

## ASSESSMENT OF THREE DIFFERENT ENDOMETRIAL CYTOLOGICAL SAMPLING METHODS IN POSTPARTUM BEEF COWS

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### ABSTRACT

The aim of this study was to assess three different cytological endometrial sampling methods used to estimate polymorphonuclear leukocytes (PMN) under high power field (HPF) microscopy and to determine subclinical endometritis in postpartum beef cows. Forty beef cows aged 3-7 years were sampled at week three and four after calving by endometrial cytology methods. The cytological sampling methods used included; cotton swab (CS), cytobrush (CB) technique, and low volume flush (LVF), respectively. The mean PMN counts at the third week was higher ( $p < 0.01$ ) (12.2 cells HPF<sup>-1</sup>) than on the fourth week (4 cells HPF<sup>-1</sup>). The average PMN counts using CB alone was significantly higher (11.3 cells HPF<sup>-1</sup>) than CS (7 cells HPF<sup>-1</sup>) and LVF (6 cells HPF<sup>-1</sup>) methods. Smears from CB had more endometrial cells (58.55 cells HPF<sup>-1</sup>) at HPF, which was significantly higher ( $p < 0.01$ ) than CS and LVF methods. Both CB and CS methods yielded more intact cells (62.4 % and 61.9 %) ( $p < 0.01$ ) than LVF (52.4 %). The prevalence of subclinical endometritis in the beef cows between 22 and 28 days postpartum using a threshold value of  $\geq 8$  % by cytobrush method was 12.5%, which is considered low. In conclusion, CB method was found to be better and effective technique in comparison to other cytological methods used in obtaining endometrial cytology samples.

**Keywords:** endometritis, postpartum, cytology, neutrophils.

### INTRODUCTION

When a cow's uterus is exposed to several types of microbial infections after parturition, it can cause severe economic losses in cattle (Sheldon *et al.*, 2006). Uterine infections can be classified as puerperal metritis, clinical metritis, clinical endometritis and subclinical endometritis (Sheldon *et al.*, 2006). Subclinical endometritis (SCE) is prevalent in high producing dairy cows and, it has been associated with decreased pregnancy per insemination, extended interval to pregnancy and increased culling rate (Gilbert *et al.*, 2005). The precise diagnosis of cows suffering from endometrial infections is hampered by the lack of consensus on an acceptable definition of endometritis which is simple and effective in cows (Gilbert *et al.*, 2005; Sheldon *et al.*, 2006). A huge portion of the problem is that most cows experience some degree of endometritis during normal uterine involution after birth. SCE is an inflammation of endometrium without the presence of mucopurulent exudates accumulating in the vagina (Sheldon *et al.*, 2006). SCE is also termed as 'cytological endometritis' (Gilbert *et al.*, 2005; Dubuc *et al.*, 2010). Dubuc *et al.* (2010) described cytological endometritis as "an elevated ratio of PMN in endometrial cytology samples obtained through cytobrush or low-volume uterine lavage."

Despite the fact that transrectal palpation of the uterus is the most common means of diagnosing

postpartum metritis and clinical endometritis, there is an agreement that this method lacks the accuracy to identify cows with subclinical endometritis and subsequent reduced fertility (LeBlanc *et al.*, 2002; Runciman *et al.*, 2008). Several methods are now used for the detection of SCE, however, only a few of these methods can be performed through the collection of endometrial cells. In these techniques, endometrial and inflammatory cells may be collected by using a guarded cotton swab (Studer and Morrow 1978), a uterine biopsy (Bourke *et al.*, 1997), uterine lavage (Gilbert *et al.*, 2005) or cytobrush (Kasimanickam *et al.*, 2004). Both cytobrush (CB) and uterine lavage (LVF) are less invasive techniques than a uterine biopsy (Kasimanickam *et al.*, 2005). Application of CB is less harmful than LVF, because the fluid (normal saline 0.9%) used in LVF produces endometrial irritation (Brook, 1993). Saline solution also extends the time required to get samples, 17% failure to get back saline and increases the alteration of cells harvested by LVF method (Kasimanickam *et al.*, 2005). However, Barlund *et al.* (2008) described CB as the most reliable method of diagnosing endometritis in cattle.

The biggest problem associated with the diagnosis of subclinical endometritis is that no consensus has been reached by previous studies about the cutoff values used to differentiate diseased from healthy cows. Furthermore, the differences in the timing of sampling and the diagnostic tests used for endometritis have been variable, making comparisons among studies almost impossible. Different threshold values for the proportion

of PMN have been suggested, these vary from 5 to 18% (Kasimanickam *et al.*, 2004; Gilbert *et al.*, 2005; Barlund *et al.*, 2008). Most of the previous studies relating to SCE were carried out in dairy cows and only very few studies were conducted in beef cows. The incidence of subclinical endometritis in beef cows in Malaysia is unknown, and there is a lack of information on it. Thus the objective of the present study is to evaluate different endometrial cytology methods in the diagnosis of subclinical endometritis in beef cows and to provide recommendations regarding the employment of these techniques.

## MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia (Ref. UPM / IACUC/AUP-R099/2015; 10 February 2016).

**Animals:** The study was conducted in 48 beef cows (Brangus and, Kedah-Kelantan), during 15-28 days post calving. The study was conducted at the Universiti Putra Malaysia (UPM) farm between October 2015 and July 2016. The beef farm is located in Serdang, Selangor (average temperature 28 °C and relative humidity about 70%) and has about 100 beef cows of different breeds. The cows are between 3 and 7 years old, with body condition scores (BCS) ranging from 2.5 to 4 (325-480 kg). The cows were managed under free grazing system and fed with concentrated feed that comprised of alfalfa, corn silage, beet pulp, cottonseed, soya bean, corn, and barley. Individual animal data such as the history of calving, lactation breed and parity were recorded. The farm used bulls with high fertility and passed a breeding soundness examination every two months for natural mating with the cows showing clinical signs of estrus.

**Examinations of cows:** All the cows were examined via transrectal palpation during weeks 3 and 4 post calving in order to evaluate uterine involution, symmetry as well as position of the uterus. The cows with abnormal vaginal discharges were excluded from the study. Out of a total of 48 cows that calved between October 2015 and July 2016 at the UPM farm, 4 cows were excluded from the study due to clinical endometritis with acute mucopurulent discharges. Additionally, 2 cows were excluded due to the failure of passage into the cervix during the cytological sampling at week 4 post calving, while fluid could not be recovered from 2 cows by LVF. Forty beef cows with clear vaginal discharges were sampled by three different endometrial cytology methods during this study.

**Endometrial cytology:** Endometrial samples were taken between days 15 and 20, and days 22 and 28 in the postpartum period to count the PMN in the endometrial

samples, and to determine the prevalence of SCE between days 22-28 in the postpartum beef cows. The vulva and perineum were cleaned and disinfected using povidone-iodine (Betadine®, MEDA Pharma S.P.A., Milan, Italy), rinsed with water and dried with a clean paper towel. In each cow, samples for endometrial cytology were initially collected by a cotton swab (CS), then cytobrush (CB) and lastly low volume flush (LVF).

**Cytobrush method:** In this method, a sterile Cytobrush Plus GT (Medscand Medical, Germany) was modified for utilization in cows (Madoz *et al.*, 2013). The handle was shortened to 2 cm and threaded to enable it to be inserted into a stainless steel rod (artificial insemination gun; 65 cm × 4 mm). The cytobrush and stainless steel rod combination were then inserted into a plastic sheath (Chemise Sanitaire, IMV Technologies, France) to avoid vaginal contamination, lubricated with a gel (Triad Sterile Lubricating Jelly, H&P Industries Inc., Mukwonago, WI, USA), and introduced into the vagina. Next, a sleeved arm was introduced into the rectum to facilitate passage of the instruments through the genital tract and os cervix. Once the device was passed into the cervix, the cytobrush was exposed and turned (360°) to get cellular materials from the adjacent endometrium. The cytobrush was then withdrawn from the genital tract, and the samples were rolled onto glass slides.

**Cotton swab:** A sterile cotton was attached to the tip of the AI gun and the cotton and stainless steel rod combination (AI gun) were then covered by a plastic sheath (Chemise Sanitaire, IMV Technologies, France) to avoid vaginal contamination. A few drops of sterile normal saline (0.9 %) was used to make the cotton swab wet before obtaining the uterine sample. The same procedure that was used in the cytobrush method for sample collection was used here. Collected samples were then rolled on a clear glass slide.

**Low volume flush (LVF):** In the LVF method between 50 and 60 mL of sterile saline (0.9 %) was infused into the uterus (Kasimanickam *et al.*, 2005) using a plastic sheath supported by an AI gun as in the other procedures above. After the instrument had been passed through the cervix region into the end of the uterus, the AI gun was withdrawn from the female genital tract, leaving just the plastic sheath. The other end of the plastic sheath was connected to a rubber piece in order to facilitate infusion of the saline solution using a 50 mL syringe. After the infusion of 50 to 60 mL of sterile saline into the uterus, the uterus was massaged for 20 seconds and then retracted to recover the fluid by negative pressure aspiration into a syringe. The recovered fluid was then transferred into a test tube and placed in an ice box until used, usually within 3 hrs. Endometrial samples were centrifuged at 3,000 rpm for 5 minutes as reported by Kasimanickam *et al.* (2005). A drop of the sediment was

streaked onto a clean microscopic glass slide and air dried.

All slides were fixed with methanol for 30 minutes and stained with 5% Giemsa stain for three minutes and dried.

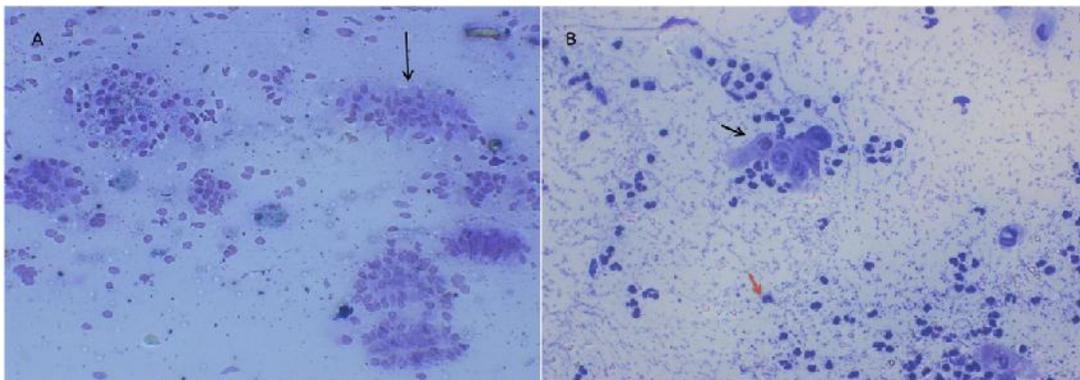
**Cytological evaluation:** All the slides were blindly evaluated by selecting ten high power field (HPF) to determine the total cellularity of endometrial cells (epithelial cells and PMN) and cellular morphology (Percentage of intact and distorted cells). All slides were also evaluated by counting 300 cells at  $400\times$  magnification (Leitz Laboulux-S, Wetzlar, Germany) to determine the PMN %. About 40 slides were selected randomly and blinded to the pathologist to get intraobserver repeatability and reduce bias. Two endometrial threshold values  $\geq 5\%$  and  $\geq 8\%$  were used to determine the prevalence of subclinical endometritis in the farm between 22 and 28 days postpartum (Gilbert *et al.*, 2005; Barlund *et al.*, 2008; Santos *et al.*, 2009; Plontzke *et al.*, 2010; Madoz *et al.*, 2013 ).

**Statistical Analyses:** All the Numerical data were tested by using the Kolmogorov–Smirnov test for normality

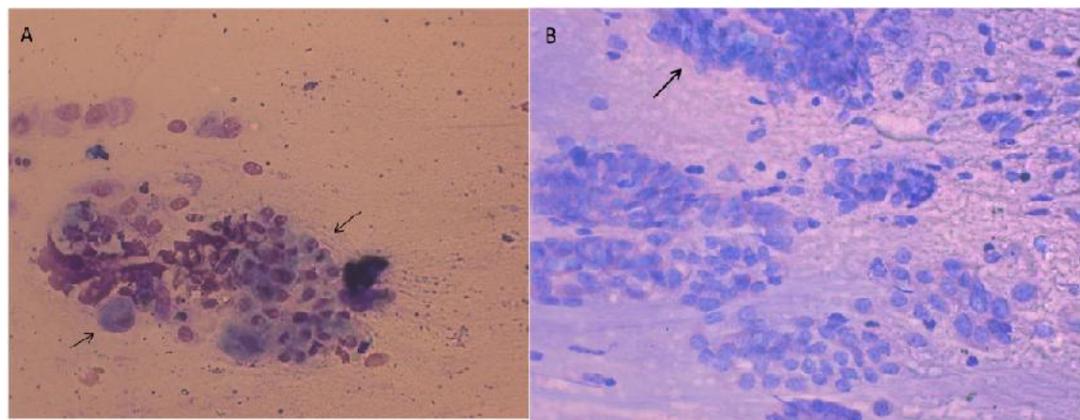
distribution with a statistical software program (SPSS version 20; IBM Corporation, NY, USA). Since the data was non-normal distribution, it was analyzed using Kruskal-Wallis test and Mann-Whitney test. The level of statistical significance was set at  $P < 0.01$ . Kappa statistic was used to assess the agreement between the clinician and the pathologist who conducted the microscopic assessment, and agreement between the three methods for SCE diagnosis. The value of agreement was poor if  $k \leq 0.2$ , fair if  $0.2 < k < 0.4$ , moderate if  $0.4 < k < 0.6$ , substantial if  $0.6 < k < 0.8$  and good if the value of  $k$  is more than 0.8 (Cocchia *et al.*, 2012).

## RESULTS AND DISCUSSION

The objective of this study was to compare the efficiency of three different endometrial cytology methods to count PMN, cellularity, and quality of endometrial cells during 15 to 28 days postpartum and also to determine the prevalence of subclinical endometritis in beef cows between day 22 to 28 postpartum.



**Figure 1:** Cytology smear obtained by CB from a healthy cow (A) and subclinical endometritis cow (B), stained with Giemsa, observed by light microscopy 400 magnification, the black arrow shows endometrial cells, and red pointed neutrophil.



**Figure 2.** Cytology smear obtained by CS from a healthy cow (A) by LVF from a healthy cow (B), stained with Giemsa, observed by light microscopy 400 magnification, the black arrow shows endometrial cells.

Based on the results observed in this study, the agreement between the clinician and pathologist was good ( $k=0.82$ ). The duration of sample collection for endometrial cytology was shorter (2-3 min.) in CB and CS than LVF (6-10 min.) method. Kasimanickam *et al.* (2005) and Cocchia *et al.* (2012) showed that the time needed to get uterine samples by CB was appropriate and quicker than LVF. Barlund *et al.* (2008) had selected CB as the reference endometrial cytological detection test because it is the most reliable method in the diagnosis of SCE and has a higher repeatability. Besides that, during the CB procedure, only one trained person was needed to get an endometrial cytological sample, whereas, LVF method always requires two people at least to get a uterine specimen. In the present study, eight cows were excluded because uterine samples could not be obtained from them due to various reasons stated previously. Unsuccessful attempts during sample collection were also reported with LVF technique resulting in a failure rate of 17% of all attempts to recover uterine fluid in Holstein cows (Kasimanickam *et al.*, 2005). All samples of endometrial cytology were obtained from cows, first with a cotton swab, then with the cytobrush, and finally followed by LVF (Figure 1 and 2). This is because LVF induces uterine irritation, thus CS and CB were done first in order to avoid alteration of the samples due to endometrial irritation resulting from LVF (Kasimanickam *et al.*, 2005; Cocchia *et al.*, 2012). Furthermore, the

amount of normal saline injected into the uterus in LVF method might also affect the PMN % and develop a false result from CB and CS methods. Interestingly, there is an argument among studies about the possibility of irritation of the endometrium by the fiber nylon of the brush during sampling (Cocchia *et al.*, 2012).

The range of PMN at weeks 3 and 4 post calving for the three different methods of endometrial cytology varied from 0-23 cell/HPF. The total mean of PMN at week 3 was higher (12.2 cells HPF<sup>-1</sup>;  $p < 0.01$ ) than week 4 (4.0 cells HPF<sup>-1</sup>) after calving (Table 1). The total average of PMN by CB method at weeks 3 and 4 was higher (11.3%;  $p < 0.01$ ) than the total average of PMN % in CS and LVF (7.0 and 6.0), respectively. The mean PMN obtained using CB technique was higher at week 3 (16.9 cells HPF<sup>-1</sup>;  $p < 0.01$ ) than week 4 (5.6 cells HPF<sup>-1</sup>), while that of CS (11.02 cells HPF<sup>-1</sup>) at week 3 was higher ( $p < 0.01$ ) than week 4 (3 cells HPF<sup>-1</sup>). Similarly, the mean PMN obtained from LVF at week 3 was also higher (8.65 cells HPF<sup>-1</sup>;  $p < 0.01$ ) than week 4 (3.5 cells HPF<sup>-1</sup>) (Table 1). At week 3, the mean PMN obtained by using CB was higher ( $p < 0.01$ ) than CS and LVF methods, and the average of PMN from CS was greater ( $p < 0.01$ ) than LVF method. At week 4 after calving, the mean PMN obtained by CB was higher ( $p < 0.01$ ) than the other methods; CS and LVF, while the LVF had a slightly higher value than the CS which was not significant (Table 1).

**Tables 1. The mean of PMN by three different endometrial cytological methods at week 3 and 4 postpartum.**

Method	W3 mean PMN/HPF $\pm$ SD	W4, Mean PMN/ HPF $\pm$ SD	Mean* $\pm$ SD
CB	16.92 $\pm$ 6.87 <sup>A</sup>	5.67 $\pm$ 1.97 <sup>a D</sup>	11.3 $\pm$ 7.56 <sup>A</sup>
CS	11.02 $\pm$ 3.97 <sup>B</sup>	3 $\pm$ 1.77 <sup>b E</sup>	7 $\pm$ 5.07 <sup>a</sup>
LVF	8.65 $\pm$ 4.68 <sup>c</sup>	3.35 $\pm$ 2.02 <sup>c E</sup>	6 $\pm$ 4.47 <sup>a</sup>
Mean*	12.2 $\pm$ 6.32 <sup>A</sup>	4 $\pm$ 2.26 <sup>a</sup>	-

CB = Cytobrush, CS= Cotton swab, LVF= Low volume fluid, PMN= Polymorphonuclear leukocytes

Capital and small letters within the rows indicate to significant differences at  $p < 0.01$  for the same method at week 3 and 4. Different capital letters within the columns indicated to a significant difference at  $p < 0.01$  between three different methods. Capital and small letters for mean\* within the rows and column indicate to significant difference at  $p < 0.01$  between weeks and three methods.

**Table 2. The endometrial cells/HPF and quality of these cells by three endometrial cytological methods.**

Method	Mean endometrial Cell / HPF	intact cell%	distorted %
CB	58.55 $\pm$ 8.92 <sup>A</sup>	62.4 <sup>c</sup>	36.7 <sup>E</sup>
CS	39.78 $\pm$ 6.60 <sup>b</sup>	61.9 <sup>c</sup>	37.8 <sup>E</sup>
LVF	36.65 $\pm$ 8.26 <sup>b</sup>	52.4 <sup>D</sup>	47.5 <sup>f</sup>

CB = Cytobrush, CS= Cotton swab, LVF= Low volume fluid and HPF= High power field.

Different letters within columns indicate significant difference at  $p < 0.01$ .

The results of this study showed a dramatic decrease in mean PMN with advancement of the postpartum period due to infiltration of PMN during physiological events at calving, which gradually declined over the stage of the uterine involution. This finding is in agreement with previous studies that showed a reduction

in the mean of PMN as the postpartum period approached the completion of histological involution (Gilbert *et al.*, 1993; Kasimanickam *et al.*, 2005), which usually occurs at 40 days post calving (Stevenson, 1997). The results of this study also showed that the mean PMN at weeks 3 and 4 was higher from the CB method than the other

two methods; CS and LVF, respectively. Due to the fiber nature of the cytobrush tip and the rigid nylon, the vertical position of the handle tip allowed more endometrial cells to be picked up from the surface of the uterus in comparison to the other two methods. Besides that, the CB method allows for a deeper penetration of the uterine endometrium resulting in more PMN (Martin-Hirsch *et al.*, 2000). Due to the soft nature of the cotton in the CS method, the number of endometrial cells and PMN was lower than in the CB method at weeks 3 and 4 (Cocchia *et al.* 2012). At the same time, the average PMN were lower in cows through LVF method than CB during 15 and 28 days postpartum, which was due to difficulties in getting infused fluid because the uterus was not fully involuted at this time, consequently only fewer cells were obtained (Kasimanickam *et al.*, 2005).

The study also showed that the mean number of total cells within the smear was significantly higher (58.55 cells/HPF) in CB method than CS and LVF methods. However, the total cells obtained from the CS method was (39.7) greater than LVF (36.6), but not significantly different (Table 2).

The mean intact cells in the CB and CS methods were higher (62.4 % and, 61.9 %;  $p < 0.01$ ) than the LVF

method (52.4 %). The distorted cell mean was higher (47.5 %;  $p < 0.01$ ) in LVF method than the other two methods, while the CS method had more average distorted cells than CB but it was not significantly different (37.8 %: 36.7%) (Table 2). In cytological studies, PMN % obtained from endometrial cytology samples is essential due to its effect as a cut-off threshold used to judge subclinical endometritis in cows (Riddle *et al.*, 2007). The efficiency of uterine smears is referred by the existence of uterine epithelial cells, while lack of the smears with a suitable count of epithelial cells and PMN is useless as these samples smears cannot be used in the detection of disease (Martin-Hirsch *et al.* 2000). This study showed that all endometrial cytological smears displayed good endometrial cellularity, and the CB technique was the one that produced a higher rate of endometrial cells in comparison to the CS and LVF methods. This result agrees with a previous study that showed the number of cells in the endocervical samples using CB method was more than CS method due to the ability of brush (CB) to pick up more quantity of cells (Trimbos and Arentz 1985).

**Table 3. The occurrence of subclinical endometritis by using two thresholds PMN % by different cytological methods 22-28 day postpartum.**

Method	Threshold 5 % PMN	Threshold 8 % PMN
CB	50 %	12.5 %
LVF	20 %	10 %
CS	12.5 %	7.5 %

CB = Cytobrush, CS= Cotton swab, LVF= Low volume fluid method.

**Table 4. Comparison of LVF and CS techniques using  $\geq 5$  % and  $\geq 8$  %PMN (22-28 d) threshold value with cytobrush technique as the reference diagnostic test for subclinical endometritis.**

Method	Threshold %	Sensitivity	Specificity	Kappa (P value)
LVF	5 %	20 %	100 %	0.21 (0.02)
CS	5 %	15 %	100 %	0.15 (0.07)
LVF	8 %	60 %	100 %	0.72 (<0.01)
CS	8 %	40 %	100 %	0.53 (<0.01)

CS= Cotton swab, LVF= Low volume fluid method.

The present study showed increased in abnormal endometrial cells in LVF in comparison with CB and CS methods. This was in agreement with previous findings by Barlund *et al.* (2008), and Kasimanickam (2005), who believed that LVF method has a harmful effect on the integrity of the cells. There are several factors that were thought to contribute to an increased rate of distorted cells by using LVF method. The extended period (3 to 6 hr) of processing the uterine samples may affect the shape and appearance of the cells and total counts in the sample. The pH of normal saline used to recover the uterine sample (4.7 and 7.0) also has a potential reason to

yield more distorted cells (Vanderwall and Woods, 2003). The centrifugation process is also another reason that can increase the possibility of obtaining distorted cells, even though 3,000 rpm for 5 minutes is considered safe for the cells. Barlund *et al.* (2008) and Kasimanickam *et al.* (2005) reported variable rate of distorted cells at centrifugation speeds of 600 g (2,400 rpm) for 15 min and 766 g for 5 minutes, respectively, while Gilbert *et al.* (2005) used a slower speed (1000 rpm for 7 min). The mean number of distorted cells in CB and CS methods were relatively high, which can be attributed to the nature of the cotton soft fiber which resulted in

adherence of the endometrial cells and destruction of the cells (Bourke *et al.*, 1997). On the other hand, Cocchia *et al.* (2012) reported increased rate of distorted cells by using cytobrush method in comparison with cotton and LVF methods, this was attributed to the nature of rigid fibers of the brush which might have induced traumatic effect to the endometrial cells during sampling resulting in the development of distorted cells (Martin-Hirsch *et al.*, 2000).

Until now, there is still no consensus among previous studies about the particular threshold value for PMN % and the precise time (range 21 to 60 days post calving) from sample collection to the diagnosis of SCE in cows. Scholars have suggested many threshold values for the proportion of PMN, ranging from 5% to 18% (Gilbert *et al.*, 2005; Kasimanickam *et al.*, 2004). The occurrence of SCE using two threshold of PNM% ( $\geq 5$  and  $\geq 8$ ) by different cytological methods are presented in Table 3. The CB method had a higher occurrence of SCE at threshold of  $\geq 5$  (50%) and lower at threshold  $\geq 8$  (12.5%). The LVF method had an occurrence of 20% and 10%, while the CS method had an occurrence of 12.5% and 7.5% at threshold levels of  $\geq 5$  and  $\geq 8$ , respectively. Due to the low rate of PMN cells obtained during this study, especially at week 4, decreased rate of SCE was noticed in these cows.

The agreement between the cytological methods; LVF, CS with CB was poor ( $k=0.20$ ) and ( $k=0.15$ ). The lack of agreement among the methods used to determine SCE rate may be due to the difference in the methods used. For example, cytobrush has the rigid fiber that allowed picking up of more endometrial cells than other methods (Trimbos and Arentz, 1985). Similarly, the lack of cotton to pick up and the pressure of texture cotton during sampling and rolling of the sample on the glass slide played a vital role in reduction of endometrial cells and PMN (Bourke *et al.*, 1997; Trimbos and Arentz, 1985). On the other hand, most of the studies used the LVF procedure at late postpartum period (more than 30-day post calving) (Barlund *et al.*, 2008; Gilbert *et al.*, 2005; Santos *et al.*, 2009), while the current study was conducted early after calving. The difficulty of recovering the fluid infusion from the uterus during its involution, especially at week 2 post calving (Kasimanickam *et al.*, 2005; Saut *et al.*, 2013), and lack of infusion fluid to cover the whole uterus (big uterus after calving) might have led consequently to the decreased rate of endometrial cells and PMN % during sampling, thus resulting in a disagreement with the others methods. For these reasons most studies considered the cytobrush method the fastest and most reliable endometrial cytological method to collect uterine samples (Barlund *et al.*, 2008).

At the same time, the present study also showed the agreement between LVF and CS methods by using CB method (PMN % threshold  $\geq 8$ ) as a reference was  $k=$

0.70 and  $k=0.52$ , respectively. The agreement between the methods by using a threshold value of  $\geq 8$  was substantial and better than using a threshold of  $\geq 5$  %, which may be due to the general reduction of mean PMN during week 4 and a decreased in the number of cows that exceeded the threshold of 8%. Cows that depended on open grazing method had less bacterial contamination and had the ability to clean their uterus early in the postpartum period (Madoz *et al.*, 2013). Barlund *et al.* (2008) indicated a good agreement;  $k=0.074$  between LVF and CB for diagnosis of endometritis in dairy cattle between 28 and 41 days postpartum. The study also showed a sensitivity (92.3 %) and specificity (93.9 %) at a threshold value of  $\geq 8$  % PMN and CB as a reference, which was similar to what was observed in the current study. In contrast to the present study, a study by Saut *et al.* (2013) showed a high rate of SCE; (42.3-22.2 % and 65.4- 59.3 % between among 21 and 28 days postpartum through CB and LVF, respectively, by using a threshold value of  $\geq 18$  % PMN, giving a sensitivity of 50-100 % and specificity of 35.3-53 %. Santos *et al.* (2009), reported in his study that endometritis was uncommon in beef cattle and had minor impacts on fertility of these cows. Besides, the prevalence of SCE in dairy cows was more than in beef cows, and it ranged between 5 and 76 % using different thresholds and diagnostic methods. A study in beef cows in the USA using a threshold value of 5.5 % through LVF method showed 88 % SCE in cows before week 2 after calving, 77 % between 2-7 weeks postpartum and 17 % after week 7 (Santos *et al.*, 2009). In comparison to this study, the rate of SCE using a threshold value of  $\geq 5$ % was 50 % using CB, 20 % using CS and 12.5 % using LVF. These differences in the prevalence of SCE may be attributed to differences in geographic area, environment and breed..

Therefore, more studies are necessary in order to demonstrate the dynamics of polymorphonuclear leukocytes in postpartum beef cows and their relationship with the occurrence of subclinical endometritis in these cows by using endometrial cytology methods.

**Conclusion:** This study reported a higher number of PMN/PHF at week 3 than 4 post calving. In addition, the CB method proved to be the best as more endometrial and PMN were obtained through this method. The rate of intact cells was higher in CB and CS than LVF methods, while the percentage of distorted cells was greater in LVF than the other two methods. The rate of SCE in beef cows was lower in comparison with other previous studies and the agreement between CB, CT and LVF ranged from weak to moderate. The cytobrush method was the best and most reliable endometrial cytological method to collect uterine samples.

**Conflict of interest:** We certify that there is no conflict of interest with any financial organization regarding the content of this manuscript.

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