Progesterone Profiles During the Estrous Cycle and Pregnancy of Grazing Bali Cows Supplemented with Concentrate

(Profil Progesteron Selama Siklus Birahi dan Kebuntingan pada Sapi Bali yang Digembalakan dengan Penambahan Konsentrat)

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Abstract. The influence of concentrate during estrous cycle and pregnancy on pattern of progesterone were studied. Seventeen calf-rearing cows were devided randomly into two groups, group A (N=9 were grazed on natural pasture as basal diet, whereas goup B (n=8) received additional 2.4 kg concentrate consists of 1.2 kg corn meal, 0.6 kg rice bran and 0.6 coconut cake. Animals from each group were closely observed for estrous characteristics i.e. estrus detection two times a day by two experienced technisians. Blood sample taken 3-times per week from jugular vein were used for assessing plasma progesterone concentrations using RIA procedure. Plasma progesterone concentrations were not influenced by concentrate supplementation neither during successive estrous cycles nor during pregnancy. The concentrations began to rise on day 4 of the cycle (1.9 ng/mL and 1.8 ng/mL) and increased to concentrations as high as 5.4 ng/mL and 5.1 ng/mL for supplemented and non-supplemented cows during the luteal phase (day 12) before returning to basal levels i.e. 0.1 ng/mL and 0.1 ng/mL, before ovulation. Following conception, plasma progesterone increased from 0,16 ng/mL and 0.21 ng/mL at estrus to 3.6 ng/mL and 2.73 ng/mL at week-1 and then rose to 5.09 and 4.57 ng/mL at week-2 for NS and S groups, respectively. Aftewards, progesterone continued to rise to 5,46 ng/mL and 5.17 ng/mL on week-3, and then reached 6.25 and 5.3 ng/mL for non-supplemented and supplemented cow, respectively. During 10 weeks pre-calving period, progesterone levels declined gradualli achieving levels 2.83 ng/mL and 2.87 ng/mL in the non-supplemented and supplemented groups, respectively, declined markedly 1 to 2 days prior to parturition and remained at these levels before reaching the basal level after parturition.

Key Words: Bali cows, progesterone, estrous cycle, pregnancy

Introduction

The activity of the bovine ovary is generally reflected by the alternating presence and absence of a corpus luteum (CL), several investigators have paid attention to the possibility of measuring progesterone. Concentrations of progesterone in bovine blood plasma above a relatively low basal level provide a reliable measure of the presence and secretory activity of luteal tissue, since other possible sources of progesterone are absent in non-pregnant animals. Cattle exhibiting regular estrous cyclicity show characteristic periodic fluctuations in progesterone concentration with a 2-week period of high levels followed by 1 week of low levels (Bulman and Wood, 1980).

It well established that progesterone plays a major role in stimulating the production of a variety of endometrial secretions necessary for the successful development of the embryo. Mann *et al*. (1998) demontrated the importance of the pattern of maternal progesteron secretion on the development of the embryo of the cow. In the ovary, the corpus luteum graviditatis is a transitive gland producing progesterone, an important hormone for the maintenance of pregnancy; insufficiency of corpus luteum graviditatis to produce progesterone during early pregnancy hinders the development of the embryonic cells to produce interferon-tau (Hansen et al., 1999).

The concentration of progesterone in blood is related to production and clearance rates. Aspects of nutrition have been shown to affect both processes. Most of the research findings focused on progesterone production suggest that subnormal progesterone levels in energy-deficient cows are sometimes a result of a decreased CL function (Villa-Godoy et al., 1988). Cows in a negative energy balance and low levels of progesterone may be at a greater risk of premature luteolysis than cows with normal progesterone levels and a positive energy balance. A low level of energy in the diet can also affect progesterone concentrations as shown by Smith (1988) who found that a low serum glucose concentration decreased LH pulse frequency and amplitude resulting in diminished progesterone production by the CL. Cumming et al. (1971) were the first to recognise that modifying the level of feed intake also altered the plasma concentration of progesterone. The cause of this response was thought to be an increase in hepatic blood flow with increased feed intake and consequently an increase in the metabolism of progesterone by the liver.

Concurrent determinations of progesterone profiles in plasma could provide an insight into the mechanisms which regulate reproductive activity during estrous cycles and pregnancy of Bali cow with two different levels of nutrition.

Research Methods

From more than 100 available multiparous Bali cows, 17 calf-rearing cows between 4-8 years of age were selected. They were rented from farmers after being mustered for age, body weight and condition score. Prior to the experiment, all cows were rectally palpated to assure a clinical state of reproductive health, i.e. involution of the uterus, presence of ovarian structures such as a corpus luteum or a follicle.

The animals were divided randomly into groups A (n=11) and group B (n=10), and were grazed on the available native pasture (approximately 15 ha) near the village and based on the prevailing system of 8 h/day grazing (08.00 - 16.00) as a basall diet. The cows in group A received no additional feed, whereas those of Group B received an additional 2.4 kg of concentrate (1.2 kg cornmeal; 0.6 kg rice bran; 0.6 kg coconut cake). However, both groups had similar opportunities to drink water and lick salt. These were available either in their individual pens or around the gathering yard on the farm. The same area was also used to observe the estrous behaviour of the cows.

Animals from each group were closely observed for reproductive performance, i.e. estrus detection took place twice a day for about 1 hr at 6 a.m. and 6 p.m. by two experienced technicians. They recorded estrous intensity as well as length of estrus and estrous cycles. Length of estrus was defined as duration between onset and end of estrous behavior. The onset of estrus behavior was defined as the first time the cow stands to be mounted by other cows, while end of estrus was identified as the last standing to be mounted without subsequent mounted during the next observation.

Blood samples collected from the jugular vein were collected three times weekly for assessing plasma progesterone concen-trations, and assayed by using the 125-I progesterone double-antibody radioimmuno-assay (RIA) method as described by FAO/IAEA (International Atomic Energy Agency). After 2 estrous cycles, a Bali bull with proven semen quality was kept with the cows around the clock. From 5 a.m. to 7 p.m. of each day, two trained technicians recorded any estrous activity in the herd. According to their observation, every cow was mated at least once or twice per estrus period. Pregnancy was verified via rectal palpation at 45-60 days after breeding. Level of progesterone also served as an indicator to support this observation. The day of conception was verified by the calving date.

Statistical analysis of plasma progesterone using multivariate analysis of variance for repeated measure data using the general linear model (GLM) procedure of SAS (1988). Studen t-test is only used to examine the differences of the animal's oestrus data i.e. estrous cycle, length of estrus, and levels of progesterone.

Results

Chemical composition of native grass and concentrate are presented in Table 1. CP and energy content of concentrate was higher than of native grass.

Based on the intensive estrus observation and measurement of progesterone concentrations during overall 26 cycles, the mean length of the estrous cycle were determined to be 22,3 and 21,1 for cows grazing on natural pasture and cows receiving supplement concentrate, respectively. Correspondingly, the length of estrus of the two groups was 18,7 hours and 20,8 hours. Both groups exhibited good intensity of estrus, with scores 2,6 and 2,9 (standing estrus behavior = 3), respectively. None of these differences was significant.

Plasma progesterone concentrations during successive estrous cycles were not influenced by concentrate supplementation. In the course of the cycle, the concentrations began to rise on day 4 of the cycle (1.9 ng/mL and 1.8 ng/mL) and increased to concentrations as high as 5.4 ng/mL and 5.1 ng/mL for supplemented and non-supplemented cows during the luteal phase (day 12) before returning to basal levels i.e. 0.1 ng/mL and 0.1 ng/mL, usually 2-4 days before ovulation. It is evident that the means progesterone concentrations measured two-day interval during estrous cylce are similar between two groups (Fig 1).

One interesting finding in this study was 29,41% of cows to be estrus and conceived without any luteal phase. 4 out of 9 unsupplemented cows (44,40%) and 1 from 8 supplemented cows (0,12%) did not appear to be normally cyclic during the period of 3-times weekly sampling but they conceived after natural mating (Fig 2 and Fig 3).

Table 1. Chemical composition of native grass and concentrate

Composition	Native grass	Concentrate
Dry matter (DM), %	23,50	89,50
Crude protein (CP), % DM	7,80	13,70
Ether extract (EE), % DM	1,56	5,10
Crude fibre (CF), % DM	38,84	13,90
Nitrogen free extract (NFE), % DM	39,10	54,00
Neutral Detergent Fibre (NDF), % DM	58,30	38,10
Ash, % DM	12,70	13,30
Ca, % DM	0,90	0,30
P, % DM	0,70	0,20
Energy, MJ/kg	13,70	16,40

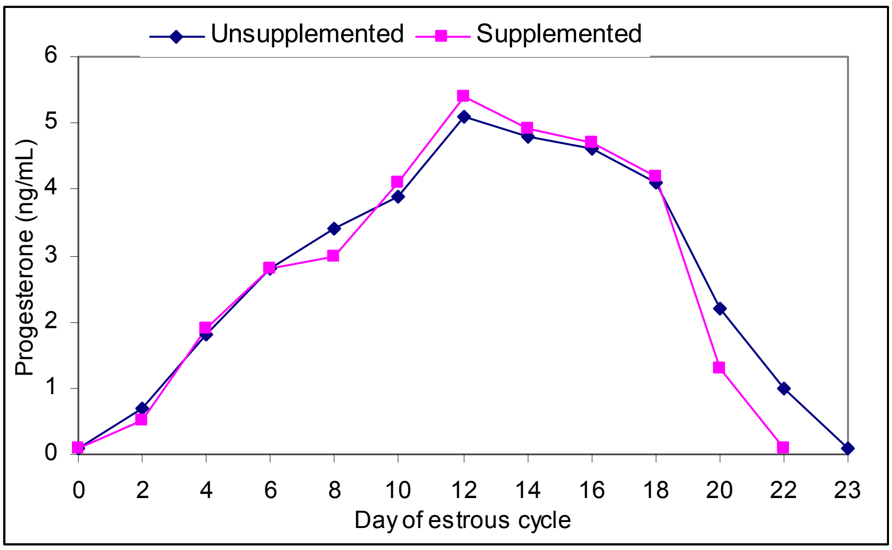

Figure 1. Mean plasma progesterone concentrations during the estrous cycle of supplemented and unsupplemented cows (Day 0 = estrus).

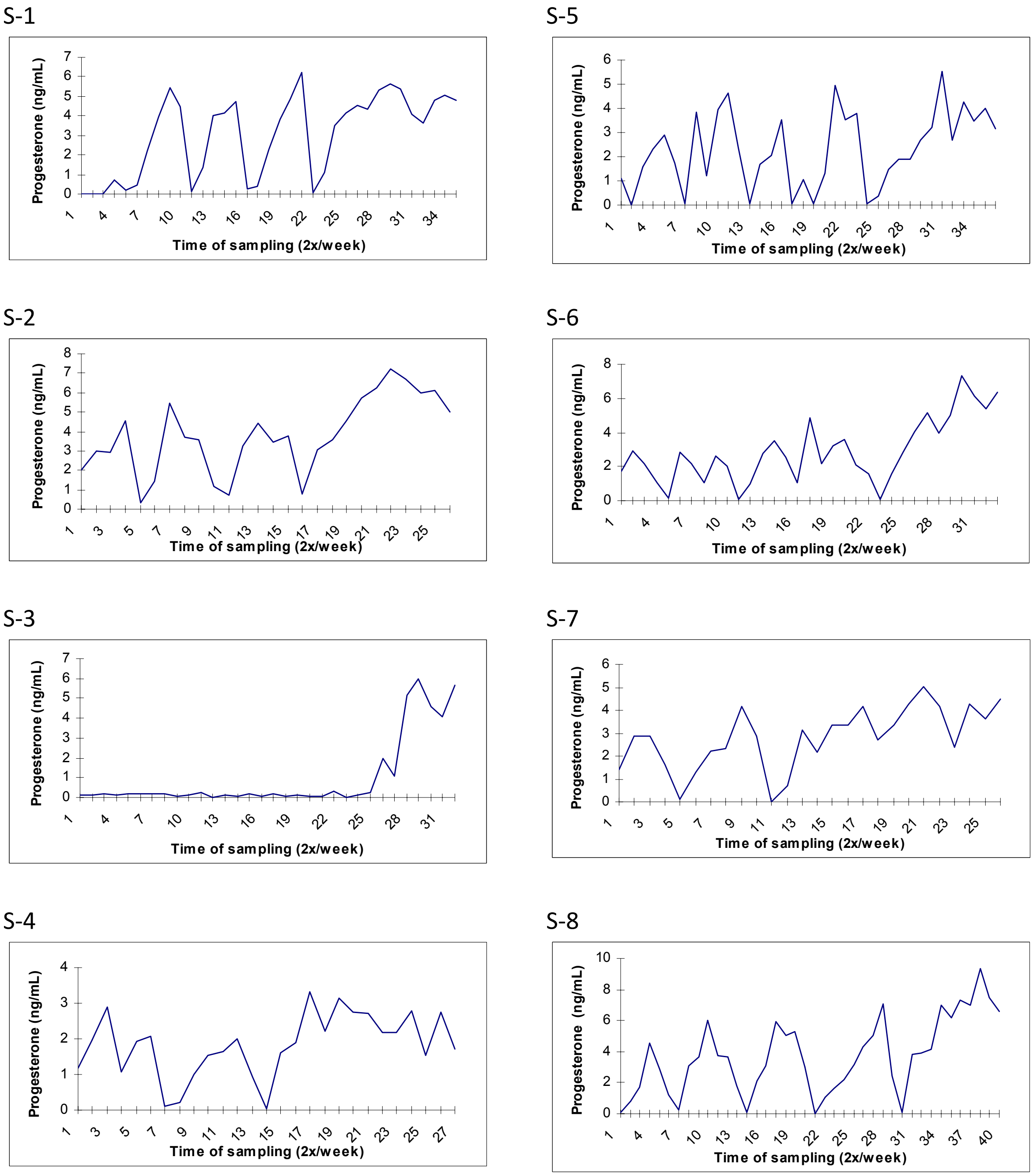

Figure 2. Individual plasma progesterone profiles during estrous cycles up conception and early pregnancies, cow S-3 did not appear normal cyclic but conceived

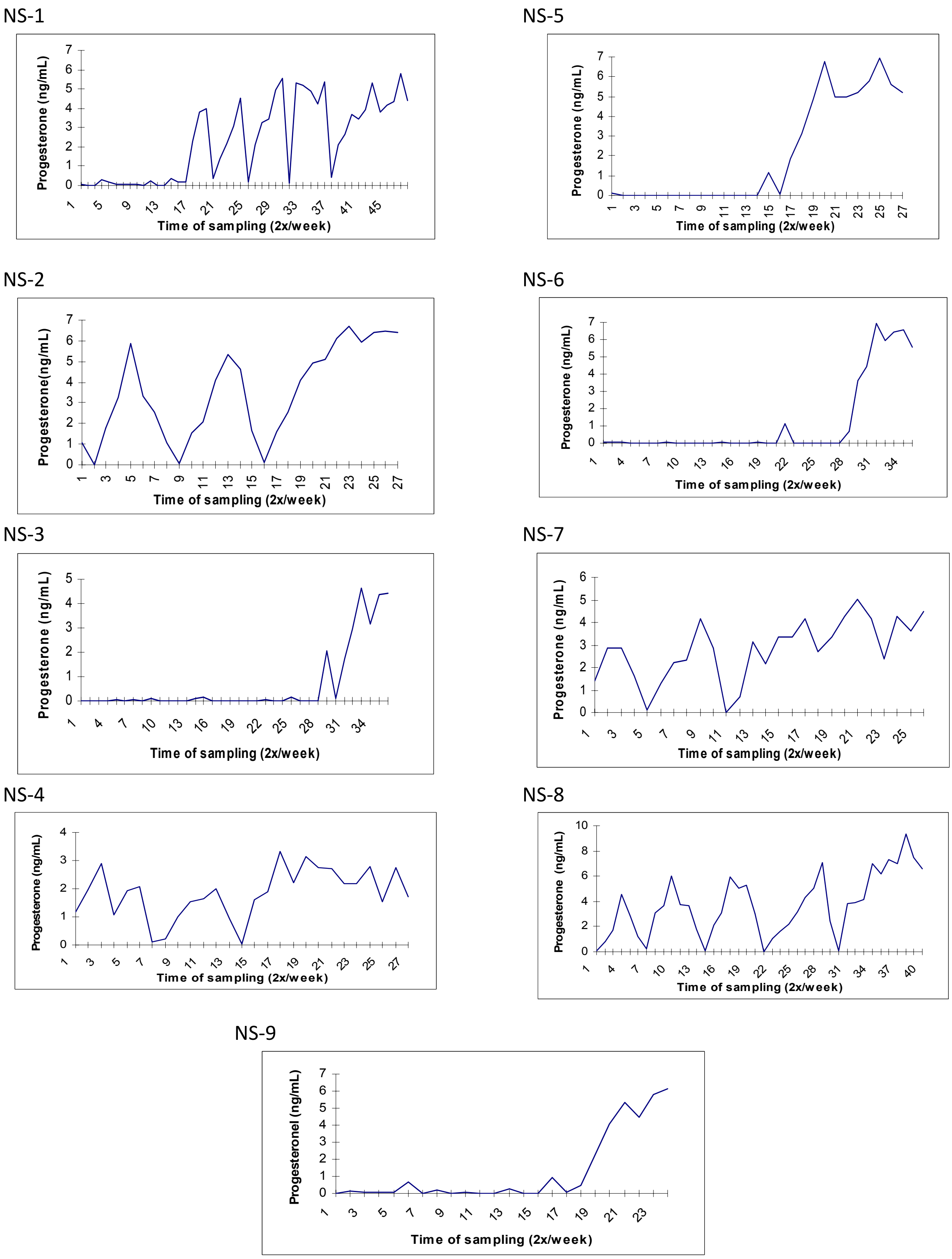

Figure 3. Individual plasma progesterone profiles during estrous cycles to conception, 4 cows (NS-3, NS-5, NS-6 and NS-9) failed to appear normal cyclics.

The progesterone profiles of the 17 cows (two groups) during pregnancy are depicted in Fig 3. All cows whose progesterone profiles after mating, remained elevated indicating pregnancy as confirmed by rectal examinattion at 45-60 days post-mating. Following conception, plasma progesterone increased from 0,16 ng/mL and 0.21 ng/mL at estrus to

3.6 ng/mL and 2.73 ng/mL at week-1 and then rose to 5.09 and 4.57 ng/mL at week-2 for NS and S groups, respectively. Aftewards, progesterone continued to rise to 5,46 ng/mL and 5.17 ng/mL on week-3, and then reached 6.25 and 5.3 ng/mL for non-supplemented and supplemented cow, respectively at 10 weeks prior to calving.

During 10 weeks pre-calving period, progesterone levels declined gradualli achieving levels 2.83 ng/mL and 2.87 ng/mL in the non-supplemented and supplemented groups, respectively. Plasma progesterone concentrations declined markedly 1 to 2 days prior to parturition and remained at these levels before reaching the basal level after parturition. Plasma progesterone concentrations between the two groups around this period were not significantly affected by supplementation even though cows fed supplement tended to have slightly lower progesterone levels (on weeks 8 and 9) pre-calving.

Discussion

Plasma progesterone concentration in Bali cows during the estrous cycle showed similar levels and patterns for supplemented and non-supplemented cows (Fig 1), in contrast to other reports which concluded that during the luteal phase in cyclic cows, underfeeding increased systemic levels of progesterone in the bovine during estros cycle (Lammoglia et al., 1998), and decreased progesterone concentrations (Hansel, 1973). The reduction of plasma progesterone levels was evidently a reflection of the smaller corpole lutea, which contained less progesterone, both in terms of total content and concentration. However, the results supports those indicating that restricting energy intake has no immediate effect on concentration of progesterone in the cow (Spitzer *et al*., 1978).

There was no significant difference in the progesterone levels of the two groups at the beginning of the luteal phase of the cycle which were 1.90 and 1.80 ng/mL for cows fed concentrate and not fed concentrate, respectively. Also, the mean concentration of progesterone at peak secretion on day 12 was similar for the two groups, i.e. 5.40 and 5.10 ng/mL for cows offered concentrate and no concentrate, respectively.

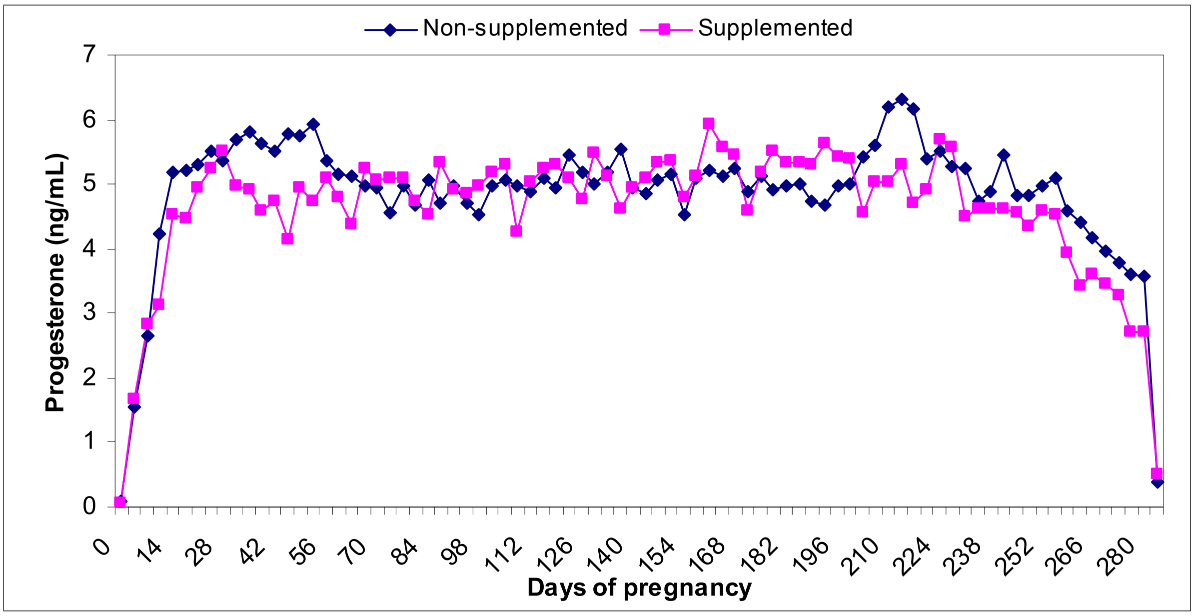

Figure 4. Mean plasma progesterone concentrations during pregnancy in non-supplemented and supplemented cows

The progesterone peaks were lower than reported by Bakry (1994) (8.95 ng/mL), but higher than reported by Mutiara (1997) (3,94 ng/mL) and Syukri (1997) (4.16 ng/mL) for the same breed in South Sulawesi. Luteolysis of the corpus luteum (CL) as sources of progesterone is caused by pulses of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) that are secreted by the endometrium around day 17-19 estrous cycle. $PGF_{2\alpha}$ induces a decrease in progesterone release from CL volume and blood flow to the CL (Acosta et al., 2002). Following an increase in luteal blood flow, $PGF_{2\alpha}$.directly stimulates the production of EDNI (endothelin-I) and Ang II (angiotensis-II) from luteal endothelial cells and luteal $PGF_{2\alpha}$ from luteal cells, and these vasoactive substances directly induce the decrease of progesterone secretion from the corpus luteum (Miyamoto and Shirasuna, 2009).

Plasma progesterone levels during estrus were 0.11 and 0.06 ng/mL for cows fed or not fed concentrate. This concentrations were similar to those reported by Mutiara (1997) and Syukri (1997), who found progesterone concentrations during estrus to be around 0.08 to 0.12 ng/mL in Bali cows, but lower than those of the *Bos indicus* breeds reported by Mukassa-Mugerwa *et al*. (1991). For normal dairy and beef cows, Lammoglia *et al*. (1997) reported average progesterone levels to be less than 1 ng/ML plasma at estrus and 5 to 9 ng/ML at the peak of the luteal phase.

The pattern of fluctuation in progesterone plasma levels during the estrous cycle of Bali cows recorded in the present study is similar to some previous observations in Bali cows (Mutiara, 1997; Syukri, 1997; Bakry, 1994), *Bos taurus* (Shotton *et al*., 1978) and *Bos indicus* (Adeyemo and Heath, 1980). The generally higher peak value of plasma progesterone in *Bos taurus* compared with Bali cows during the luteal phase was probably due to the difference observed in the ovarian sizes. Assuming that the ovarian size reflects the size of the corpus luteum during the luteal phase, it can be said that, generally, the corpus luteum was larger in the Bos taurus cows. However, it seems that the breed difference in plasma progesterone concentration was not high enough to explain the difference in the sizes of ovaries at luteal phase between *Bos taurus* and Bali cows. This probably suggests that not all parts of the luteal tissue might be utilized in progesterone secretion at all times (Adeyemo and Heath, 1980).

Among cows in two groups, different progesterone patterns were recorded. Fig 2 and Fig 3 show progesterone patterns of cows number S-1, NS-3, NS-5, NS-6, and NS-9 did not appear to be normally cyclic, indicated neither estrus signs nor elevated plasma progesterone concentrations. Plasma progesterne concentrations remained basal (<0.1 ng/mL) until ovulation and conceived after natural mating.

Based on plasma progestrone changes and published length data, conception could have occurred for individual cow. The length of gestation in the non-supplemented group of cows was 283 ± 0.8 days, in the 8 supplemented group was 282 ± 0.6 days, and in the total of 17 cows was 282.6 ± 0.5 days. Previous estimates of Banteng gestation length have been based on times observed matings. Present results falls within the range of 285 to 300 days reported for the Banteng (Lekagul and McNeely, 1977), but similar to reported by Asa *et al*. (1993).

The progesterone profiles of pregnancy for two groups showed similar patterns to investigation of Banteng cattle in Henry Doorly Zoo (Asa *et al*., 1993) but different in magnitude. Serum progesterone concentrations increased to the maximum range of 10 ng/mL by the ninth week of gestation, and declined during the last 10 weeks, higher than the maximum levels of the present results i.e. 6.33 ng/mL.

The conceptus (embryo proper and trophobalst) releases signals to maintain production of progesterone by the corpus luteum during early pregnancy (Thatcher *et al*., 2001; Spencer and Bazer, 2002), in which interferon (IFN)-tau is a major paracrine signal that is produced by the bovine conceptus and acts on the endometrium to elicit secondary responses that are necessary to maintain pregnancy (Thatcher *et al*., 1995).

In the first month of pregnancy the steroidogenic cells of the corpus luteum graviditatiis are very active cells, is indicated both by progesterone receptor expression in cells and mRNA expression that could be show evidence that cells produce progesterone intesively (Tamane *et al*., 2004). The cells activity of pregnancy corpus luteum in the early stage of pregnancy from 8 to 20 days is supported by interferon-tau secreted by the embryo trophoblast binuclear cells, which blocks arahcidonic acid transfer into $PGF_{2\alpha}$ and stimulates progesterone synthesis in the large luteal cells. In most animals, the placenta formation is complete and starts its function on about day-45 of pregnancy, while the function of corpus luteum is maintained even until day-200 of pregnancy (Niswender *et al*., 2000). However, the bovine placenta produces progesterone contributes only marginally to peripheral maternal plasma levels, and its capacity to maintain pregnancy in the absence of luteal progesterone production is restricted to a short phase between days 180 and 250 (Pimentel *et al*., 1986). In cattle, concentration of progesterone in placental tissue rise considerably within the fifth and sixth month of gestation, reaching maximal values in the cotyledons of about 50 ng/g wet tissue in the eighth and ninth month (Tsumagari *et al.,* 1994; Hartung, 1995).

The decline in progesterone during the week before parturition agrees with previous report (Mukasa-Mugerwa *et al*., 1991). Although the levels of progesterone in the present study were not of the same magnitude as reported by previous worker for beef cattle, the trend of the pattern of prepartum progesterone was. This difference may be due to the breed. Furthermore, Stabenfeld *et al*. (1970) reported a gradual decrease in serum progesterone during the last 2 weeks of gestation, preceding the marked progesterone decline immediately prepartum, but they provided no statistical verification of that observation. The prepartal decline of peripheral progesterone prior to parturition in cattle is a result from a switch in placental steroidogenesis from progesterone to estrogens (Mason *et al*., 1989).

The data presented here are in agreement with the “progesterone block“ theory of initiation of parturition. The first major change in serum steroid hormones is a 10-fold increase in estrogen during the month before parturition, to a peak at about 2 days before parturition. Parturition removes the negative feedback action of progesterone and estradiol on the hypothalamo-hypophysis axis, allowing for recovery of gonadotropin function. If basal levels of progesterone were increased during the early postpartum period, luteal function might be postponed due to the negative feedback from the hypothalamo-hypophysis axis.

In conclussion, supplementation with concentrate (1.2 kg coermeal + 0.6 kg rice bran + 0.6 kg coconut cake) in Bali cows grazing on natural pasture failed to influence progesterone concentrations and patterns during estrous cycle and pregnancy.

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