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Research Article

Progesterone and Biochemical Profile of Ettawa-Saanen Crossbreed Goats in Turi Area, Yogyakarta-Indonesia

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Abstract

Background and Objective: Reproductive rate is a major factor contributing to the efficiency of milk and meat production. Genetic, environmental and physiological factors can affect the reproductive efficiency of goat. This research aimed to determine levels of the progesterone and blood profiles from Ettawa-Saanen crossbreed goats in Indonesia during normal oestrus cycle. **Materials and Methods:** Eight Ettawa-Saanen crossbreed goats (*Capra hircus*) with Body Score Condotion (BCS) 2-3, known the cycle and the oestrus detection were studied by vaginal smear methods. Investigations were done by comparing progesterone levels and biochemical blood profiles of goats with natural oestrus cycles and goats with oestrus synchronization. Blood samples were collected from the jugular vein on days 0, 3, 12 and 15 of oestrus as the representative sample of each phase of the oestrus cycle. Progesterone concentration in the blood was determined using enzyme-linked immune sorbent assay and the biochemical blood serum profile was measured using spectrophotometry. Values obtained were expressed as Mean \pm SEM and were subjected to one-way analysis of variance (ANOVA) followed by SPSS version 16.0. **Results:** The serum progesterone level of crossbreed goats was significantly higher ($p < 0.05$) in the luteal phase of the oestrus cycle than in the follicular phase. The serum progesterone concentration increased gradually from the mean basal value of 0.083 ± 0.15 ng mL⁻¹ on day-0 to 0.084 ± 0.11 on day-3 and reached the peak value of 0.23 ± 0.11 on day-12, which was maintained until day-15. The biochemical blood serum profile (glucose, albumin, cholesterol and blood urea nitrogen levels; $p > 0.05$) showed no significant difference in each phase of the oestrus cycle. **Conclusion:** It was concluded that the progesterone level was significantly different in each phase of the oestrus cycle and peaked during the dioestrus phase.

Key words: Progesterone, glucose, albumin, cholesterol, blood urea nitrogen, oestrus cycle, Ettawa-Saanen crossbreed goats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reproductive rate is a major factor contributing to the efficiency of milk and meat production. Genetic, environmental and physiological factors can affect the reproductive efficiency of goats. Measuring the progesterone levels during different physiological stages in animals is considered one of the most important parameters used to determine fertility status^{1,2}. The progesterone (P4) levels in peripheral blood of mammals provides valuable information about their reproduction status^{3,4}. Levels of progesterone were shown to determine variations in oestrus cycles of goats⁵. Several researchers have reported the concentrations of plasma progesterone during oestrus cycle of goats for several breeds⁶⁻⁹. Variations in progesterone levels in the blood regulate the oestrus cycle, which in turn affect cardiovascular and serum biochemical indices^{5,10,11}. Blood biochemical values are important tools to measure the health status of the animals and are also important as indicators of metabolic activity in lactating animals^{12,13}. Blood biochemical values has been shown in various conditions of the animal's health due to differences such as sex, season and oestrus cycle phase^{5,14,15}. Investigation of progesterone levels and biochemical blood profiles during the oestrus cycle in goats with natural oestrus cycle or without oestrus synchronisation is rare. Further, to our knowledge, there are no studies of progesterone levels and biochemical blood profiles of Ettawa-Saanen crossbreed goats in Indonesia, especially for the natural oestrus cycles without a synchronisation procedure. The purpose of the present study was to determine the progesterone, albumin, glucose, urea and cholesterol levels, as well as the relationship between these blood parameters and progesterone levels during the oestrus cycle in Ettawa-Saanen crossbreed goats without oestrus synchronisation.

MATERIALS AND METHODS

Experimental animals, location and period of research: This experiment was conducted using Ettawa-Saanen crossbreed goats (*Capra hircus*) maintained on a goat farm in Turi, Sleman Yogyakarta, Indonesia. The research was carried out from March to May 2016. The goats were under semi-intensive maintenance, housed in pens and fed 1.5 kg head⁻¹ day⁻¹ concentrate feed and 3 kg head⁻¹ day⁻¹ fresh forage and legume (*Calliandra haematocephala* and *Pennisetum purpureum* 'Mott'), with water offered *ad libitum*. The experiment was carried out using 8 mature (2-3 years of age) lactating goats with body weights varying between 35 and

40 kg and Body Score Condition (BCS) 2-3. The experiment was conducted with during the natural oestrus cycle without oestrus synchronisation.

Oestrus identification: Vaginal smears were conducted to determine the oestrus cycle of Ettawa-Saanen crossbreed goats¹⁶. Vaginal smears were collected from each goat at every day over a 60-day period to determine the timing of each phase of the oestrus cycle¹⁷. To strengthen oestrus cycle determination, the pH of the vagina was also determined. The vaginal smear and pH measurements were carried out every day. The smear collection procedure was adopted from a previous study^{16,17}. The epithelial cells were classified into superficial, intermediate and parabasal cells by using the Grunet criterion, to determine the status of oestrus phase cycle.

Blood preparation: Blood samples were collected from the eight goats following the phases of oestrus, namely, oestrus (E/day-0), metoestrus (M/day-3), dioestrus (D/day-12) and proestrus (P/day-15). Samples were collected 8 h after feeding. Blood preparation was adopted from Gaafar *et al.*⁹.

Determination of hormone concentration in plasma: The concentration of progesterone in plasma was determined using a solid phase competitive Enzyme-Linked Immune Sorbent Assay (ELISA, DRG, Germany). Each well of ELISA microtitration plate was coated with a monoclonal antibody against progesterone. The procedure was adopted from Astuti *et al.*¹⁸.

Determination of biochemical levels in plasma: Plasma total albumin, cholesterol, glucose and urea were measured using a UV spectrophotometer (Microlab 300).

Data analysis: Values obtained were expressed as Mean \pm SEM and were subjected to one-way analysis of variance (ANOVA)⁵. The statistical package used was SPSS version 16.0. The values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Level of non-pregnant progesterone in goats during the oestrus cycle: The serum progesterone level (Table 1) in goats was significantly higher ($p < 0.05$) in the luteal phase of oestrus cycle than in the follicular phase. The serum progesterone concentration increased gradually from the mean basal value of 0.083 ± 0.15 ng mL⁻¹ on day-0 to 0.084 ± 0.11 on day-3 and

Table 1: Biochemical profile and progesterone level of Ettawa crossbreed goats during the phases of oestrus cycle

Parameter	Oestrus phases				Mean
	Oestrus (Day-0)	Metoeustrus (Day-3)	Dioestrus (Day-12)	Proestrus (Day-15)	
Glucose (mg dL ⁻¹)	70.960±8.82	71.320±9.20	73.18±5.37	67.93±7.87	70.85±7.87
Albumin (mg dL ⁻¹)	37.600±7.8	36.400±3.5	37.30±2.9	36.90±2.8	37.02±4.56
Cholesterol (mg dL ⁻¹)	76.750±15.12	76.870±15.13	78.74±11.15	77.19±11.20	77.19±12.20
BUN (mg dL ⁻¹)	49.090±8.69	49.680±3.52	51.94±5.33	50.93±7.4	50.41±6.33
Progesterone (ng mL ⁻¹)	0.083±0.15 ^a	0.084±0.11 ^b	0.23±0.11 ^c	0.23±0.11 ^d	0.15±0.135

^{abcd}Total Means with different superscripts within a column differs significantly ($p < 0.05$). Values obtained were expressed as Mean \pm SEM

reached the peak 0.23 ± 0.11 on day-12. These levels were maintained on day-15.

Biochemical blood profile concentrations of goat serum in relation to oestrus phase: The biochemical blood profile serum concentration (Table 1) showed no significant differences in the serum level from the glucose, albumin, cholesterol and Blood Urea Nitrogen (BUN) levels for phases of the oestrus cycle.

In this study, a minimum level of non-pregnant progesterone serum was detected during oestrus days in goats and increased sharply during the dioestrus phase. The serum progesterone (Table 1) concentration increased gradually from the mean basal value of 0.083 ± 0.15 ng mL⁻¹ on day-0 to 0.084 ± 0.11 on day-3 and reached the peak 0.23 ± 0.11 on day-12; this level was maintained on day-15. In Ettawa-Saanen crossbreed goats, the pattern and levels of progesterone concentration increased in metestrus and proestrus phase which in luteal phase and then declined after the luteal phase. This is similar to the results in other goat breeds^{1,2,5,6,7}. Progesterone concentrations remained at the basal level throughout oestrus as shown in previous studies^{1,2,9,19}. Khanum *et al.*¹ reported a mean progesterone concentration during the follicular phase of the oestrus cycle as 0.1 ± 0.03 ng mL⁻¹ in dwarf goats and in the luteal phase as 3.0 ± 0.9 - 7.7 ± 0.6 ng mL⁻¹. This was different from the result of this study, which showed very low progesterone levels of 0.23 ± 0.11 ng mg⁻¹ during the luteal phase and 0.083 - 0.084 ± 0.11 ng mL⁻¹ during the follicular phase. The relatively low progesterone levels found in this study are similar to the findings in West African Dwarf goats²⁰, Nigerian Red Sako to goats²¹ and parturient ewes²² and sows²³. This low progesterone serum concentration may be because of the short life span and low-level functionality of the corpora lutea²¹. The low levels of progesterone circulation in this study were influenced by urea levels in plasma. Previous studies have shown cattle with high urea plasma had low plasma progesterone levels of approximately 30%²⁴. Progesterone levels were reported lower in cattle with higher blood urea levels^{25,26}. High levels of urea would inhibit binding

Table 2: Comparison level of albumin (mg dL⁻¹) in various resources

Albumin level in this research	35.38-38.66 (mg dL ⁻¹)
Pugliese <i>et al.</i> ³⁹	32.5-49
Lloyd ⁴⁰	15.0-28.6
Jovanovic <i>et al.</i> ⁴¹	21.0-33.0
Kraft and Durr ⁴²	27.0-39.0
Fernandez <i>et al.</i> ⁴³	20.0-28.0
Janku <i>et al.</i> ⁴⁴	36.7-42.7

of gonadotropin in the corpus luteum, which can be associated with the low levels and overall decrease of progesterone²⁷.

The most important indicators of energy are glucose, cholesterol and triglycerides^{28,29}. Blood glucose is one of the key nutrients affecting ovarian activity in farm animals. The concentration of glucose in the blood of animals may influence the rate of steroidogenesis and gonadotropin synthesis and secretion^{30,31}. In this study, glucose serum concentration (Table 1) was not significantly different in each phase of oestrus. This pattern of results was similar to that in dairy cows³². The glucose level was more significantly influenced by the age, onset of puberty, pregnancy stage and lactation stage^{33,34}. Notably, the range of glucose levels in the present research was normal at about 50-75 mg dL⁻¹ for the dairy goat in the lactating stage³⁵.

The mean serum albumin concentration (Table 1) in this study was relatively constant during the different phases of the oestrus cycle. This result was similar to those in previous studies⁵. The mean of the serum albumin concentration in this study was also similar to the range reported in previous studies^{5,36,37}. This indicates that the serum albumin level did not change during follicular growth. Similar findings were reported by Collins *et al.*³⁸ in mares. Albumin values did not significantly change during the oestrus cycle. The data range of albumin in the serum is shown in Table 2.

In this study, the albumin level was similar to the normal albumin reference range of 27-39 mg dL⁻¹³⁵. Albumin is the main plasma protein synthesised in the liver representing 50-65% of the total blood protein⁴⁵. Kaltenbach and Dunn⁴⁶ and Khan *et al.*⁴⁷ state that progesterone has an effect on the synthetic regulation of some amino acids, resulting in increased protein demand for Gonadotrophin Releasing

Hormone (GnRH) and Luteinizing Hormone (LH) during the early luteal phase. The high levels of albumin caused the increasing demand for biosynthesis of GnRH and LH amino acids to initiate ovulation.

Cholesterol is a source of energy and is a precursor of steroid hormones and bile acids and is also required for normal cell function⁴⁸. The relationship between serum cholesterol and fertility has been investigated by several researchers⁴⁹. Samarutel *et al.*⁵⁰ reported that serum cholesterol concentrations were significantly higher $p < 0.05$ in cows with ovarian disorders than in healthy cows. Guzel and Tanriverdi⁵¹ found a positive association between cholesterol levels and reproductive performance in cows. In this study, the serum cholesterol concentration (Table 1) was not significantly increased but slightly increased in the early luteal phase and declined in the late luteal phase. This pattern was similar to that reported by Tarumi *et al.*⁵², who reported that the total serum cholesterol level increased after oestrus and declined in the late luteal phase. Additionally, the total serum cholesterol levels tended to increase before the second oestrus. This tendency is similar to that in humans⁵³ and dairy cows^{54,55}. The normal level of cholesterol in dairy goats in the lactating stage is about 80-130 mg dL⁻¹³⁵, while in this study, the cholesterol level was slightly lower than the reference range, because of the differences in the area of this research and the reference.

The high level of plasma urea observed in the luteal phase was also observed in Red Sokoto goats⁵, Alpine goats²⁷, buffalo⁵⁶ and dairy cows⁵⁷. The serum BUN concentration in this study was also not significant in each phase of the oestrus cycle. The urea concentration in this study was higher than the normal range of 21.4-42.8 mg dL⁻¹⁵. According to Pugliese *et al.*³⁹, high urea levels can occur when low energy diets are provided because of decreased protein synthesis microbes⁵⁸. The high concentration of plasma urea nitrogen may cause elevated ammonia and urea concentration in the pre-ovulatory follicles and uterine fluid⁵. This situation may affect fertility by impairing embryo viabilities in lactating cows^{5,58,59}. Serum urea concentration was the lowest during the late oestrus phase, which is the phase of ovulation and embryo development⁵.

We believe the data obtained from the present study are the first reference values for Ettawa-Saanan crossbreed goats in Indonesia. This study can increase our understanding of this breed's parameters, which will help researchers in the future. The data collected from this study can be used for monitoring and evaluating health status at the farmer level and can also help improve the management of the breed as well.

CONCLUSION

Concentrations of the progesterone hormone were found to affect the phases of the oestrus cycle. Progesterone levels were found to be significantly different in each phase of the oestrus cycle and peaked during the dioestrus phase. In this study, no significant differences were observed in the biochemical blood profile levels according to the phase of oestrus cycle.

SIGNIFICANCE STATEMENT

The current study aimed to explore the level of progesterone and biochemical blood profiles of Ettawa-Saanan crossbreed goats during the normal oestrus cycle. This research was expected to result in data to be used in further research studies of Ettawa-Saanan crossbreed goats, particularly with regard to physiological variables such as steroid hormone and biochemical blood profiles.

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