

## EFFECTS OF IMMUNOPOTENTIATORS ON POSTPARTUM REPRODUCTIVE PERFORMANCE IN SAHIWAL COWS

A. Sattar, L.A. Lodhi<sup>1</sup>, Z.I. Qureshi<sup>1</sup>, I. Ahmad<sup>1</sup> and N.A. Naz<sup>2</sup>

Research Institute for Physiology of Animal Reproduction, Bhunikey (Pattoki), Distt. Kasur

<sup>1</sup>Department of Animal Reproduction, University of Agriculture, Faisalabad

<sup>2</sup>Livestock Production Research Institute, Bahadurnagar, Okara

### ABSTRACT

A total of 32 Sahiwal cows in the last trimester of pregnancy were used to determine the effect of different immunopotentiators on their postpartum reproductive performance. The cows were divided into four groups. Group-1 served as untreated control while groups-2, 3 and 4 were treated with levamisole hydrochloride (0.5 mg/Kg body weight orally), vitamin E-selenium (10 ml intramuscularly per cow) and BCG vaccine (0.5 ml subcutaneously per cow), respectively. These immunopotentiators were given twice with one week interval at about 60 days prior to expected date of calving. Various reproductive parameters during subsequent postpartum period, *i.e.*, placental expulsion period (hours), uterine involution period (days), postpartum estrus interval (days), number of services per conception and service period (days) were studied. It was noted that duration of all these parameters (mean  $\pm$  SE) were longest in cows of untreated control group (10.56  $\pm$  0.57, 52.38  $\pm$  1.38, 125.38  $\pm$  25.79, 2.63  $\pm$  0.50 and 286.88  $\pm$  39.04, respectively) and shortest in the cows of vitamin-E selenium treated group (8.88  $\pm$  0.30, 31.13  $\pm$  0.52, 70.00  $\pm$  15.92, 1.50  $\pm$  0.19 and 115.00  $\pm$  19.75, respectively). Two reproductive parameters, *i.e.*, postpartum estrus interval and number of services per conception were non-significantly lesser in the cows of treated groups as compared to those of untreated control. Three reproductive parameters, *i.e.*, placental expulsion period, uterine involution period and service period were reduced significantly ( $p < 0.05$ ) in vitamin-E selenium and BCG treated groups as compared to control. The cows of levamisole hydrochloride treated group showed significant ( $p < 0.05$ ) reduction only in uterine involution period as compared to untreated control. All the treated groups differed non-significantly amongst each other for all reproductive parameters except uterine involution period.

**Keywords:** BCG vaccine, cow, immunopotential, levamisole, reproduction, selenium, vitamin E

### INTRODUCTION

Pakistan has about three per cent annual growth rate of human population whereas the livestock sector of the country has the modest growth rate which is not keeping pace with the population's increasing demand of livestock products. Hence, an enhanced livestock production is indispensable to meet the ever increasing milk and meat demand in the country. Longer calving interval in cows is an important factor lowering their reproductive efficiency.

Reproductive efficiency is economically important in dairy animals because it affects the average amount of milk produced per animal per day and the replacement frequency (Dijkhuizen *et al.*, 1985). Successful attempts to improve reproductive efficiency by administering immunopotentiators like levamisole hydrochloride, vitamin-E selenium and BCG during late gestation in sows (Purswell *et al.*, 1988), Nili-Ravi buffaloes (Qureshi *et al.*, 1997) and Holstein-Friesian cows (Flesh *et al.*, 1982) has been reported previously. However, little information is available for Sahiwal

cows, especially under environmental conditions of Pakistan. Levamisole hydrochloride, in addition to its anthelmintic activity, stimulates bone marrow regeneration and enhances maturation of lymphocytes *in vivo*; whereas in *in vitro* it acts by enhancing blast transformation of lymphocytes (Brunner and Muscoplat, 1980). Vitamin E-selenium is reported to improve humoral immune responses to bacterial and viral antigens (Larsen, 1988). BCG stimulates humoral and cellular immune responses to unrelated antigens in addition to its own antigenic determinants (Ben-Efrain and Diamantstein, 1975).

The present study was carried out to evaluate the efficacy of three reported immunopotentiators, levamisole hydrochloride, vitamin E + selenium (vitamin E-Sel) and *Bacille Calmette Guerin* (BCG) vaccine, in improving the postpartum reproductive performance of Sahiwal cows.

### MATERIALS AND METHODS

A total of 32 adult Sahiwal cows in the last trimester of pregnancy were selected at the Livestock

Production Research Institute (LPRI), Bahadurnagar, Okara, Pakistan. They were randomly divided into 4 groups, having eight animals in each group. All the experimental animals were kept under similar managerial conditions and were offered good quality seasonal green fodder *ad libitum*. In addition, each animal was given 2 kilogram of concentrate daily.

The cows in group-1 served as untreated control. Those in group-2 were treated orally with levamisole hydrochloride (Shahani Laboratories, Faisalabad, Pakistan) at dose rate of 0.5 mg/Kg body weight while in group-3 each cow was administered with Etosol-SE 10 ml intramuscularly (Aesculaap bv, Boxel, Holland-each ml containing Natrii selenis pentahydricum 1.5 mg and dl-alfa tocopherolacetate 50 mg). Similarly, each cow in group-4 was injected with BCG vaccine 0.5 ml subcutaneously (Pasteur Merieux, Lyon, France). The immunopotentiators were given twice with one week interval at about 60 days prior to expected date of calving. After parturition, the period of expulsion of fetal membranes was recorded. Rectal palpation was performed twice a week until involution was complete. Estrus was confirmed by rectal palpation and they were inseminated using frozen thawed semen of the same bull.

Data were derived for the mean values ( $\pm$  SE) of the placental expulsion period (hours), uterine involution period (days), postpartum estrus interval (days), number of services per conception and service period (days). The data were subjected to one-way analysis of variance to assess the significance of variation among the four groups (Steel and Torrie, 1984). Least significant difference test (LSD) was applied to compare treatment means. Results were considered significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The values (mean  $\pm$  SE) of various postpartum reproductive parameters are given in Table-1. It was noted that duration of all these parameters was longest

in cows of untreated control and shortest in those of vitamin-E Sel treated group. Two reproductive parameters, *i.e.*, postpartum estrus interval and number of services per conception were non-significantly lesser in the cows of treated groups as compared to those of untreated control. Three reproductive parameters, *i.e.*, placental expulsion period, uterine involution period and service period reduced significantly ( $p < 0.05$ ) in vitamin-E sel and BCG treated groups as compared to control. The cows of levamisole hydrochloride treated group showed significant ( $p < 0.05$ ) reduction only in uterine involution period as compared to untreated control. All the treated groups differed non-significantly amongst each other for all reproductive parameters except uterine involution period.

In the present study, levamisole hydrochloride improved the reproductive performance but the results were not statistically significant. Debowy *et al.* (1985) reported that prepartum treatment of pregnant sows with levamisole hydrochloride (0.5 mg/Kg) decreased the morbidity and mortality in neonatal pigs. Treatment of dairy cows and first calf dairy heifers with an immunopotentiating dose (2.5 mg/Kg) of levamisole in late gestation resulted in decreased postpartum metritis and mastitis and increased survival of the calves (Oyndia and Nelken, 1978; Flesh *et al.*, 1982). Qureshi *et al.* (1997) treated prepartum Nili-Ravi buffaloes with levamisole hydrochloride and resultantly there was only non-significant decrease in all the reproductive parameters as compared to untreated controls and these findings were in line with the present study except uterine involution period which differed significantly ( $p < 0.05$ ) as compared to control group. Rehman *et al.* (1989) reported that levamisole was more effective if repeated at an interval of 3-4 weeks. The response to levamisole differ from species to species due to differences in their physiological mechanisms (Purswell *et al.*, 1988). The different dose and frequency of administration of levamisole along with the different experimental species, may account for the smaller effect of levamisole in the present study.

**Table 1: Postpartum reproductive traits (mean  $\pm$  SE) after prepartum immunopotentiation in Sahiwal cows.**

Reproduction traits	Group-1 (Control)	Group-2 (Levamisole treated)	Group-3 (Vit E-Sel treated)	Group-4 (BCG treated)
Placental expulsion period (hours)	10.56 $\pm$ 0.57 <sup>a</sup>	9.94 $\pm$ 0.38 <sup>ab</sup>	8.88 $\pm$ 0.30 <sup>b</sup>	9.06 $\pm$ 0.41 <sup>b</sup>
Uterine involution period (days)	52.38 $\pm$ 1.38 <sup>a</sup>	40.88 $\pm$ 0.85 <sup>b</sup>	31.13 $\pm$ 0.52 <sup>c</sup>	33.38 $\pm$ 0.86 <sup>c</sup>
Postpartum estrus interval (days)	125.38 $\pm$ 25.79 <sup>a</sup>	118.75 $\pm$ 41.51 <sup>a</sup>	70.00 $\pm$ 15.92 <sup>a</sup>	87.88 $\pm$ 15.94 <sup>a</sup>
Services per conception (No.)	2.63 $\pm$ 0.50 <sup>a</sup>	1.88 $\pm$ 0.30 <sup>a</sup>	1.50 $\pm$ 0.19 <sup>a</sup>	1.75 $\pm$ 0.31 <sup>a</sup>
Service period (days)	286.88 $\pm$ 39.04 <sup>a</sup>	210.75 $\pm$ 47.34 <sup>ab</sup>	115.00 $\pm$ 19.75 <sup>b</sup>	165.88 $\pm$ 37.38 <sup>b</sup>

Values bearing different superscripts in the same row differ significantly ( $P < 0.05$ ).

Pregnant animals are more susceptible to selenium deficiency than non-pregnant animals (Awad *et al.*, 1985) leading to increased incidence of prepartum and postpartum reproductive disorders. The shorter expulsion period of fetal membranes in the vitamin E-Sel treated cows in this study might be due to improved uterine muscular function (Youssef *et al.*, 1985). The significant reduction in service period in the cows treated with vit E-Sel compared with the control in the present study is supported by the findings of Allen *et al.* (1975), Vleet *et al.* (1977) and Hemken *et al.* (1978). These workers reported that service period was significantly ( $p < 0.05$ ) shorter in cows treated with prepartum injection of vitamin E-Sel. There was a non-significant reduction in postpartum estrus interval in the cows treated with vit E-Sel compared with the control in the present study which is also supported by the findings of above three workers but the difference reported by them was statistically significant ( $p < 0.05$ ). Similarly, Qureshi *et al.* (1997) treated prepartum Nili-Ravi buffaloes with vitamin E-selenium which resulted into significant ( $p < 0.05$ ) decrease in uterine involution period, postpartum estrus interval, service period as compared to untreated controls while, in the present study, placental expulsion period, uterine involution period and service period differ significantly ( $p < 0.05$ ) as compared to untreated control. Cortese (1988) and Roger *et al.*, (1991) reported a significant ( $P < 0.05$ ) decrease in number of services required per conception following prepartum immunopotentiality by vitamin E-Sel treatment in cows but in the present study but this difference was statistically non-significant. The results of the present study differ from those of Trinder *et al.* (1973) and Gwazdauskas *et al.* (1979), who reported that prepartum injection of vitamin E-Sel did not improve the postpartum reproductive performance in term of postpartum estrus interval and service period. This difference may be due to difference in the pretreatment selenium status of the cows and the frequency of injections (Awad *et al.*, 1985).

Immunopotentiality with BCG vaccine prior to calving resulted in improvement in all the reproductive parameters under study. Qureshi *et al.* (1997) treated prepartum Nili-Ravi buffaloes with BCG which resulted in significant ( $p < 0.05$ ) decrease in postpartum estrus interval and service period while, in the present study, placental expulsion period, uterine involution period and service period were reduced significantly ( $p < 0.05$ ) as compared to untreated control. The mechanism by which BCG improves the reproductive performance is not known but it has been reported to increase nonspecific resistance against various unrelated pathogens, *e.g.*, bacteria, viruses or parasites, (Salvin *et al.*, 1975) and to potentiate humoral and cell-mediated immune responses to protein antigens (Wendell and Harris, 1982). It is possible that BCG may have increased the uterine resistance to infection by enhancing both cell-mediated and humoral immunity

against microflora which might otherwise have reduced the conception rate and increased the service period.

Since the number of pregnant cows available for the present study was small, no definite conclusion can be made. However, a beneficial effect may be obtained by prepartum immunopotentiality using levamisole hydrochloride, vitamin E-Sel or BCG. Additional work involving a larger number of animals is suggested.

## REFERENCES

- Allen, W. M., R. Bradley, S.B. Berrett, W.H. Parr, K. Swammack, C.R.O. Barton and A. Macphea, 1975. Degenerative myopathy with myodegeneration in yearling cattle. *British Vet. J.*, 131: 292-308.
- Awad, Y. L., R. H. Youssef and M. F. Mikhail, 1985. Influence of prepartum selenium treatment on the postpartum reproductive pattern of Egyptian buffalo and on the born calves. *Egyptian J. Vet. Sci.*, 22: 55-64.
- Ben-Efrain, S. and T. Diamantstein, 1975. Mitogenic and adjuvant activity of a methanol extraction residue (MER) of tubercle bacilli on mouse lymphoid cells *in vitro*. *Immunolo. Commun.*, 4: 565-577.
- Brunner, C. J. and C. C. Muscoplat, 1980. Immunomodulatory effects of levamisole. *J. Amer. Vet. Med. Assoc.*, 176: 1159-1162.
- Cortese, V., 1988. Selenium and reproductive performance in dairy cattle. *Agric. Pratic. Nutrition/Reproduction*, 9: 5-7.
- Debowy, J., T. Garbulinski, B. Obminska-Domeradika and M. Switala, 1985. Levamisole, Chlormethine and imuthiol (DTC) as immunostimulating agents in swine. *Schmiedeberg's Archives of Pharmacology*, 330: R55.
- Dijkhuizen, A. A., J. Stelwagen and J. A. Renkema, 1985. Economic aspects of reproductive failure in dairy cattle. I. Financial loss at farm level. *Prev. Vet. Med.*, 3: 251-263.
- Flesh, J., W. Harel and D. Nelken, 1982. Immunopotentiating effect of levamisole in the prevention of bovine mastitis, fetal death and endometritis. *Vet. Rec.*, 111: 56-57.
- Gwazdauskas, F.C., T.L. Bibb, M. L. McBilliard and J. A. Lineweaver, 1979. Effect of prepartum selenium vitamin E injection on time for placenta to pass and on productive function. *J. Dairy Sci.*, 62: 978.
- Hemken, R. W., D. Olds, R. L. Botts and L. S. Bull, 1978. Selenium injection prior to calving and prevention of retained placenta. *J. Dairy Sci.*, 61: 209.
- Larsen, H. J., 1988. Influence of selenium on antibody production in sheep. *Res. Vet. Sci.*, 45: 4.

- Oyndia, F. J. and D. Nelken, 1978. Prevention of bovine mastitis by treatment with levamisole. *Israel J. Med. Sci.*, 14: 394-396.
- Purswell, J. B., D. L. Dawe, J. Brown and J. B. Williams, 1988. Effect of levamisole on immune function and reproductive performance in first litter gilts. *American J. Vet. Res.*, 49: 856-859.
- Qureshi, Z. I., L. A. Lodhi and A. Sattar, 1997. An apparent effect of immunopotential during late gestation on the postpartum reproductive performance of Nili-Ravi buffaloes (*Bubalus bubalis*). *Vet. Res. Commun.*, 21(5): 375-380.
- Rehman, A. S., F. Adel and M. S. Jagannath, 1989. Role of levamisole in the induction of cell mediated immunity in experimental caecal coccidiosis. *Indian Vet. J.*, 66: 706-710.
- Roger, D. H., K. D. Brenda, G. Cheryl and E. D. Jazen, 1991. Effect of prepartum parenteral supplementation of pregnant beef cows with selenium/vitamin E on cows and calf plasma selenium and productivity. *Canadian Vet. J.*, 32: 113-115.
- Salvin, S. B., E. Ribi and D. L. Garnger, 1975. Migration inhibitory factor and type II interferon in the circulation of mice sensitized with Mycobacterial components. *J. Immunology.*, 114: 354-359.
- Steel, R. G. D. and J. H. Torrie, 1984. Principles and Procedures of Statistics. A biometrical approach, 2<sup>nd</sup> ed., McGraw Hill, Tokyo.
- Trinder, N., R. J. Hall and C. P. Renton, 1973. The relationship between intake of selenium and vitamin E on the incidence of retained placenta in dairy cows. *Vet. Rec.*, 93: 641.
- Vleet, J. F., R. R. Crawlay and H. E. Amstutz, 1977. Myodegeneration associated with selenium vitamin E deficiency in a pregnant heifer. *J. American Vet. Med. Assoc.*, 171: 443.
- Wendell, W. D. and S. C. Harris, 1982. Interferon induction in healthy and tumour bearing dogs by cell walls of *Mycobacterium bovis* strain of *Bacille Calmette-Guerin*. *American J. Vet. Res.*, 43: 1232-1237.
- Youssef, R. H., Y. L. Awad and M. F. Mikhail, 1985. Effect of prepartum selenium treatment on retained placenta in buffaloes and on their subsequent fertility. *Egyptian J. Vet. Sci.*, 22: 65-71.