The_profile_of_vaginal_epithelial_shelly.pdf

by Shelly Salmah

FILE	THE_PROFILE_OF_VAGINAL_EPITHELIAL_SHELLY.PDF (758.39K)		
TIME SUBMITTED	10-MAY-2019 08:14AM (UTC+0700)	WORD COUNT	4951
SUBMISSION ID	1127998669	CHARACTER COUNT	26652

The profile and percentage of vaginal epithelial cell numbers during the estrous cycle in Bali cattle

IOP Conf. Series: Earth and Environmental Science **247** (2019) 012006 doi:10.1088/1755-1315/247/1/012006

The profile and percentage of vaginal epithelial cell numbers during the estrous cycle in Bali cattle

Jasti Rahayu[1], Selly Salmah[2], Muhammad Yusuf[3], Bone Ramadhan[4], Dwi Kesuma Sari[1*]

[1]Study Program of Veterinary Medicine, Faculty of Medicine, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia
[2]Department of Histology, Faculty of Medicine, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia
[3]Department of Food Crops, Horticulture and Animal Husbandry , Jl. Jambu No. 34, Sengkang, Wajo Regency, South Sulawesi 90918, Indonesia
[4]Department of Animal Production, Faculty of Animal Science, Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia

Email: dwiksari@vet.unhas.ac.id

Abstract. The aim of this study was to observe the percentage of the number and the diameter of vaginal epithelial cells during the estrous cycle in Bali cattle. Six female Bali cattle were used in this research. Sample collection and estrous detection were observed at 06.00-07.00 am and 17.00-18.00 pm for 28 days. The method of this research used vaginal smear with Giemsa staining and then observed by microscope. Image Raster 3.0 was used to measured cells diameter and data were analysed using Independent T-Test. Based on the results of this study, the vaginal epithelial cells during the estrous cycle were intermediate cells, parabasal cells, superficial cells, and cornification cells. The percentage of intermediate cell numbers increased in the proestrus phase and decrease in the estrous phase. The percentage of parabasal cell numbers increased in metestrus and diestrus phase along with decreased in proestrus phase and not found in the estrous phase. The percentage of superficial and cornification cell numbers increased in the estrous phase and last stage of the proestrus phase along with decreased in the metestrus phase and not found in the diestrus phase. In measuring the diameter of the vaginal epithelial cells, superficial cells have a larger cytoplasmic diameter and the smallest diameter of the cytoplasm was parabasal cells. Whereas in measuring the nucleus diameter and diameter ratio of the cytoplasmic nucleus, parabasal cells have a larger diameter and cornification cells have smallest nucleus diameter and cytoplasmic nucleus diameter ratio.

1. Introduction

Bali cattle is one of the original Indonesian cattle and be in demand by the community. High economic value make this cattle has an important meaning because can produce a wide variety of resource needs, especially as meat producing source, manure, skin, and bones that can be utilized.

The condition of Bali cattle farms is currently experiencing a shortage of supply because population growth of Bali cattle is not balanced with the increasing needs of the community. Another problem frequently encountered is the reproductive disorders. Reproductive disorders have a major contribution in increasing population decline and are known as the main cause of low animal health

status [1]. Slow estrous detection by farmers can also be one of cause of the Bali cattle reproduction. This is because the slow detection of estrous can extend the calving to calving interval and calving to conception interval and decrease the economic value. Visual observations often used by farmers to detect estrous, in general it can be recorded well but the use and efficiency are controversial [2]. Therefore, other methods are needed that can be used to detect estrous in confirming optimal mating time in Bali cattle. In connection with this problem, the way can be done is by looking at vaginal cytology using vaginal smear method.

The method of vaginal smear is very important and needed in comparative observations that require a deeper understanding, especially the problem of reproductive organs [3]. This method is carried out in a qualitative way, namely monitoring the estrous cycle by taking and identifying epithelial cells and leukocytes from the animal's vagina. Through vaginal smear, various levels of differentiation on vaginal epithelial cells can be studied [4]. Observations of vaginal cytology during the estrous cycle have been carried out in sheep [5] goat [6] and pig, but observations of vaginal cytology in cattle little known [7]. The aim of this study was to observe and calculate the percentage of numbers and the diameter of vaginal epithelial cells during the estrous cycle in Bali cattle so that can help evaluate reproductive status, detect estrous and determine the optimal mating time.

2. Materials and Methods

Six female Bali cattle were used in this research, age over 2 years, have normal estrous cycle and were not pregnant. In addition, other materials used in this research are Giemsa stain, aquadest, methanol absolute, gloves, drop pipette, cotton swab, object glass, slide box, tissue, marker, rope, digital thermometer, and binocular microscope.

Sample collection and estrous detection were observed at 06.00-07.00 am and 17.00-18.00 pm for 28 days. Vaginal smears were obtained by introducing a cotton swab into Bali cattle vagina with circular motion. The cotton swab transferred to an object glass which was previously fixed using methanol absolute. Sample of vaginal smear was stain using Romanowski method with Giemsa staining for 10-15 minutes and after the samples are stained, the sample then rinsed with water and after being dried observed by the microscope.

Observation and data collection for the identification of epithelial cells in vaginal smear was carried out using a 40x10 magnification microscope which was connected with the Optilab. Data collection for calculate the percentage of vaginal epithelial cell numbers, was done by observing 100 epithelial cells for each sample at several different points then the epithelial cells were classified as parabasal cells, intermediate cells, superficial cells and cornification cells and each type of epithelial cell was multiplied by 100%. Cytoplasmic diameter measurements and cell nucleus diameters were calculated using Image raster 3.0 with 4 fields of view for each cell.

The description of vaginal epithelial cells is determined from the results of examination of epithelial cell morphology during the estrous cycle. Data from the analysis of epithelial cell numbers are grouped according to the estrous cycle phase and tabulated in percentages (0-100%) then analyzed descriptively. Data from measurement of cytoplasmic diameter and nucleus diameter are calculated based on formula the nucleus diameter divided by cytoplasmic diameter (N/C) for cell diameter ratio. Independent T-Test was used to see the percentage difference in the number of epithelial cells, nucleus diameter, cytoplasmic diameter and cell diameter ratio during the estrous cycle in Bali cattle. The difference is stated to be significant when $P<0.05$.

3. Result and Discussion

3.1. Clinical symptoms of estrous

The clinical symptoms of estrous are the determining factors for the successful breeding of the animal. Clinical symptoms of estrous were observed in 6 samples of Bali cattle applied in figure 1 and table 1.

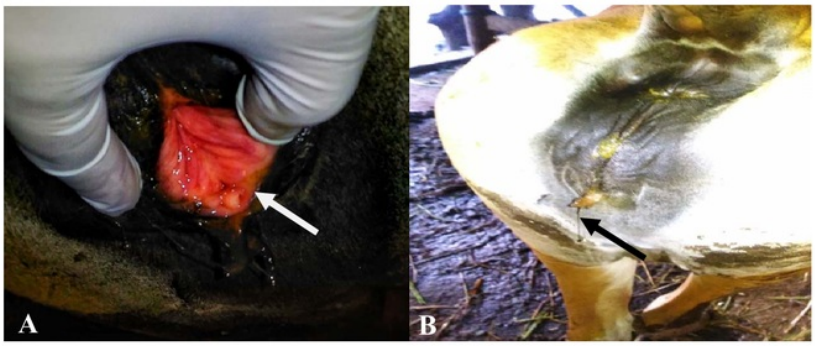

Figure 1. Clinical symptoms of estrous observed form of mucus from the vagina (A) and mucus hanging on the vulva (B)

Table 1. Clinical symptoms of estrous were observed in 6 samples of Bali cattle during 28 days

Cattle code	Clinical Symptoms of Estrous				
	Mucus on the Vagina	Vulva Swelling	Body Rubbing	Agitate	Mooing sound
1.	+	+	-	+	+
2.	-	-	+	+	-
3.	-	-	+	+	-
4.	+	+	-	+	+
5.	-	-	-	-	-
6.	-	-	-	-	-

(+) : observable estrous
(-) : unobservable estrous

Clinical symptoms when estrous based on figure 1 can be seen the mucus from the vagina and mucus hanging on the vulva looks clear and thickens towards the end of estrous. The mucus has a function to control and accelerate the process of sperm migration [8]. Swelling of the reddish vulva occurs due to increased blood flow to the reproductive tract and associated genital organs [9]. Other clinical symptoms such as agitate, body rubbing, and frequent mooing sounds are influences from the nervous system [10]. Based on table 1, the clinical symptoms of estrous observed between cows with each other have differences. Differences in clinical symptoms of estrous between individuals can be caused by the influence of hormonal mechanism, especially estrogen which tends to increase during the estrous phase. Moreover being caused by hormones, appearance of estrous symptoms in an animal depend on the species, breed, age, and time of measurement [11].

3.2. The profile of vaginal epithelial cells

The profile of vaginal cells in one estrous cycle is influenced by hormonal factors that will show cell changes. Based on the results of observations of Bali cattle's vaginal smear using a microscope with 40x10 magnification and connected with Optil[illegible]t was found that different cell types were varied. In one estrous cycle found 4 types of cell namely parabasal cells, intermediate cells, superficial cells and cornification cells can be seen in f
igure 2.

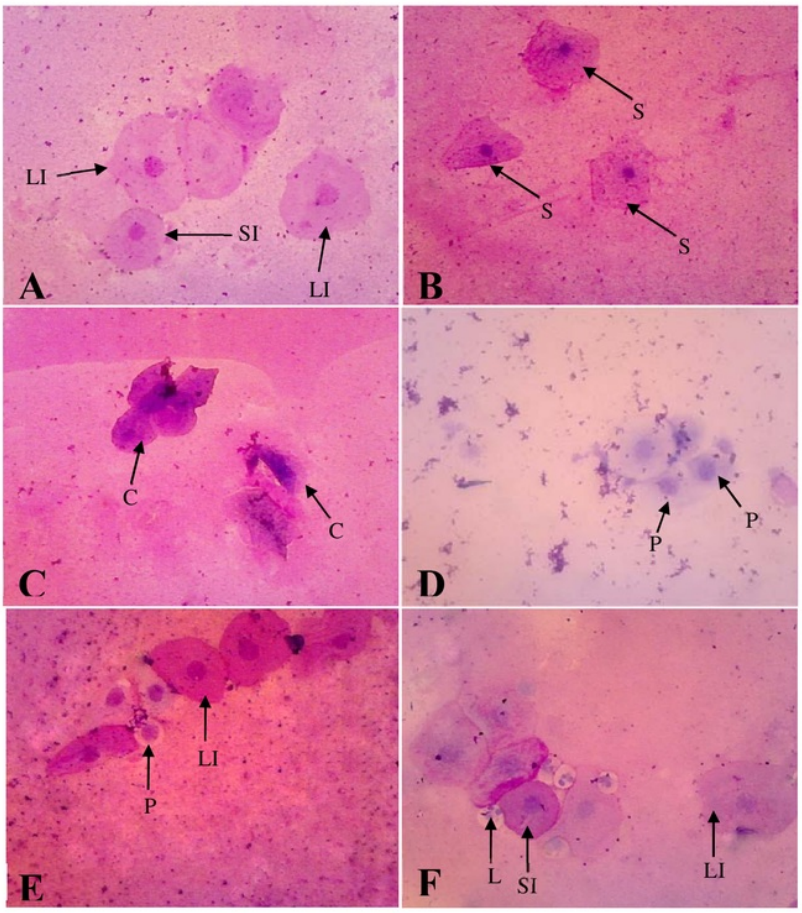

Figure 2. The profile of vaginal epithelial cells in Bali cattle during the estrous cycle (A) Proestrous, (B) and (C) Estrous, (D) Metestrous, (E) and (F) Diestrous (P: Parabasal; SI: Small Intermediate; LI: Large Intermediate; S: Superficial; C: Cornification; L: Leukocyte)

3.2.1. Parabasal. Parabasal cells are epithelial cells that have a small size, round or oval in shape with larger nuclei than cytoplasm. In general, cytoplasm of parabasal cells is dark, thick and basophilic. Parabasal cells are the youngest cells found in the estrous cycle. These cells are found in the diestrus, metestrus and anestrus phases, but are not commonly found during the estrous phase [12].

*3.2.2. Intermediet.*Intermediate cells are the oldest vaginal cell types of parabasal cells but are younger than superficial cells, which have a variety of shapes and sizes. Intermediate cells have a diameter of about 2-3 times greater than parabasal cells. Intermediate cells are divided into two groups, small intermediate cells and large intermediate cells. Small intermediate cells have a slightly round or oval shape with a clear and prominent nucleus. Large intermediate cells have a polygonal in shape with small nuclei. During the metestrus phase intermediate cells have angular or even nucleated cytoplasm. These intermediate cells are generally found in all estrous cycles except in the estrous phase [13].

3.2.3. Superficial. Superficial cells are epithelial cells that have the largest size among other epithelial cells, have a polygonal or irregular shape, flat, uneven edges and no nuclei or pyknotic (the nucleus looks small and dark). Superficial cells that are not nucleated often experience cornification or keratinization which serves to protect the vaginal mucosa from irritation during copulation. Superficial cells can be found in large numbers in the estrous phase and are not found in the diestrus or anestrus phase [14].

3.2.4. Cornification. Cornification cells are the oldest vaginal cell type than parabasal cells, intermediate cells, superficial cells, and incomplete nucleus characteristics. The presence of cornification cells occurs because of the high estrogen concentration during estrous resulting in thickening of the vaginal wall and resulting epithelial cells undergoing clotting and detachment from the vaginal epithelial wall. Cornification cells can be found in large amounts in the estrous phase and are not found in the diestrus or anestrus phase [15].

3.3. The percentage of vaginal epithelial cell numbers in Bali cattle

The results from observations of Bali cattle's vaginal smear during 28 days with the distribution of intermediate cells, parabasal cells, superficial cells and cornification cells can be seen in table 2.

Table 2. Distribution of average the percentage of vaginal epithelial cell numbers in Bali cattle during 28 days observation

Cell type	Day 1-7 Mean±SD	Day 8-14 Mean±SD	Day 15-21 Mean±SD	Day 22-28 Mean±SD
Intermediet (%)	41.04±24.08	40.76±7.48	38.78±21.49	42.90±6.22
Parabasal (%)	16.73±16.08	57.88±5.18	23.83±16.47	49.85±9.54
Superficial (%)	27.21±14.29	1.02±1.41	24.85±18.96	4.71±7.3
Cornification (%)	15.38±13.68	1.21±1.87	12.85±16.22	2.59±5.04

SD: Standard Deviation

The distribution of average the percentage of the vaginal epithelial cell numbers during 28 days observation in diagrams form can be seen in the following diagram (1) :

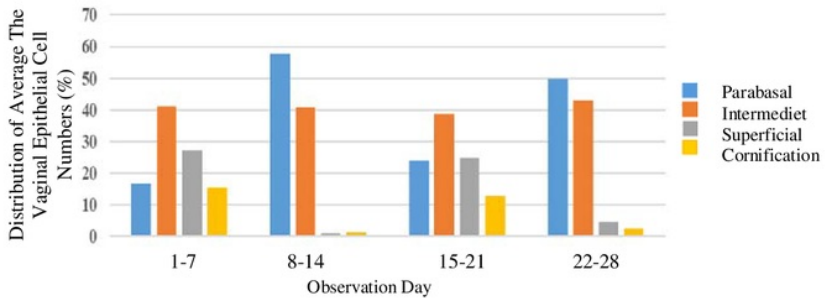

Diagram 1. Distribution of average the percentage of vaginal epithelial cell numbers in Bali cattle during 28 days observation

Based on the results of statistical tests using Independent T-Test showed that average the percentage of the intermediate cell numbers in six samples Bali cattle observed had non-significant difference (P>0.05) during the observation day. This shows that the resulting cytology of vaginal smear composition is unclear and not distinctive so this study can only be used to determine the estrous phase and cannot be used to determine the optimal mating time. In parabasal cells the results of the Independent T-Test test showed a significant difference (P <0.05) from average the percentage of parabasal cell numbers in six samples Bali cattle on day 1 until day 7 with day 8 until day 14 and day

22 until day 28, but did not differ significantly (P>0.05) with day 15 until day 21. On day 8 until day 14 average the percentage of the parabasal cell numbers had a significant difference (P <0.05) with day 15 until day 21 but not significantly different (P> 0.05) with day 22 until day 28. On day 15 until day 21 with day 22until day 28 average the percentage of parabasal cell numbers had a significant difference (P<0.05).

The results Independent T-Test for differences in average the percentage of superficial cell numbers showed that there were significant differences (P<0.05) from average the percentage of superficial cell numbers in six samples Bali cattle on day 1 until day 7 with day 8 until day 14 and day 22 until day 28, but did not differ significantly (P> 0.05) from day 15 until day 21. On day 8 until day 14 average the percentage of superficial cell numbers had a significant difference (P<0.05) with day 15 until day 21, but not significantly different (P>0.05) with day 22 until day 28. On day 15 until day 21 with the day 22 until day 28 average the percentage of superficial cell numbers had a significant difference (P <0.05). In cornification cells, based on the results of the Independent T-Test showed that there was a significant difference (P <0.05) from average the percentage of cornification cell numbers in six samples Bali cattle on day 1 until day 7 with day 8 until day 14 and day 22 until day 28 but not significantly different (P> 0.05) with day 15 until day 21. On day 8 until day 14 average the percentage cornification cell numbers did not have a significant difference (P> 0.05) with day 15 until day 21 day and day 22 until day 28. On day 15 until day 21 with day 22 until day 28 average the percentage of cornification cell numbers did not have a significant difference (P> 0.05).

Intermediate cells have an increasing average percentage of cell numbers in the proestrus phase, in mouse deer (*Tragulus javanicus*) the proestrus phase occurs when the percentage of intermediate cells progressively increases [4]. During the proestrus phase, intermediate cells experience an increase in number compared to other cells [14]. Intermediate cells can also be found in the metestrus and diestrus phases and decrease in numbers in the estrous phase but can still be found. On the cytology picture of Etawa crossbreeding goat's vagina, a number of intermediate cells can be found throughout the estrous cycle [16].

The average percentage of parabasal cell numbers has increased in the metestrus and diestrus phases (luteal phase) and decreases in the proestrus phase and not even found in the estrous phase (follicular phase). Parabasal cells in sheep vaginal cytology dominate before estrous phase followed by intermediate cells and superficial cells [17]. In cytology experiments with timor deer (*Cervus timorensis*) parabasal cells are dominant in the luteal phase [12]. Smear preparation in diestrus phase are found many white blood cells and nucleated epithelial cells that are scattered and homogeneous [18].

The results of the average percentage of superficial cell numbers and cornification in the proestrus and estrous phase increase and decrease even not found in the metestrus and diestrus phase. A high percentage of superficial cells indicates that animals are in the estrous phase [12]. In dog's vaginal smear, found superficial cells dominate during the estrous phase but not all cells undergo cornification [14]. The number of superficial cells in dog vaginal cytology was around 90% and 5% for total parabasal cells [19]. The percentage of these cells is higher than the results of research conducted. For Bligon goats, in the estrous phase full cornification cells are found and superficial cells dominate, whereas parabasal cells and intermediate cells are not found. Superficial cells and cornification cells were only found in estrous phase for mouse deer (*Tragulus javanicus*) [4].

The difference in the average percentage of epithelial cell numbers can be influenced by individual differences, environmental conditions, and hormonal status [9,12]. The presence of dominating cornification epithelium can caused by high estrogen concentrations at the time of estrous resulting in thickening of the vaginal wall and resulting epithelial cells undergoing clotting and detachment from the vaginal epithelial wall [15]. Superficial cells found in the Aceh cattle estrous phase are caused by an increase in estrogen hormones, thus activating the uterine wall and hypersecretion of epithelial cells in the uterus and vagina so that superficial cells become exfoliated. However, when the hormone progesterone increases in the metestrus and diestrus phase, superficial epithelial cells will experience significant shrinkage so that the dominating cells shift to parabasal cells and intermediate cells [20].

3.4. Diameter size of Bali cattle vaginal epithelial cells

Results of measurement of cytoplasmic diameter, nucleus diameter, and diameter ratio of cytoplasmic nucleus of intermediate cells, parabasal cells, superficial cells and cornification cells can be seen in table 3, 4 and 5 below:

Table 3. The results of observation cytoplasmic diameter of Bali cattle vaginal epithelial cells

Cell Type	Cytoplasmic Diameter Mean ±SD
Intermediet	47.95±6.66 µm
Parabasal	26.59±2.15 µm
Superficial	75.25±1.97 µm
Kornifikasi	46.24±4.3 µm

SD: Standard Deviation

Based on data analysis using Independent T-Test, the results of cytoplasmic diameter measurements of vaginal epithelial cells (table 3) showed that there were significant differences ($p<0.05$) between the diameter of intermediate cells cytoplasm with parabasal cells and superficial cells, but did not have significant differences ($p>0.05$) with cornification cells. Cytoplasmic diameter of parabasal cells with superficial cells and cornification cells had a significant difference ($p<0.05$) and also the cytoplasmic diameter of superficial cells with cornification cells had a significant difference ($p<0.05$). The average diameter of the superficial cytoplasm is greater than other epithelial cells which is 75.25 µm followed by the cytoplasmic diameter of intermediate cells 47.95 µm, then cornification cells 46.24 µm and the smallest cytoplasmic diameter is parabasal cells which is 26.59 µm.

Table 4. The result of observation nucleus diameter of Bali cattle vaginal epithelial cells

Cell Type	Nucleus Diameter Mean ±SD
Intermediet	14.24±0.82 µm
Parabasal	14.53±0.51 µm
Superficial	13.02±0.77 µm
Kornifikasi	0±0 µm

SD: Standard Deviation

Based on data analysis using Independent T-Test, the results of measurements of the nucleus diameter of vaginal epithelial cells (table 4) showed that there was no significant difference ($p>0.05$) between the diameter of intermediate cell nucleus with parabasal cells and superficial cells, but had significant differences ($p<0.05$) with the nucleus diameter of the cornification cell. The nucleus diameter of parabasal cells with superficial cells and cornification cells has a significant difference ($p<0.05$) and also the nucleus diameter of superficial cells with cornification cells has a significant difference ($p<0.05$). The average nucleus diameter of parabasal cells is greater than that other epithelial cells, which is 14.53 µm followed by intermediate cell nucleus diameter 14.24 µm, and superficial cell nucleus diameter 46.24 µm. The nucleus diameter of the cornified cell is 0 µm, this is because the epithelial cells undergoing cornification do not have a nucleus. The nucleus loss in cornified cells is caused by the keratinization process followed by the desquamation of superficial cells because the keratinized substance is formed which prevents the diffusion of nutrients from the capillaries into the vaginal tissues [20].

Based on data analysis using the Independent T-Test, the results of the nucleus cytoplasmic diameter ratio of vaginal epithelial cells (table 5) showed that there were significant differences ($p<0.05$) from the average diameter ratio of nucleus cytoplasmic vaginal epithelial cells. The average diameter ratio of nucleus cytoplasmic parabasal cells is greater than other epithelial cells which are 0.54 µm and then followed by the intermediate cell nucleus cytoplasmic diameter ratio 0.3 µm, then the diameter ratio of nucleus cytoplasmic superficial cells was 46.24 µm and the smallest nucleus

cytoplasmic diameter ratio was cornification cell that is 0 µm. In dog's vaginal parabasal cells have high nucleus cytoplasmic diameter ratio (N/C) [19].

Table 5. The result of observation nucleus cytoplasmic diameter ratio (N/C) of Bali cattle vaginal epithelial cells

Cell Type	Nucleus Cytoplasmic Diameter Ratio Mean ±SD
Intermediet	0.3±0.03
Parabasal	0.54±0.03
Superficial	0.17±0.006
Kornifikasi	0±0

SD: Standard Deviation

Estrogen hormone affects the cytodifferentiation of stratified squamous epithelium through cellular receptors which results in increased numbers of cells in the body [21]. Increasing size of the nucleus diameter was related with deoxyribonucleic acid (DNA) increase which functions as cell replication. Vaginal epithelial cells have different sizes for each phase [22]. Variations in vaginal epithelium may be related to physiological status and hormonal activity of animals [23].

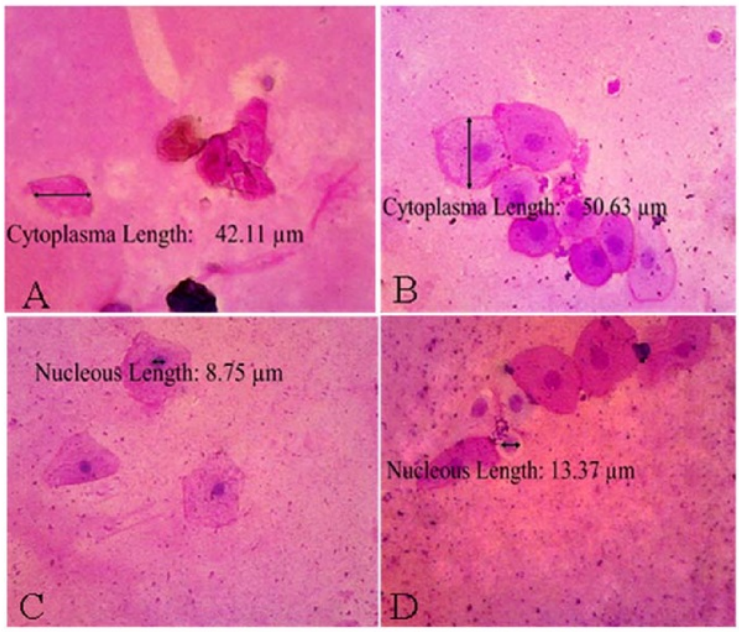

Figure 3. Comparison of cytoplasmic diameter and nucleus diameter (A:Cornification cell; B: Intermediet cell; C:Superficial cell; D: Parabasal cell)

Conclusion

Based on the results of this study, the profile of Bali cattle vaginal epithelial cells during the estrous cycle found intermediate cells, parabasal cells, superficial cells and cornification cells. The percentage of intermediet cell numbers increase in proestrus phase and decrease in estrous phase. The percentage of parabasal cell numbers increase in metestrus and diestrus phase along with decrease in proestrus

phase and not found in estrous phase. The percentage of superficial and cornification cell numbers increase in estrous phase and last stage of proestrus phase along with decrease in metestrus phase and not found in estrus phase. The diameter of the vaginal epithelial cells, superficial cells have a larger cytoplasmic diameter and the smallest diameter of the cytoplasm is parabasal cells. Whereas in measuring the nucleus diameter and diameter ratio of the nucleus cytoplasmic, parabasal cells have a larger diameter and cornification cells have smallest nucleus diameter and nucleus cytoplasmic diameter ratio.

5. Acknowledgement

The authors would like to highly appreciate to the staff of the diagnostics laboratory Clinic of Veterinary Education Hasanuddin University, the Farmers and Department of Agriculture and Animal Husbandry Bantaeng regency for giving permission to use facilities, animals and barn during this research.

References

[1] Putro P P 2009 *Dampak Crossbreeding Terhadap Reproduksi Induk Turunannya : Hasil Studi Klinis* Lokakarya Crossbreeding Sapi Potong di Indonesia: Aplikasi dan Implikasinya terhadap Perkembangan Ternak Sapi di Indonesia Lustrum VIII (Yogyakarta: Fakultas Peternakan UGM)

[2] Fahey J, K O'Sullivan, J Crilly and J F Mee 2002 The effect of feeding and management practices on calving rate in dairy herds *Anim. Reprod. Sci.* **74** 133-150

[3] Megawati D, Sutarno and Shanti L 2005 Siklus Estrous dan Struktur Histologis Ovarium Tikus Putih (Rattus norvegicus, L.) Setelah Pemberian Monosodium Glutamat (MSG) Secara Oral *J. Bio Smart* **7**(I) 47-52

[4] Najamudin, Rusdin, Sriyanto, Amrozi S, Agungpriyono dan T L Yusuf 2010 Penentuan Siklus Estrous Pada Kancil (*Tragulus Javanicus*) Berdasarkan Berdasarkan Sitologi Vagina *J. Vet.* **11** 81-86

[5] Ola S I , Sanni W A and Egbunike G 2006 Exfoliative vaginal cytology during the oestrous cycle of West African dwarf goats *Reprod. Nutr. Dev.* **46** 87–95

[6] Mayor P, Galvez H, Guimaraes D A, Lopez Gatius F and Lopez Bejar M 2005 Serum estradiol-17β, vaginal cytology and vulval appearance as predictors of estrous cyclicity in the female collared peccary (*Tayassu tajacu*) from the eastern Amazon region *Anim. Reprod. Sci.* **97** 165-174

[7] Mingoas J P K and L L Ngayam 2009 Preliminary Findings on Vaginal Epithelial Cells and Body Temperature Changes During Oestrous Cycle in Bororo Zebu Cow *Int. J. Biol. Chem. Sci.* **3**(1) 147-151

[8] Fatet A, Pellicer Rubio M T and Leboeuf B 2010 Reproductive cycle of goats *J Anireprosci* **124** 211-219

[9] Saara C S, S G Clark, R V Knox and M A Tamassia 2011 Vulvar Skin Temperature Changes Significantly During Estrous in Swine as Determined by Digital Infrared Thermograph *J. Swine Health and Prod.* **19**(3) 152

[10] Ramli, Mauridatun, Tongku N Sieragar, C N Thasmi, Dasrul, Sri Wahyuni dan Arman Sayuti 2016 Hubungan Antara Intensitas Estrous dengan Konsentrasi Estradiol pada Sapi Aceh pada Saat Inseminasi *J. Med. Vet.* **10**(1)

[11] Widiyono, Irkham, Prabowo, Purnomo P, Sarmin, Pudji A, C M Airin 2011 Kadar Estradiol dan Progesteron Serum, Tampilan Vulva dan Sitologi Apus Vagina Kambing Bligon Selama Siklus Birahi. *J. Vet.* **12**(4) 263-268

[12] Nalley W M M, R Handarini, M Rizal, R I Arifiantini, T S Yusuf and B Purwantara 2011 Penentuan siklus estrous berdasarkan gambaran sitologi vagina dan profil hormon pada Rusa Timor *J. Vet*. **12** 98-106

[13] Johnston S D, M R Kustritz and P Olson 2001 *Canine and Feline Theriogenology* (Philadelphia:

W.B. Saunders comp) Pp 32-40
[14] Bowen R A 2006 *Techniques for Preparing a Canine Vaginal Smear* (Colorado State University : Animal Reproduction and Biotechnology Laboratory)
[15] Busman H 2013 Histologi Ulas Vagina dan Waktu Siklus Estrous Masa Subur Mencit Betina Setelah Pemberian Ekstrak Rimpang Rumput Teki *Prosiding Semirata* (Lampung: FMIPA Universitas Lampung)
[16] Azmi 2013 Penentuan Estrous Melalui Gambaran Sitologi Ulas Vagina Dan Hubungannya Dengan Gejala Klinis Estrous Pada Kambing Peranakan Etawah *Skripsi* (Bogor: Fakultas Kedokteran Hewan IPB)
[17] Solis G, Aguilera J I, Rincon R M, Banuelos R and Arechiga C F 2008 Characterizing cytology (ECV) in ewes from 60 d of age through parturition *J Anim Sci* **82** Suppl.1
[18] Karlina Y 2003 *Siklus Estrous Dan Struktur Histologi Ovarium Tikus Putih (Rattus Norvegicus) Setelah Pemberian Alprazolam* (Surakarta: Fakultas Matematika Dan Ilmu Pengetahuan Alam Universitas Sebelas Maret)
[19] Beimborn, Valerie R.Heather L. Tarpley. Perry J. Bain. Kenneth S. Latimer 2003 *The Canine Estrous Cycle: Staging Using Vaginal Cytological Examination* (Athena: The University of Georgia)
[20] Siregar, T N J Melia, Rohaya, C N Thasmi, D Masyitha, S Wahyuni, J Rosa, Nurhafni, B Panjaitan and Herrialfian 2016 Determining Proportion of Exfoliative Vaginal Cell during Various Stages of Estrous Cycle Using Vaginal Cytology Techniques in Aceh Cattle *Vet Med Int.* 2016: 3976125
[21] Donald P M, R George, G Sriram, B Kavitha and B Sivapathasundharam 2013 *Hormonal changes in exfoliated normal buccal mucosal cells* (http://www.jcytol.org/article.asp?issn=0970 9371;year=2013; volume=30;issue=4;spage =252; epage=256;aulast=Donald. Accessed on Thursday, 30 August 2018)
[22] Singh M, Sircar K, Tandon A 2014 The role of tobacco as an etiological agent for oral cancer: Cytomorphometrical analysis of the buccal mucosa in tobacco users *J Dent. Res.* **11**(6) 649-55
[23] Hussin A M 2006 The vaginal Exfoliative Cytology of Awassi Ewes During Post-parturient Periods *Iraqi J. Vet. Med.* **30**(2) 130-137

The_profile_of_vaginal_epithelial_shelly.pdf

ORIGINALITY REPORT

%12	%8	%5	%7
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.veterinaryworld.org Internet Source	%1
2	www.newcov.tv Internet Source	%1
3	www.ijsrp.org Internet Source	%1
4	www.ijalel.org Internet Source	%1
5	Tongku N. Siregar, Juli Melia, Rohaya, Cut Nila Thasmi et al. "Determining Proportion of Exfoliative Vaginal Cell during Various Stages of Estrus Cycle Using Vaginal Cytology Techniques in Aceh Cattle", Veterinary Medicine International, 2016 Publication	<%1
6	Submitted to Syiah Kuala University Student Paper	<%1
7	ejournal.undip.ac.id Internet Source	<%1

8	Submitted to Coventry University Student Paper	<%1
9	Saleh H.M. Abdalla, Lynne Boddy. "Effect of soil and litter type on outgrowth patterns of mycelial systems of Phanerochaete velutina", FEMS Microbiology Ecology, 1996 Publication	<%1
10	ojafr.ir Internet Source	<%1
11	media.neliti.com Internet Source	<%1
12	www.bioone.org Internet Source	<%1
13	article.sciencepublishinggroup.com Internet Source	<%1
14	ludwigsdoodles.com Internet Source	<%1
15	Submitted to Universiti Teknologi MARA Student Paper	<%1
16	businessdocbox.com Internet Source	<%1
17	ijlr.org Internet Source	<%1
18	manuelcastillo.es	

	Internet Source	<%1
19	Submitted to University of Witwatersrand Student Paper	<%1
20	Submitted to University of Queensland Student Paper	<%1
21	Submitted to Mount Ida College Student Paper	<%1
22	ksep.or.kr Internet Source	<%1
23	Submitted to iGroup Student Paper	<%1
24	Submitted to UIN Maulana Malik Ibrahim Malang Student Paper	<%1
25	Peter Wellsbury, R.John Parkes. "Acetate bioavailability and turnover in an estuarine sediment", FEMS Microbiology Ecology, 1995 Publication	<%1
26	e-journal.unair.ac.id Internet Source	<%1
27	dspace.stir.ac.uk Internet Source	<%1
28	hal.archives-ouvertes.fr	

	Internet Source	<%1
29	Submitted to The University of the South Pacific Student Paper	<%1
30	www.ajol.info Internet Source	<%1
31	academic.oup.com Internet Source	<%1
32	W. Kuhnz, T. Louton, M. Hümpel, D.J. Back, N.M. Zaman. "Influence of high doses of vitamin C on the bioavailability and the serum protein binding of levonorgestrel in women using a combination oral contraceptive", Contraception, 1995 Publication	<%1
33	Submitted to University of Sunderland Student Paper	<%1
34	Submitted to Universiti Malaysia Kelantan Student Paper	<%1
35	Submitted to University of North Florida Student Paper	<%1

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	< 5 WORDS
EXCLUDE BIBLIOGRAPHY	ON		