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Diagnosing Subclinical Endometritis in Postpartum Murrah Buffaloes Using Cytobrush Technique

Nitin Kumar Bajaj*, Salil Kumar Jain, Madhu Swamy and Omprakash Shrivastava

Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, M.P., INDIA

**Corresponding author: NK Bajaj; Email:drnitinbajaj@gmail.com*

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ABSTRACT

A total of 150 postpartum (28 to 45 days) apparently healthy buffalo with normal calving history and free from peripartum disorders from college livestock farm and organized dairy farms in and around Jabalpur were screened. After recording history all the animals were subjected to gynaeco-clinical examination, White side test and endometrial cytology by cytobrush technique. On the basis of polymorphonuclear cell (PMN) percentage the animals were divided into different groups of normal, clinical and sub-clinical endometritic buffaloes. Endometrial cytology by cytobrush technique in different groups (normal, clinical and sub-clinical endometritic buffaloes) revealed PMN percentage to be 4.34±1.85, 35.35±3.43 and 21.17±0.45%, respectively. The difference between the groups was significant ($p<0.05$). Fibroblasts count was recorded as 0.20±0.09 and 12.33±1.61% in sub-clinical and clinical endometritic buffaloes while fibroblasts were not observed in endometrial smears of normal buffaloes. The difference was significant ($p<0.05$) between the groups. It was concluded that endometrial cytology by cytobrush technique was easy and accurate method to diagnose subclinical endometritis in postpartum Murrah buffaloes.

Keywords: subclinical endometritis, endometrial cytology, Whiteside test

The current world buffalo *(Bubalus bubalis)* population is 194 million (FAO, 2010). Of these, 97.13% are in Asia and the Pacific region, whereas 57% of it is in India (110.58 million). Buffalo contributes 12.8% of world milk supply. In India, buffalo accounts for 33% of the milk producing animals and 45% of overall milk production of the country.

Buffalo milk is higher in nutrients (e.g. fat, lactose, protein and minerals) and contains less water than cow milk (FAO, 2010). While, the world cattle population over the last two decades has increased by less than one per cent per year, the buffalo population has gone up by two per cent per year, with higher rise in India (3.5%). The higher population growth rate of buffalo can be attributed to better feed conversion efficiency as compared to the local cattle.

However, the milk production is only profitable when it is free from losses that occur due to reproductive disorders resulting in increased calving intervals, decreased conception rate and decreased calf crop per animal. The annual incidence of postpartum uterine infections ranges from 10-50% in dairy cattle (Lewis, 1997) and 20-75% in dairy buffaloes (Usmani *et al*., 2001). Among various uterine affections, endometritis is most commonly encountered under field or farm conditions in buffaloes (Agarwal and Tomer, 2003).

Postpartum sub-clinical endometritis is defined as an endometrial inflammation occurring 21 days or more after parturition without any clinical signs whereas clinical endometritis is indicated by the presence of purulent/ mucopurulent discharge (Sheldon *et al*., 2006). Studies on clinical and sub-clinical endometritis reported the prevalence of these diseases ranging from 18 to 37% (Drillich *et al*., 2005) and 12 to 94% (Barlund *et al*., 2008), respectively while LeBlanc *et al*. (2012) quoted the incidence of metritis between 10 and 20%, clinical endometritis as 15% and sub-clinical or cytological endometritis as 15% in postpartum dairy cattle.

Routine methods for diagnosing endometritis involve uterine biopsy, lavage and swab but these techniques causes irritation and distortion of cells. However, recent research studies are concentrated on sophisticated diagnosis of endometrial alterations beyond clinical signs of endometritis. Keeping this in view, the present study was planned with an objective to diagnose sub-clinical endometritis endometrial cytology using cytobrush technique i*n postpartum Murrah buffaloes.*

MATERIALS AND METHODS

A total of 150 postpartum (28 to 45 days) apparently healthy buffalo with normal calving history and free from peripartum disorders from college livestock farm and organized dairy farms in and around Jabalpur were screened. After recording history all the animals were subjected to gynaeco-clinical examination, White side test and endometrial cytology by cytobrush technique.

Sample collection

After proper restraining, the buffaloes were subjected to evacuation of rectum through back racking. The perineal region and vulva were washed with savlon and water and later on disinfected with spirit swab. The vulvar lips were pulled apart by an assistant and the modified cytobrush assembly (Madoz *et al*., 2014; Singh, 2014) specially fabricated for buffaloes was introduced in the vagina. The assembly consisted of stainless steel catheter and a stylette attached with cytobrush (Hi-Cytobrush, HI-MEDIA Laboratories Limited, Mumbai, India) and covered with chemie (sterile sanitary over-sheath, 21", IMV Technologies, France).

On reaching the external os of the cervix, the outer sanitary over-sheath was perforated and the assembly was introduced into the cervix and then to the body of uterus. After assuring its location, the stylette was advanced so as to expose the cytobrush into the lumen of body of uterus where it was gently rotated clockwise and anticlockwise. Gentle pressure was applied on its tip against the uterine body per rectum for proper contact to obtain cellular material from endometrium. The cytobrush and inner stylette were then retracted back into the outer catheter to its normal position and the whole assembly was withdrawn from the reproductive tract. The threshold cut off values for diagnosis of sub-clinical endometritis by endometrial cytology were >18 % PMNs between day 20-33 days postpartum and >10% PMNs between day 34-47 days postpartum as described by Kasimanickan *et al*. (2004).

Staining Method for endometrial cytology

Immediately after removal from reproductive tract, the cytobrush was rolled over on clean microscopic glass slide, air dried and transported to the laboratory for examination). The slide was exposed to ready to use modified Wright's Giemsa stain solution (Wright's-Giemsa stain, Modified, M/s Sigma-Aldrich Inc., USA) for 2 minutes on staining rack. The stain was then diluted with equal volume of triple glass distilled water and kept for 5 minutes. The slide was then washed with triple glass distilled water and air dried. After drying slides, were screened for the presence of endometrial cells and polymorphonuclear cells (PMN cells) or neutrophils. Three hundred cells were counted under the microscope at 400X and 1000X (Oil immersion) and per cent PMN cells were calculated. This data served to classify the health status of uterus along with nature of discharge (clear, purulent or mucopurrulent) as clinical or sub-clinical endometritis or normal (without inflammation or healthy).

Statistical analysis

The data was analysed statistically by as per the standard method described by Snedecor and Cocharn (1994).

RESULTS AND DISCUSSION

The detailed results of endometrial cytology are outlined in table 01. On the basis of characteristics of cervico-vaginal mucus, Whiteside test, per-rectal examination and endometrial cytology, 50.67 (76/150) % buffaloes were found to be suffering from endometritis.

On the basis of PMN percentage the animals were divided into different groups, i.e., normal, clinical and sub-clinical endometritic buffaloes group. The incidence of sub-clinical and clinical endometritis was recorded as 26.00 (39/150) and 24.67 (37/150) %, respectively. Endometrial cytology by cytobrush technique in different groups (normal, clinical and sub-clinical endometritic buffaloes) revealed polymorphonuclear cell (PMN) percentage to be

Table 1: Endometrial cytology in normal, clinical and sub-clinical endometritic postpartum buffaloes

Groups	Endometrial cytology				
	PMN*	Endometrial cell*	Fibroblast*	RBC*	PMN %
Normal (n=06)	$13.00^{c} \pm 5.56$	$286.67^{a} \pm 5.70$	$0.00^{c} \pm 0.00$	0.33 ± 0.33	$4.34^{c} \pm 1.85$
Clinical endometritis (n=06)	$106.67^{a} \pm 10.30$	$164.50^{c} \pm 10.98$	$12.33^{a} \pm 1.61$	0.00 ± 0.00	$35.56^{a} \pm 3.43$
Sub-clinical endometritis (n=30)	$65.13^{b} \pm 1.36$	$233.47^{b} \pm 1.57$	$0.20^{b} \pm 0.09$	1.93 ± 0.61	$21.17^{b} \pm 0.45$

The means with the same superscript within the column did not differ significantly ($p>0.05$).

*Numbers per 300 cells counted.

4.34±1.85, 35.35±3.43 and 21.17±0.45%, respectively. The difference between the groups was significant ($p<0.05$). Fibroblasts count was recorded as 0.20±0.09 and 12.33±1.61 % in sub-clinical and clinical endometritic buffaloes while fibroblasts were not observed in endometrial smears of normal buffaloes. The difference was significant ($p<0.05$) between the groups.

Polymorphonuclear cells (PMN) are the predominant inflammatory cell types found in intrauterine fluid accumulations and determination of the relative proportion of PMN has been shown to be predictors of reproductive performance in the postpartum cows (Kasimanickam *et al*., 2005).

Endometrial cytology by cytobrush in the present study revealed significant difference ($p<0.05$) in PMN cell percentage in normal, clinical and sub-clinical endometritic buffaloes. It is obvious from the results of present study that there was a dramatic increase in the percentage of PMN cells in clinically endometritic and sub-clinically endometritic buffaloes as compared to normal buffaloes. However, the PMN percentage in sub-clinically endometritic buffaloes was lower than in the clinically endometritic buffaloes. This clearly reflects that in endometritis whether it is infected or moderately infected, there is an influx of PMN cells in the uterine lumen indicating an inflammatory process. Corroborating the findings of bacterial count in clinically endometritic and sub-clinically endometritic buffaloes wherein it was observed that the clinically endometritic buffaloes had a significantly higher bacterial count as compared to sub-clinical endometritic buffaloes also supports the findings that the PMN cells were higher in clinically endometritic buffaloes as compared to sub-clinically endometritic buffaloes. Thus the difference in the infected and moderately infected response is obvious. Ghasemi (2011) also reported that >18 % PMNs on endometrial cytobrush cytology was the lowest percentage of PMN associated with an elevation of inflammatory cytokines (IL-6, IL-8, TNF-α).

The cut off threshold of PMN (>18 % PMNs between day 28-33 and >10% PMNs between day 34 to 47 postpartum) percentages by cytobrush technique in the present study are higher than what has been reported earlier where the most appropriate threshold was >8 % PMNs for defining endometritis-positive disease status in cows sampled between 28 to 41 (Barlund *et al*., 2008) and 25 (Dourey *et al*., 2011) days postpartum using 150-270 day pregnancy status as the outcome, respectively. Kasimanickam *et al*. (2004) reported that a threshold of >18 % PMNs was most appropriate for cows examined between 20-33 DIM and, that >10% PMNs should be used for cows examined between day 34 and 47 DIM. Although the DIM ranged between 28 and 41 in the present study, the mean was 34.26±0.73 DIM which supports for a higher threshold to differentiate between PMN influx associated with bacterial infection rather than the PMN influx associated with the normal uterine involution process.

CONCLUSION

It was concluded that the endometrial cytology by cytobrush technique is highly accurate and simple technique to diagnose subclinical endometritis in postpartum Murrah buffaloes.

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