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Research Article
Blood and Hormonal Profile Association with the Length of Estrous Cycle in Saanen Etawah Crossbreed Goat

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Abstract

Background and Objective: In mammals the prevalence for short estrous cycle (SEC) was increased which may cause reproductive failures. So the aim of the study was to identify the hormonal and blood metabolite profiles of Saanen-Etawah cross-breed does (SECD) which has a short estrous cycle (SEC) and normal estrous cycle (NEC). **Materials and Methods:** The does (n = 32) were divided into 2 groups, according to the length of the estrous cycle: SEC (<17 days) and NEC (17-19 days). Blood drew in each phase of the estrus cycle. All the data were analyzed using one-way ANOVA and Student t-test. **Results:** The results showed that there was a significant difference ($p<0.05$) between albumin, cholesterol and progesterone levels in SECD with SEC and NEC. The albumin and progesterone level in SECD with SEC were significantly lower rather than in SECD with NEC but had higher cholesterol level rather than the SECD with NEC. **Conclusion:** In conclusion, there was a significant difference in some blood profile and hormonal between SEC and NEC does.

Key words: Saanen-Etawah, crossbreed goats, metabolite profiles, steroid hormones, blood profile, short estrous cycle, normal estrous cycle

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

INTRODUCTION

The incidence for short estrous cycle (SEC) was increasing in mammals[1-5]. The SEC was accompanied by abbreviated luteal phase, so animals with potential for SEC at the breeding time would not maintain pregnancy and triggered reproductive failures. Wilks *et al.*[1] suggested that short luteal phase in mammals were caused by an altered ratio of LH and FSH, resulting in the ovulation of an immature follicle incapable of sustaining luteal function. In ruminants, short cycle due an inadequate luteal phase occurs in some specific physiological situation. It also clear that the SEC is due to the shortened lifespan of the corpus luteum (CL), the CL regressed at 6-7 days after the previous estrous[6]. Follicular function prior to short estrous cycle is clearly defective and pre-ovulatory circulating estrogen concentration was reduced in each of the physiological situations that result in a SEC due to the shortened luteal phase. The estrous cycle is regulated by hormones of the hypothalamus, the pituitary, the ovaries and the uterus[7]. These cycles were usually accompanied by low rates of conception because CL regress when the embryo was still not capable of producing sufficient interferon t (IFN-t, hormone responsible by maternal-fetal recognition) to block luteolysis, leading to early embryonic death[8].

The assessment of the nutritional and health status in ruminants could be by determining certain blood metabolic concentration[9]. Metabolites concentrations indicated the extent of the metabolism of energy, proteins and other nutrients in the ruminants[10]. Changes in the circulation metabolites were an important indicator of the metabolic status of a ruminant and luteal function. Inadequate of the CL as demonstrated by deficiency secretion of both estrogen and progesterone had a basic in defective follicular maturation[11]. Previous research suggested that normal blood levels of various biochemical constituents were indispensable for the normal function of various systems of the body including reproductive system[6]. The present study aimed to elucidate the association between certain biochemical blood serum and steroid hormones in short and normal estrous cycle in Saanen-Etawah cross-breed does (SECD). Information about blood metabolite and hormonal level in SECD also was very limited. The aims of the present study were to identify the differences between blood metabolite and hormonal level between SECD with SEC and NEC (normal estrus cycle).

MATERIALS AND METHODS

Animal and blood sampling: The study was used eight SECD (Saanen Etawah Crossbreed Does) raised by local farmer located in Turi, Sleman, Yogyakarta. The research was carried out from March-May, 2017. The does were maintained with semi-intensive maintenance were housed in pens and were fed with 1.5 kg/head/day concentrate feed and 3 kg/head/day fresh forage and legume (*Calliandra haemotocephala* and *Pennisetum purpureum* cv. Mott) and water was offered *ad libitum*. The study was carried out 32 matured (2-3 years of age) lactating does with body weight varying 35-40 kg and BCS 2-3. The experiment was performed with natural estrus cycle without estrous synchronization. Blood sample collection were initiated in each phase of the estrous cycle which determined by using vaginal smear and vaginal acidity[12]. Blood samples were collected from *vena jugularis* and the serum blood preparation was adopted the step from the previous publication[12]. The estrous cycles were classified as short (<17 days) and normal (17-25 days).

Determination of hormone concentration in serum: The concentration of progesterone and estrogen in serum were determined a solid phase competitive enzyme-linked immune sorbent assay (ELISA, DRG, Germany). Each well of ELISA micro-titration plate was coated with monoclonal antibody against progesterone and estrogen. The procedure adopted from Sitaresmi *et al.*[12].

Determination of selection blood metabolite level: Serum total albumin, cholesterol, glucose and urea were measured using the UV spectrophotometer (Microlab, 300)[12].

Statistical analysis: The data analysis using t-test between SEC and NEC to find the significant differences and also using one way ANOVA to analyzed the significant differences in each phase of the estrous cycle at $p<0.05$ and $p<0.01$.

RESULTS

Blood biochemistry: Serum biochemical constituents (Mean±SE) in does with SEC and does with NEC were given in Table 1. Mean concentrations of serum glucose in does showed not significantly different ($p<0.05$) between two groups but the glucose level in does with SEC showed

Table 1: Blood and hormonal profile in Short Estrous Cycle (SEC) goat and Normal Estrous Cycle (NEC) goat (mean±SE)

Parameters	SEC (n = 16)	NEC (n = 16)
Glucose (mg dL^{-1})	72.619±7.96	68.938±10.49
Albumin (mg dL^{-1})	3.680±0.59*	4.079±0.41*
Cholesterol (mg dL^{-1})	82.056±9.49**	65.169±7.70**
Urea (mg dL^{-1})	50.644±7.33	48.631±6.56
Estrogen (pg dL^{-1})	45.201±19.11	46.319±16.68
Progesterone (ng dL^{-1})	0.099±0.06**	0.203±0.15**

*p<0.05, **p<0.01

Table 2: Blood and hormonal profile in Short Estrous Cycle (SEC) goat and Normal Estrous Cycle (NEC) goat in each phase of estrous cycle (mean±SE)

	Estrous		Metestrous		Diestrous		Proestrus	
Parameters	SEC	NEC	SEC	NEC	SEC	NEC	SEC	NEC
Glucose	70.650±11.39	67.450±9.76[e]	72.650±10.66	78.850±13.50[f]	67.320±5.54	70.325±2.13[g]	73.975±5.92**	59.125±1.86**[h]
Albumin	3.890±1.14	3.903±0.35	3.443±0.34*	4.365±0.53*	3.795±0.28	3.838±0.24	3.592±0.31*	4.210±0.37*
Cholesterol	86.200±11.05*	65.250±3.29*	82.600±11.76*	61.550±6.61*	75.450±6.51	70.975±9.65	83.975±7.88**	62.900±8.92**
Urea	50.025±11.91	47.625±3.04	51.025±2.58	46.725±5.91	49.500±3.96	54.650±10.08	52.025±9.97	45.525±1.32
Estrogen	62.593±24.16	61.044±7.07[e]	39.201±14.54	33.122±4.73[f]	28.981±5.50	29.053±3.61[g]	50.029±12.97	62.058±7.47[h]
Progesterone	0.050±0.04	0.145±0.19	0.058±0.03	0.140±0.15	0.136±0.02	0.247±0.14	0.153±0.05	0.280±0.13

*p<0.05, **p<0.01

higher rather than the does with NEC. The data showed the albumin serum from does with SEC was significantly ($p<0.05$) lower (3.680±0.59 mg dL^{-1}) rather than does with NEC (4.079±0.41 mg dL^{-1}). The cholesterol serum from does with SEC was significantly ($p<0.05$) higher (82.056±9.49 mg dL^{-1}) rather than does with NEC (65.169±7.70 mg dL^{-1}). No significant difference in the concentration of blood urea nitrogen in SEC does and NEC does was observed in the present study.

The recent study also found the significantly different ($p<0.0.5$) in the biochemical variable in each phase of the estrous cycle between SEC and NEC does (Table 2). The data of glucose showed significant different ($p< 0.01$) between SEC and NEC does in proestrus phase. The data of albumin showed significant different ($p<0.05$) between SEC and NEC does in metestrous and proestrus phases. The data of cholesterol showed significant different ($p<0.05$) between SEC and NEC does in estrous, metestrous and proestrus phases (Table 2).

Levels of estradiol 17-b and progesterone: The data showed a significant different ($p<0.05$) of estrogen level between SEC and NEC in SECD (Table 1). The progesterone in the SECD with SEC (0.099±0.06 ng mL^{-1}) was significant lower rather than in the SECD with NED (0.203±0.15 ng mL^{-1} in NED) but the estrogen in the SECD with SEC was bit lower rather than SECD with NED.

DISCUSSION

Blood biochemical investigation provides valuable information and an indication about the general health of animals and also important indicators of animal nutritional status[13]. Mean concentrations of serum glucose (Table 1), it does with SEC and NEC showed not significantly different ($p<0.05$) between two groups but the glucose level in does with SEC showed higher rather than the does with NEC. The higher level of glucose in SEC, it was the effect of low progesterone level in SEC. Progesterone raised the level of insulin in the bloodstream, which insulin is a hormone that is secreted to decrease glucose level. Also inadequate of utilization glucose thus reduce the hypothalamic release of GnRH, leads to a decrease in LH release and eventually delays or prevents ovulation[14].

Albumin, the major serum protein, binds a wide variety of lipophilic compounds including steroids, other lipophilic hormones and various phytochemicals and xenobiotics that bind to receptors for steroids and other lipophilic hormones[15]. The data showed the albumin serum from does with SEC was significantly ($p<0.05$) lower rather than does with NEC. The difference data might could be an indicator that low albumin could cause SEC in SECD. Lack of albumin caused the onset of weak libido, calm, anestrus, repeat breeding, early embryonic death. It also the higher albumin level could bind to progesterone and prevent its detrimental effect on the endometrium and prevent the premature luteinization.

The data showed the cholesterol serum from does with SEC was significantly ($p<0.05$) higher rather than does with NEC. The higher level of progesterone in NEC does rather than SEC does lead the stimulation with estrogen to produce lipoprotein lipase, the protein which the cell uses to break down triglycerides and resulting in a lower triglyceride level in the bloodstream together with estradiol. The other reasons were the decrease in low-density lipoprotein (LDL) cholesterol induced by estrogen by an increase in LDL clearance[16]. It was similar with the previous study which declared that many of the adverse effects of progesterone on lipid and lipoproteins, such as decreasing HDL cholesterol levels were thought to be due to activation of the androgen receptor[17]. Another reason is the estradiol reduced the plasma cholesterol synthesis by inhibition of HMG CoA reductase, which is the first enzyme in cholesterol biosynthesis[18]. Samarutel *et al.*[19] reported that serum cholesterol was significantly higher ($p<0.05$) in animals with the ovarian disorder than the healthy animal.

No significant difference in concentration of blood urea nitrogen in SEC does and NEC does was observed in the present study. The values showed in SEC does were apparently higher but not significantly different and its similar with the previous study[20].

In the NEC, serum estradiol from preovulatory follicle might induce uterine progesterone receptor which was required to establish progesterone dominance of subsequent ovulation. The dominance of progesterone resulted in inadequate uterine progesterone receptor synthesis and the estrogen concentration would decrease like the result in this study slightly lower in SEC rather than NEC even though not significantly different. The uterus might lose progesterone dominance earlier which this would be initiated the positive feedback loop between oxytocin and PGF_{2a} earlier in the SEC[21]. In cows exhibiting a SEC, decreased serum concentration in estradiol have been observed at estrous compared with cows having a subsequent NEC[22] and it was similar with current results of the study.

The data showed a significantly different ($p<0.05$) of progesterone level between SEC and NEC in SECD, the progesterone in SECD with SEC was lower rather than SECD with NED. The CL formed is smaller in the SEC, 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 3b-HSD and sTAR[23]. During luteal regression, initially decreased in the concentration of progesterone do not appear to be due to loss of steroidogenic luteal cells. Vascularization could influence development individual capillary network, while it can receive nutrients and oxygen by passive diffusion from the stroma blood vessel. The decreased secretion of progesterone caused decreased luteal blood flow by PGF2a and thus may reduce delivery of nutrients and substrate of steroidogenesis. It also similar with Chohan *et al.*[24] which suggested that the difference in progesterone level during SEC and NEC due to the luteal failure during SEC.

In the SEC, the CL could demine early because prostaglandin excreted from uterus quickly. In day 6 after estrous the uterus more sensitive to the luteolytic effect of prostaglandin[25]. The decreased concentration estradiol in SEC may, therefore, result in inadequate uterine progesterone receptor synthesis. Thus, the uterus may lose progesterone dominance earlier following ovulation. This would allowed premature synthesis of oxytocin receptor and thereby initiate the positive feedback loop between oxytocin and prostaglandin earlier in the uterus. Oxytocin stimulates the uterus to secrete prostaglandin, in turn, prostaglandin cause release of oxytocin from the ovary and make high amplitude pulse of prostaglandin during luteal regression[21]. Also, lack of progesterone in the uterus may allow the synthesis of endometrial oxytocin receptor. Thus, the uterus becomes responsive to oxytocin and a positive feedback loop between prostaglandin and luteal oxytocin develops.

CONCLUSION

There was significantly different in some blood profile and hormonal between SEC and NEC does. Which the short estrous in the animal could be caused by the low progesterone level in does. The circumstantial evidence presented supports this assumption but it is hoped to confirm subsequently this hypothesis by clinical examination.

SIGNIFICANCE STATEMENT

This study discovered the information about blood metabolite and hormonal level in SECD with NEC and SEC that can be beneficial for base data in other research using SECD. This study will help the researchers to uncover the critical areas of reproductive abnormalities.

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