

CLINICAL AND BIOCHEMICAL STUDIES ON ENDOMETRITIC REPEAT BREEDING COWS FOLLOWING TREATMENT WITH LEVAMISOLE

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ABSTRACT

The study was aimed to determine the efficacy of levamisole treatment in crossbred repeat breeder cows suffering with endometritis. Twelve such crossbred cows were administered levamisole @ 2.5 mg/kg body weight subcutaneously on day 0 (estrus) and subsequently on day 2 and 4 of the cycle. Serum biochemical parameters and progesterone profile were studied in the plasma samples collected during estrus, before and after treatment. Following levamisole treatment 66.67% repeat breeding cows were treated which was subsequently corroborated by negative White side test & pH (\hat{A} 8.0) of cervico-vaginal mucus. A significant ($P < 0.05$) increase in mean total protein (6.88 ± 0.27 vs. 5.17 ± 0.21 g/dl) and globulin (4.41 ± 0.29 vs. 2.79 ± 0.20 g/dl) along with a significant decrease (0.57 ± 0.05 vs. 0.88 ± 0.09) in the mean A/G ratio was recorded in cows at post-treatment estrus, while the mean activity of serum aspartate aminotransaminase and progesterone levels decreased. Following treatment 25% of the animals conceived at 1st post treatment estrus with an overall conception of 50%.

Key words: Cow, endometritis, immunomodulation, levamisole, repeat breeder

Bacterial endometritis is considered to be the most common cause of repeat breeding in bovines (Maurya *et al.*, 1992). The indiscriminate use of antibiotic in treatment of uterine infections has resulted in emergence of resistant bacterial strains making further use of such therapy ineffective (Arora *et al.*, 2000). Recently regulation of reproduction by immune intervention has emerged and levamisole due to its potent thymomimetic action has been reported to result in systemic immunomodulation, thus potentiating both systemic and uterine defense mechanism, favoring clearance of infection (Biswal *et al.*, 2014) and improving subsequent conception rate. Levamisole, a non-specific immune-stimulant, also stimulates sub-normal phagocytosis (Vandeplasseche, 1984) and cell mediated response in bovines (Tawfik and El-Garhy, 1988). It is known that administration of a single dose of levamisole @ 2.5 mg/kg (one-third of the anthelmintic dose) results in immunomodulatory effect for about 48 h (Anderson, 1984), however, Einstein *et al.* (1994) observed more effective immune response using intermittent treatment with same dose. The present study aimed to determine the impact of levamisole treatment on recovery, biochemical indices, estrus progesterone levels and conception in repeat breeder cows with endometritis.

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MATERIALS AND METHODS

A total of 12 crossbred repeat breeder cows with endometritis aged between 4-10 years and calved 6-24 months ago were included in the present experiment. All the animals were examined per rectum and only those cows with normal genitalia were selected for further treatment. Diagnosis of endometritis and evaluation of recovery post-treatment was done by studying the physico-chemical properties of the cervico-vaginal mucus (CVM) *viz.* appearance, pH and white side test. The appearance of CVM was noted in respect of its cleanliness and transparency. The pH of CVM was assessed by a narrow range pH paper. White side test (WST) was performed using 5% NaOH and the colour reaction (if any) was recorded. Levamisole (Lemasol[®]-75, Zoetis India Limited) was administered @ 2.5mg/kg body weight subcutaneously in the brisket region on day 0 (presented estrus), 2 and 4th day of the estrus cycle.

Blood samples (~6 ml) were collected randomly from six cows at the presented estrus and the during the next estrus following levamisole treatment. Biochemical parameters *viz.*, total protein, albumin, total cholesterol, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), calcium and phosphorus were estimated in serum using diagnostic kit and semi-automatic

analyzer. Progesterone estimation was done by radioimmunoassay (Kamboj and Prakash, 1993). Statistical analysis was done using SPSS 16.0 software.

RESULTS AND DISCUSSION

In 33.33% (4), 16.67% (2) and 50% (6) of the selected cows a transparent, translucent and cloudy discharge, respectively was observed at the time of estrus, however, after levamisole treatment only 16.67% (2) cows showed a cloudy discharge. Mean pH of the discharge also decreased (7.28 ± 0.18 vs. 7.67 ± 0.19). Although reports on pH of CVM following levamisole treatment in cattle endometritis are not available, however, Biswal *et al.* (2014) reported a significant decrease in bacterial count after levamisole treatment which is positively correlated to pH (Kumar *et al.*, 2015). A positive colour reaction to WST has been observed as an indicator of the uterine infection (Popov, 1969), therefore, recovery from endometritis in these animals was judged on the basis of negative WST of cervico-vaginal mucus at the post-treatment estrus. Four (33.33%) and eight (66.67%) cows, respectively, showed mild and moderate reaction to WST before treatment while after levamisole treatment, only 4 (33.33%) cows showed mild color reaction to WST and the remaining eight (66.67%) were negative to WST. This could possibly be because of the property of levamisole to stimulate cell-mediated immune reactivity by potentiating the rate of T-lymphocyte differentiation, the sensibility to antigens and mitogens and activity of phagocytes (Brunner and Muscoplat, 1980) which, in turn, might have cleared the infection thereby resulting in a negative WST.

Following treatment with levamisole in the present study, 66.67% (8/12) cows recovered from endometritis and 25% conceived at 1st post treatment estrus with an overall 50% conception rate after three successive inseminations. Similar to that of present study, Saini *et al.* (1999) and Kswain *et al.* (2011) reported an overall conception rate of 50 to 60% after levamisole treatment in cattle. A variable response and recovery which was observed in the present study could possibly be attributed to the dependence of the immuno-stimulatory effect of levamisole on the dose rate, timing of administration and the condition of the animal under treatment (Symoens and Rosenthal, 1977). Tizard (1998) reported that the effect of levamisole is greatest in animals with depressed T-cell function and a little or no effect on the immune system of normal animals.

Table 1
Pre-and post-treatment serum biochemical indices and progesterone concentration (Mean±SE) in endometritic repeat breeder cows

Parameter	Pre-treatment	Post-treatment	P-value
Total protein (g/dl)	5.17±0.21 ^a	6.88±0.27 ^b	0.002
Albumin (g/dl)	2.38±0.09 ^a	2.47±0.06 ^a	0.405
Globulin (g/dl)	2.79±0.20 ^a	4.41±0.29 ^b	0.006
A:G Ratio	0.88±0.09 ^a	0.57±0.05 ^b	0.046
Cholesterol (mg/dl)	161.96±4.53 ^a	150.83±7.50 ^a	0.079
AST (U/L)	40.91±7.25 ^a	35.24±1.01 ^a	0.469
ALT (U/L)	17.62±1.60 ^a	17.64±2.32 ^a	0.993
Calcium (mg/dl)	7.93±0.80 ^a	9.96±0.93 ^a	0.120
Phosphorus (mg/dl)	6.96±0.49 ^a	6.19±0.68 ^a	0.363
Progesterone (ng/ml)	0.28±0.08 ^a	0.26±0.07 ^a	0.825

Different superscripts (a and b) in a row indicate significant difference ($P \leq 0.05$)

Serum biochemical indices and progesterone concentration during estrus before and after levamisole treatment in repeat breeder cows are depicted in Table 1. A significant ($P < 0.05$) increase in the post-treatment mean total protein (6.88 ± 0.27 vs. 5.17 ± 0.21 g/dl) and globulins (4.41 ± 0.29 vs. 2.79 ± 0.20 g/dl) as compared to pre-treatment levels was observed. The mean albumin level increased non-significantly post-treatment (2.47 ± 0.06 vs. 2.38 ± 0.09 g/dl) while a significant decrease (0.57 ± 0.05 vs. 0.88 ± 0.09) in the mean A/G ratio at post-treatment was recorded. The significant increase in total serum globulin after levamisole treatment is suggestive of its general immunomodulatory effect. The increase in the post-treatment mean serum total protein could be mainly due to increase in globulin and to a lesser extent due to increase in albumin which was evident by an increase in globulin and decrease in the mean A/G ratio. Albumin has been reported as a negatively reacting acute phase protein i.e., its concentration decreases in case of inflammation and infection (Eckersall and Bell, 2010) and its elevation after levamisole treatment (relative to the pre-treatment level) although non-significant, could be possibly because of diminishing acute phase responses i.e. decrease in inflammation, infection and uterine tissue damage post therapy. Mean serum cholesterol levels and AST levels decreased after the treatment. The decrease in AST might be attributed to substantial remission of infective process and restoration of damaged uterine tissue.

There was a slight decrease in the mean level of progesterone, which was probably due to the fact that two animals which had higher progesterone level at presented estrus had low levels at subsequent estrus

after treatment. It may be because of improved endometrial function due to treatment of endometritis which resulted in optimum synthesis and release of PGF_{2α} from the endometrium of the affected animals. Thus, it can be concluded that levamisole is effective in decreasing the severity and/or resolving endometritis and thus may be used as an alternative or adjunct to antibiotic therapy.

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