

Original Research Article

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## Study on Puerperal Endometritis in Crossbred Cows using Endometrial Cytology, pH and Whiteside Test of Genital Discharge and their Association with Fertility

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### ABSTRACT

The present study was conducted on early postpartum (PP, n=30) crossbred cows under field conditions to evaluate their endometrial cytology, pH and Whiteside test score of genital discharge in relation to fertility post-treatment. The puerperal discharge aspirated was categorized based on the quality into four classes as bloody chocolate coloured, bloody with white flakes, turbid purulent and clear watery blood tinged. Endometrial cytology was collected using cytobrush assembly. The cows yielding bacterial growth from the discharge were treated (Gr.I, n=19) with sensitive antibiotics (i/m) and cows not yielding bacterial growth served as control (Gr II, n=11). The treated cows were inseminated at subsequent estruses and followed for repeating to estrus for 3 cycles and/or pregnancy. The subclinical endometritis was diagnosed using cytobrush technique with mean PMN% (cut off value  $\geq 18$ ) of  $22.66 \pm 1.86$ ,  $22.00 \pm 2.17$ ,  $22.80 \pm 6.34$  and  $30.50 \pm 5.93$  in cows yielding bacterial isolates and having bloody chocolate colour, bloody white flakes, turbid purulent, and clear watery blood tinged puerperal discharges, respectively. In cows not yielding bacterial isolates, the mean PMN % were  $8.75 \pm 5.06$  and  $3.00 \pm 1.96$  for bloody chocolate coloured, and clear watery blood tinged puerperal discharges, respectively. The mean Whiteside test (WST) scores recorded were  $1.33 \pm 0.33$ ,  $1.80 \pm 0.20$ ,  $2.20 \pm 0.49$  and  $1.83 \pm 0.40$  for above four classes of puerperal discharges in cows with bacterial isolates (Gr I). These values for bloody chocolate coloured and clear watery blood tinged discharges in cows with no bacterial growth were  $0.00 \pm 0.00$  and  $0.14 \pm 0.14$ , respectively (Gr. II). The mean pH values for bloody chocolate colour, bloody with white flakes, turbid purulent, and clear watery blood tinged discharges were  $6.58 \pm 0.28$ ,  $7.21 \pm 0.27$ ,  $7.55 \pm 0.22$  and  $6.67 \pm 0.19$  in cows with bacterial isolates, with corresponding post-treatment pregnancy rates of 33.33, 20.00, 40.00 and 83.33 %, with an overall pregnancy rate of 47.36 %. While the mean pH values for cows not yielding bacterial isolates and having puerperal discharge of bloody chocolate coloured, and clear watery blood tinged characteristics were  $7.49 \pm 0.24$  and  $7.39 \pm 0.23$ , respectively. The pregnancy rates in cows under these categories of control group were 75.00 and 57.14%, respectively, with an overall pregnancy rate of 63.63%. It was concluded that the cytobrush technique (PMN%) for endometrial cytology and Whiteside test of genital discharge were good tools for ruling out the subclinical genital infections in early postpartum cows.

#### Keywords

Crossbred cows,  
Puerperal period,  
Cytobrush,  
Endometrial  
cytology, pH,  
White side test,  
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Fertility.

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## **Introduction**

The postpartum period constitute an important period in the reproductive life of bovine. In dairy cows, resumption of ovarian activity plays an important role in subsequent fertility (Darwash *et al.*, 1997). Decreased reproductive efficiency of dairy animals drastically affects economy of dairy farming. Puerperal complications lead to ascending infection and puerperal endometritis extending the days open. It is estimated that the uterus of approximately 40 % of cows is still contaminated beyond three weeks postpartum (Sheldon *et al.*, 2008). Therefore, there is a need to concentrate more on postpartum uterine health in dairy animals to sustain dairy industry, as endometritis is associated with lower conception rates, longer service period, and higher culling rate (LeBlanc *et al.*, 2002). Endometrial cytology and physical characteristics of genital discharge are considered as effective laboratory tools to predict the uterine health and infertility in cattle (Rangnekar *et al.*, 2002; Madoz *et al.*, 2013). Hence, this study was aimed to evaluate the endometrial cytology, as well as physical characteristics, pH and white side test score of genital discharge in early postpartum cows in relation to fertility post-treatment.

## **Materials and Methods**

This investigation was carried out during August 2016 to February 2017 on crossbred cows with puerperal infection (25±2 days postpartum, n=30) presented at Artificial Insemination Centres of Chikhodra and Bedva villages of Anand district in Gujarat.

### **Physical properties and culture of puerperal discharge**

The genital discharge samples collected using aspiration technique of Panangala *et al.*,

(1978) were soon observed for colour by direct examination and were categorized into four classes as bloody chocolate colour, bloody with white flakes, turbid and purulent, and clear watery blood tinged. The samples were subjected to pH estimation using digital pH meter. For white side test the discharge samples (2 ml) were subjected to heating with equal volume of 5% sodium hydroxide in glass tubes up to boiling point, and after cooling the intensity of colour changes were graded from score 1 to 4 for colourless (normal), light yellow colour (mild infection), yellow colour (moderate infection) and dark yellow colour (severe infection) (Kumar *et al.*, 2015). The samples were also cultured on blood agar for bacterial isolation, identification and antibiotics sensitivity spectrum as per Bauer *et al.*, (1966).

### **Collection and evaluation of endometrial cytology using cytobrush assembly**

The sterile cytobrush assembly (specially fabricated for collection of bovine endometrial cytology comprising intrauterine catheter and a stylette attached with cytobrush) was introduced aseptically into vagina and then through the cervix, body of the uterus and up to the mid uterine horn. The stylette was pushed to expose cytobrush and then screwed gently in clockwise direction. Gentle pressure was applied on uterine horn by the left hand via the rectum to ensure proper contact of cytobrush with the endometrium. The inner stylette was then withdrawn into the outer catheter to its normal position and then the whole catheter was withdrawn from the reproductive tract. Immediately after removing the cytobrush from uterine horn, it was rolled on clean glass slide. The slide was fixed with methyl alcohol, air dried and stained with Field stain (Eosin and Methylene blue). The slide was washed in running tap water, air dried and were observed under microscope with 400X

magnification and then oil immersion to determine the percentage of neutrophils (PMN %). The whole slide was assessed and representative area was selected to determine the PMN. A total of 100 endometrial and PMN cells were counted in each specimen and the per cent PMN cells count was assessed. The puerperal or subclinical endometritis was defined by the presence of  $\geq 18$  per cent neutrophils (PMN) in endometrial cytology samples (Galvao, 2012).

### **Antibiotic therapy and fertility**

The cows yielding bacterial growth were subjected to treatment by i/m route for 3-5 days using antibiotics sensitive on *in vitro* test (treatment Gr. I: n=19). They were followed for recovery from genital infections and subsequent estrus, breeding and pregnancy results. Whereas the cows not yielding any bacterial growth (control, Gr. II: n=11) were injected with 10 ml of normal saline by i/m route as placebo, were inseminated and followed for repeating to subsequent estrus and/or pregnancy. The observations made on PMN%, white side test score and pH of puerperal discharge, and the fertility results of cows were analyzed statistically.

## **Results and Discussion**

### **Physical characteristics and pH of puerperal discharge**

Out of 30 postpartum cows subjected to collection of puerperal discharge, 19 samples yielded bacterial isolates, comprised of bloody chocolate coloured (15.78%; 3), bloody with white flakes (26.31%; 5), turbid purulent (26.31%; 5), and clear watery blood tinged (31.57%; 6) quality. The mean pH values for respective quality discharges were recorded as  $6.58 \pm 0.28$ ,  $7.21 \pm 0.27$ ,  $7.55 \pm 0.22$  and  $6.67 \pm 0.19$ . The post-treatment pregnancy rates in cows under these categories were recorded to be 33.33, 20.00, 40.00 and 83.33

%, respectively. The mean pH values recorded in puerperal discharges of remaining cows (n=11) not yielding bacterial isolates and having bloody chocolate coloured and clear watery blood tinged discharge were  $7.49 \pm 0.24$  and  $7.39 \pm 0.23$  with pregnancy rates of 75.00 and 57.14%, respectively (Table 1).

The mean pH values recorded in the puerperal cows yielding bacterial isolates and covered under treatment group having bloody discharge with white flakes and turbid purulent quality were significantly ( $P < 0.05$ ) higher ( $7.21 \pm 0.27$  and  $7.55 \pm 0.22$ ) and alkaline as compared to the pH values found in bloody chocolate coloured and clear watery blood tinged puerperal discharges ( $6.58 \pm 0.28$ , and  $6.67 \pm 0.19$ ). These findings were supported by Boiter *et al.*, (1980) and Bhat *et al.*, (2015), who opined that pH of genital discharge increases as the growth and multiplication of organisms occur in the genitalia resulting in infection and inflammation. The cows not yielding bacterial isolates and having bloody chocolate coloured and clear watery blood tinged puerperal discharges (control group) evinced higher pregnancy rates (63.63%; 7/11) as compared to animals showing prevalence of bacterial isolates (treatment group; 47.36%; 9/19).

### **Whiteside test scores of puerperal discharge**

The mean white side test (WST) scores recorded for puerperal discharges in the cows yielding bacterial isolates and having bloody chocolate colour, bloody with white flakes, turbid purulent, and clear watery blood tinged characteristics (treatment Group I, n=19) were  $1.33 \pm 0.33$ ,  $1.80 \pm 0.20$ ,  $2.20 \pm 0.49$  and  $1.83 \pm 0.40$ , respectively.

The cows having turbid purulent puerperal discharge had the highest WST score as compared to other categories, but the

differences were not significant. The post-treatment pregnancy rates in cows under these categories were recorded to be 33.33, 20.00, 40.00 and 83.33 %, respectively (Table 1). The mean WST scores obtained for cows not yielding bacterial isolates (Group II) and having bloody chocolate coloured and clear

watery blood tinged puerperal discharges were 0.00±0.00 and 0.14±0.14 with pregnancy of 75.00 and 57.14%, respectively. The present finding of 63.33 % postpartum cows being found positive for WST is in close agreement with the observation made by Markandeya *et al.*, (2010).

**Table.1** Relationship of discharge characteristics and pregnancy rates in postpartum crossbred cows under treatment (n=19) and control (n=11) groups

Group I (Treatment): Cows yielding bacterial isolates (n=19)				
Puerperal discharge	PMN %	pH	Whiteside test score	Pregnancy rate* (%)
Bloody, chocolate coloured (3)	22.66±1.86 <sup>bc</sup>	6.58±0.28 <sup>a</sup>	1.33±0.33 <sup>b</sup>	33.33 (1)
Bloody with white flakes (5)	22.00±2.17 <sup>bc</sup>	7.21±0.27 <sup>abc</sup>	1.80±0.20 <sup>b</sup>	20.00 (1)
Turbid and purulent (5)	22.80±6.34 <sup>bc</sup>	7.55±0.22 <sup>c</sup>	2.20±0.49 <sup>b</sup>	40.00 (2)
Clear, watery, blood tinged (6)	30.50±5.93 <sup>c</sup>	6.67±0.19 <sup>ab</sup>	1.83±0.40 <sup>b</sup>	83.33 (5)
Group II (Control) Cows yielding no bacterial isolates (n=11)				
Bloody, chocolate coloured (4)	8.75±5.06 <sup>ab</sup>	7.49±0.24 <sup>c</sup>	0.00±0.00 <sup>a</sup>	75.00 (3)
Clear, watery, blood tinged (7)	3.00±1.96 <sup>a</sup>	7.39±0.23 <sup>bc</sup>	0.14±0.14 <sup>a</sup>	57.14 (4)

Means bearing different superscripts (a, b, c) differ significantly (p<0.05) within column.

\* Pregnancy rates differed significantly (p<0.05) within the column ( $\chi^2$ : 3.12).

### Endometrial cytology

The mean per cent PMN cells recorded in cows yielding bacterial isolates (Gr. I) and having puerperal discharges of bloody chocolate colour, bloody with white flakes, turbid purulent, and clear watery blood tinged characteristics were 22.66±1.86, 22.00±2.17, 22.80±6.34 and 30.50±5.93, respectively. The mean per cent PMN cells in cows of Group II not yielding bacterial isolates and having puerperal discharges of bloody chocolate colour and clear watery blood tinged characteristics were 8.75±5.06 and 3.00±1.96, respectively (Table 1). The results indicated that the cows yielding bacterial growth had comparatively higher PMN % values as compared to the cows not yielding bacterial growth, suggestive of immune response to the bacterial invasion during the period of puerperium. McDougall *et al.*, (2011) also reported that PMN (%) was a better predictor

of reproductive performance than either intrauterine bacteriology or gross vaginal inflammation score. Thus, the reflection of endometrial health in the form of PMN % in endometrial cytology can be a very good tool for confirmative clinical diagnosis for monitoring the postpartum uterine health.

The PMN value found in cows under study with different type of puerperal discharges were above the cut off value ( $\geq 18$ ) indicative of prevalence of subclinical endometritis, as reported by Galvao (2012). The present findings were in agreement with the reports of Kasimanickam *et al.*, (2004) and Baranski *et al.*, (2013), who found positive endometrial cytology score in dairy cows during the period from 22 to 42 days postpartum. The post-treatment pregnancy rates in cows with the puerperal discharges having bloody chocolate colour, bloody with white flakes, turbid purulent, and clear watery blood tinged

characteristics with respect to PMN (%) recorded were 33.33 (1/3), 20.00 (1/5), 40.00 (2/5) and 83.33 (5/6) %, respectively, with the overall pregnancy rate of 47.36 (9/19) %. These findings were in line with Kasimanickam *et al.*, (2004) and McDougall *et al.*, (2011).

It was concluded that about 63.33 % postpartum cows harbour bacterial contaminants in their uterus till 25-30 days postpartum and show varying qualities of puerperal discharges, and that cytobrush technique (PMN%) and Whiteside test are good tools for ruling out the subclinical genital endometritis in early postpartum cows with good fertility, if treated with antibiotics based on sensitivity results of isolates.

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