

Exfoliative vaginal cytology and vaginal acidity profile in Ettawa-Saanen grade does

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Abstract

The knowledge of the reproductive physiology of estrus cycle is important for animal management. The stage of the estrus cycle was predicted through the morphologic, endocrine and secretary changes occurring in the ovaries and the tubular genitalia during the estrus cycle, which had been associated with the level of steroid sex hormone. Vaginal smear is a simple technique to determine the stages of estrus cycle and it is a useful tool in determining optimum standing heat in does. The aim of this study was to determine the proportion of exfoliative vaginal cell during the various stages of estrus cycle using vaginal smear techniques in Ettawa-Saanen grade does. Thirteen healthy Ettawa-Saanen grade does with average weigh 35-40 kg, age

of 3-4 years and had period of estrus 16-18 days in lactating period were used. All does were in natural estrus cycle without synchronization. A vaginal smear and vaginal pH were collected from swabbed vaginal epithelium in does. Smear was stained with 3% Giemsa and observed microscopically. Progesterone level of the collected blood serum from vena jugular was assayed by enzyme linked immunosorbent assay (ELISA). All data were collected on day 0, 3, 12 and 15 of estrus. Period of estrus was predicted from the last three periods of the earlier recording of estrus cycles. The result showed the proportion of vaginal cell was significant different in each phase of estrus cycle. Proportion of parabasal, intermediate, and superficial epithelium cell in the particular period of estrus cycle were 0; 8.2375 ± 6.1301 ; 91.7624 ± 6.1302 in estrus phase, 2.29 ± 6.87 ; 38.56 ± 31.39 ; 59.14 ± 34.37 in met-estrus phase, 83.22 ± 17.66 ; 15.08 ± 14.91 ; 1.70 ± 3.75 in diestrus phase, 9.56 ± 18.13 ; 70.57 ± 31.86 ; 19.87 ± 26.77 proestrus phase respectively. The vaginal pH showed in estrus phase were 7.17 ± 0.54 , in met-estrus were 6.17 ± 0.30 , in diestrus phase were 5.79 ± 0.43 , and in proestrus phase were 5.92 ± 0.474 . Progesterone levels showed in estrus phase were 0.083 ± 0.15 , in metestrus phase were 0.084 ± 0.11 , in diestrus phase were 0.23 ± 0.11 and in proestrus phase 0.23 ± 0.11 . In conclusion dominant proportion of superficial cell, a high vaginal pH value and the lowest level of progesterone that occurred in estrus period might be used as the base for determining optimal time for insemination.

Key Words: Vaginal cytology; estrus cycle; Ettawa-Saenen grade does, pH vagina, progesterone.

1 Introduction

The changes during normal estrus cycle is related to the basic concept of the ovulation process, regression of the corpus luteum, pregnancy and birth. (1). The application of vaginal cytology as a useful tool for estrus detection in modern breeding stations has been described for some species and breeds of animals (2). In the normal cycling female livestock, morphologic, endocrine and secretory changes occurring in the ovaries and the tubular genitalia during

the estrus cycle usually depict the stages of the cycle, these changes have been associated with levels of steroid sex hormones (3). Vaginal cytology changes during estrus cycle have been studied in goat (3), cow (4), swine (5) and ewes (6). The morphology of exfoliated cells has been found very useful to determine the physiological and pathological status of female animal as well as a tool for hormonal bioassay in several animal species (7,8). The variations of vaginal mucosa at each phase of estrus cycle was occurred under the effect of estrogen and progesterone hormones that secreted from the ovary in the absence of infection (3,9). Exfoliated cells in the vaginal lumen are the result of rising peripheral estrogen which causes the vaginal wall thickens As the outermost layer moves further from the vascular supply, the cells keratinize and detach from the wall. The relative proportion of different types of vaginal epithelial cells can be used as a marker of the endocrine environment (9). Thus, the exfoliated cells are a normal occurrence during estrus cycle of animal (7,10,12). Moreover the vaginal cytology may be used clinically to evaluate the hormonal status or to characterize the reproductive stages of animal (6). And also during the different stages of estrus cycle make the variation of vaginal pH values (13) and until right now there have been a limited number of studies on the pH values of vaginal secretions in the does. However, there has been no systemic study of vaginal cytology and vaginal pH of Ettawa-Saanen grade does in Indonesia especially in natural estrus cycle without a synchronization procedure. Thus, the purpose of the present study was to evaluate morphologic characteristics of the epithelial cells and pH values from the vagina along with measurement of serum progesterone concentration during estrus cycle that has not being studied before in Ettawa-Saanen grade does in Indonesia.

2 Literature Review

Vaginal exfoliative cytology is reported to be a sensitive indicator of the stage of estrus cycle in many species presumably reflecting the balance between the influence of estrogen and progesterone (14). The vaginal epithelial cells were classified according to their location in the vaginal mucosa as parabasal, intermediate and superficial cells (15). When the female is in proestrus stage, mostly

nucleated and some cornified epithelial cells are present. As the stages of the cycle advances to estrus, mostly cornified epithelial cells are present. If the cycle is not interrupted by pregnancy, pseudopregnancy or other phenomena, metestrus will begin. Metestrus is a brief stage when corpora lutea form but fail to fully luteinize due to still a lack of progesterone. In this stage is seen in the form of cornified epithelial cells in the vaginal smear and some nucleated epithelia cells will also be present in late metestrus. Diestrus is the longest phase in the estrus cycle, vaginal smear during this stage show primarily epithelial cells (16). Exfoliated cells in the vagina lumen are the result of rising peripheral estrogen which cause the vaginal wall thickens. As the outermost layer moves further from the vascular supply, the cell keratinize and detach from the wall (7). During the different stages of the sexual cycle, the pH values of the vaginal secretions are influenced by the steroid hormone level in the female organism (17). Many of the remaining control mechanism, as well as the biological role of this vaginal parameter are not fully understood yet (18). Those changes occur in the genital tract in order to facilitate sperm transportation and fertilization (19).

Assessment of progesterone levels during different physiological stages in animals is considered as one of the most important parameters (20). The progesterone levels in peripheral blood of mammals provide valuable information about their reproduction status and also levels of progesterone determined have variation to regulate the estrus cycle (21,23). The progesterone concentrations remained at the basal levels throughout the estrus or in follicular phase and will increase gradually in luteal phase due to the growth and development of the corpus luteum (24,25).

3 Methodology/Materials

3.1 Experimental Animals, location and period of research

The experiment was conducted on Ettawa-Saanen grade dairy goat (*Capra hircus*) maintained in a goat farm in Turi, Sleman, Yogyakarta, Indonesia. The research was carried out from March to May 2016. The does were maintained with semi-intensive mainte-

nance, housed in pens and fed with 1.5 kg/head/day concentrate feed (54% pollard, 20% copra meal, 15% corn meal, 10% soybean meal and 1% mineral premix) and 3 kg/head/day fresh forage and legume (50% *Calliandra haematocephala* and 50% *Pennisetum purpureum* cv. Mott), water were offered ad libitum. The experiment was carried out on 13 mature (3-4 years of age) lactating does with body weight varying 35-40 kg and BCS 3-3.5. The experiment was performed with natural estrus cycle without estrus synchronization.

3.2 Estrus Identification

Vaginal epithelium smears were taken to determine estrus cycle on Saanen-Ettawa grade does (26). Vaginal smear was collected from each doe at 2-3 day intervals over a 60 day period of each stages of estrus cycle determination so those data can predict the next estrus cycle for recording (3), also measuring the vaginal pH is done to strengthen the estrus cycle determination [26]. Vaginal smear, vaginal temperature and the vaginal pH measurement was carried out also each 2-3 day intervals.

3.3 Vaginal smear and cytology

For determining the estrus stages, vaginal smear was collected from does with the aid of vaginal swabs which consisted of clean, soft and gentle pure cotton buds. The vulva and perineum were rinsed with clean water and gently wiped with tissue paper. Each doe was well restrained in standing position by assistant and the swab was gently inserted into the anterior vaginal with the right hand while the left thumb and forefinger were used to expose the vulva lips. At the anterior vagina, the swab was gently and briskly rolled against the vaginal mucosa and carefully withdrawn. The swab was immediately smeared on glass slide. The smears were stained with Giemsa stain (3,9). The cells encountered in the vaginal smear were categorized by percentage of parabasal, intermediate and superficial cells. Identification of vaginal epithelial cells was performed by microscopic observation (Gx100), based on their morphological and stained characteristics (3,5,9). The percentage of vaginal cells was calculated by divided the number of each type of cell by the total number of cells seen within 3 microscopic fields.

The smear collection procedure was adopted from previous research (3,14). The epithelial cells were classified into superficial, intermediate and parabasal cell using the Grunet criterion to determine the status of estrus phase cycle.

3.4 Determination of vagina; pH

The vaginal pH levels were measured with pH Merck paper by 0-14 indicator. The pH paper was dipped into the mucus vaginal. The changing color of the paper was compared to the attached standard value (13).

3.5 Measurement of serum progesterone

Blood samples were collected from the thirteen does as follows the phase of estrus which are estrus (E /day-0), metestrus (M /day-3), diestrus (D/day-12), and proestrus (P/day-15) 8 hours after feeding time. Blood preparation was adopted the step from Khadiga et al (15). Blood sample (5 ml) was collected from each doe, serum was recovered by centrifugation (15 minutes at 3,000 rpm) and stored at -20C until the serum progesterone concentration was 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. The procedure was adopted from Astuti et al (27).

3.6 Statistical analysis

Data were analyzed using a statistical program (SPSS, version 17.0). Vaginal cells percentages, vaginal pH values and mean progesterone concentration among the stages of estrus cycle were analyzed by ANOVA. Significance was assigned at $p < 0.05$.

4 Results and Findings

4.1 Exfoliative vaginal cytology in Ettawa-Saanen grade doe

Vaginal cytology is an important aid for diagnosing the sexual cycle. The typifying of morphologic and morphometric characteristics of the epithelial cells from the vagina was accordance with the study of previous researchers (6)(28). Several types of cells were observed in the mucosal surface of vagina during the estrus phase, in this study total of three types of vaginal cells were identified and illustrated in figures (Fig. 1a, 1b, 1c). Parabasal (p) cells were round, small, oval shape with large prominent nuclei. Intermediate cells (i) has polygonal shape with a small nuclei or cytoplasmic ratio with size 2-3 times larger compared to the parabasal cells. Superficial cells (s) has irregular shape with a clear side, with or without nuclei (keratinized) (6). Figures (Fig. 2a, 2b, 2c, 2d) were described exfoliative vaginal cytology during each phase of estrus cycle, superficial cells dominated in estrus phase (Fig. 2a) which are keratinized cells and will still appeared in the 3 or more following days of estrus. Superficial cells appeared to be associated with the proestrus, estrus and early metestrus phases which similar in Ola et al [3] study. These phases are controlled by estrogen and known to be characterized by high concentrations of endometrial cytoplasmic estrogen receptors in response to the high circulating estrogen from the pre-ovulatory and newly ovulated Graafian follicles. Intermediate cells were dominated the majority of the smear especially on metestrus and proestrus phases (Fig. 2b, 2d). The intermediate and parabasal cells were more conspicuous each day which correspond to the luteal phase controlled by progesterone (3). These cyclical relationship between the exfoliated cells and the ovarian steroid have been severely established for small ruminant and other species (3)(6)(7)(12).

The percentages of vaginal epithelial cells according to the stages of estrus cycle were presented in Table 1. Based on the standard characteristic of epithelial cell in proestrus, estrus, metestrus and diestrus phases, the propotion of parabasal, intermediate and superficial cells was found with measurement (Table 1) of 9.56 ± 18.13 , 70.57 ± 31.86 , 19.87 ± 26.77 in proestrus, 0, 8.24 ± 6.13 , 91.76 ± 6.13

in estrus, 2.29 ± 6.87 , 38.57 ± 31.39 , 59.14 ± 34.37 in metestrus and 83.22 ± 17.66 , 15.08 ± 14.91 , 1.70 ± 26.77 in diestrus respectively and significant different ($p < 0.05$) for each phase in estrus cycle. Vaginal epithelial cells proportion was similar with previous research reported (1)(3)(29). The epithelium vagina was sensitive to estrogen concentration during its development. The receptor of estrogen can be obtained on vagina tissue (30). In estrus phase, estrogen increased the uterus wall activity due to the hypersecretion in epithelial cell of the uterus and vagina, and trigger the exfoliation of vagina epithelial cells. Increased plasma estrogen concentration cause thickening of the vaginal mucosa becomes keratinized squamous epithelium (31). Epithelial cells which undergoes the cornification and keratinization to become superficial cells serves to protect the vaginal mucosa from irritation while copulation. The exfoliated and leucocyte cells is increase sharply as the result of the increasing vaginal mucus secretion (3). During metestrus to the diestrus, the intermediate and parabasal cells is increased under progesterone dominance (18). In the metestrus and diestrus phase population of superficial cells decreased and happened vice versa as the increased of intermediate and parabasal cells (1).

4.2 Dynamic of vaginal pH in Ettawa-Saanen grade doe during estrus cycle

The vaginal pH is caused by the condition of the biophysics and biochemistry of cervical mucus controlled by hormonal changes during estrus cycle (28). This mechanism explained by Noakes [43], that each of different stages of estrus cycles produces a different pH values as well. The vaginal pH showed in estrus phase (Table 1) were 7.17 ± 0.54 , in met-estrus were 6.17 ± 0.30 , in diestrus phase were 5.79 ± 0.43 , and in proestrus phase were 5.92 ± 0.474 , those data was similar with other research in cows and does (32) [44]. The presence of estrogen is a signals to functioning the ovarian cycle and discharge of cervical mucus. Estrogen level during estrus phase is closely associated with cervical mucus conditions (33) which pH values of the mucus on heat estrus is more alkaline due to the increased levels of estrogen, which influence the levels of sodium chloride and water content on the cervix become higher (34). The vaginal pH values is important because the cervical mucus is the

transport medium for sperm. The pH 7.0-8.5 is optimal condition of the viability and motility of sperm, whereas level pH below 6 lead to reducing the sperm motility (32,33).

4.3 Level of non-pregnant progesterone in Ettawa-Saanen grade doe during the estrus cycle

The serum progesterone level (Table 1) in does were significantly higher ($P < 0.05$) in luteal phase of estrus cycle than the follicular phase. The serum progesterone concentration increased gradually from the mean basal value of 0.083 ± 0.15 ng/ml on day-0 to 0.084 ± 0.11 on day-3 and reached the peak 0.23 ± 0.11 on day-12 and those level were maintained on day-15. In the present study, minimum level of non-pregnant progesterone serum were detected on the day of estrus in goats and sharply increased on the day of diestrus. The serum progesterone (Table 1) concentration increased gradually from the mean basal value of 0.083 ± 0.15 ng/ml on day-0 to 0.084 ± 0.11 on day-3 and reached the peak 0.23 ± 0.11 on day-12 and those level were maintained until day-15. The increasing patterns of the levels of progesterone and then declining after the luteal phase in Ettawa-Saanen grade does is similar to the pattern found in other breeds of goat. The progesterone concentrations remained at basal level throughout the estrus as observed by others (24)(20)(25)(35). Previous research (29) reported that mean progesterone concentration during follicular phase of estrus cycle is 0.1 ± 0.03 ng/ in Dwarf does and in luteal phase is $3.0 \pm 0.9-7.7 \pm 0.6$ ng/ml which was different with this study that showed a very low progesterone level during the luteal phase, only 0.23 ± 0.11 ng/mg and on follicular phase the level of progesterone is $0.083-0.084 \pm 0.11$ ng/ml. The relatively low progesterone level on this study have been similar with observation in West African Dwarf does [44], Nigerian Red Sakoto doe (36), in parturient ewes (37) and sows (38). These low progesterone serum concentration may also due to the short life span and low level functionally of the corpora luteal (36) and all the sample had <21 days estrus cycle. The low levels of circulating progesterone in this study also caused by the high level of urea blood (> 40 mg/dl) in plasma as well as with the previous research in cows that conclude the plasma progesterone level was reported to be approximately 30% lower progesterone level in cow with high

urea plasma (39). Plasma progesterone level were reported lower in cows fed high dietary protein which correlated had higher level of blood urea (39,40) and urea inhibits the binding of gonadotropin to plasma membrane in corpus luteum and associated with reducing circulating of progesterone (41) that caused short life span of corpus luteum and depressed the progesterone level in plasma.

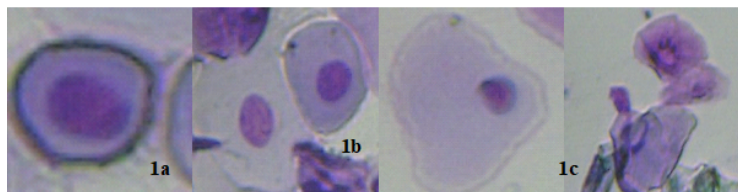


Figure 1. Various epithelial cell in cytologi vaginal. 1a) Parabasal cell; 1b) Intermediate cell and 1c) Superficial cell (with and without keratinized)

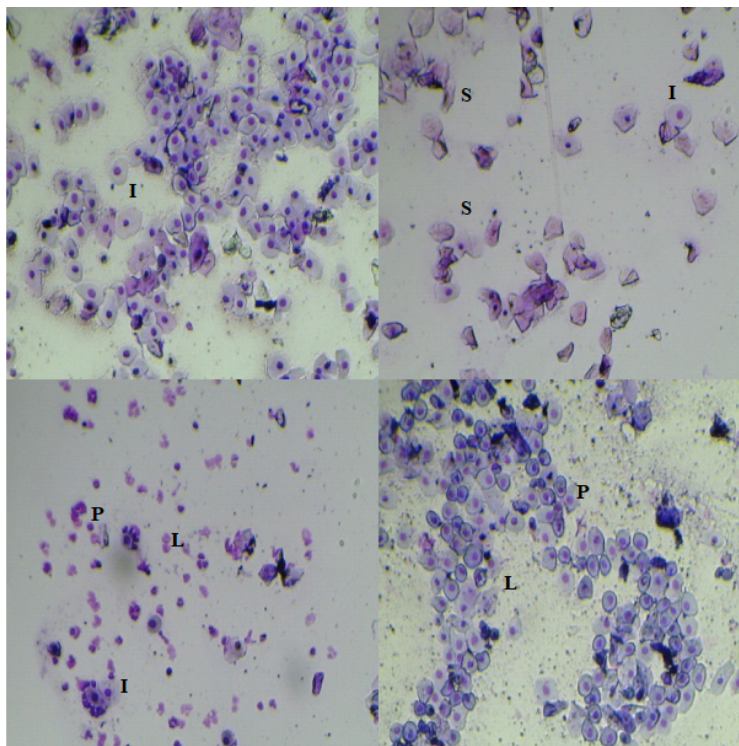


Figure 2. Natural Gas Demand (mmscfd)⁴

Table 1. Proportion of the Ettawa-Saanen Grade does vaginal epithelial cell during the estrus cycle, level P4 and pH values .

Estrus Phase	Parabasal	Intermediete	Superficial	Progesterone
	0 ^a	8.2375 ± 6.1301 ^b	91.7624 ± 6.1302 ^c	0.083±0.15
Estrus				
	2.2919± 6.8729 ^a	38.5684 ± 31.3992 ^b	59.1396 ± 34.3741 ^c	0.084±0.11
Met-Estrus				
	83.2192±17.6567 ^a	15.0788±14.9094 ^b	1.7031 ± 3.7532 ^c	0.23±0.11
Diestrus				
	9.5573±18.1271 ^a	70.5684 ± 31.8610 ^b	19.8743±26.7703 ^c	0.23±0.11
Proestrus				
Mean	23.7668 ± 37.1729	33.1132± 33.5846	43.1199± 41.2328	0.14±0.12

^{a,b,c}The different superscript in the same column indicates significant differences ($P < 0.05$).

5 Conclusion

Based on this findings, the morphologic changes, progesterone levels, and vaginal pH value can be used to determine the phase of the estrus cycle in the Saanen-Etawa breed does. The difference condition of epithelium cells, progesterone levels, and vaginal pH values in each phase of estrus is occurred do to the hormonal changes. The estrus phase was marked by the dominant proportion of superficial cell, high vaginal pH value and the lowest level of progesterone.

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