The Bangladesh Veterinarian (2025) 42(1 – 2): 28 - 37

Prediction of genital health status of cows by exfoliative vaginal cytology

PK Paul[1], MF Islam, MM Afroj[1], MB Zaman[1], MS Tousif[1], KNHB Nabil[1], MS Hossain[1], O Oishi[1] and BF Zohara*

Department of Medicine, Surgery and Obstetrics, Faculty of Veterinary and Animal Sciences, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

Abstract

Exploration of exfoliative vaginal cytology (EVC) was conducted to detect subclinical uterine infection (SUI) in clinically normal dairy cows and to evaluate the impact of anoestrus and repeat breeding (RB) on reproductive performance. Fifty indigenous and crossbred cows were examined during the first visit (V1). Clinically normal cows (n = 23) were selected based on the absence of disease signs during external examination. Genital discharge was assessed through visual inspection for abnormalities, with or without a history of anoestrus or repeat breeding. All cows were re-examined at the second visit (V2) and subsequently monitored for a minimum of eight months, until they either became pregnant or were culled. EVC was used to establish diagnostic criteria for SUI, based on factors associated with reduced pregnancy rates. At V1, a positive EVC result defined as >45% polymorphonuclear leukocytes (PMN) or an equivalent proportion of exfoliative vaginal cells was significantly associated with lower pregnancy rates and was considered indicative of SUI. At V2, a positive EVC result of >65% PMN or a similar proportion of exfoliative vaginal cells also identified cows with SUI. Cows showing various types of vaginal discharge with exfoliative vaginal cells, particularly those with mean PMN values >45%, were predominantly in anoestrus. In contrast, cows with PMN values >60% were classified as repeat breeders, compared to those without SUI. Given that EVC involves vaginal swab collection and cytological staining, it offers a diagnostic approach with predictive value for pregnancy risk. The findings suggest that SUI, as diagnosed through EVC, is associated with reduced pregnancy rate in dairy cows. (*Bang. vet.* 2025. Vol. 42, No. 1 – 2, 28 – 37)

Introduction

Cattle are an important source of animal protein and income, playing a vital role in poverty reduction and employment generation. According to the National Economic Report (2020) of Bangladesh, livestock contributes 14% to indigenous trade and

[1]Postgraduate students, Department of Medicine, Surgery & Obstetrics, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

*Corresponding author:- E-mail: fatemazohara@hstu.ac.bd

DOI: https://doi.org/10.3329/bvet.v42i1.86506

Received: 20 July 2025; **Accepted:** 27 September 2025; **Published:** 24 December 2025

commerce, with the total value of livestock products in that year amounting to 432,151 billion Taka (1 US Dollar = 121 Bangladeshi Taka). The annual production rates are approximately 9,920,000 metric tonnes of milk; 7,510,000 metric tonnes of meat, and 1,770 million eggs. Livestock provides about 80% of the total protein demand of the population.

However, in light of the rapidly growing population, current production rates remain insufficient. A major constraint is production loss due to reproductive disorders (Sheldon *et al.*, 2004).

Exfoliative vaginal cytology (EVC) has gained attention as a tool for detecting subclinical infections of the uterus and vagina. It is recognised as a sensitive indicator of the stage of the oestrous cycle in many species, presumably reflecting the balance between oestrogen and progesterone (Reddy *et al.*, 2011). It has been used to diagnose reproductive stages and predict abnormalities during the periparturient period (Deguillaume *et al.*, 2012). Vaginal epithelial cells are classified according to their origin within the vaginal mucosa into parabasal, intermediate, superficial, and keratinised (Mayor *et al.*, 2005). Inflammatory conditions affecting the genital tract cause characteristic changes in vaginal cytology.

Understanding of normal cell clusters in the bovine vagina will assist cytological diagnosis of reproductive failure in cows. Cytological criteria for diagnosing subclinical endometritis continue to be refined, with the postpartum interval for sampling being a key variable (Chapwanya *et al.*, 2008). The severity of inflammation is assessed by determining the number of polymorphonuclear leukocytes (PMN) cells per 100 cells (PMNs plus endometrial cells) (Chapwanaya *et al.*, 2008). It has been reported that 8% PMNs or more is associated with a significantly lower pregnancy rate at 150 days postpartum. Although the specificity of this threshold was high (89.9%), sensitivity was low (12.9%), indicating that pregnancy rate is often affected by other factors.

Therefore, this study was designed to explore the relationship between exfoliative vaginal cells, genital tract infections, and reproductive disorders.

Materials and Methods

***Study area and period*:** The study was conducted on smallholder dairy farms in Sadar Upazila (Sub-district), Dinajpur district, from January to December 2020.

***Selection and grouping of animals*:** Dairy households were selected based on the presence of at least one milking or dry cow, or a heifer. A total of 88 households and one small farm were included for initial data collection. Based on a questionnaire survey, 50 animals were selected with discharge or reproductive disorder for the final study. Information on reproductive disorders was collected through interviews with the farm owners and/or workers using a structured questionnaire. The dairy cattle were examined to identify any clinical signs. Cows were grouped according to the type of vaginal discharge (clear white, opaque white, blood-tinged, or blackish) and the presence of reproductive disorders (anoestrus or repeat breeding).

***Sample collection*:** Swab samples were collected directly from the vagina using a sterile cotton bud, rolled onto a clean glass slide and air-dried.

***Methods of vaginal smear and cytology*:** The smears were stained with Leishman stain (Protocol-Hema 31, Biochemical Sciences Inc., Swedesboro, NJ, USA; Bowen, 2006). Cells were categorised as parabasal, intermediate, superficial, keratinised, and PMN, counting a minimum of 100 cells at 400× magnification (Leitz Laborlux-S, Germany) based on morphology and staining characteristics (Valerie et al., 2003). The percentage of each type of vaginal cell was calculated as the number of that cell type divided by the total number of cells observed.

***Data management and analysis*:** Data were entered into Microsoft Excel spreadsheets and analysed using the Statistical Package for the Social Sciences (SPSS), version 20.0 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics were used to summarise the results. Differences in vaginal cell counts between discharge types were compared using ANOVA, while comparisons between reproductive disorder groups were made using the Independent *t*-test. A *P*-value of less than 0.05 was considered statistically significant.

Results and Discussion

Clinical signs and Reproductive disorders

A total of 50 cows were selected with discharge or reproductive disorder (Table 1). Vaginal discharge was found in 22 animals, 12 clear white, 4 opaque white and 6 blood-tinged. Anoestrus was seen in 39 and repeat breeding syndrome in 11. In cows with anoestrus, 12 had abnormal discharge and in cows with repeat breeding 10 had abnormal discharge

Cytological characteristics

The five types of vaginal cells are illustrated in Fig. 2. Parabasal cells were small, round or nearly round and had a high nuclear to cytoplasmic ratio (Fig 1): intermediate cells were oval with large, prominent nuclei or polygonal with a small nuclear/cytoplasmic ratio were (Fig. 2). Superficial cells were polygonal and distinctly flat, with pyknotic nuclei (very small and dark) (Fig. 3). Keratinized or cornified cells were without nuclei, seen in large sheets or strings (Fig. 4). Normal bacterial flora was present and organisms often were attached to the superficial cells. Basically, streptococcus and staphylococcus were found most in cows with abnormal discharge (Fig. 2).

Table 1: Clinical findings in animals

Animal No.	Inflammation	Discharge	Reproduction history	Bacterial load
1	+	Clear white	Anoestrus	++
2	-	No	Anoestrus	+++
3	+	Clear white	Anoestrus	++
4	-	No	Anoestrus	+
5	-	No	Anoestrus	+++
6	+	Clear white	Anoestrus	+++
7	+	Blood tined	Repeat breeding	+++
8	+	Clear white	Anestrus	+
9	-	No	Repeat breeding	++
10	-	No	Anoestrus	+
11	+	Opaque white	Repeat breeding	-
12	+	Blood tined	Repeat breeding	+++
13	+	Blood tined	Repeat breeding	+++
14	-	No	Anoestrus	++
15	-	No	Anoestrus	+
16	-	No	Anoestrus	++
17	-	No	Anoestrus	-
18	+	Opaque white	Repeat breeding	+
19	-	No	Anoestrus	+
20	-	No	Anoestrus	++
21	+	Blood tined	Repeat breeding	+
22	+	Clear white	Anoestrus	+
23	+	Clear white	Anoestrus	+
24	+	Clear white	Anoestrus	-
25	+	Clear white	Anoestrus	+
26	-	No	Anoestrus	++
27	-	No	Anoestrus	+++
28	-	No	Anoestrus	+
29	-	No	Anoestrus	++
30	-	No	Anoestrus	++
31	-	No	Anoestrus	+
32	+	Clear white	Anoestrus	++
33	-	No	Anoestrus	+
34	+	Clear white	Anoestrus	+
35	-	No	Anoestrus	++
36	-	No	Anoestrus	++
37	+	Blood tined	Repeat breeding	+++
38	-	No	Anestrus	++
39	+	Clear white	Repeat breeding	+
40	+	Opaque white	Anoestrus	+++
41	-	No	Anoestrus	+++
42	+	Clear white	Anoestrus	++
43	-	No	Anoestrus	+
44	-	No	Anoestrus	+
45	-	No	Anoestrus	++
46	-	No	Anoestrus	++
47	-	No	Anoestrus	++
48	+	Opaque white	Repeat breeding	+++
49	-	No	Anoestrus	+++
50	+	Blood tined	Repeat breeding	+++

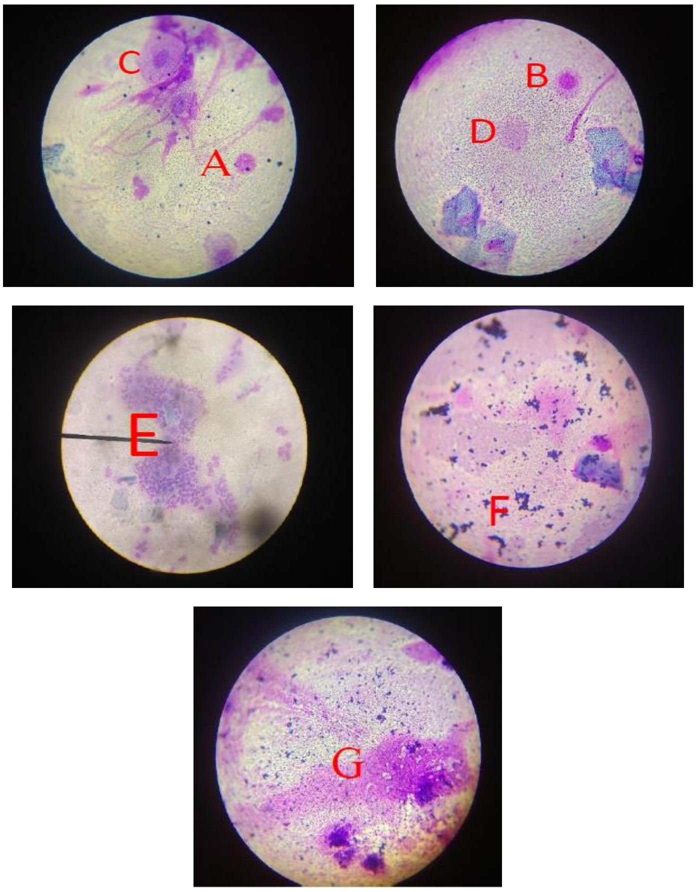

Fig. 1: Vaginal cytology of anoestrus and repeat breeder cows with abnormal discharge.

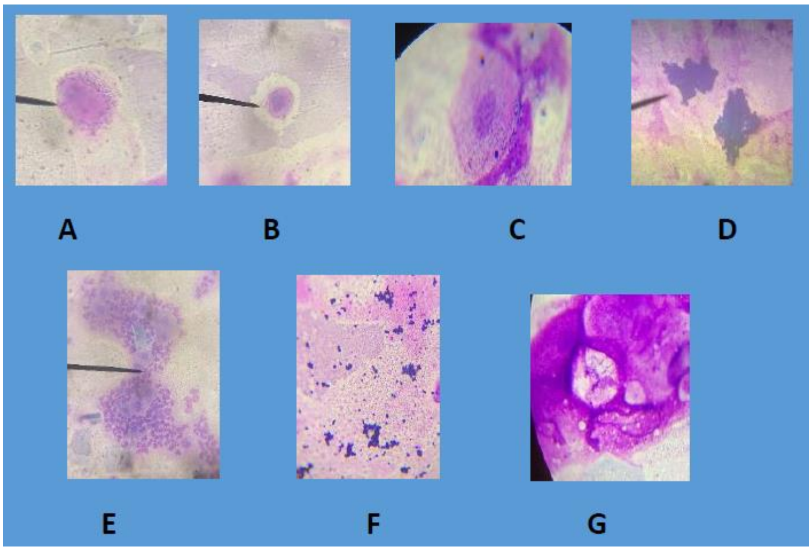

Fig. 2: Specification of different vaginal exfoliative cells and others (A: Para basal cell, B: Intermediate cell, C: Superficial cell, D: Keratinized, E: PMN infiltration, F: Bacterial foci, G: Goblet cell).

Average values of vaginal exfoliative cells on the basis of vaginal discharge are presented in Table 2. There was no significant difference of the para basal cells, intermediate cells, superficial cells and keratinized cells on the basis of different discharge. The mean value of PMN cells was significantly different in relation to type of discharge. Among all groups, the highest value of PMN was in case of blood-tinged discharge.

Table 2: Average value of different vaginal exfoliative cell on the basis of vaginal discharge

Vaginal cells	Clear white	Opaque white	Blood-tinged	No discharge	Level of significance
Para basal	28.2 ± 6.2	22.8 ± 6.5	22.7 ± 3.7	25.6 ± 6.7	0.307
Intermediate	20.6 ± 5.2	17.5 ± 4.7	15.7 ± 3.9	18.8 ± 7.3	0.487
Superficial	17.9 ± 5.8	13.3 ± 5.9	14.0 ± 5.4	15.9 ± 6.0	0.463
keratinized	3.6 ± 1.7	4.5 ± 2.7	5.5 ± 3.5	4.3 ± 3.4	0.698
PMN	51.5 ± 10.4	50.0 ± 5.2	64.8 ± 10.1	45.3 ± 13.8	0.010

Level of significance is considered P<0.05

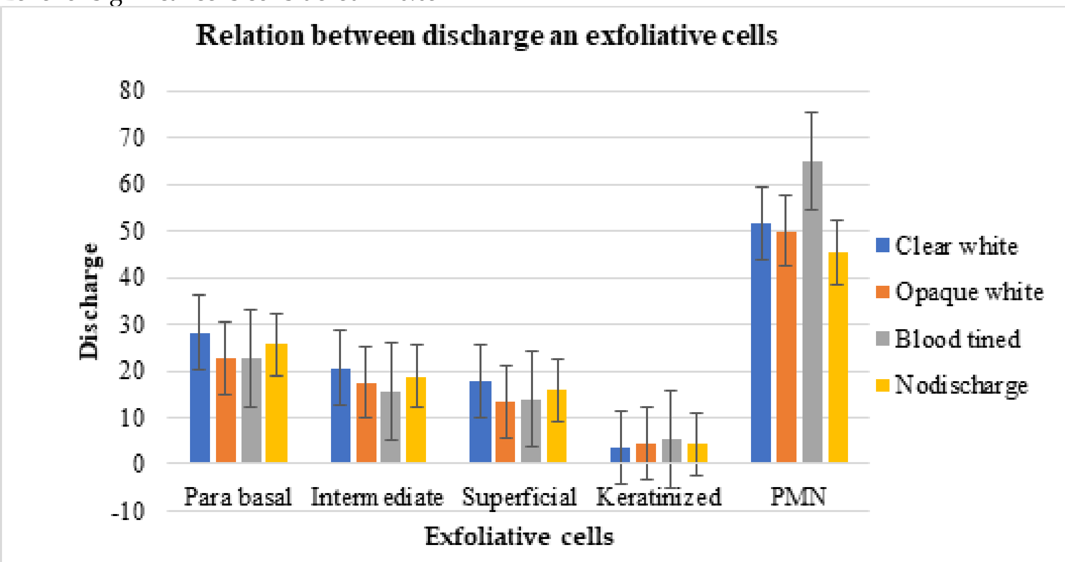

Fig. 3: Relation between discharge and exfoliative cells.

The average values of exfoliative cell types, based on reproductive disorders, are presented in Table 3. No significant differences were seen in the proportions of parabasal, intermediate, superficial, or keratinised cells between the reproductive disorder groups. However, a significant difference was in PMN values across the groups, with the highest PMN percentage in cows affected by repeat breeding.

Table 3: Average value of different exfoliative cells on the basis of reproductive disorder

Exfoliative cell	Anoestrus	Repeat breeding syndrome	Level of significance
Para basal	26.0 ± 6.9	24.1 ± 5.5	0.393
Intermediate	19.3 ± 6.8	16.5 ± 4.0	0.190
Superficial	16.2 ± 6.0	14.9 ± 5.3	0.530
Keratinized	4.0 ± 2.6	5.5 ± 4.1	0.155
PMN	46.5 ± 12.7	59.6 ± 11.7	0.004

Level of significance is considered P<0.05

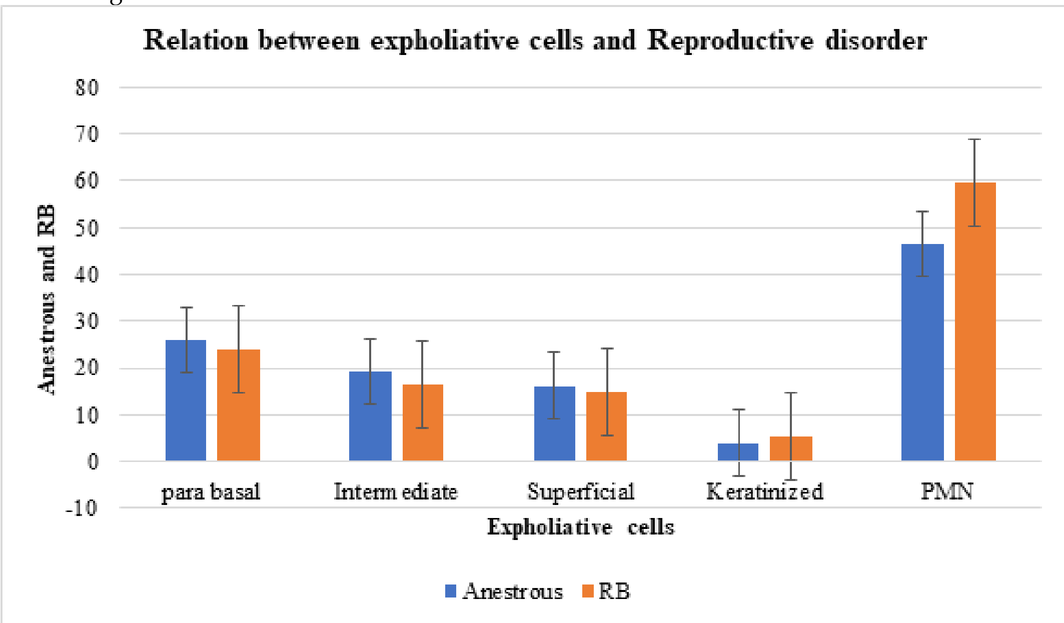

Fig. 4: Relation between exfoliative cells and reproductive disorder.

A total of 22 cows exhibited abnormal vaginal discharge, categorised as clear white, opaque white, or blood-tinged. Similar discharges have been reported by others (Kurude *et al.*, 1993; Dimitrov *et al.*, 2016). In the present field survey, two reproductive disorders were identified: anoestrus (n = 39) and repeat breeding syndrome (n = 11). Previous studies (Lafi *et al.*, 1997; McDougall *et al.*, 2007) have reported pyometra and endometritis in association with anoestrus and repeat breeding.

The mean percentage of parabasal cells in cows with clear white, opaque white, blood-tinged, and no discharge was 28.2 ± 6.2, 22.8 ± 6.5, 22.7 ± 3.7, and 25.6 ± 6.7, respectively. In anoestrus and repeat breeding cows, the mean values were 26.0 ± 6.9 and 24.1 ± 5.5, respectively, consistent with other reports (Rahayu *et al.*, 2019). Previous studies have shown that the average percentage of parabasal cells increases during the luteal phase and decreases during prooestrus, being absent during oestrus.

In sheep, parabasal cells dominate before oestrus, followed by intermediate and superficial cells (Solis *et al.*, 2008).

The mean percentage of intermediate cells for cows with clear white, opaque white, blood-tinged, and no discharge was 20.6 ± 5.2, 17.5 ± 4.7, 15.7 ± 3.9, and 18.8 ± 7.3, respectively. For anoestrus and repeat breeding cows, values were 19.3 ± 6.8 and 16.5 ± 4.0, respectively. Other studies (Rahayu *et al.*, 2019) have reported higher intermediate cell counts (45 – 60%), suggesting variation in results. Intermediate cell percentages typically increase during the prooestrus phase, as noted in mouse deer (*Tragulus javanicus*) (Najamudin *et al.*, 2010).

Superficial cell percentages for cows with clear white, opaque white, blood-tinged, and no discharge were 17.9 ± 5.8, 13.3 ± 5.9, 14.0 ± 5.4, and 15.9 ± 6.0, respectively. For anoestrus and repeat breeding cows, values were 16.2 ± 6.0 and 14.9 ± 5.3, respectively, which are in line with previously reported ranges (2 – 12%) (Rahayu *et al.*, 2019). The proportion of superficial cells increases during prooestrus and oestrus and declines or disappears during metoestrus and dioestrus (Nalley *et al.*, 2011).

The mean percentage of keratinised cells for cows with clear white, opaque white, blood-tinged, and no discharge was 3.6 ± 1.7, 4.5 ± 2.7, 5.5 ± 3.5, and 4.3 ± 3.4, respectively. In anoestrus and repeat breeding cows, values were 4.0 ± 2.6 and 5.5 ± 4.1, respectively, comparable to other findings (Rahayu *et al.*, 2019). High proportions of keratinised epithelial cells may result from elevated oestrogen concentrations during oestrus, causing thickening of the vaginal wall and sloughing of epithelial cells (Busman, 2013).

PMN percentages for cows with clear white, opaque white, blood-tinged, and no discharge were 51.5 ± 10.4, 50.0 ± 5.2, 64.8 ± 10.1, and 45.3 ± 13.8, respectively. In anoestrus and repeat breeding cows, values were 46.5 ± 12.7 and 59.6 ± 11.7, respectively. PMN values were consistently high, in agreement with previous reports (>20%) (Dimitrov *et al.*, 2019). Elevated PMN percentages (45 – 65%) have been associated with subclinical or clinical inflammation of the reproductive tract (Anderson *et al.*, 1985; Klucinski *et al.*, 1990). PMN infiltration is positively correlated with bacterial growth in the uterus and may impair uterine involution (Mateus *et al.*, 2002). Gilbert *et al.* (2005) reported that PMN presence 40 – 60 days postpartum was associated with SUI and poorer reproductive outcomes. In the present study, cows with SUI had higher PMN counts in the presence of vaginal discharge compared to clinically normal cows. However, the absence of discharge did not necessarily indicate absence of inflammation, as some cows without discharge still had PMN counts of approximately 45%.

Evaluating PMN values in relation to vaginal discharge suggests that cows with abnormal discharge particularly blood-tinged were more likely to have repeat breeding syndrome, whereas cows with abnormal discharge or no discharge but PMN counts between 45 – 64% were more often affected by anoestrus.

Conclusions

The highest PMN percentage was in blood-tinged discharges. No significant differences in the mean values of vaginal cell types were detected among cows with any type of abnormal genital discharge and reproductive disorders. In anoestrus cows, the mean percentages of parabasal, intermediate, superficial, and keratinised cells were 26.0 ± 6.9, 19.3 ± 6.8, 16.2 ± 6.0, and 4.0 ± 2.6, respectively. In repeat breeding cows, these values were 24.1 ± 5.5, 16.5 ± 4.0, 14.9 ± 5.3, and 5.5 ± 4.1, respectively. The highest mean PMN value was recorded in repeat breeding cows (59.6 ± 11.7) compared to anoestrus cows (46.5 ± 12.7).

The characteristics and proportions of different vaginal exfoliative cells, based on reproductive disorders and types of genital discharge, can provide valuable information for predicting genital health status and detecting subclinical infections in cows. Further studies with larger sample sizes and microbial analyses are recommended to improve diagnostic accuracy.

References

Anderson HL, Hamida NA, Frank A, Whitmore HL, Gustafsson BK 1985: Collection and phagocytic evaluation of uterine neutrophilic leucocytes. *Theriogenology* **24** 205–315.

Bowen RA 2006: *Techniques for Preparing a Canine Vaginal Smear.* Colorado State University, Animal Reproduction and Biotechnology Laboratory, USA.

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, Indonesia).

Chapwanaya C 2008: Uterine disease in dairy cows: Classification, diagnosis and key roles foe veterinarian. *Irish Veterinary Journal* **61** 183–186.

Deguillaume L, Geffr_e A, Desquilbet L, Dizien A, Thoumire S, Vorni_ere C, Constant F, Fournier R, Chastant-Maillard S 2012: Effect of endocervical inflammation on days to conception in dairy cows. *Journal of Dairy Science* **95** 1776–1783.

Dimitrov D, Manov V, Ralchev I, Hristov K, Popov G 2016: *Tradition and Modern Veterinary Medicine* **1** 27-32.

Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M 2005: Prevalence of endometritis and its effect on reproductive performance of dairy cows. *Theriogenology* **64** 1879–1888.

Rahayu J, Salmah R, Yusuf M, Ramadhan B, Sari DK 2019: The profile and percentage of vaginal epithelial cell numbers during the estrous cycle in Bali cattle. *IOP Conference Series: Earth and Environmental Science* **247** 012006.

Klucinski W, Targowski SP, Winnicka A, Miernisk-Degorska E 1990: Immunological induction of endometritis model investigations in cow. *Zentralbl Veterinarmed* **37** 148–153.

Kurude NP, Jalnapurkar, Mantri AM 1993: Exfoliative vaginal cytology and serum progesterone levels in normal and abnormal oestrus cycle of cow. *Indian Journal of Animal Reproduction* **14** 1013.

Lafi SQ, Khamas WA, Hailat AM, Darraji AI, MA Fathalla 1997: Vaginal cytology in small ruminants. *Indian Veterinary Journal* **74** 662-665.

Mateus L, Lopes da Costa L, Carval H, Serra P, Silva JR 2002: Blood and intrauterine leukocyte profile and function in dairy cows that spontaneously recovered from postpartum endometritis. *Reproduction in Domestic Animal* **37** 176–181.

Mayor P, Galvez H, Guimaraes DA, Lopez-Gatius F, Lopez- Bejar M. 2005: Serum estradiol-17β, vaginal cytology and vulval appearance as predictors of estrus cyclicity in the female collared peccary (*Tayassu tajacu*) from the eastern Amazon region. *Animal Reproduction Science* **97** 165-174.

McDougall S, Macaulay R, Compton C 2007: Association between endometritis diagnosis using a novel intravaginal device and reproductive performance in dairy cattle. *Animal Reproduction Science* **99** 9–23.

Najamudin, Rusdin, Sriyanto, Amrozi S, Agungpriyono dan TL Yusuf 2010: Penentuan Siklus Estrous Pada Kancil (*Tragulus Javanicus*) Berdasarkan Berdasarkan Sitologi Vagina. *Journal of Veterinary* **11** 81-86.

Nalley WMM, Handarini R, Rizal M, Arifiantini RI, Yusuf TS, Purwantara B 2011: Penentuan siklus estrous berdasarkan gambaran sitologi vagina dan profil hormon pada Rusa. *Timor Journal of Veterinary* **12** 98-106.

Reddy KCS, Raju KGS, Rao KS and Rao KBR, 2011: Vaginal cytology, vaginoscopy and progesterone profile: breeding tools in bitches. *Iraqi Journal of Veterinary Science* **25** 51-54.

Sheldon IM, Bushnell M, Montgomery J, Rycroft AN 2004a: Minimum inhibitory concentration of some antimicrobial drugs against bacteria causing uterine infections in cattle. *Veterinary Record* **155** 383–387.

Solis G, Aguilera JI, Rincon RM, Banuelos R, Arechiga CF 2008: Characterizing cytology (ECV) in ewes from 60 days of age through parturition. *Journal of Animal Science* **82** **(**Suppl.1).

Valerie R, Beimborn M, Heather L, Tarpley, Perry JB and Kenneth SL. 2003: The Canine Estrous Cycle: Staging Using Vaginal Cytological Examination. *Veterinary Clinical Pathology Clerkship Program*, The University of Athens, Greece. pp 6.