

## THERAPEUTIC MANAGEMENT OF REPEAT BREEDING DUE TO SUB-CLINICAL ENDOMETRITIS IN CROSSBRED COWS

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**Abstract:** Thirty two (32) repeat breeding crossbred cows were selected on the basis of history and per rectal examination. All animals were subjected to White Side Test before and after antibiotic treatment. Cervical mucus was collected from white side positive animals and was used for in- vitro selection of most sensitive antibiotic, physico-chemical studies (appearance, consistency, pH and White Side Test), before and after antibiotic treatment. White side positive animals were treated with most sensitive Levofloxacin antibiotic (@30ml intra uterine) for five days and out of 32 animals, 28 cows become White Side negative in next estrus. Blood samples were collected before and after antibiotic treatment for the estimation of Hb, PCV, TLC, TEC of all selected animals. Out of Thirty two animals, twenty eight animals become white side negative (87.5%) after antibiotic treatment. The levels of Hb, PCV and TEC increased after antibiotic treatment and the total leukocyte count ( $\times 10^3/\mu\text{l}$ ) has significantly decreased ( $12.26 \pm 0.58$  vs.  $7.43 \pm 0.45$ ). All the white Side negative cows ( $n=28$ ) were inseminated. Out of 28 inseminated cows 16 (57.14%) become pregnant. On the basis of above finding it is concluded that Levofloxacin combination is effective in treating sub-clinical endometritis and it has also improved general health condition of the animals which might be helpful in conception of repeat breeder cows.

**Keywords:** Sub-clinical endometritis, Repeat breeding, Levofloxacin, Recovery rate.

### Introduction

The repeat breeding cow has normal or nearly normal estrous cycles and estrous periods and has been bred two or more times by fertile bull/semen yet failed to conceive. The clinical examination of the animal may fail to reveal any definite lesion or condition to explain the failure of conception (Roberts, 1971). The incidence of repeat breeding reported is 5-32% in cows and 6-30% in buffaloes (Gupta *et al.*, 2005).

Abattoir studies have also reported that 50% of genital tract had evidence of endometritis (Hartigan *et al.*, 1972), though only 12.5% had shown gross lesions. So, subclinical endometritis is a main contributor to the repeat breeder syndrome of bovines (Noakes *et al.*,

2001) and it is a major source of economic loss in dairy herds causing infertility in cattle and buffaloes.

It is difficult to diagnose a case of sub-clinical endometritis under field conditions from normal cyclic cows. White side test on cervical mucus could diagnose sub-clinical endometritis which is one of the major causes of reproductive failure in cattle. Various antimicrobials have been suggested for treatment of endometritis. Fluoroquinolones are exceptionally important and rapidly developing group of antimicrobial drugs introduced into medicine for wide variety of infections (Purohit and Sharma, 2007). The present study reports the efficacy of intrauterine levofloxacin in sub-clinical metritis, diagnosed on the basis of white side test.

### **Materials and methods**

Experimental animals comprised of thirty two (n=32) apparently healthy repeat breeding crossbred cows, aged about 3- 8 years. All cows were examined per-rectally to rule out any developmental defect of genitalia. They were kept under uniform feeding and managerial conditions at Instructional Dairy Farm, Nagla, G.B. Pant University of Agriculture and Technology, Pantnagar- 263 145 (Uttarakhand). All the cows were subjected to White Side Test to rule out possibility of mild infection/endometritis. Cervical mucus was collected at the time of estrus by recto-vaginal technique. After restraining the animal in the insemination crate, the vulvar and perineal regions were washed with mild antiseptic solution. The uterus was gently massaged per rectum and cervical mucus flowing out was collected in sterilized test tube (Nair and Kharche, 1988). This mucus was used for pH, White Side Test and also used for selection of sensitive antibiotics in vitro. Following antibiotic treatment at subsequent estrus, again cervical mucus was collected for white side test and also for pH examination. Estrual cervical mucus was subjected to white side test as described by Popov (1969). About 2 ml cervical mucus was mixed with 2 ml of 5 % sodium hydroxide (NaOH) solution in a test tube and boiled on the flame of spirit lamp. The appearance of light yellow or yellow colour was taken as a positive indication of subclinical endometritis. Samples showing no colour change after boiling with equal amount of 5 % sodium hydroxide were considered as normal.

For determination of pH of cervical mucus, pH paper strips (Hi Media Laboratories Ltd., Mumbai) ranges of 6.5 to 10.5 were used. pH paper strip was touched with the cervical mucus and the colour matched with standard colour present on the strip and pH noted. For selection of most sensitive antibiotic only 20 samples were cultured on nutrient agar media

(HI-Media Ltd. Mumbai) and obtained mixed bacteria were used for determination of *in-vitro* antibacterial activity. Five antibiotics (Hi Media Laboratories Ltd., Mumbai), namely enrofloxacin (10 mcg), cefotaxim (10 mcg), Levofloxacin (5 mcg), tetracycline (10 mcg) and ciprofloxacin (10 mcg) were used for the selection of most sensitive one by standard disc diffusion technique (Bauer *et al.*, 1966). White side positive animals were treated with most sensitive (Lenovo AP containing Levofloxacin @30ml intra uterine) antibiotic for five days. Blood samples were collected from the animals at the time of estrus before and after giving them antibiotic treatment and measured hemoglobin, packed cell volume, total erythrocyte count. All White side negative animals (n=28) were inseminated out of which 16 (57.14%) become pregnant. The data were analyzed statistically and subjected to the test of significance as per the methods described by (Snedecor and Cochran, 1989).

### Result and Discussion

Antibiogram pattern of 20 samples (Table 1; Plate 1) obtained from cervical mucus of 20 repeat breeding crossbred cows suffering from subclinical endometritis revealed that 85% samples were susceptible to levofloxacin followed by ciprofloxacin(75%) and enrofloxacin (70%). Cefotaxim and tetracycline were found to be least sensitive with only 30% and 25% sample susceptibility, respectively. Out 32 animals only from 20 samples were taken for *in-vitro* antibacterial sensitivity test to know the most effective antibiotic in herd. Results of the study revealed that the most effective antibiotic was levofloxacin followed by ciprofloxacin, enrofloxacin, cefotaxim and tetracycline. Bhattacharyya *et al.* (2011) reported that intra uterine fluoroquinolones (Levofloxacin and Ciprofloxacin) are very much effective in treating sub-clinical endometritis in cows.

**Table 1: Results of antibiogram of bacterial samples obtained from cervical mucus of repeat breeding crossbred cows**

S. No.	Antibiotics used	Total no of samples	No of sensitive samples(>10mm) inhibition zone	Sensitivity (%)
1	Levofloxacin	20	17	85
2	Ciprofloxacin	20	15	75
3	Enrofloxacin	20	14	70
4	Cefotaxim	20	6	30
5	Tetracycline	20	5	25



**Plate 1: Antibiotic sensitivity test, showing zone of inhibition**

The efficacy of treatment increases by the addition of anti-protozoal with fluoroquinolones. The host defenses against infections enhanced by Vitamin E through improving phagocytic cell function and fertility. Treatment with Vitamin E might have improves oxygen utilization, endometrial health. It also provides assistance for metabolism as a cellular antioxidant for effective innate defense that may enhances fertility. Bhattacharyya *et al.* (2011) also reported 78.26% recovery rate in his study by using levofloxacin combination. Kumar *et al.* (2014) also found 83.34% recovery rate with the levofloxacin combination.

The mean pH of cervical mucus before and after antibiotic treatment was  $7.87 \pm 0.10$  and  $6.94 \pm 0.65$ , respectively. In this study the mean pH value declined significantly from pre treatment to post treatment towards normal range.

The pH of cervical mucus before treatment was alkaline (more than 7.6) indicating infection. A number of worker reported that higher pH towards alkaline side is associated with uterine infection (Singh *et al.*, 2004). This increase in pH may be caused due to metabolites of bacteria and inflammatory exudates in estrual cervical mucus (Salphale *et al.*, 1993). Increased pH is not suitable for survival of spermatozoa and embryo in the uterus (Sheldon *et al.*, 2006), however, during infection bacterial toxin binds directly to uterine epithelial and stromal cell to stimulate the release of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). The role of PGE<sub>2</sub> is luteotropic and it may prevent luteolysis (Bogan *et al.*, 2008). After treatment with Levofloxacin and Tinidazole combination, discharges were clear and transparent at next

estrus with pH of (6.94\*±0.65) in next estrus. Similar results were reported with ciprofloxacin combination by Singh *et al.*, 2009. The pH reduction may be due to lowering in bacterial count and subsequently inflammatory process in the uterus after therapy (Shaktawat, 2005).

**Table 2: Haematological findings of the treated and untreated animals**

Blood parameter	Group	
	Before antibiotic treatment	After antibiotic treatment
Haemoglobin (gm %)	8.41±0.14	10.48±0.183
Packed cell volume (%)	27.16±0.36	31.13±0.45
Total erythrocyte count(10 <sup>6</sup> /ml)	6.24±0.05	7.62±0.11
Total leukocyte count(10 <sup>3</sup> /μl)	12.26±0.58*	7.43±0.45

Mean bearing \* differ significantly at 5% level.

Roberts (1971) reported that anaemia (reduced Hb) in repeat breeding cows may be associated with reproductive disorders. The mean value of haemoglobin increased after antibiotic treatment due to improvement of general body condition, but statistically there was no significant difference (Table 2). Our reported value lies within normal range. Radiostits *et al.* (2007) reported normal range as 8.0 to 15.0 g/dl. The mean packed cell volume increased after treatment but statistically there was no significant difference. Normal packed cell volume in healthy cows was reported from 30 to 40% (Benzamin, 2007). Thrall (2004) reported that in inflammatory disease, erythropoietin is diminished presumably because of inflammatory cytokines leading to lowered erythropoiesis and ultimately lowered packed cell volume value in blood. The mean erythrocyte count (x10<sup>6</sup>/ml) before and after antibiotic treatment were 6.24±0.05 and 7.62±0.11, respectively (Table 2). The mean value has increased after treatment but there was no significant difference. Ahmad *et al.* (2003) observed significantly (P<0.05) higher total erythrocyte count in normal cyclic cows than repeat breeding cows.

The mean TLC showed a significant decrease (P<0.05). Normal TLC value ranges from 4.0 to 12.0×10<sup>3</sup>/μl (Radostits *et al.*, 2007). Leukocytosis occurs as a result of infection in the body. The degree of leukocytosis depends upon several factors including nature of the causative agent, severity of infection, resistance of animal and localization of inflammatory response (Benjamin, 1978).

On the basis of above finding it is concluded that Levofloxacin combination is effective in treating sub-clinical endometritis and it has also improved general health condition of the animals which might be helpful in conception of repeat breeder cows.

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