesterone produced from CL plays an important role in various reproductive functions, including regulating cycle length, pregnancy formation and pregnancy maintenance (Rekawiecki *et al.* 2008 and Skarzynski *et al.* 2013) [16, 42, 48], but in this study cattle were not mated and progesterone levels experience a gradual decline after reaching the peak from the 15th day to the 21st day of the cycle. Furthermore, the level of the hormone progesterone decreases to reach the lowest level on posluteolisis days 22 and 23 of the estrous cycle. The results of this study are in line with those described by Day and Geary (2005) [11] and Stevenson (2017) [51, 52] that if on the 16th day there is no sign of pregnancy, then the next day CL will decay by itself as a result of the secretion of PGF2α by the uterus. At the same time, progesterone levels will also decrease rapidly about 4 days before estrus (proestrus) and remain low during estrus and for 2 days after estrus this coincides with an increase in estrogen levels.

Conclusion

The administration of a single injection of Dinoprost intramuscularly in Bali cattle which has functional CL is able to accelerate the occurrence of estrous induction and produce a maximum estrus response. The highest levels of the hormone estrogen during estrus range from: 55.71±0.65 to 56.97±1.19pg/ml, while the lowest levels of the hormone progesterone when estrus ranges from: 0.35±0.10 to 0.43±0.03 ng/ml. During the estrous cycle, prolongation of peak levels of progesterone in the luteal phase and three surges of estrogen hormone levels are found.

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