

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.906.097>

Effect of Certain Immunomodulators on Subclinical Endometritis in Cattle

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ABSTRACT

Forty sub-clinical endometritic (repeat breeding) cows were assigned to four groups, ten in each. Experimental design envisaged administration of Levamisole S/C, three injections at weekly interval @ 1ml/30kg body weight (group I), Restobal, a herbal drug 50 ml twice daily per animal orally for 10 days (group II), PHA-M @ 0.5 mg per cow through I/U once on the day of estrus (group III) and control (group IV) with no treatment. All the experimental cows were inseminated in the following estrus. Blood and uterine samples from each experimental animal were collected for evaluation of hematological parameters like haemoglobin concentration, total leucocyte count (TLC), differential count (DC) and uterine bacterial load during pre and post treatment period, consequent to various therapies as per standard procedures. The haemoglobin concentration (gm/dl), TLC values in different experimental groups did not reveal any significant difference at pre and post treatment period either within or between groups. In all the experimental groups the post treatment uterine bacterial count significantly receded ($p < 0.01$) compared to their pre treatment values and also differed significantly ($p < 0.01$) within and between the groups. The overall conception rate was highest (70 per cent) in group III subjected to therapy of PHA-M followed by 60%, 50% and 30% in Levamisole (group I), Restobal (group II) and control (group IV), respectively. Clinical management of sub-clinical endometritis (repeat breeding) by immunomodulating agents like PHA-M has got clinical importance.

Keywords

Endometritis,
Immunomodulators

Article Info

Accepted:
15 May 2020
Available Online:
10 June 2020

Introduction

Repeat breeding has been recognized as one of the obstinate perplexing and most worrisome infertility problem encountered in dairy cattle throughout the world and the major sub fertile condition which adversely affect farmer's economy by ultimately lowering the calf crop and milk production. Sub-clinical endometritis, often associated with repeat breeding, is associated with the

presence of >18% polymorphonuclear (PMN) cells in uterine cytology samples collected 21–33 days postpartum, or >10% PMNs in samples collected at days 34–47. Cows with subclinical endometritis do not have uterine discharge, however, the severity of the disease is still considered sufficient to impair reproductive performance. Difficulty in controlling predisposing factors under field conditions as well as high cost of treatment, emergence of microbial resistance to

antibacterial drugs, lack of laboratory facility for culture and sensitivity test (CST) and uterine biopsy in tropical country like India has drawn more attention towards the therapeutic management of subclinical endometritis cows. In the present experiment drugs which stimulate the natural uterine defense mechanisms through immunomodulation were administered through intrauterine and oral route to subclinical endometritic cows and the therapeutic efficacies of these drugs were evaluated on the basis of haematological parameters and successful conception rate.

Materials and Methods

The present experiment was conducted in repeat breeding cows due to subclinical endometritis at estrus presented in the Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, O.U.A.T., Bhubaneswar. Forty repeat breeding Jersey cows in between 1st to 5th parity suffering from subclinical endometritis (screened by White Slide test) were selected. Rectal palpation of internal genitalia of the individual animals was conducted to exclude cows with cervicitis, salpingitis, cystic ovarian degeneration and other palpable genetic, hereditary or acquired defects from the experiment. Both internal and external signs of estrus were confirmed. The selected animals were divided into 4 groups with 10 animals in each. Group-I cows were administered Levamisole (kalmisole) a thymomimetic agent, @ 1ml/30kg body weight, S/C for three occasions at weekly interval. Group-II cows were administered with Restobal, a herbal drug @50 ml/animal twice daily, orally for 10 days and group-IV with PHA-M @ 0.5 mg per cow through I/U once on the day of estrus keeping Group-IV as control. The animals were given sexual rest following treatment. Collection of blood and uterine sample was done on the day of estrous

both pre and post treatment for estimation of different haematological parameters, study of bacterial count of uterine discharge. The conception rates in consonance to various treatment protocols were assessed to evaluate the comparative efficacy of various immunomodulating agents. Results were statistically analysed as per Snedecor and Cochran (1994).

Results and Discussion

Studies on subclinical endometritis (SCE) found prevalence in the range between 12 and 94 % (Barlund *et al.*, 2008, Gilbert *et al.*, 2005, Hammon *et al.*, 2006, Kasimanickam *et al.*, 2005 and Raab, 2004) compared to 18- 37 % for clinical endometritis. However, with antibacterial drugs destroying uterine defense (Oxender and Seguin, 1976 and Massera *et al.*, 1980), antiseptics general irritant nature (Vandeplasseche, 1981) and PGF₂ α that requires presence of corpus luteum for its effect (Whittier *et al.*, 1989), the progressive awareness of treatment failure by conventional therapy has necessitated adopting an alternative therapy for clinical management of subclinical endometritis and has focused attention on alternative therapies which stimulate the natural uterine defense mechanisms through immunomodulation (Gilbert, 1992).

Various hematological parameters like Hb concentration, total leucocyte count (TLC), differential count (DC) and uterine bacterial load were evaluated during pre and post treatment periods (Table-1). Analysis of variation of haemoglobin concentration (gm/dl) and TLC revealed significant difference, neither between nor within pre and post treatment values. The post treatment values of lymphocyte per cent, except control, were significantly higher than pretreatment counter parts. The Restobal treated animals (group I) in this study also showed

significantly higher post treatment lymphocyte count which is comparable to Swain (2009) where he used an herbal immunomodulator (Immulite). Neutrophil per cent showed a reverse trend incurring a lower value in post treatment stage compared to pre treatment estimations. The pre and post treatment values for neutrophil revealed significant difference ($p < 0.01$) except in group III where it was not significant. The neutrophil value in different treatment protocol both in pre and post treatment stage are within normal physiological range which constitute between 15 to 45 per cent (Runnells *et al.*, 1965 and Chauhan, 1995) in bovines.

The post treatment eosinophil count per cent in all the experimental groups including control decreased marginally in the post treatment sampling but it was not different significantly from pre treatment value. The monocyte count in did not show significant difference either between or within groups. The haemoglobin value in the present study corroborates with the findings of Kumar *et al.*, 1986 (11.71 ± 71) and Prabha and Singh, 2000 (10.18 ± 0.09 gm/dl). As reported by Awasthi and Kharche (1987), the haemoglobin values did not change significantly between fertile and infertile cows.

On the contrary Kekan *et al.*, (2005) reported significantly lower haemoglobin values in repeat breeding cows. The present observation of total leukocyte count in post treatment period is at par with that of Chauhan (1995) ($4-12 \times 10^3/\text{cmm}$). The present values are in accordance with the findings of Behera (2007) and Swain (2009) consequent to immulite treatment. The significant elevation of lymphocyte count in PHA-M treated animals (group II) might be due to systemic immunomodulation. The observed neutrophil value in the present study is in agreement with report of Ahamed *et al.*,

(2003) and Kekan *et al.*, (2005). It could be presumed that the significant decrease in neutrophil count might be due to application of various immunomodulatory drugs which have modulated innate immune system to augment uterine defense mechanism. In control cows the marginal depression of neutrophil count might be due to action of estrogen which favours migration to uterine lumen which has reflected in differential count. The present findings of the monocyte count corroborate with the observation made by Mohanty (2006), Behera (2007) and Swain (2009) where they recorded similar values for monocyte count following Immulite (a herbal immunomodulator) or PGF 2α application. In the present study the eosinophil count in the post treatment period corroborates with Behera (2007) and Swain (2009) where these workers recorded lower value in eosinophil count in the post treatment sampling irrespective of drug used. The treatment with sexual rest might have suppressed mast cells in preventing histamine like substances responsible for allergy.

The uterine bacterial load (millions/ml) in pre treatment sample did not differ significantly within groups whereas post treatment bacterial count differed significantly ($p < 0.01$). In all the experimental groups the post treatment bacterial count significantly receded ($p < 0.01$) compared to their pre treatment values. The test of significance of post treatment uterine bacterial load between various experimental groups recorded highly significant difference ($p < 0.01$) except group I. The percentages of reduction of bacterial load were calculated to be highest for Group II followed by Group I and Group III, control. This suggested PHA-M is more potent in reducing bacterial load compared to other experimental groups. The overall conception rate was highest in group III. However, no significant variation in conception rate to be observed by chi-square analysis.

Table.1 The differential count (in per cent), bacterial count and conception rate of all the experimental groups including control in pre and post treatment period

Parameters	Group I (Levamisole)		Group II (Restobal)		Group III (PHA-M)		Group IV(Control)	
	Pre treatment	Post treatment	Pre treatment	Post treatment	Pre treatment	Post treatment	Pre treatment	Post treatment
Hb (gm/dl)	10.8 ± 0.07	0.88 ± 0.05 10.88±0.05	10.70 ± 0.08	10.76 ± 0.12	10.9 ± 0.04	11.00 ± 0.05	10.76± 0.10	10.70± 0.08
TLC (10³/cm)	7.35 ± 0.68	7.1 ± 0.64	7.13 ± 0.66	7.02 ± 0.57	7.04 ± 0.22	6.96 ± 0.24	7.52 ± 0.19	7.41 ± 0.18
Lymphocyte (%)	54.30± 0.89 ^c	58.07 ± 0.96 ^d	53.50 ± 0.57 ^c	56.10 ± 0.48 ^d	55.40 ± 0.21 ^c	58.60 ± 0.35 ^d	55.20 ± 0.78	56.60± 0.64
Neutrophil(%)	36.40 ± 0.46 ^c	34.60 ± 0.36 ^d	36.20 ± 0.37 ^a	34.90 ± 0.30 ^b	35.50 ± 0.43 ^c	32.60 ± 0.38 ^d	35.70± 0.45	35.00± 0.24
Eosinophil(%)	6.80 ± 0.23 ^c	5.20 ± 0.17 ^d	7.80 ± 0.24	7.00 ± 0.24	6.50 ± 0.45	6.40 ± 0.43	6.90 ± 0.30	6.30 ± 0.25
Monocyte (%)	2.50 ± 0.18	2.13 ± 0.55	2.50 ± 0.16	2.00 ± 0.24	2.60 ± 0.15	2.40 ± 0.21	2.20 ± 0.13	2.10 ± 0.09
Utr. bacterial load ×10⁶/ml	8.91 ± 0.99 ^c	0.36 ± 0.05 ^d	8.60 ± 0.65	1.73 ± 0.65	8.84 ± 0.54	0.342 ± 0.06	8.831± 0.36	3.99 ± 0.37
Reduction in bacterial load (%)			79.89		96.13		54.82	
Conception Rate (%)			50		70		30	

* P < 0.05, ** P < 0.01, NS - Not significant

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How to cite this article:

Sahoo, H.S., A.K. Barik, D.N. Mohanty, P.C. Mishra, A.K. Nahak and Jena, B. 2020. Effect of Certain Immunomodulators on Subclinical Endometritis in Cattle. *Int.J.Curr.Microbiol.App.Sci*. 9(06): 759-764. doi: <https://doi.org/10.20546/ijcmas.2020.906.097>