

## Comparison of the cytobrush, cytotape and uterine lavage techniques in healthy postpartum cows

ANCHAL RANA\*, MADHUMEET SINGH, PRAVESH KUMAR and AKSHAY SHARMA

Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh 176 062 India

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The poor reproductive performance of dairy herds has become a major concern worldwide. Sub-clinical endometritis (SCE), a prevalent condition in postpartum dairy cows, is one of the most common causes of reducing conception rate, profitability and sustainability of dairy farm. SCE is a chronic, unapparent inflammation process of endometrium with a relatively high proportion of polymorphoneutrophils (PMNs) in the uterus, which suppress the fertility of affected cow (Sheldon *et al.* 2009). The condition affects approximately 30% of lactating dairy cows, with the prevalence ranging from 11 to >70% in some herds and cause substantial economic losses due to decrease in both fertility and milk production (Gilbert *et al.* 2005, Barlund *et al.* 2008). As SCE cannot be detected by simple visual inspection and trans-rectal method, complementary examinations are necessary for its diagnosis.

Sub-clinical endometritis in dairy cows has been one of the most controversial topics discussed among many researchers due to the lack of a diagnostic gold standard. But nowadays improved diagnostic methods such as ultrasonography, endometrial cytology, optical density and leukocyte esterase test have been proposed to enhance diagnostic accuracy (Wagener *et al.* 2017). In both field and research setups endometrial cytology is the preferred technique to diagnose SCE, mainly for reasons of simplicity and low cost (De Boer *et al.* 2014). But among cytological methods, a technique that yields well-preserved cells representative of a large uterine surface area without causing harm to the reproductive tract is required for consistent and reliable cytological results. Keeping this in view, the present study was designed to compare the accuracy of three different techniques of endometrial cytology in healthy cows during postpartum period.

**Animals:** The present study was carried out on 21 apparently healthy postpartum dairy cows (Jersey and Jersey-crossbred) calved normally (no pre, during or postpartum complication) from July 2018 to March 2019 at Livestock dairy farm, CSKHPKV Palampur. These cows were examined at weekly interval from day 14 to 42 after parturition.

\*Corresponding author e-mail: anjlirana02@gmail.com

**Sample collection:** In each animal, samples for endometrial cytology were collected, first by cytotape (CT) followed by a cytobrush (CB) and finally by a uterine lavage (UL) method at weekly interval from day 14 to 42 postpartum of which no cow was in estrus at the time of examination. Cytotape assembly was a modified version of cytobrush assembly developed by authors. The stainless steel rod of the cytobrush assembly was cut (approx. 4 cm) from the top so that more proportion of the stylet (having tape) could come out from the rod to collect cytology sample and the tip of the stylet was made blunt to avoid any injury. Thus, the cytotape assembly was made up of stainless steel rod, stainless steel stylet (blunt tip) and paper/surgical tape (3M Micropore™). A 2.5 cm sterile clean piece of paper tape was rolled on the top of the stylet tip (towards blunt tip) and then was retracted back into the rod of the instrument. Then the assembly was placed in a sanitary plastic sheath to protect it from vaginal contamination. Under per-rectal guidance, the assembly was introduced into the vagina after cleaning the vulvar area. At external os of the cervix, sheath was perforated and cytotape assembly was introduced into the body of the uterus. Sample was taken by rolling the stylet having tape, on the wall of uterine body with gentle pressure of index finger through rectum. The cytotape was retracted into the tube of the instrument prior to removal from the uterus. Smear was formed by gently rolling the tape on clean glass slide.

In all cows, after sample collection with CT, endometrial sample for cytology was collected by cytobrush (Fig. 1) (Barlund *et al.* 2008).

Finally, for the collection of PMN cells by uterine lavage

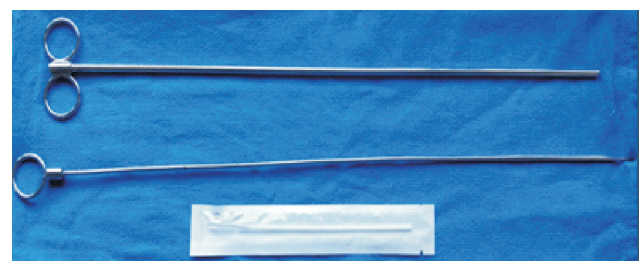


Fig. 1. Cytobrush assembly

method, 30–40 ml of sterile normal saline (Sodium Chloride 0.9%, Abbott Laboratories Ltd., Saint Laurent, Quebec) was infused via 50 ml sterile disposable syringe (Dispo Van; Hindustan Syringes and Medical Devices Ltd., India) into uterus through Foley's catheter of which 15–20 ml of fluid was recovered. After trans-rectal massage of uterus for 5–10 seconds, fluid was aspirated and collected into sterile centrifuge tube. Aspirated fluid was centrifuged at 1000 rpm using conventional centrifuge machine (Remi R-303) for 8 minutes. Supernatant was discarded and smear was formed with the remaining pellet and allowed to air dry.

**Staining technique and cytological evaluation:** Prepared slides were air dried, fixed in methanol for 15 minutes and then stained with Giemsa stain for 45 minutes. All the slides were evaluated by optical light microscope at 40 × and 100 × magnifications. Cells were counted in a total of 10 fields and the percentage of epithelial cells and PMNs were assessed at 40× magnification. Slides prepared by CB, CT and UL technique were compared on the basis of percentage of neutrophils, total cellularity, red blood cells (RBC's) contamination and quality of smear (percentage of intact cells). Other parameters (total cellularity, quality and RBC contamination) were assessed at 100× magnification in 10 high power fields.

To evaluate total cellularity, the numbers of cells were estimated and classified as no cells, low (<50), moderate (50–100) and high cells (>100). Quality was evaluated by estimating the percentage of intact cells leading to the categorization in three different groups: very good (>75% intact cells), good (50–75% intact cells), or bad (<50% intact cells). Finally, the RBC contamination was evaluated by estimating the quantity of erythrocytes and subsequent classified as no RBCs, low (disperse erythrocytes), moderate (high number of erythrocytes), and high RBCs (strong background of erythrocytes) (Pascottini *et al.* 2015).

**Statistical Analysis:** Categorical variables were compared using contingency table and Pearson chi square test, whereas continuous variables were compared by using one-way analysis of variance (ANOVA) with SAS (Statistical Analysis Software), SAS® 9.2 TS Level version 2M2 for windows (USA).

Postpartum dairy cows (21) were included in present study. In total 315 smears were analyzed, all of which were readable and acceptable for further analysis. PMN percentage obtained by three different techniques of

Table 1. Polymorphonuclear neutrophils (PMN) percentage (Mean±SEM) obtained by different methods of endometrial cytology at weekly interval from postpartum dairy cows

Days postpartum	Polymorpho neutrophils (PMNs%)		
	Mean±SEM		
	Cytobrush	Cytotape	Uterine lavage
14	17.66±1.27	16.89±0.88	16.26±0.93
21	13.75±0.96	15.13±1.02	13.63±0.83
28	11.43±1.06	11.04±0.89	10.55±0.80
35	9.69±0.92	9.30±0.93	9.27±0.90
42	7.87±0.67	7.86±0.71	8.01±0.65

endometrial cytology has been shown in Table 1.

The present study recorded no difference between all three methods in % PMN cells recovered. This finding is similar to Kasimanickam *et al.* (2005) and Jayaram (2018) who compared cytobrush technique with uterine lavage method whereas Pascottini *et al.* (2015) and Vinita *et al.* (2018) compared cytobrush and cytotape technique and reported the similar results. Cellularity is a critical element in cytologic specimens and is closely related with the threshold used to define inflammation. The quality, total cellularity and RBC's contamination of the slides harvested by cytobrush, cytotape and uterine lavage technique have been shown in Table 2 and Fig. 2.

Perusal of the Table 2 indicated cytotape method to be the best for obtaining the cells after making smears on the basis of quality, total cellularity and RBC's contamination of the slides. In present study, it was observed that cytobrush and cytotape methods yielded similar total cellularity. However, significantly more cells were obtained by cytobrush ( $P<0.01$ ) and cytotape ( $P<0.01$ ) methods when compared to uterine lavage, as more number of smears were categorized in the category of low cellularity (<50) in later technique. Hence, the least number of total cells were obtained by uterine lavage method, as it yielded diluted sample of luminal contents (Kasimanickam *et al.* 2005, Barlund *et al.* 2008, Cocchia *et al.* 2012). Cytobrush and cytotape yielded high cellularity than lavage probably because of the soft bristles of cytobrush, when rolled over the endometrium surface collected more cells, whereas in case with cytotape, it did not have absorbent property, so most of the cellular material was adhered to the glass slide when the tape was rolled on it (Pascottini *et al.* 2015).

Table 2. Quality, total cellularity and RBCs contamination in the samples taken by cytobrush, cytotape and uterine lavage method

Technique	Quality			Cellularity			RBCs contamination			
	Bad	Good	Very Good	Low	Moderate	High	None	Low	Moderate	High
	Intact cells			Total cells						
	(<50%)	(50–75%)	(>75%)	(<50)	(50–100)	(>100)				
Cytobrush (n=100)	–	22 <sup>a</sup>	78 <sup>a</sup>	11 <sup>a</sup>	62 <sup>a</sup>	27 <sup>a</sup>	11	40 <sup>x</sup>	37 <sup>x</sup>	12 <sup>x</sup>
Cytotape (n=100)	–	18 <sup>a</sup>	82 <sup>a</sup>	14 <sup>a</sup>	67 <sup>a</sup>	19 <sup>a</sup>	17	52 <sup>xa</sup>	22 <sup>xa</sup>	9 <sup>xa</sup>
Uterine Lavage (n=100)	–	57 <sup>b</sup>	43 <sup>b</sup>	33 <sup>b</sup>	51 <sup>b</sup>	16 <sup>b</sup>	–	43 <sup>yb</sup>	42 <sup>yb</sup>	15 <sup>yb</sup>

<sup>a,b</sup>( $P<0.01$ ), <sup>x,y</sup> ( $P<0.05$ ).

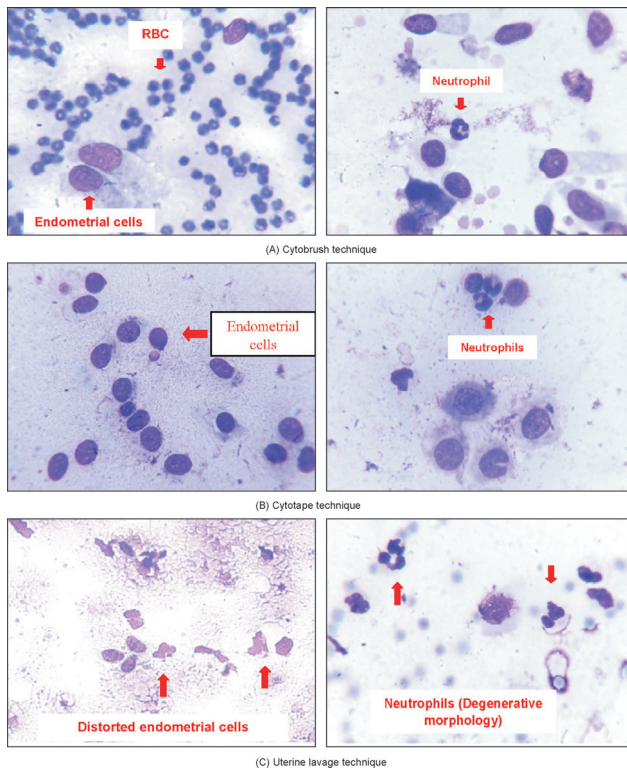


Fig. 2. Cytology smears obtained by cytobrush, cytotape and uterine lavage technique, stained by Giemsa stain and observed under light microscope ( $\times 100$ ). (A) cytobrush sample with more RBC contamination, (B) cytotape sample with more intact cells and less RBC contamination, (C) uterine lavage sample showing high percentage of distorted cells or cells with degenerative morphology.

Cocchia *et al.* (2012) reported that debris were present in a higher percentage of uterine lavage smears, on the other hand CB and CT provided clearer smear.

RBC's contamination was more in cytobrush and uterine lavage techniques whereas distorted cells were significantly ( $P < 0.00001$ ) more frequent in smears obtained by uterine lavage method as compared to other two methods. Similar results were reported by other researchers where the highest distortion was reported in cells following uterine lavage method probably due to centrifugation procedure (Kasimanickam *et al.* 2005, Barlund *et al.* 2008, Overbeck *et al.* 2011).

Uterine lavage requires the presence of 2 to 3 assistants and takes more time to perform than other two methods (Barlund *et al.* 2008, Cocchia *et al.* 2012). Presence of high RBC in uterine lavage samples was demonstrated by Kasimanickam *et al.* (2005), who compared cytobrush and uterine lavage technique in cows whereas Cocchia *et al.* (2012) compared all the three techniques in mare. This may be related to trauma resulting from the manipulation of the uterus and infusion rod while attempting to recover fluid. The failure to recover fluid and possible trauma to the uterus indicate that the lavage technique is inconsistent and possibly detrimental (Kasimanickam *et al.* 2005).

In present study, cytotape yielded more percentage of intact cells and less RBC contamination. Similar findings

were reported by Pascottini *et al.* (2015) and Vinita *et al.* (2018) who compared cytobrush with cytotape technique and reported lower percentage of distorted cells and RBC contamination in cytotape technique. Rigidity of the brush bristles and fragmentation of cells can lead to distortion of cells when the brush is rolled on the microscope slides. Contrarily, the endometrial cells can be easily detached from the tape and scattered on the glass slides, thus, resulting in a small percentage of distorted cells (Pascottini *et al.* 2015; Vinita *et al.* 2018).

In conclusion, present study revealed that, although all the three techniques provided diagnostic sample, however, cytotape method proved to be the best method, followed by cytobrush technique.

## SUMMARY

This study was conducted to compare the accuracy of three different techniques of endometrial cytology to diagnose sub-clinical endometritis in dairy cows. Postpartum dairy cows (21) with history of normal calving were selected for obtaining endometrial samples at weekly interval from day 14 to 42 postpartum. In each cow, samples were obtained in a sequential manner, first with the cytotape, then with the cytobrush followed by uterine lavage. Smears were stained with Giemsa stain method. Parameters used to evaluate these techniques were; presence of polymorphoneutrophils (%), total cellularity, red blood cell contamination and quality of the smears. No difference was recorded between the three techniques in % polymorphoneutrophils recovered. All techniques provided diagnostic samples; however, cytotape yielded the highest quality sample. On the other hand, cytobrush and cytotape methods yielded similar total cellularity ( $P = 0.3781$ ), uterine lavage yielded significantly ( $P < 0.01$ ) less number of total cells than other two techniques. Cytotape produced more intact cells than cytobrush and uterine lavage ( $P < 0.05$ ) and red blood cells' contamination was found high in cytobrush and uterine lavage techniques ( $P < 0.05$ ). Distorted cells were significantly ( $P < 0.01$ ) more frequent in smears obtained by uterine lavage. Also, uterine lavage technique is time consuming and required 2–3 assistant than other two techniques. Hence, cytotape was found to be comparatively the best technique to obtain endometrial cytology samples.

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