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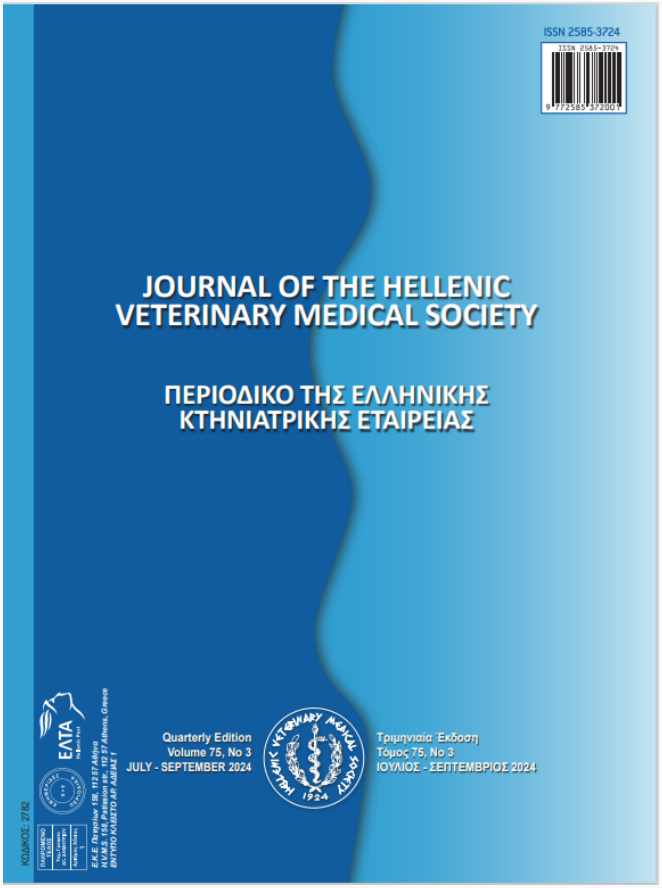

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Research article
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Vaginal cytology during the estrous cycle and early pregnancy of ewes

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ABSTRACT: Changes in vaginal epithelial cells and blood progesterone and estrogen concentrations were compared between estrous cycle and early pregnancy in multiparous Sanjabee ewes. Twenty non-pregnant ewes were synchronized with intravaginal insertion of a controlled internal drug release (CIDR) device which was in place for 7 days, intramuscular administration of a GnRH analog (Alarelin acetate; 12.5 μg) at the time of CIDR insertion (Day -7), a PGF2α analog (D-cloprostenol sodium; 125 μg) and 500 I.U. of human chorionic gonadotropin (hCG) at the time of CIDR removal (Day 0). At Day 0, all ewes were introduced to four fertile rams and observed for estrous signs. Ewes exhibiting estrous signs (n=14) were divided into two groups; group 1 was not allowed to mate (the CYCLING group) and group 2 mated (the PREGNANT group; transrectal ultrasound scanning was performed at Day 35, for pregnancy diagnosis). Vaginal smears and blood samples were taken daily and every other day, respectively, from the beginning of estrus for 20 days for vaginal epithelial cells evaluation and hormone assays. Based on the results, during Days 0 and 1 of the estrous cycle, only the percentages of intermediate cells differed between the groups ($P<0.05$). During Days 2 to 4, there was no difference in the cell populations between the groups ($P\geq 0.05$). During Days 5 to 16, the percentages of all cell types (except the parabasal cells) differed between the groups ($P<0.05$). During Days 17 to 20, the percentages of all types of vaginal epithelial cells differed between the groups ($P<0.05$). Progesterone concentration increased gradually from Days 0 to 14 of the estrous cycle in both groups; however, it decreased significantly afterward in the CYCLING group. Estrogen concentration changes showed an opposite pattern to that of progesterone in the study groups. Collectively, vaginal cytology can be used as a useful tool in assessing hormonal and physiological characteristics of the female reproductive system and thus provides a more accurate understanding of the physiology of estrous cycle and early pregnancy than clinical observation in ewes, and could be used to improve reproductive management.

Keywords: early pregnancy; estrogen and progesterone; estrous cycle; Sanjabee ewe; vaginal cytology.

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INTRODUCTION

Understanding the reproductive physiology of the estrous cycle is important in flock management (Zohara et al., 2014a). Vaginal exfoliative cytology is a sensitive indicator of the estrous cycle stage in many species and presumably reflects the balance between the influence of estrogen (E2) and progesterone (P4) (Sharma and Sharma, 2016). It is well known that the female reproductive tract is a target for the steroid sex hormones and that the endometrium and the vaginal epithelium are especially influenced by sex hormones which determine their development and function (Pérez-Martínez et al., 1999). In normal cycling female livestock, morphologic, endocrine, and secretory changes occurring in the ovaries and the tubular genitalia during the estrous cycle usually depict the stages of the cycle (Ola et al., 2006). Characterization of different phases of the estrous cycle in animals is considered to be very important in reproductive studies, particularly in seasonal breeds of animals (Zarkawi and Soukouti, 2001). In other words, vaginal epithelial cells and serum P4 concentration changes during the estrous cycle can be a useful tool for the definition of the stages of the estrous cycle (Zohara et al., 2014a) and in this regard, the vaginal smear is a simple technique to determine the stages of the estrous cycle (Sitaresmi et al., 2018).

Vaginal cytology analysis has been used to evaluate the different stages of estrous cycle of several species and its application as a useful tool for estrus detection in modern breeding stations has been described for some species and breeds of animals such as bitches, ewes, does, pigs and cows (England, 1992; Pérez-Martínez et al., 1999; Mota-Rojas et al., 2005; Ola et al., 2006; Mingoas and Ngayam, 2009; Leigh et al., 2010; Leigh et al., 2013; Zohara et al., 2014b). Vaginal cytology presents a direct correlation with the animal's hormonal state and provides essential information about the female reproductive tract conditions (Ribeiro et al., 2019). The vaginal epithelial cells have been classified according to their location in the vaginal mucosa as parabasal, intermediate and superficial cells (Mayor et al., 2007). It has been reported that when the female is in the proestrus stage, mostly nucleated and some cornified epithelial cells are present. At estrus, mostly cornified epithelial cells are present. During metestrus, cornified epithelial cells are found in the vaginal smear, whereas some nucleated epithelial cells will also be present in late metestrus. Diestrus is the longest phase in the estrous cycle during which primarily epithelial cells are found in vaginal smears (Sharma and Sharma, 2016).

Vaginal cytology may be used to clinically evaluate the hormonal status, and/or to characterize the estrous cycle stages in the ewes (Zohara et al., 2014a) and therefore, the optimum time for mating in species, in which the changes are fairly consistent (Sharma and Sharma, 2016). Accurate monitoring of estrus is crucial for the success of timed mating or artificial insemination programs (Mayor et al., 2007). Assessment of P4 concentration during different stages of the estrous cycle in animals is considered one of the most important parameters (Zarkawi and Soukouti, 2001). The P4 concentration remains at the basal levels throughout the follicular phase and will increase gradually in the luteal phase due to the growth and development of the corpus luteum (Sitaresmi et al., 2018). The relative proportion of different types of vaginal epithelial cells can be used as a marker of the endocrine environment as under the influence of estrogen, the epithelial cells accumulate a large amount of glycogen and undergo cell proliferation in the basal and parabasal layers, and more superficial squamous cells are produced (Sharma and Sharma, 2016). Exfoliated cells in the vaginal lumen are the result of increased peripheral estrogen concentrations that causes stratification of the vaginal wall. As the outermost layer moves away from the vascular supply, the cells keratinize and detach from the wall. Thus, the exfoliated cells are normally present during the estrous cycle of animals (Sitaresmi et al., 2018).

To the best of our knowledge, there is no comparative study in this particular breed of sheep evaluating the pattern of changes in the epithelial cells of the vagina along with the changes in serum concentrations of E2 and P4 of the pregnant and non-pregnant ewes during the period following estrus. The objective of the present study was to investigate if the changes in vaginal epithelial cells differ between the estrous cycle and the first 20 days of the gestation period (the peri-implantation period).

MATERIALS AND METHODS

Farm and Animals

The study was conducted on the educational and research farm of Razi University consisting of about 200 sheep located in Kermanshah, the capital city of Kermanshah province, west of Iran during March and April, 2022. The animals were maintained on natural grazing and in barns with open-air and roofed buildings, had access to fresh water freely, fed a diet that

had been adjusted to provide their requirements. The reproductive tract and mammary glands of the ewes were examined by inspection and ultrasound for the evidence of any abnormalities before initiation of the experiment. Twenty multiparous non-pregnant ewes that were clinically normal were enrolled in the experiment.

Experimental design

All the ewes were synchronized by intravaginal insertion of a controlled internal drug release (CIDR) device and an intramuscular injection of 12.5 μg of a gonadotropin releasing hormone (GnRH) analog (Alarelin acetate, Vetaroline, 10 ml vial, 5 μg/ml, Abureyhan pharma Co., Veterinary Division, Tehran, Iran) at the same time. Seven days later the CIDR was removed and 125 μg of a prostaglandin F2α analog (D-cloprostenol sodium, Vetaglandin®, 10-ml vial, 0/075 mg/mL, Aburaihan pharmaceutical Co., Tehran, Iran) and 500 I.U. of human chorionic gonadotropin (hCG; PDpreg® 5000 I.U./vial, API, BSV, Germany) were administered intramuscularly to all animals in order to induce ovulation as hCG has luteinizing hormone (LH) effect in ruminants (Cabrera et al., 2019). Then the ewes were introduced to four fertile rams and observed for estrous signs for five days. The ewes (n=14) that exhibited estrous signs (Day 0) were randomly allocated to the following groups: group 1 in which the ewes were not allowed to mate (CYCLING group; n=5) and were immediately separated from the rams; and group 2 in which the ewes were allowed to mate (PREGNANT group; n=9).

Vaginal smear and cytology

Vaginal smears were collected daily from all the ewes beginning on Day 0 (the day of estrus onset) until Day 20 with the aid of vaginal swabs with a clean, soft and pure cotton as described by Sitaresmi et al. (2018). Briefly, the vulva and perineum were rinsed with clean water and wiped with tissue paper. Then a speculum was inserted into the vagina of the ewes. A swab was inserted into the anterior vagina and gently and briskly rolled against the vaginal mucosa and carefully withdrawn. The swab was immediately smeared on a glass slide, air-dried, and immediately fixed with 100% ethanol. Three smears were prepared from each animal, and stained with Giemsa for microscopic evaluation. The cells identified in the vaginal smears by light microscopy (Gx100) were categorized as parabasal, intermediate, superficial, and keratinized cells, and also neutrophils based on their morphological and stained characteristics. The percentage of each type of vaginal cells was calculated as the number of each type of cell divided by the total number of cells (100 cells) counted within 10 microscopic fields in a Z manner on the corresponding slides, divided by the total number of cells observed within the same microscopic fields.

Blood sampling for hormone assay

Blood samples (8 ml) were collected from the jugular vein of each ewe into sterile vacuum tubes lacking anticoagulant agent every other day beginning on Day 0 until Day 20 for hormone assay. Serum was recovered by centrifugation (15 minutes at 3,000 rpm) and stored at -20 °C until the serum E2 and P4 concentrations were determined by a solid-phase competitive enzyme-linked immune sorbent assay (ELISA) using commercially available kits (ELISA, Monobind, Inc. Lake Forest, CA, USA).

Pregnancy diagnosis

Transrectal ultrasonography was performed in all animals 35 ± 2 days after CIDR removal to determine pregnancy status using a real-time B-mode scanner equipped with a 5.0 and 7.5 MHz linear rectal probe adapted on a PVC support to facilitate manipulation in the rectum. Data of four ewes out of nine in the PREGNANT group that were diagnosed as non-pregnant were excluded from the study and the remaining five ewes that were diagnosed pregnant were considered as the PREGNANT group. All animals in the CYCLING group (n=5) that were not allowed to mate were confirmed to be non-pregnant and considered as the CYCLING group.

Statistical analysis

Data were analyzed using SAS® software (Statistical Analysis System, Release 9.4. Cary, NC, USA: SAS Inst. Inc.). Changes in serum E2 and P4 concentrations were evaluated using repeated-measures ANOVA. Changes in the percentages of the vaginal epithelial cell types were evaluated using GLM. A comparison between the mean values was performed using LSD. The level of significance was set at $P<0.05$.

RESULTS

Vaginal cytology

The mean cell counts of the epithelial cells and neutrophils and the mean concentrations of E2 and P4 were compared between the groups from Days 0 (the

day of estrus onset) to 20. The results of the vaginal cytology are presented in Table 1.

No parabasal cells were observed on Days 0-1 in both groups; these cells were increased on Days 2-4 and decreased on Days 5-16 in both groups; however, they increased significantly in CYCLING group on days 17-20 compared to PREGNANT group. Intermediate cells were increased (P<0.05) on Days 0-1 in PREGNANT group; however, on Days 17-20, they increased (P<0.05) in the CYCLING group. Superficial cells presented the highest percentage on Days 0-1 in both groups and then decreased until Days 17-20 in PREGNANT group or until Days 5-16 in CYCLING group; they were increased significantly on Days 17-20 in CYCLING group. The keratinized cells presented the second higher percentage on Days 0-1 in both groups, and there was no significant difference between the groups. Then a constant decrease was noted in PREGNANT group until Days 17-20, whereas in CYCLING group they were significantly increased on Days 5-16 and then significantly decreased on Days 17-20; the differences between the groups were significant on days 5-20. Concerning neutrophils, they were constantly increased from Days 0-1 to Days 17-20 in the PREGNANT group, while in the CYCLING group a significant drop was recorded on Days 17-20.

Hormone assay

The variations in serum concentrations of E2 and P4 in the PREGNANT and CYCLING ewes are presented in Table 2. Progesterone concentration increased significantly in both groups until Day 10 and remained constant until Day 20 in the PREGNANT group, but in the CYCLING group, P4 began to decrease significantly from Day 16 to reach below 1 ng/mL on Day 18. The difference in P4 concentration between the groups was significant on Day 2 and again

Table 1 Results of vaginal cytology (mean ± SD) during the estrous cycle (CYCLING, n=5) and the first 20 days of pregnancy (PREGNANT, n=5) in Sanjabee ewes

Cell type	Ewe status	Days 0-1	Days 2-4	Days 5-16	Days 17-20
Parabasal	PREGNANT	0	2.1 ± 1.1^{Da}	0.9 ± 0.2^{Gb}	0.5 ± 0.1^{Eb}
	CYCLING	0	3.6 ± 0.8^{Db}	1.3 ± 0.8^{Gb}	20.3 ± 3.0^{Ca}
Intermediate	PREGNANT	8.1 ± 0.9^{Cb}	15.6 ± 1.8^{Ca}	12.2 ± 1.2^{Ea}	9.3 ± 1.0^{Db}
	CYCLING	4.8 ± 1.0^{Dc}	14.3 ± 1.6^{Cb}	5.5 ± 1.7^{Fc}	26.0 ± 3.1^{Ba}
Superficial	PREGNANT	51.7 ± 4.6^{Aa}	31.4 ± 2.9^{Ab}	21.5 ± 2.9^{Cc}	16.3 ± 2.8^{Cd}
	CYCLING	58.9 ± 3.5^{Aa}	31.9 ± 2.5^{Ab}	17.4 ± 8.3^{Dd}	29.3 ± 4.4^{Bb}
Keratinized	PREGNANT	33.0 ± 1.3^{Ba}	26.0 ± 2.5^{Bb}	23.0 ± 3.62^{Cc}	20.4 ± 4.9^{Cc}
	CYCLING	28.5 ± 8.4^{Bb}	25.1 ± 4.4^{Bb}	40.6 ± 5.5^{Aa}	13.7 ± 4.0^{Dc}
Neutrophil	PREGNANT	7.2 ± 4.6^{Cd}	24.9 ± 3.1^{Bc}	42.4 ± 5.7^{Ab}	53.6 ± 7.1^{Aa}
	CYCLING	7.8 ± 3.6^{Cc}	25.2 ± 4.1^{Bb}	35.2 ± 4.6^{Ba}	10.6 ± 1.5^{Dc}

$^{a-f}$ Different lower-case superscripts in the same row differ significantly (P<0.05).
$^{A-F}$ Different upper-case superscripts in the same column differ significantly (P<0.05).

Table 2 Serum estrogen (pg/ml) and progesterone (ng/ml) concentrations (mean ± SD) during estrous cycle (CYCLING, n=5) and the first 20 days of pregnancy (PREGNANT, n=5) inSanjabee ewes

Days	Progesterone		Estrogen	
	PREGNANT	CYCLING	PREGNANT	CYCLING
0	0.32 ± 0.04^{e}	0.40 ± 0.12^{e}	4.28 ± 0.41^{a}	4.48 ± 0.41^{a}
2	1.34 ± 0.23^{d}	$2.00\pm0.20^{d*}$	2.76 ± 0.28^{b}	$1.88\pm0.34^{c*}$
4	2.88 ± 0.15^{c}	2.68 ± 0.37^{cd}	1.92 ± 0.40^{c}	1.60 ± 0.23^{c}
6	3.48 ± 0.23^{c}	3.28 ± 0.19^{c}	1.62 ± 0.26^{d}	$1.04\pm0.15^{d*}$
8	5.68 ± 0.18^{b}	5.90 ± 0.10^{b}	1.21 ± 0.31^{d}	$0.88\pm0.07^{e*}$
10	6.54 ± 0.18^{a}	6.50 ± 0.26^{a}	1.20 ± 0.28^{d}	$0.76\pm0.09^{e*}$
12	7.02 ± 0.28^{a}	7.30 ± 0.18^{a}	1.10 ± 0.20^{e}	$0.78\pm0.08^{e*}$
14	7.10 ± 0.28^{a}	7.36 ± 0.34^{a}	0.98 ± 0.19^{e}	$0.36\pm0.18^{f*}$
16	7.22 ± 0.26^{a}	$3.70\pm0.21^{c*}$	0.94 ± 0.21^{e}	$2.40\pm0.12^{b*}$
18	7.14 ± 0.27^{a}	$0.69\pm0.11^{e*}$	0.78 ± 0.08^{ef}	$4.35\pm0.26^{a*}$
20	7.16 ± 0.29^{a}	$0.32\pm0.11^{d*}$	0.66 ± 0.11^{f}	$4.78\pm0.33^{a*}$

* Means with different superscripts in the same row differ significantly (P<0.05).
$^{a-f}$ Means with different superscripts in the same column differ significantly (P<0.05).

from Day 16 to Day 20 ($P<0.05$). Estrogen concentration was the highest on Day 0 in both groups. Then, it decreased significantly to reach the lowest levels on Day 20 ($P<0.05$). In the CYCLING group, the E2 concentration decreased significantly after Day 0 to reach the lowest levels on Day 14 ($P<0.05$), and then started increasing significantly from Day 16 to reach the highest levels on Day 20 ($P<0.05$).

DISCUSSION

Changes in vaginal epithelial cells and serum estrogen and progesterone concentrations were compared for the first time between PREGNANT and CYCLING Sanjabee ewes during the first 20 days after the beginning of estrous. The morphologic characteristics of the vaginal epithelial cells were in accordance with the study of other researchers and categorized into four types, i.e., parabasal, intermediate, superficial, and superficial keratinized cells and polymorphonuclear neutrophils (Zohara et al., 2014a).

On Days 0 and 1, no parabasal cell was found in the vaginal smears of the ewes in both groups. Mayor et al. (2007) reported that parabasal cells were diminished in collared peccary females during estrus. On Days 2 to 16, the percentages of parabasal cells were similar in both groups. In the study by Zohara et al. (2014a) 3.5% of parabasal cells were reported in ewes, which is similar to that observed in the present study. On Days 17 to 20, the percentage of the parabasal cells increased significantly in the CYCLING group, as the E2 concentration increased significantly from Day 16 to day 20. Sharma and Sharma (2016) reported that under the influence of E2, the epithelial cells undergo cell proliferation in the basal and parabasal layers, and more superficial squamous cells are produced.

On Days 0 and 1, the percentage of the intermediate cells was significantly greater in the PREGNANT ewes than that in the CYCLING ewes. On Days 2 to 4, the percentage of the intermediate cells increased significantly in both groups, however, the difference between the groups was not significant. In the study of Zohara et al. (2014a) the percentage of intermediate cells in metestrus was reported to be 15.8%, which is similar to those found in both groups of the present study. On Days 5 to 16, the percentage of the intermediate cells decreased in both groups, but the decrease was significant only in the CYCLING ewes. In the study of Zohara et al. (2014a) the percentage of the intermediate cells during diestrus was 4.8% in non-pregnant ewes which is similar to the 5.54% found in the CYCLING ewes of the present study; in contrast, in the PREGNANT ewes, this percentage (12.2%) was significantly greater than that of the CYCLING ewes. On Days 17 to 20, the percentage of the intermediate cells significantly decreased in the PREGNANT ewes, but it significantly increased in the CYCLING ewes. These findings are in contrast to that of Siregar et al. (2016) who reported that the proportion of intermediate cells were similar during proestrus, estrus, metestrus, and diestrus.

The highest percentage of superficial cells was recorded on Days 0 and 1, when the ewes in both groups were exhibiting estrous signs and the E2 concentration was more than 4 pg/ml. In this stage, the percentage of the superficial cells differed significantly from those of other cell types in the same and other stages of the estrous cycle. This result was in agreement with those of Leigh et al. (2010), Zohara et al. (2014a) and Siregar et al. (2016) who reported significantly more superficial cells in the vaginal smears of West African dwarf goats, Bangladesh indigenous ewes and Indonesia Aceh cattle during the estrus phase, respectively. Siregar et al. (2016) reported that increasing concentrations of estradiol in proestrus and estrus phases may be related to the high proportion of superficial cells. In contrast to these findings, Ribeiro et al. (2019) reported a predominance of intermediate cells in the vaginal smears during the induced estrus of Santa Inês nulliparous and multiparous ewes.

In the present study, the maximum mean serum E2 concentration was measured in both groups on Days 0 and 1. It has been reported that the number and proportion of vaginal superficial cells increase in proestrus and estrus under the high E2 concentration (Zohara et al 2014a; Siregar et al 2016) and decrease as the estradiol levels decrease (Siregar et al 2016). Mayor et al. (2007) reported a high proportion of superficial cells in the vaginal smears of collared peccary females when the animals were in estrus and concluded that superficial cells in proportions of 45%, and superficial plus intermediate cells in proportion of higher than 60% could be taken as an indicator of estrus. Similarly, Leigh et al. (2010) suggested that an increase in the percentage of superficial cells may be used to predict estrus in the West African dwarf doe. In another study, it was concluded that the dominant proportion of superficial cells during the estrus period in Aceh cattle might be used as the base for determining the optimal time for insemination (Siregar

et al., 2016). The results of the present study showed that during the estrus phase (Days 0 and 1), superficial and keratinized superficial cells together were at their highest rate compared to other cell types in both groups, as previously reported by Zohara et al. (2014a).

On Days 2 to 4, the percentage of the superficial cells significantly decreased in both groups, concurrently with a decrease in the E2 concentration and an increase in the P4 concentration; however, the difference between the groups was not significant. On Days 5 to 16, the superficial cells significantly decreased in both groups concurrently with a decrease in the E2 concentration; the decrease in superficial cells and E2 concentration in the CYCLING ewes was significantly greater than that in the PREGNANT ewes. Siregar et al. (2016) also reported that the number and proportion of vaginal superficial cells decrease in parallel with the decrease in the estradiol levels. Zohara et al. (2014a) found 26.3% and 11.5% superficial cells in metestrus and diestrus smears of ewes, respectively, which are less than those found in the present study (31.43% and 31.86% in metestrus and 21.53% and 17.42% in diestrus in the PREGNANT and CYCLING ewes, respectively). According to Siregar et al. (2016) the proportion of the superficial cells was higher during proestrus (31.16) and increased during estrus (46.09), and showed significant differences with metestrus and diestrus phase. In their study, the proportion of superficial cells during metestrus and diestrus was 21.67 and 21.54, respectively. The superficial cells significantly decreased in the PREGNANT ewes and increased in the CYCLING ewes, on Days 17 to 20 compared to Days 5-16. According to Sharma and Sharma (2016), under the influence of E2, more superficial squamous cells are produced. It has been demonstrated that the number and proportion of vaginal superficial cells increased under the high E2 concentration (Zohara et al 2014a; Siregar et al 2016) and decreased as the estradiol levels decreased (Siregar et al 2016). According to the findings of the present study, on days 17 to 20, the concentration of E2 decreased and increased significantly in the PREGNANT and CYCLING ewes, respectively.

The keratinized cells were the second most frequent cell population in both groups on Days 0 and 1, which is in agreement with the findings of Zohara et al. (2014a). As evidenced by Siregar et al. (2016), the keratinized cells are the result of cornification or keratinization of the superficial epithelial cells which are anuclear and serve to protect the vaginal mucosa from irritation at the time of copulation. In the present study, on Days 2 to 4, the percentage of the keratinized cells decreased in both groups as the E2 concentration decreased and the P4 concentration increased. The decrease was significant only in the PREGNANT ewes. In the study of Zohara et al. (2014a) it was found that the percentage of the keratinized cells in the metestrus phase of ewes was 26.8% which is similar to those found in both groups of the present study. Similar to the finding of Zohara et al. (2014a), in the present study, on Days 5 to 16, during which the E2 concentration decreased and the P4 concentration increased further in both groups, the percentage of the keratinized cells further decreased significantly in the PREGNANT ewes; on the contrary, in the CYCLING ewes, the percentage of the keratinized cells increased significantly, which may be due to the gradual increase in the E2 concentration and the decrease in P4 concentration at the end of this period. On Days 17 to 20, the percentage of keratinized cells decreased in both groups, but the decrease was significant only in the CYCLING group. The authors have no explanation about the unusual decrease in the percentage of keratinized cells in the CYCLING group during Days 17 to 20 despite the increase in the E2 concentration. A larger scale experiment including a greater number of ewes may be needed in order to clarify this enigma.

The percentage of neutrophils was similar in both groups on Days 0 to 4. Zohara et al. (2014a) reported that neutrophils were generally absent during the estrus phase of the cycle in ewes and that only one vaginal smear showed a small number of neutrophils (2.1% compared to 7.18%and 7.83% in the PREGNANT and CYCLING groups of the present study, respectively). Furthermore, Zohara et al. (2014a) reported that in metestrus, neutrophils (27.5%) were predominant in the vaginal smears of ewes in addition to other cell types; although similar percentage of neutrophils were observed in the current study, superficial cells were the predominant type in both groups on Days 2 to 4. The percentage of neutrophils increased significantly from Days 0 to 16 in both groups as the P4 level rose above 1 ng/ml and that of E2 decreased in both groups. Zohara et al. (2014a) reported that, when the serum P4 level was elevated during the diestrus phase, vaginal smears were dominated by neutrophils (40.2%), a finding similar to that of the present study (42.38% and 35.17% in the PREGNANT and CYCLING ewes, respectively).

It has been documented that very low concentration of P4 immediately after ovulation (Day 0) and during the period of corpus luteum formation, is followed by increasing concentration between Days 3 and 7, which then plateau up until approximately Day 12, remains relatively constant from Days 8 to 14 and subsequently shows rapid decline reaching a nadir prior to the next estrus and ovulation (Bartlewski et al., 1999). Similar observations have been reported in Western White Face and Finn ewes (Davies et al., 2006). In the current experiment, the P4 level on the estrous day was below 0.50 ng/ml in both groups. In the PREGNANT group, the maximum level of P4 during the study period was 7.22 ± 0.26 ng/ml and was observed on Day 16. In the CYCLING group, the maximum level of P4 was 7.36 ± 0.34 ng/ml, which was observed on Day 14 of the study period. In the current study, the P4 concentration on Days 0, and Days 4 to 14 did not differ significantly between the groups, but the differences on Days 16 to 20 were significant. It has been demonstrated that there are differences in the overall mean circulating concentrations of P4 amongst prolific and non-prolific breeds of sheep (Davies et al., 2006).

The oestrogen concentration decreased in both groups after estrus, and as expected, reached again the highest value on Day 20 (next estrus) in CYCLING group. It has been documented that during each follicular wave in sheep, serum concentrations of estradiol peak at the end of the growth phase of the largest follicle of the wave (Barrett et al., 2007).

CONCLUSION

In conclusion, the results of this study showed that 17 to 20 days after exhibiting estrus, the percentages of the parabasal, intermediate and superficial cells in the anterior vagina were significantly higher and those of the keratinized cells and neutrophils were significantly lower in the CYCLING ewes compared to those in the PREGNANT ewes, while the serum estrogen level was significantly higher in the CYCLING ewes compared to that in the PREGNANT ewes and the serum progesterone level was lower in the CYCLING ewes compared to that in the PREGNANT ewes. Changes in the vaginal epithelial cells and serum estrogen and progesterone concentrations of indigenous ewes could be used to improve reproductive management and facilitate the application of breeding programs and artificial insemination.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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None

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