Cytological Characteristics of Mucose Cell and Vaginal Temperature and pH During Estrous Cycle in Local Sheep

Siti Darodjah Rasad* and Rangga Setiawan

Animal Reproduction & AI Laboratory, Animal Husbandry Faculty, Universitas Padjadjaran
Jl. Raya Bandung-Sumedang KM 21, Campus Jatinangor, Sumedang–Indonesia
***Corresponding author email: s.d.rasad@unpad.ac.id**

Abstract. The aim of this study was to examine the cytological characteristics of mucous cell and vaginal temperature and pH during estrous cycle in 30 local sheep. Synchronization of estrous was using vaginal sponge impregnated with 20 mg progesterone prior to observations of cytology of cells from vaginal mucose through vaginal swabs to measure vaginal temperature and pH. Vaginal swabs were collected daily at 07.00 am for a week. Vaginal temperature and pH were measured twice a day at 07.00 am and 15.00 pm for a week after vaginal swabs. The smears of the swab were prepared on glass slide and stained with Giemsa. The study used descriptive statistical method and the parameters were cytology of vaginal cells and vaginal temperature and pH during estrous cycle. Vaginal epithelial cells classfied into parabasal, intermediate and superficial cells were counted and determined the percentage during proestrous, estrous and diestrous. Observation on vaginal temperature showed that the highest temperature (39.08±0.28°C) was obtained on the third day. It can be concluded that during estrous phase, cytology of vaginal mucose cell was dominated by superficial cell and both vaginal temperature and pH increased.
Key words : cytology, vaginal temperature, vaginal pH, estrous cycle, sheep

Abstrak. Tujuan dari penelitian ini adalah untuk menguji karakteristik sitologi dari sel-lendir, suhu dan vaginanya pH selama siklus estrus pada domba lokal. Sebanyak 30 ekor domba lokal yang disinkronisasi dengan spons vagina yang mengandung 20 mg hormon progesteron. Sebelum dilakukan pengamatan sitologi sel dari mucose vagina melalui apusan vagina, dilakukan pengukuran suhu dan pH vagina. Apusan vagina dilakukann setiap hari pada pukul 07.00 pagiselama seminggu. Pengukuran Suhu vagina dan pH dilakukan dua kali sehari, pada pukul 07.00 WIB dan 15.00 WIB selama satu minggu setelah apusan vagina. Metode statistik yang digunakan adalah deskriptif dengan parameter yang diteliti adalah sitologi sel vagina, temperatur dan pH vagina selama siklus estrus. Pengamatan sel epitel vagina; Parabasal, sel intermediate dan keratin diamati dan dilakukan penghitungan persentase sel yang muncul selama pro-estrus, estrus dan di-estrus. Pengamatan suhu vagina tertinggi diperoleh pada hari ketiga yaitu 39,08±0,28°C. Dari hasil penelitian dapat disimpulkan bahwa selama fase estrus, sitologi sel mucose vagina didominasi oleh sel superfisial dan suhu vagina serta pH vagina meningkat.
Kata Kunci : sitologi, temperatur, pH vagina, siklus estrus, domba

Introduction

Behavioral estrous in livestock animal like sheep or cattle can be detected from the exterior behavior or changes in vaginal cytology during estrous cycle. During ovulation, there are changes in ratio of cornification of the epithelial cells and basal cell (Zaid, 2011), and change in behaviour, body temperature and physical properties of genitals fluids of female cattle due to reproductive hormone status during estrous cycle (Redden et al., 1993). Evaluation of estradiol levels, vaginal cytology, and the appearance of the vulva can be used as a predictor of the estrous cycle in female (Mayor et al., 2006). During estrous, sheep will be affected by estrogen and the symptom of estrous will appear visually from the behavior or changes in the vulva of the sheep. However, sheep often experience reproductive disorder like silent estrous which is a phenomenon of ovulation without signs of estrous, especially common in young sheep. Silent estrus often occurs during the first estrus cycle, which may also be due to estrogen deficiency. In these conditions, the female sheep will not show the symptoms of estrus so it cannot be detected visually. Determination of this estrus cycle in

addition to visual observations can be done by the vaginal smear method. Through this method the changes of cytology of epithelial cell and polyneclear leucocyte cells in the vaginal mucus can be seen (Vidal et al., 2013).

To date, research on the characteristics of vaginal cytology during estrous cycle in local sheep is still limited. Therefore, the objective of this research is to examine the characteristics cytology of vaginal mucous cell, temperature and pH during estrous cycle in local sheep.

Materials and Methods

The descriptive research used 31 local sheep for synchronization using vaginal sponge impregnated with 20 mg progesterone hormone before vagina swab as the sample. Upon sponge removal, vaginal swab was conducted every day at 7 am for a week. Estrous phase was justified through estrous symptom and cervix dilatation using speculum and then evaluated the condition of the cervix. Vaginal temperature and pH was measured twice a day (at 7 am and 3 pm) for a week using thermometer and pH meter. Smears of the vaginal swab were then prepared on glass slide and stained with Giemsa.

Parameters of the research were (1) cytology cell of mucous vagina. Vaginal epithelial cells classified into parabasal, intermediate and superficial cells were counted and the percentage during proestrous, estrous and diestrous was determined. Diestrous was characterized by the absence of superficial cells in the epithelial vaginal, while progressive increase in percentage of intermediate/ superficial cells in epithelial vagina characterized proestrous and superficial/ cornification cells in most vaginal epithelial vagina indicates diestrus; (2) temperature vagina, and (3) pH vagina.

Statistical analysis was conducted by data tabulation which consisted of day of observation, mean of epithelial cell of mucose vagina (%), percentage of estrous identified of sheep through dominancy of cell (%), mean of temperature vagina (°C) and mean of pH vagina. Epithelial cell was counted from the percentage of parabasal cell, intermediate, superficial cell and ceratine cell with the formula:

$$\% \text{ cell counted} = \frac{\text{Number of cell counted}}{\text{Total number of cell}} \times 100\ \%$$

Description of the data, was analyzed through mean of sample and standard deviation.

Results and Discussion

Estrous detection through cytology of mucose cell of vagina

The observation of epithelial cells percentage of vaginal mucosa in local sheep consisted of parabasal cell, intermediate cells, superficial cells and ceratine cells is presented in Table 1.

Table 1 explains that vaginal swab on day 1 is dominated by intermediate cell, indicated higher percentage than the other cells. Day 2 shows declining percentage of intermediate, parabasal and ceratine cells, but increasing superficial cell percentage. On day 3, superficial cell percentage was still higher (41.03%) than the other cells, therefore increasing for 2 days straight. According to Widiyono et al. (2011) superficial cell percentage of goat could increase from proestrous to estrous phase and tend to decline after estrous phase because of estradiol change in blood. Day 4 indicates a decline in superficial cell percentage, an increase in while ceratine cell and a stable intermediate cell percentage. On day 5, ceratine cell was high, in contrast with the declining superficial cells, and it prolonged to day 6 and 7. Parabasal cell and intermediate cell began to increase, conversely with superficial

and ceratine cell. Changes of cytology cells in vaginal mucosa of local sheep are presented in Figure 1.

Cytology changes of vaginal mucosa cell occurred because of changes of hormonal level during estrous activities in ovary. In estrous phase, level estrogen in blood was high, this could be impact to increasing of uterus activities and make hyper secretion, ceratination of epithelial vagina cell and then increased of mitotic cell and proliferation of epithel cell (Popalayah et al., 2013). Ceratination process was important to protect epithelium of vagina from pathogenic invasion between cell or tissues (Hussin et al., 2014). Endogenous bacteria could be metabolized glycogen in cell to lactic acid and could be decline of pH vagina for the protecting of the pathogenic bacteria.

This observation about cytologies cell of mucose vagina was supported through observation of dilatation of cervix. This justification of cervix dilatation was done to observed peak of estrous and the dominance of cell could be detected. Observation of cervix dilatation is shown in Figure 2.

Table 1. Average Percentage of Epithelial Cells of Mucose Vaginal Identified as a Sheep Estrous

Day	Epithelial Cell of mucose vagina				Number of Sheep estrous
	Parabasal	Intermediate	Superficial	Ceratine	
	……%……				
1	26.61	34.71	22.58	16.10	13
2	20.83	31.36	32.48	15.33	26
3	22.14	23.46	41.03	13.37	52
4	22.59	23.34	31.62	22.46	19
5	23.27	21.58	17.95	37.21	16
6	21.16	22.19	26.81	29.84	13
7	25.87	30.51	22.86	20.77	13

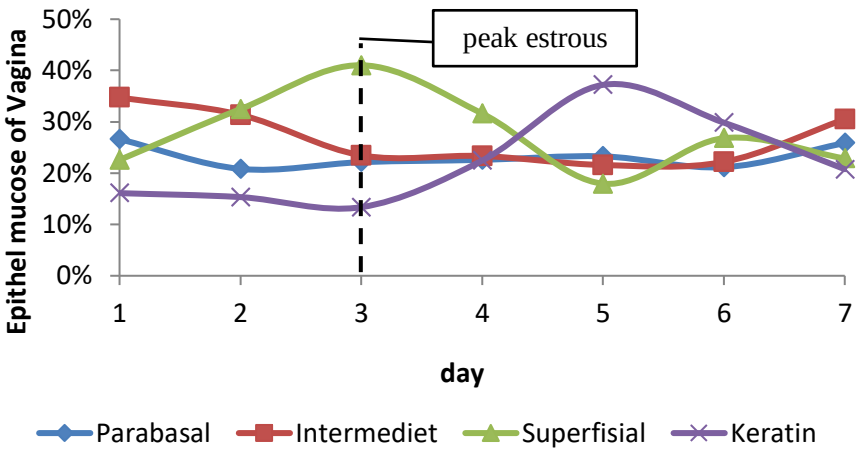

Figure 1. Cytology cells of vaginal mucose of sheep Local

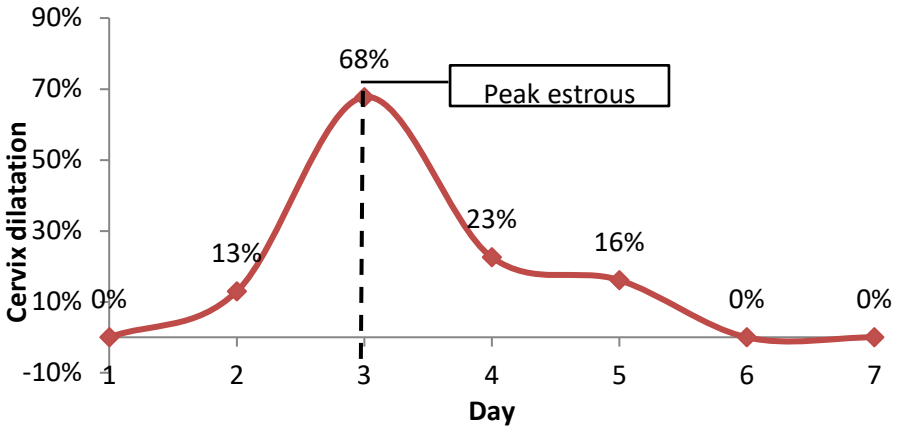

Figure 2. Percentage of Cervix Dilatation

Cervix dilatation is a character of sheep estrous. Based on cervix dilatation observation, the number of female sheep with open cervix or dilated the cervix tend to increase from Day 1 after removal of sponge vaginal to Day 3, then tend to decline until day 7. Cervix dilatation occurred because of relaxation of cervix and normally done when estrous as a effect of increasing of estradiol level in blood, oxytocin receptor (OxR), cyclooxygenase-2 (COX-2), prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$)(Kershaw et al., 2005). Rodriguez-Pinon et al. (2014) mentioned that estradiol and LH will be increased during estrous. In pre ovulation, estrogen could be increased sensitivity of cervix as an effect of oxytocin activities and OxR. Oxytocin could be initiate cervix to produce in vitro $PGF_{2\alpha}$ and could be affected to mediate COX-2 to catalyzed arachidonic acid to the prostaglandin. Prostaglandin activity then could be effect of cervix dilatation.

In Figure 2 showed, that increasing number of sheep (31 sheep) at day 3 (68%) was cervix dilated condition. According to Vivanco (1986), sheep will be estrous 2 days after vaginal sponge removed, and began at day 3 estrous was done. Feradis (2010) mentioned that estrous phase could be done 24–36 h after met-estrous phase.

Table 1 showed that day 3 almost sheep were in estrous phase based on superficial cell in vaginal mucose cell and began day 4 until day 7, the percentage of lamb in estrous phase tend to decline to anestrous phase (Figure 3).

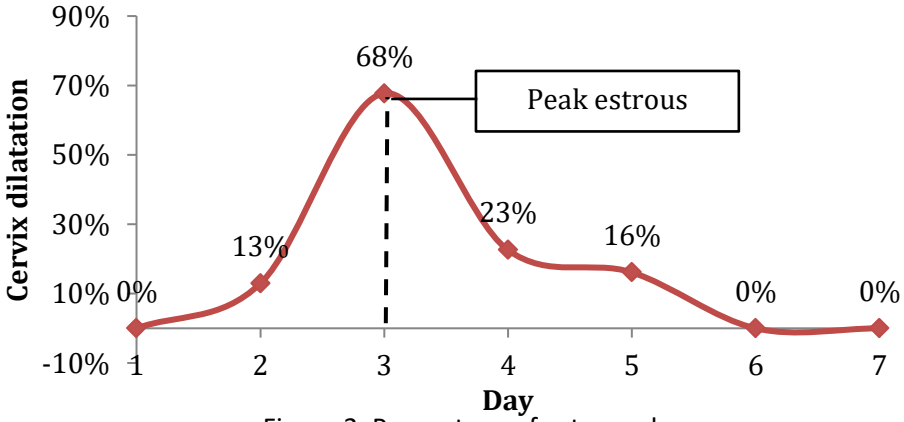

Figure 3. Percentage of estrous sheep

Temperature was measured twice a day (in the morning and evening) for a week after removing vaginal sponges, as presented in Table 2.

Based on Table 2, the average temperature of the vagina sheep on the first day was 38.96±0.32℃, but on the second day the temperature was decline. Otherwise, on the third day when the peak of estrous predetermined through cervical dilatation (Figure 3) was high, and the next day the temperature declined. Vaginal temperature on the third day also showed as a higher temperature than the other days.

On day 5, the temperature was high after declined on day 4, increased on day 6 and continued to decrease on day 7. The temperatures would increase at the estrous phase, and decreased again at the time of ovulation, the rebound during the luteal phase (Khalifa et al., 2010).

Observation indicated that vaginal temperature during estrous increased on Day 2 from 38.85±0.29°C to 39.08±0.28°C on day 3, about 48h after vaginal sponge removal that was defined as estrous occurence time. The average change of vaginal temperature of local sheep is described in Figure 4.

Vaginal temperature up to 24h before estrous increased by 0.23°C. Previous study on goats by Popalayah et al. (2013) showed that vaginal temperature during estrous increased by 0.4°C for 6h to 18h and decreased gradually from 24h to 36h before the end of estrous.

Table 2. Temperature Vagina of Local Sheep

Day	Morning Temperature	Evening Temperature	Average
	..... (℃).....		
1	38.75±0.32	39.17±0.43	38.96±0.32
2	38.78±0.40	38.92±0.25	38.85±0.29
3	39.06±0.32	39.09±0.30	39.08±0.26
4	38.97±0.24	39.00±0.24	38.98±0.22
5	39.05±0.33	39.05±0.32	39.05±0.30
6	39.09±0.25	38.94±0.28	39.01±0.23
7	39.01±0.33	38.95±0.31	38.98±0.28

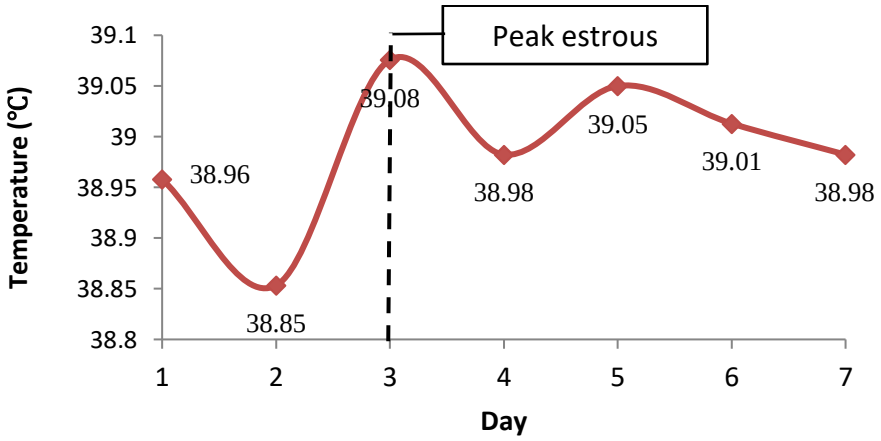

Figure 4. Average of temperature vagina changes of sheep

Vaginal temperature of sheep increased during estrous phase is the influence of hormonal factors. In the connective tissue of vagina, there are many blood vessels that could form extensive plexus (Hardjopranjoto, 1995). High estrogen at the time of estrous due to increasing blood circulation in the vaginal area and increased fluids in cells resulted in swelling vulva and vagina. Swelling in the vulva and vagina is due to the increased blood flow by estrogen activities and its receptor were found in blood vessels and clinically lowering vascular resistance (Heffner and Danny**,** 2005)**.**

Estrous detection based on pH Vagina

Vaginal pH conditions during observations could be seen in Figures 5. In that graphic could be seen pH changes for a week. Based on Figure 5, the higher pH of vagina was in day 3, and this according to the estrous cycle that occurs where the peak estrous was on day 3 after removed of vaginal sponge. At day 2, pH vaginal tend to increase until day 3, and then continuously decline until day 5. It was happened that at day 6 and day 7 the pH vaginal increased back again until maximum 7 and last day of observation was 6.9, because normally pH vaginal is acidic.

According to animal behavior since estrous phase, normally at that time the female in estrous phase, it was happened increasingly the body temperature as well pH vaginal. That condition closed related with hormonal activities during estrous period.

Based on data during observation, it could be cleared that pH vaginal was influenced of reproduction behavior and physiological changes in the reproductive organs can be seen during estrous cycle which is a manifestation of the increased estrogen in the blood. Toelihere (1993) suggest that elevated levels of estrogen lead into estrous female animals that marked the emergence of symptoms of lust like vulva swollen, red, warm, slimy, and silent when ridden by a male. Effect of estrogen results in changes in the physicochemical properties of the mucus of the cervix so that a change in viscosity (Darodjah, 2002)**.** Decrease in vaginal pH will return coincided with the process of ovulation (Khalifa et al., 2010)**.** Rezac et al. (2001) also reported that at the end of estrous, vaginal mucus is alblaskaline pH. A decrease in vaginal pH depends on the addition of ions in the vagina, such as hydrogen, sodium and chloride, as well as a decrease in pH is also caused by the accumulation of glycogen and protein (Khadiga et al., 2005). Blaszczyk et al. (2004) mentioned that the decrease in pH coincides with the release of LH in ovulation process.

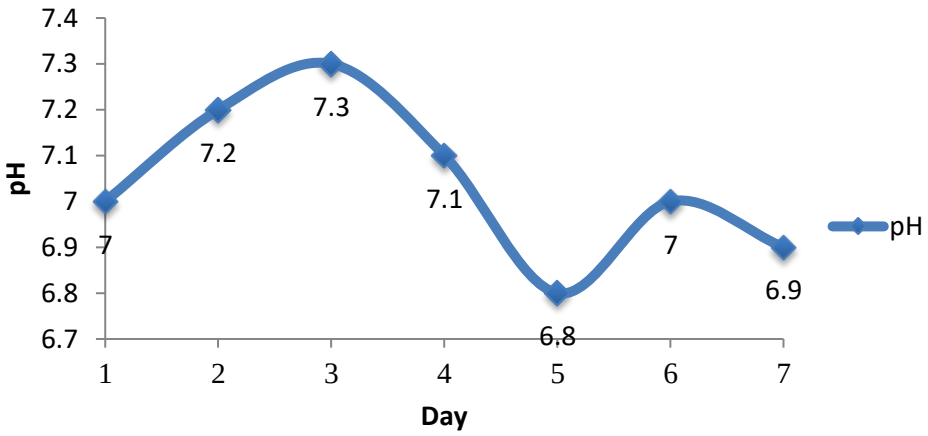

Figure 5. Average data of vaginal-pH changes during observation

Conclusions

The study conclude that cytology cells from the vaginal mucus of local sheep during the estrous phase were dominated by superficial cells. During estrous period, vaginal temperature and pH of local sheep increased.

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