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Research Article
Estrus Detection Through Vaginal pH in Saanen Etawah Crossbreed Goats

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Abstract

Background and Objective: In goats, weak estrus behavior is potentially caused by a decline in reproductive efficiency. In the present study, aim was to measure the vaginal pH to determine the accuracy of lust detection in Saanen Etawah crossbreed goats. **Materials and Methods:** Thirteen Saanen Etawah crossbred does (*Capra hircus*) with body condition score of 2-3 were used. The dose were fed concentrate, provided forage and water *ad libitum*. The experiment was conducted during the natural estrous cycle. Vaginal smears were collected from each dose every day over a 60 days period to determine the time of each phase of the estrous cycle. The vaginal pH was measured using pH indicator paper; simultaneously, the blood was sampled from the caudal vein into anticoagulant-coated tubes following estrous cycle, covering estrus (E/day-0), metestrus (M/day-3), diestrus (D/day-12) and proestrus (P/day-15). The concentration of plasma estrogen and cortisol was determined by solid phase competitive enzyme-linked immune sorbent assay and the correlation between vaginal pH with each phase of estrous cycle and estrogen level was found. **Results:** The changes in vaginal pH were confirmed with estrogen and cortisol profile at each estrous phase. The highest vaginal pH and estrogen content were found during estrus, whereas, the lowest vaginal pH and estrogen content was found during diestrus. Furthermore, the cortisol level was significantly different in each phase of the estrous cycle; the highest level of cortisol was observed during proestrus. **Conclusion:** The correlation between vaginal pH and estrous phase indicates that the vaginal pH can be a useful parameter for estrus detection.

Key words: Vaginal pH, plasma estrogen, estrous phase, plasma cortisol, estrus detection

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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

Goat production has an important role in generating income, particularly for small-holder farmers. In Indonesia, there are several goat breeds that are used for milk production, such as Etawah, Etawah crossbreed and Saanen dairy goat. Some valuable characteristics of goats are: they breed quickly and the litter size is greater than 1. However, their reproductive performance is low in field[1].

The detection of estrus remains the major problem. To improve estrus detection, various factors have to be observed. On one hand goat has to express estrus and on the other hand the farmer has to detect it[2]. The detection of estrus based on visual changes is often difficult due to silent heat. One of the attempts to facilitate the detection of lust is by observing the changes in vaginal pH.

During estrous cycle, the vaginal pH is influenced by the secretion of steroid hormones. Studies have reported that dynamic vaginal pH changes occur in each phase of the estrous cycle. Antonov *et al.*[3] reported that the vaginal pH in bitch was the lowest during ovulation. Similar changes occur in mares and goats; a decrease in vaginal pH has been considered to be characteristic and indicative of ovulation[4,5]. Rasad and Setiawan[6] reported that during the estrus phase, both the vaginal temperature and pH increase in local sheep and they were a manifestation of increased estrogen level in the blood. Estrogen attained higher levels at estrus phase and decreased within 3-4 days post estrus[6-8]. When an animal is in the estrus phase, they become restless and this condition is closely related to cortisol level. However, elevation in cortisol level during this phase did not exceed the physiological levels during rest stage, but showed an increase at the time when estrus behavior was the maximum[9]. Previous studies have reported that an elevation in the level of glucocorticoid, such as cortisol titers, associated with chronic stress can lead reproductive failure[10]. Therefore, studies on reproduction dysfunction mainly focus on the suppressive effects of cortisol. However, cortisol also plays a critical role in the promotion of healthy reproductive function[11-13]. Under physiological condition, a short-term increase in cortisol level can have a positive effect on reproductive function.

Studies on the characteristics of vaginal pH and estrogen and cortisol levels during the estrous cycle in Saanen Etawah crossbreed goats are limited. Therefore, the aim of this study was to observe the changes in vaginal pH during the estrous cycle and its association with the level of estrogen.

MATERIALS AND METHODS

Experimental animals: Thirteen female lactating Etawah-Saanen crossbreed (SAPERA) dose with 2-3 body condition score were used in this study. The dose were under semi-intensive condition in a goat farm in Turi, Sleman, Yogyakarta, Indonesia. The dose were housed in pens and fed 1.5 kg head day^{-1} concentrate and 3 kg head day^{-1} fresh forage and legumes (*Calliandra haematocephala* and *Pennisetum purpureum* 'Mott'), with *ad libitum* water. The experiment was conducted during the natural estrous cycle.

Ethnic approval: All animal procedures were performed according to the guidelines for the care and use of experimental animals of Faculty of animal Science, Universitas Gadjah Mada.

Ethical clearance for the use of experimental animals has been stated in a declaration letter and signed by the dean of the faculty.

Estrous detection and sampling: Vaginal smears were collected from each dose every day over a 60 days period to determine the time of each phase of the estrous cycle. When each cycle was determined, the vaginal pH was measured along with blood collection at estrus (E/day-0), metestrus (M/day-3), diestrus (D/day-12) and proestrus phases (P/day-15). The pH of the vaginal mucus was determined using a paper pH indicator (Merck®, Darmstadt, Germany) by gluing pH indicator strip on the vaginal mucous and then matched with standard pH 1-14. This vaginal pH measurement was adopted from the method reported by Antonov *et al.*[3].

Blood sample (10 mL) was collected from the caudal vein of does into anticoagulant-coated tubes. The blood samples were immediately placed on ice and centrifuged at 3500 rpm (1372 g) for 15 min at 4°C after sampling. The plasma was decanted and stored at -20°C until further analysis.

Determination of hormone concentration in the plasma: The plasma concentration of estrogen and cortisol was determined by solid phase competitive enzyme-linked immune sorbent assay (ELISA) with a commercially available kit (DiaSys Diagnostic System, Holzheim, Germany), according to the manufacturer's instruction.

Data analyses: The data obtained are expressed as mean±standard error of the mean (SEM) and were subjected

to the one-way analysis of variance (ANOVA). The statistical package used was SPSS version 16.0 (IBM, Chicago, IL, USA). Probability at $p \leq 0.01$ was considered statistically significant.

RESULTS

There were significant differences in the vaginal pH at each stage of the estrous cycle. The highest vaginal pH was observed during estrus (10.26±1.36) and the vaginal pH tended to decrease to the next stage of the cycle (Table 1). Similar changes were observed in the estrogen level. High levels of estrogen were observed during estrus 59.14±13.41 pg mL^{-1} and tended to decrease during metestrus and diestrus. The lowest estrogen level was observed in diestrus. However, on day 15 (proestrus), the level of estrogen increased again 58.43±10.83 pg mL^{-1} until reaching the maximum level on the day of estrus (Table 1).

The level of cortisol also exhibited significant difference in each phase of the estrous cycle. The level of cortisol was the highest during proestrus. The lowest cortisol level was observed during metestrus, which started to increase again during diestrus (Table 1).

DISCUSSION

The data showed significant different ($p<0.05$) in all of variable which were vaginal pH, estrogen and cortisol level in each phase of estrus cycle, with the highest level of vaginal pH and estrogen were in estrus phase but the highest cortisol level was in proestrus phase.

In Saanen Etawah crossbreed goats, the vaginal pH increased during estrus and then decreased in the subsequent phase, similar to that observed in other goat breeds[5]. The decrease in vaginal pH immediately after estrus was found to be characteristic and indicative of ovulation[4,5]. Moreover, vaginal mucus was watery and colorless during estrus, indicating changes in ion composition. Gaafar *et al.*[14] reported that the decrease in vaginal pH depends on the accumulation of ions, such as hydrogen, sodium and chloride, in the vagina. Besides that, the accumulation of glycogen and protein also contributes to a decrease in vaginal pH[14,15].

Table 1: Vaginal pH and estrogen and cortisol levels during the estrous cycle in Saanen Etawah crossbreed goats

	Parameters		
Estrous cycle stage	Vaginal pH (n = 13)	Estrogen (pg mL^{-1}, n = 13)	Cortisol (ng mL^{-1}, n = 13)
Estrus	10.26±1.36[a]	59.14±13.41[a]	6.23±5.43[a]
Metestrus	7.97±0.95[b]	33.21±9.28[b]	4.79±3.39[b]
Diestrus	7.74±1.20[c]	27.57±4.40[c]	6.66±3.90[c]
Proestrus	7.69±0.77[d]	58.43±10.83[d]	16.11±6.09[d]

[a-d]Means with different superscripts within a row differ significantly ($p<0.05$)

In the Saanen Etawah crossbreed goats, the increase in estrogen level occurred during estrus and then decreased during the luteal phase, which is similar to that observed in other goat breeds[5,8]. Based on the observation, the visual signs and changes in animal behavior were observed during estrus, including slightly swollen vulva, vaginal discharge, reddened vulva, constant tail wagging and mounting on another dose or let another doe mount her. The changes in behavior during estrus are a manifestation of increased estrogen level in the blood. The hormonal system that regulates estrus is centered on the gonadotropin hormone of the anterior pituitary gland and the ovarian hormones (FSH and estrogen). The changes in FSH and estrogen levels resulted in physiological changes in the does, which manifested in physical changes that included swelling and reddening of the vulva[16].

Moreover, the results of the present study showed an increase in the level of cortisol during proestrus and decrease during metestrus, which is similar to that observed in other animals[5]. Increase in the level of cortisol during proestrus has an important role in promoting receptive behavior, stimulating gonadotropins, facilitating ovulation and reducing the damage caused by inflammation[17,18]. In estrous cycle, the level of cortisol increased during the follicular phase, culminating with a peak around ovulation (during proestrus). The level of cortisol then decreased quickly at the beginning of luteal phase or during the metestrus phase when the progesterone level increased and remained low throughout most of the luteal phase[13]. This was similar to the findings in other species, such as elephants[13], musk shrews[19], humans[20], pandas[21] and sheep[22]. The increase in cortisol level is not due to the overall upregulation of adrenal activity, but due to a change in the amplitude of the circadian rhythm[20]. The decrease in vaginal pH along with the appearance of symptoms of lust explains that the vaginal pH affects reproduction behavior and physiological changes in the reproductive organs, which is regulated by an increase in the level of estrogen and cortisol in the blood[6-8]. All of the data could be used as base informations for the future study in Saanen Etawah crossbreed goes (SAPERA) and the vaginal pH method could be another method for identifying estrus cycle in animal.

CONCLUSION

The present study demonstrated that the vaginal pH is related to the estrous phase. Therefore, vaginal pH could be a useful parameter for lust detection in goats and other livestock.

SIGNIFICANCE STATEMENT

This study is relevant and makes a significant contribution to the field because it demonstrates the correlation between vaginal pH with each phase of estrous cycle and estrogen level. The highest vaginal pH and estrogen level were found during estrus. The cortisol level was significantly different in each phase of the estrus cycle. The correlation between vaginal pH and estrous phase indicates that the vaginal pH can be a useful parameter for estrus detection.

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