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## Diagnosis of clinical endometritis in graded Murrah buffaloes

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#### Abstract

A study was conducted on 50 graded murrah buffaloes with the history of repeat breeding to diagnose the endometritis by various diagnostic methods. The buffaloes were divided into five groups (n=10). The cervico-vaginal mucus were collected for estimation of pH, White side test, Bacterial load and uterine flushings were collected for PMNL (Polymorphonuclear leukocyte) cell count. The overall mean values of the pH, bacterial load and PMNL cell count were  $8.13 \pm 0.00$ ,  $77.12 \pm 0.50 \times 10^6/\text{ml}$ ,  $23.76 \pm 0.40$  respectively. The study was concluded that PMNL cell count and Bacterial load could be reliable methods to diagnose the endometritis in buffaloes.

**Keywords:** endometritis, buffaloes, diagnosis, white side test, bacterial load

#### 1. Introduction

Endometritis is termed as inflammation of the uterine endometrium and the underlying glandular tissues. During parturition, the physical barrier of the cervix, vagina and vulva is compromised in providing opportunity to bacteria to ascend the genital tract. Normal uterine defense mechanism is essential to prevent colonization of invading bacteria (Hussain, 1989) [5]. However, certain organisms may dominate the existing uterine defense mechanism and colonize to cause endometritis. The buffaloes that cannot eliminate the infection may subsequently develop endometritis. The bacteria colonize and penetrate into the uterine epithelial layer and produces toxins. The endotoxins released by gram negative bacteria, these endotoxins passes into peripheral blood circulation, prevent the secretion of the Gonadotropin releasing hormone from the hypothalamus and the luteinizing hormone from the anterior pituitary (Sheldon *et al.* 2004) [8]. Diagnosis of endometritis and immediate treatment is essential to minimize the economical losses by reducing the inter calving period in dairy farms.

#### 2. Materials and Methods

##### 2.1 pH

The cervico-vaginal mucus samples were collected in sterile tubes with the help of 20 ml syringe and AI sheath on the day of estrus for pH estimation by digital pH meter (SYSTRONICS Digital pH Meter 335).

##### 2.2 White side test

1ml of cervical mucus was transferred into sterile glass test tube and 1ml of 5 per cent sodium hydroxide solution was added. The mixture was heated at boiling temperature for 10 minutes and observed the colour reaction to White side test. If the colour changes to yellow, it was considered as positive for endometritis (Pateria and Rawal, 1990) [7].

##### 2.3 Bacterial load

The bacterial count was performed by the pour plate technique (Diliello, 1979) [3]. One ml of cervico vaginal mucus was mixed with 9 ml of sterile Normal saline solution, so as to attain 1: 10 dilution. Likewise tenfold serial dilutions were made in sterile test tubes. Plate count agar was prepared, autoclaved and kept ready in a molten state at 45 to 50 °C. 1 ml of serially diluted sample was drawn from each test tube and charged in separate labeled petriplate. 15-20 ml of molted plate count agar was poured into each petridish and mixed thoroughly by rotating in a circular fashion. The plates were incubated at 37 °C aerobically for 24 hours and individual colonies were counted with the aid of colony counter. Plates showing isolated and discrete colonies ranging from 30 to 300 in number were counted.

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The total number of bacteria in one ml of cervico vaginal mucus was calculated as follows. Bacterial colony forming unit per ml = No. of colonies x dilution rate

#### 2.4 PMNL Cell count

Uterine flushing was collected by aspiration of fluid aseptically by a sterile two-way, 20 gauge Foley's catheter as per the procedure described by Anderson *et al.* (1985) [1]. The catheter was fixed trans-cervically by inflating the balloon with 10-15 cc of air and then stylet was withdrawn, subsequently 20-25ml of sterile phosphate buffer saline (PBS) was infused into the uterus and massaged gently for mixing with uterine content. After 2 minutes, the uterine contents were flushed out aseptically with gentle pressure and allowed to flow into a sterile vial.

The uterine flushing fluid was centrifuged at 1000 rpm for 15 minutes, after discarding supernatant, cytological smears were prepared from sediment on clean glass slide. The smears were air dried, fixed with methanol for 2 minutes and stained with Giemsa stain for 25- 30 minutes. Then the slide was washed with running water and air dried. The stained smears were observed under oil immersion at 100x magnification, 200 cells were counted for per cent of PMN cells (Methai, 2000) [6].

### 3. Results and Discussion

The incidence of endometritis is 10.28% in the present study. The mean pH values of cervico-vaginal mucus collected from endometritic buffaloes in all the five groups are presented in table.1. The pH of cervical mucus of all the experimental groups ranged from 7.90 to 8.25. The overall mean pH of cervical mucus of all the groups was  $8.13 \pm 0.00$  (Alkaline).

All the experimental buffaloes had abnormal uterine discharges of mucus containing flakes of pus, mucopurulent and completely purulent with light yellow (32%), yellow (48%) and dark yellow (20%) colour reaction to white side test. The positive result of white side test could be explained on the basis of number of leucocytes and cellular debris present in the cervical mucus. The normal cervical mucus has less number of leucocytes, which doesn't cause any change of colour in white side test. Whereas, in clinical endometritis, the cervical mucus contains increased number of leucocytes which causes colour reaction to white side test (Deori, 2002) [2]. Another reason for colour reaction might be due to ribonucleic acid present inside the nucleus of white blood cells which reacts with 5% NaOH to produce Yellow colour (Gupta *et al.*, 2011) [4].

The mean bacterial loads of all the groups are presented in table.1. The overall mean bacterial load of all the groups are  $77.12 \pm 0.50 \times 10^6$ . The overall mean per cent of PMNL cell count of all the groups are  $23.76 \pm 0.40$ .



Fig 1: Showing PMNL cell-Giemsa stain

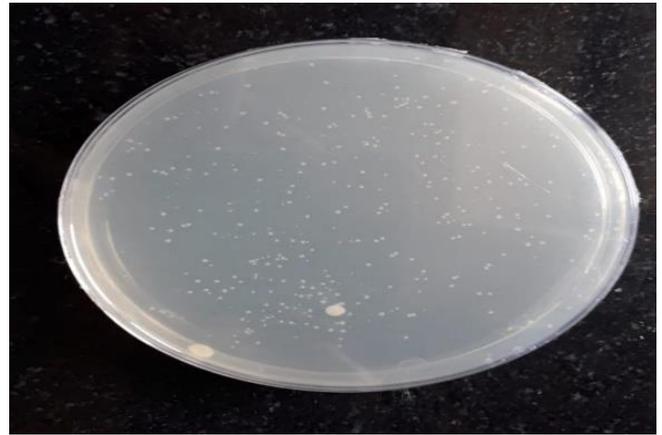


Fig 2: Bacterial load-Plate count agar

Table 1: Mean pH, Bacterial load and PMNL cell count.

Group	pH	Bacterial load (X10 <sup>6</sup> /ml)	PMNL Cell count
G-I	8.14±0.01	75.3±1.29	23.50±1.50
G-II	8.11±0.02	76.6±1.11	23.10±0.67
G-III	8.13±0.01	78.2±1.32	24.10±0.78
G-IV	8.12±0.01	77.1±0.91	23.70±0.73
G-V	8.15±0.01	78.4±0.89	24.40±0.61

### 4. Conclusion

The present study concluded that the bacterial load and PMNL cell count could be the reliable methods to diagnose the endometritis in buffaloes.

### 5. References

- Anderson KL, Hemeida NA, Frank A, Whitmore HL, Gustafsson BK. Collection and phagocytic evaluation of uterine neutrophilic leukocytes. *Theriogenology* 1985;24:305-317.
- Deori S. Use of Bacterial modulins in Treatment of Endometritis cows (Doctoral dissertation, IVRI, Izatnagar, U.P, India 2002).
- Diliello LR. *Clinical Microbiology*. Published by Avi Publishing Co. INC Westport, Connecticut 1979.
- Gupta JP, Shyma KP, Mohandas AC, Sneharaj RK. White side test to study subclinical endometritis in crossbred cattles. *Bhartya Krishi Anushandhan Patrika* 2011;26(3, 4):123-125.
- Hussain AM. Bovine uterine defense mechanisms: a review. *Zentralblatt fur Veterinarmedizin. Reihe B. Journal of veterinary medicine. Series B* 1989;36(9):641-651.
- Metahai A. Efficacy of Lipolysaccharides and autologous plasma in the treatment of endometritis in cows. PG Thesis, TANUVAS, Madras 2000.
- Pateria AK, Rawal CVS. White side test for subclinical metritis in buffaloes. *IJAR* 1990;11:142.
- Sheldon IM, Dobson H. Postpartum uterine health in cattle. *Animal reproduction science* 2004;82:295-306.
- Solmon Raju KG, Naidu KV, Rao KS. Cellular composition of uterine flushings and bacterial load of cervical mucus in healthy and endometritis affected buffaloes. *Indian Journal of Animal Sciences (India)* 2006.