Exfoliative vaginal cytology of Saanen goat (*Capra hircus*) during estrus cycle

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Abstract. The exfoliative cytology of the vaginal was evaluated in Saanen goat during the estrus cycle by vaginal smear method. Its a simple technique to determine of estrus cycle stages and a useful tool to determine or identify the estrus phase. The aim of this study was to determine the proportion of exfoliative vaginal cell during the stages of estrus cycle using vaginal smear techniques in Saanen goat. Twenty Saanen goat with weigh 45-55 kg, 3-4 years and had a period of estrus 19-22 days in dry period were observed. All goats were in natural estrus cycle without synchronization. A vaginal smear and vaginal pH were collected from swabbed vaginal epithelium. The result showed the proportion of vaginal cell was significantly different in each phase of estrus cycle. The proportion of superficial cell was dominated in estrus phase. Also, the highest pH values were significantly higher in estrus phase rather than in the other phases. In conclusion, the dominant proportion of superficial cell and a high vaginal pH value which occurred in follicular phase might be used as a marker to determine the optimal time for goat insemination.

1. Introduction

The reproductive process such us ovulation, the life span of corpus luteum, pregnancy and partum were related to the estrus cycle [1]. The Vaginal smear is an application which used for estrus identification and already reported in some animals [2]. Estrus behavior could be discovered either by extrinsic behavior or the exfoliative vaginal cytology cells [3]. 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 [4]. Exfoliative vaginal cytology have been studied in goat [4], cow [5], swine [6] and ewes [7]. The vaginal smear was used to ascertain the physiological and health status as well as an instrument for endocrine bioassay [8, 9].

Steroid sex hormones which released by ovary enhanced the exfoliative of vaginal mucosa in the estrus cycle [10,11]. Exfoliated cells in the vaginal epithelium have ensued from estrogen enhancement which causes the dense of the vaginal wall. As the outermost layer, the epithelium budes further from the vascular supply and exfoliated became keratin cells and easy to lose from the duct. The vaginal epithelial cells were identified relate with the location in the vaginal mucosa as parabasal, intermediate and superficial cells [9]. When the female is in proestrus, 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 was detect in the form of cornified epithelial cells in the vaginal smear and some nucleated epithelial cells will also be present in late metestrus. Diestrus is the longest of the stages, vaginal smear during this stage show primarily epithelial cells [10].

The relative population range of the variations of vaginal epithelium might be used as an endocrine indicator [8, 11, 14–16]. The vaginal smear was utilized to assess the endocrine condition also to identification the reproductive phase in female [7]. The phases of estrus cycle caused pH alterations under the effect of estrogen [15], those studies about vaginal pH still limited especially in goat. There has been no systemic study of vaginal cytology and pH vaginal in Saanen especially with natural estrus cycle which without a synchronization procedure. Thus, the purpose of the present study was to identify morphologic characteristics of the epithelial cells and pH values from the vagina during estrus cycle that not being studied before in Saanen goat.

2. Material and methods

2.1. Experimental animals, location and period of research

The experiment was conducted in Saanen goat (Capra hircus) which maintained at goat farm in Animal Breeding Centre Baturaden Purwokerto, Indonesia. The research was carried out from August to October 2017. The experiment was carried out on 20 mature (3-4 years old) in dry period with body weight varying from 45-55 kg and BCS 3-3.5. The goats were housed in same pens and fed, and water was offered ad-libitum. The experiment was performed with natural estrus cycle without estrus synchronization.

2.2. Estrus identification

Vaginal smears were taken to determine estrus cycle on Saanen goat, the smear was collected from each goat at 2-3 day intervals over a 60 day period to determine each phase of estrus cycle so those data can predict the next estrus cycle for recording [4], to strengthen estrus cycle determination, measuring the pH of vagina [16]. Vaginal smear, vaginal temperature measurement and measuring the pH was carried out also each 2-3 day intervals [17].

2.3. Vaginal smear method

For determining the 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 stained with Giemsa stain [4, 11]. The cells encountered in the vaginal smear were categorized as 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 [4, 6, 11]. The percentage of vaginal cells was calculated as the number of each type of cell divided by the total number of cells seen within 3 microscopic fields. The smear collection procedure was adopted from previous research [4, 20]. The epithelial cells were classified into superficial, intermediate and parabasal cell using the Grunet criterion to determine the status of estrus phase cycle.
2.4. 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 [15].

2.5. Statistical analysis

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

3. Result and discussion

3.1. Exfoliative vaginal smear in Saanen

Vaginal smear was a method for identify estrus cycle. Vaginal smear was reported to be as delicate indicator in the estrus cycle, which also could be a tool which represents the balance of estrogen and progesterone level in the blood [18]. Epithelium cells in vaginal were classified according to present of nuclei which were parabasal, intermediate and superficial cells [9]. The variations and characteristic of the vaginal epithelial cells were reported in previous study [7] the variations of cells in this study were identified and illustrated in figures (Fig. 1-3). Parabasal (p) (Fig. 1) cells were round, small, oval shape with large prominent nuclei. Intermediate cells (i) (Fig. 2) have polygonal shape with a small nuclei or cytoplasmic ratio and have size 2-3 times compared to the parabasal cells. Superficial cells (s) (Fig. 3) have a shape and distinctly flat with or without nuclei (keratinized) [7].

Based the study, the figures (Fig. 4a, 4b, 4c, 4d) were explained the cell of vagina morphological been changed during estrus cycle, in the estrus phase, keratin superficial cells were dominated (Fig. 4a) and those cells would still settle until metestrus phase which also mentioned with previous study [4]. In these phases was where the estrogen controlled and were 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. The epithelium vagina was sensitive for estrogen concentration during its development. The receptor of estrogen can be obtained on vagina tissue [19]. Enhanced activation of estrogen leaded the activeness of uterus wall and made the vaginal wall thicken, which trigger the exfoliative vagina epithelial cells trough keratinized squamous epithelium [20]. Estrogen mediated action in reproductive track were tightly regulated, through the most commonly estrogen receptor 1 (ESR1). Under the estrogen influence, the vaginal epithelium cyclically exhibits cell proliferations and differentiation [21] trough induced cell hypertrophy and regulates genes involved in maintaining cellular integrity and secretions of mucins [22]. All those mechanisms were purposed to protect the vaginal mucosa from irritation at the time of copulation. The sharp increased the count of exfoliated and leucocyte cells as the result of the increased vaginal mucus secretion [4]. During metestrus to the proestrus intermediate cells and parabasal cells increased under progesterone dominance [23]. In the metestrus and diestrus phase population of superficial cells decreased and happened vice versa on the increased of intermediate and parabasal cells [1]. Intermediate cells were dominated the majority of the smear especially on metestrus and diestrus phases (Fig. 4b-4d). The intermediate and parabasal cells were more conspicuous in the smears from other days which correspond to the luteal phase controlled by progesterone [4]. Parabasal cells were observed dominated in proestrus phase. These cyclical relationship between the exfoliated cells and the ovarian steroid have been severely established for small ruminant and other species [4, 7, 8, 16].

The percentages of vaginal epithelial cells according to the stages of estrus cycle were presented in Table 1. Based on the standard and characteristic of epithelial cell in proestrus, estrus, metestrus and diestrus phases, the proportion of parabasal , intermediate and superficial cells was found with measurement (Table 1) of 79.33±16.11, 10.57±1.09, 9.56±14.32 in proestrus, 0, 11.45±8.14, 88.03±12.87 in estrus, 0, 53.22±22.66, 44.39±41.28 in metestrus and 21.01±8.93, 77.49±28.55,

Table 1. Based on the standard and characteristic of vaginal epithelial cells percentage and the occurrence of parabasal, intermediate and superficial cells in estrus cycle.

| Phase     | Parabasal (%) | Intermediate (%) | Superficial (%) |
|-----------|---------------|------------------|-----------------|
| Proestrus  | 79.33±16.11   | 10.57±1.09       | 9.56±14.32      |
| Estrus    | 0             | 11.45±8.14       | 88.03±12.87     |
| Metestrus  | 53.22±22.66   | 44.39±41.28      | 21.01±8.93      |
| Diestrus   | 77.49±28.55   |                  |                 |

The changing of vaginal pH was analyzed during estrus cycle [14] and the changes were compared to the attached standard value [15]. The 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 [15].
3.01±19.95 in diestrus respectively and significant different (p<0.05) for each phase in estrus cycle. Vaginal epithelial cells proportion was similar report by previous research [1, 4, 27].

**Figure 1-3.** Various epithelial cell in cytology vaginal. 1) Parabasal cell; 2) Intermediete cell and 3) Superficial cell (with and without keratinized)

**Figure 4.** Photomicrographs of cytology vaginal from Saanen grade goat at (4a) pro-estrus, predominantly consisting of nucleated epithelial cells; (4b) estrus, with anucleated cornified cells; (4c) metestru, consisting of the three types of cell, leukocytes, cornified, and nucleated epithelial cells; and (4d) diestrus, consisting predominantly of leucocytes. Parabasal cell (P), Intermediete cells (I), leucocytes (L), superficial cell (S).

**Table 1.** Propotion of the Saanen Goat vaginal epithelial cell during the estrus cycle and pH values

| Estrus phase | Parabasal       | Intermediate   | Superficial   | pH               |
|--------------|-----------------|----------------|---------------|------------------|
| Estrus       | 0a              | 0.11±8.14b     | 88.03±12.87c  | 8.06±0.55b       |
| Met-estrus   | 0e              | 53.22±22.66c   | 44.39±11.28b  | 66.22±0.40f      |
| Diestrus     | 21.01±8.93b     | 77.49±28.55c   | 3.01±19.95a   | 6.19±0.22e       |
| Proestrus    | 79.33±16.11c    | 10.57±1.09b    | 9.56±14.32a   | 6.82±0.31g       |

abc The different superscript in the same row indicates significant differences (p<0.05)
edef The different superscript in the same column indicates significant differences (p<0.05)

3.2. **Dynamic of vaginal pH in Saanen goat during estrus cycle**

The vaginal pH is often caused by the condition of the biophysics and biochemistry of cervical mucus controlled by hormonal changes during estrus cycle [24]. This mechanism explained by Noakes [noakes 2003], that each of different stages of estrus cycles produces a different pH values as well. The pH vaginal showed in estrus phase (Table 1) were 8.06 ± 0.55, in met-estrus were 6.22 ± 0.40, in diestrus phase were 6.19±0.22, and in proestrus phase were 6.82±0.31, those data was similar with other research in cows and does [25]. The presence of estrogen signals the functioning of ovarian cycle and discharge of cervical mucus. Estrogen level during estrus phase is closely associated with cervical mucus conditions [26] which pH values of the mucus on heat estrus is more alkaline due to the increased levels of estrogen and influence the levels of sodium chloride and water content on the cervix are rising [27]. The vaginal pH values is important because cervical mucus is the transport medium for sperm. The pH of 7.0-8.5 is optimal condition of the viability and motility of sperm, whereas level pH below 6 lead to the reducing motility of sperm [25,26]. Also endogenous bacteria would metabolized the cell glycogen and produced lactic acid which caused the declined pH vagina with the purpose as the barrier from pathogenic microbial [3].
4. Conclusion
In conclusion dominant proportion of superficial cell and a high vaginal pH value that occurred in follicular phase that might be used as the base for determining optimal time for insemination. Also, the highest pH values was in estrus phase rather than in the other phases.

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