

Profiles of Steroid Hormones on Follicular and Luteal Phase in Saanen Etawah Crossbred (SAPERA) Does

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ABSTRACT

Reproductive rate may be a major issue conducive to the potency of animal production such as milk and meat. Determining estrogen, progesterone also cortisol during the phase of estrus in goats is supervised one of the most necessary parameters used to examine reproductive status. The purpose of this study was to determine the changes of estrogen, progesterone and cortisol concentrations in follicular and luteal phases. Thirteen does of Saanen Etawah crossbred (SAPERA) with body condition score of 2-3 were used. The does were fed using concentrate, forage with ad libitum water. Data collection was done without treatment during natural estrus cycle. The phase of the estrus cycle were determined using vaginal smears that were collected from each does every day over a 60 days period. Simultaneously, the blood was sampled following estrus cycle, follicular and luteal phase. The concentration of plasma estrogen, progesterone and cortisol was determined by solid phase competitive enzyme-linked immune sorbent assay. All the data were subjected to the Student T-Test. The result shown the concentration of steroid hormones significantly different in the phase of estrus cycle. The estrogen and cortisol level were significantly ($p < 0.05$) higher in follicular phase. The progesterone level was significantly higher in luteal phase. In conclusion, the concentrations of steroid reproductive hormones in SAPERA goats were related with the stage of estrus cycle.

Keywords: Cortisol; Estrus phases, Estrogen, ELISA, Progesterone.

1. INTRODUCTION

Goat was known to have a vital role in actuating domestic financial gain, notably for farmers in small scale. There are several goat breeds in Indonesia that are used for milk production one of the new breed which recently developed were Saanen Ettawah crossbred dairy goats [1]. Reproductive rate were considered as a major issue conducive to the potency of animal production such as milk and meat. Factors such as genetic, environment and physiologic will affect the reproductive potency of dairy goats [2]. Determining the steroid reproductive hormones such as estrogen, progesterone, and cortisol during the phase of estrus in goats is supervised one of the most necessary parameter used to examine reproductive status [3]. Estrogen increased higher concentration at the end of follicular

phase and lowered within 3-4 days post ovulation or in the early luteal phases [4], but this condition was opposite with the progesterone levels [5], [6]. The progesterone level tends to be in the lowest concentration in follicular phase and increased sharply in luteal phases [7]. Hypothalamus pituitary gonadotropins (HPG-axis) is the key regulation of the reproductive axis. Those organs pulsatile determines the pattern of secretion of gonadotropins follicle stimulating hormones (FSH) and luteinising hormones (LH) which affected the ovarium environment [8]. The pulsatile of FSH and LH tends to stimulate the ovum cycles, which this ovum cycles caused the variations of estrogen and progesterone levels along with the formations of dominant follicle and corpus luteum [9]. In the reproduction context, cortisol as glucocorticoids (GCs) are often considered to have negative impacts. However, some studies declared that

the fluctuation of cortisol across the estrus cycles in short term would increase and promote healthy ovarian function [10]. The estrus behaviour in goat was closely related with another steroid hormone there is cortisol, in past studies shown that the cortisol levels were elevated during the follicular stages [11]. Studies on the determining of steroid hormones during the follicular and luteal phases were limited especially in natural estrus cycle without synchronizations. Therefore, the purpose of this study was to determine changes of the estrogen, progesterone and cortisol concentrations in follicular and luteal phases.

2. MATERIALS AND METHOD

2.1. Experimental Animals

Thirteen females lactating SAPERA does (BCS 2-3) were used in present study. The does were kept under the managements of the traditional farmers in Yogyakarta. The does were kept in pens and fed with 1.5 kg head/day concentrate and 3 kg head/day fresh legumes also forages. *Ad libitum* water was also given during present study. The data collection was done without treatment during natural estrus cycle.

2.2. Estrus Detection and Sampling

The estrus cycle of SAPERA can be determined by vaginal smears method [12]. The phase of the estrus cycle were determined using vaginal smears that were collected from each does every day over a 60 days period [13]. The obtained smears would showed epithelial cells that were classified into superficial, intermediate and parabasal cells from the Grunet criterion. The collection timing of blood samples was based on the stages of estrus cycle which were follicular phase and luteal phase. The blood samples collected 8 hours after feeding time [14].

2.3. Determination of Hormones Concentration

The estrogen, progesterone and cortisol concentrations was determined by 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 the steroid hormones [15].

2.4. Data Analyses

The data obtained were expressed with mean±SEM and subjected to the Student T-Test. The statistical package used was SPSS version 25.0 (IBM, Chicago, IL, USA). Probability at $p < 0.05$ was considered statistically significant.

3. RESULT AND DISCUSSION

There were significant differences in the concentrations of steroid hormones in follicular and luteal phase of estrus cycle (Table 1). The estrogen level in follicular phase was significantly higher ($p < 0.05$) rather than in luteal phase. Similar changes were observed in cortisol levels, the cortisol level in follicular phase was significantly higher ($p < 0.05$) rather than in luteal phase. The progesterone level in luteal phase was significantly higher ($p < 0.05$) rather than in follicular phase.

Table 1. Comparison the level of steroid hormones in follicular and luteal phase

| Estrus phase | N | Follicular phase | Luteal Phases |
|----------------------|----|-------------------------|-------------------------|
| Estrogen (ng/ml) | 13 | 59.86±2.07 ^a | 29.31±0.97 ^b |
| Progesterone (pg/ml) | 13 | 0.04±0.01 ^b | 0.14±0.11 ^a |
| Cortisol (ng/ml) | 13 | 10.74±6.15 ^a | 6.15±3.91 ^b |

^{ab} total means with different superscripts within a column differs significantly ($p < 0.05$)

Generally, ovarian cycle can be divided into follicular and luteal phases. The follicular phase correlate with the wave of follicle development which could supplies the ovulation and implicates the follicle maturation which are influenced by gonadotropins until ovulation [16].

The data from present study showed significant different ($p < 0.05$) in all variable which were estrogen, progesterone and cortisol levels in follicular and luteal phases. The obtained estrogen hormone level was significantly higher ($p < 0.05$) in the follicular phase compared to its level in the luteal phase (Table 1) and this result was similar with the previous studies [1], [17]. The rises of estrogen concentrations was cognate with follicular development under the leverage of HPG axis activity [7]. The culmination level of estrogen generally accord to the follicle development when approaching ovulation [12], [18]. At the early follicular phase, the anterior pituitary would secrete FSH which stimulates the development of follicles as the alteration from primary follicle to the dominant follicle caused the increased of peripheral estrogen which produced in those follicle especially in theca cells beside from the other glands [19]. Following with the rise of estrogen also stimulated the negative feedback for FSH released and enhanced the LH pulsatile that causes ovulation, after the ovulation the follicle transform into the corpus luteum and the granulosa cells started to produce progesterone and execute the estrogen released and caused the estrogen tend to low in luteal phases [12]. It has been known that estrogen hormone was responsible for the estrus

behavior, duct development in the mammary glands and the secondary sexual characteristic.

In the opposite, in this study, the progesterone hormone level was significantly higher ($p < 0.05$) in luteal phase compared to follicular phase which similar with some previous study [20]. In the cyclic does, the progesterone levels shorten to a minimum levels during the follicular and gradually rise to the maximum concentration in the luteal phases [1], [21]. The progesterone concentration constantly remains low during the follicular until the ovulation and begins slowly increased after the ovulation as the corpus luteum formed and development [5] or in the other words the progesterone levels was related with the commencement of corpus luteum [20]. The high of progesterone level following the ovulation would obstructs the release of GnRH, FSH and LH to prepare the uterus for the possible pregnancy and suppress estrus behavior in does. If the failure to establish pregnancy occurs, it would cause the secretions of the prostaglandin in the uterus and enhanced the corpus luteum regression and stimulated the ovarium to start a new estrus cycle and depressed the progesterone level in follicular phases [22]. The progesterone data in this study relatively low which in the luteal phase tend under 1 ng/ml but this result was similar with other findings in Nigerian Red Sakoto goats [23] and sows [24]. This low progesterone serum concentration might be affected by the shorten life span of corpus luteum which also found in this study with the length of estrus for 13 goats were under 18 days.

The present study showed the cortisol escalation during the follicular phase, this phenomenon has important role in promoting receptive behavior, stimulating gonadotropins, facilitating ovulation and reducing the inflammation during estrus phase [13] but still in under the threshold physiological level. The short-term increased of cortisol might affecting reproduction function in positive way [12]. Cortisol was decreased and remains low significantly ($p < 0.05$) in the luteal phase which similar from prior study in elephants [10], musk shrews, humans [25], pandas [26] and sheep [27]. The actions of cortisol was under leverage from locally expressed enzymes which onus to activate or inactive cortisol especially the estrogen level [11]. The previous study exclaimed cortisol helps to coordinate important events in estrus cycles through a combination of agonistic and antagonistic effect. During the luteal phase the cortisol tend to low may be required for proper implantation and luteal function [25]. The increased of progesterone tends to activate of 11β HSD to inactivates cortisol. Data and information above were expected to be used as base information for the subsequent study in SAPERA does.

4. CONCLUSION

The present study showed that the concentrations of steroid reproductive hormones in SAPERA goats were related with the stage of estrus cycle which were follicular and luteal phase.

AUTHORS' CONTRIBUTION

SK, PIS, and DTW contemplated and executed the field work for data collection, also drafted the manuscript paper. SK and PIS did the literature search and statistical analysis. All authors executed the interpretation of analyzed data and revised the manuscript. The research design was supervised by DTW and YS. All of the involved authors had reviewed and approved the final version of manuscript.

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