

## EFFECT OF PRE-PARTUM VITAMIN E AND SELENIUM TREATMENTS ON REPRODUCTIVE PERFORMANCE OF BALADY COWS AND IMMUNE ACTIVITY OF THEIR OFFSPRING

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### SUMMARY

Responses of late pregnant Balady cows to pre-partum treatment with selenium (Se) and vitamin E (Vit E) were evaluated by investigating uterine health and reproductive performance of the dams, and by determining immune responses and growth of their offspring. Twenty late-pregnant Balady cows were divided into two homogenous groups, according to their age, parity and weight. In addition to basal ration fed to group I (control), animals of group II were supplemented with oral vit E (400 IU/ cow/ day) and injected with Se (5mg / cow /Day), and then weekly until the end of the experiment. Serum samples were harvested biweekly, starting 6 weeks pre-partum until 15 weeks postpartum from all cows as well as from their neonatal calves before colostrum feeding and biweekly until weaning. The serum samples

were used to determine selenium in dams and their offspring and for IgG and IgM in calves.

Serum selenium in cows and calves was significantly elevated ( $P<0.05$ ) by selenium supplementation compared to control.

Administration of Se and vit E significantly reduced the duration of udder edema, incidence of retained placenta and metritis and facilitated the calving. The service period, days open, the interval from calving to uterine involution, first estrus, first ovulation, and the number of services per conception were also decreased compared to those in non-treated cows.

Body weight gain of calves was favorably influenced after the pre-partum treatment of their dams with Se and vit E.

Radial immunodiffusion results revealed that IgG level was significantly increased in the group treated with Se and vit E.

It was concluded that pre-partum treatment of late-pregnant Balady cows with vitamin E and selenium is beneficial in improving reproductive performance of dams and growth rate of their offspring and to some extent, the immune response of calves.

## INTRODUCTION

The role of vitamin E as an antioxidant and free radical scavenger is well established, and has the advantage of being nontoxic at required levels (McCay and King, 1980). Selenium was implicated in some body functions; also it is connected with immunity (Larsen, 1993). Selenium is an essential nutrient for proper functioning of various reproductive characteristics in mammalian female. In cattle, Segerson et al. (1981) indicated optimum fertility (100%) in dams receiving supplemental selenium and vitamin E and adequate nutrition. Deficiencies of selenium and vitamin E increase the incidence of retained placenta, metritis, cystic ovaries and mastitis, in addition to weak and dead born calves (Jenkins and Hidiroglu, 1972; Harrison et al., 1984 and Smith et al., 1984).

Vitamin E and selenium intake were negatively related to rate of clinical mastitis in dairy cows

(Harrison et al., 1984 and Weiss et al., 1989). Awad et al., (1985) recorded favorable influences of the pre-partum selenium treatment on the reproductive pattern of Egyptian buffaloes and on their newborn calves.

Selenium level of calf serum at birth was reported as reflecting the maternal selenium supplementation (Perry et al., 1978).

Recently, El-Gaafarawy et al. (2000) concluded that the role of vitamin E and selenium supplemented to the neonatal Balady calves until weaning, as an immunocompetence antioxidant, was non-specific humoral immunostimulant and improving growth rate.

The present investigation was carried out to study the effect of pre-partum vitamin E (lipophilic antioxidant vitamin) and selenium (antioxidant mineral) treatment on some reproductive performance parameters in the Balady cows and the immune activity of their offspring.

## MATERIALS AND METHODS

This study was carried out at El-Serw station belongs to the Animal Production Research Institute, Ministry of Agriculture. Twenty pregnant Balady cows, 6 weeks before the expected calving date were chosen and allocated into two homogeneous groups (10 animals each), according to their age, weight and parity. The animals ranged

from 2nd 4th parity with average body weight at calving of 370.6 Kg. The cows were loose-housed in separate open shaded yards. All the cows were fed on dry feed composed of concentrate mixture plus rice straw to cover their requirements calculated according to NRC (1978).

The animals were watered freely. Cows were machine-milked twice daily in a herringbone parlor. Animals in-group one served as control, while animals in-group two received the basal ration supplemented with 400 IU vitamin E (as tocopherol acetate) and injected intramuscularly with 5 mg selenium (sodium selenite solution) per cow/ day, starting 6 weeks pre-partum until calving, and then every week to the end of the experiment (15 weeks postpartum).

Three weeks before the expected calving and after parturition, udder edema was observed and recorded. After parturition, the number of animals that did not expel the placenta within 24 hours was recorded. Calving ease was given a score from 1 to 3 in which 1 was an easy delivery with less than two hours, while 2 was delivery in two to four hours and 3 was delivery requiring assistance.

Uterine and ovarian palpation per rectum, to determine the uterine involution and first ovulation, was performed weekly from the first week after parturition until mating. Pregnancy was determined by rectal palpation starting two months af-

ter mating. Metritis was determined by a veterinarian on the basis of an abnormal vaginal discharge.

The following information were recorded: the intervals from parturition to complete uterine involution, first ovulation, first detected estrus and conception (days open).

Cows were observed for vaginal discharge and estrus was detected three times daily at 800, 1200 and 1600 h. Cows were served not earlier than the 40th day postpartum.

The birth weight of newborn calves was registered. The calves were left with their dams during the first three days of their life to receive colostrum freely. Then the calves were fed individually on their dams' milk at the rate of 10% of the body weight given in two meals for six weeks. After that, the milk allowances were reduced gradually until weaning.

Calf starter and hay were available in front of the calves from the beginning of the third week of age. Body weight of the calves was recorded weekly for 15 weeks until weaning. Blood samples were collected from the calf's jugular vein at birth, one week and then every two-week interval until weaning. The blood was centrifuged at 3000 rpm for 15 minutes and the obtained clear serum was stored at 20°C until analyzed. Determination of levels of immunoglobulins (IgG and IgM) was done by bovine radial immunodiffusion (RID) kit

according to the procedure outlined by manufacture (The Binding Site Ltd, Birmingham, UK) according to Mancini et al. (1965).

Selenium content in blood, milk, concentrate mixture and starter was determined by the fluorometric method of Olsen et al. (1975) with some modification. The hydroxylamine ethylenediaminetetraacetic acid buffer solution of the AOAC (1980) was used instead of that of Olsen et al. (1975).

## RESULTS AND DISCUSSION

### Selenium concentration of milk and rations:

Selenium content in concentrate mixture, rice straw, milk, starter and clover hay is given in table 1. The selenium concentrations did not reach 0.1 g. This rate was much lower than the recommended rate (0.3 g) for dairy cows (Podall et al., 1992).

Table (1): Selenium concentration ( $\mu\text{g/ml}$ ) in milk, starter, concentrate mixture, Rice straw and clover hay Mean  $\pm$  S.E.

Item	Se concentration
Milk (treated dams)	0.067 $\pm$ 0.014
Milk (control dams)	0.040 $\pm$ 0.021
Starter	0.097 $\pm$ 0.003
Concentrate mixture	0.073 $\pm$ 0.008
Rice straw	0.021 $\pm$ 0.006
Clover hay	0.048 $\pm$ 0.004

### Selenium level in blood serum of dams and their offspring:

Selenium level in blood serum of dams and their offspring during the experimental period is presented in table 2. Cows treated with selenium and vitamin E had significantly ( $P < 0.05$ ) higher concentrations in their serum than those in control group.

Table (2): Selenium concentration ( $\mu\text{g/ml}$ ) in blood serum of treated and control Balady pregnant cows and their offspring Mean  $\pm$  S.E.

Experimental period (week)	Control group	Treated group
<b>Dams:</b>		
-6	0.023 $\pm$ 0.007	0.021 $\pm$ 0.004
-4	0.025 $\pm$ 0.005 <sup>b</sup>	0.054 $\pm$ 0.009 <sup>a</sup>
-2	0.028 $\pm$ 0.003 <sup>b</sup>	0.056 $\pm$ 0.008 <sup>a</sup>
0 (Parturition)	0.029 $\pm$ 0.008 <sup>b</sup>	0.063 $\pm$ 0.009 <sup>a</sup>
1	0.030 $\pm$ 0.007 <sup>b</sup>	0.077 $\pm$ 0.007 <sup>a</sup>
3	0.035 $\pm$ 0.008 <sup>b</sup>	0.072 $\pm$ 0.008 <sup>a</sup>
5	0.029 $\pm$ 0.006 <sup>b</sup>	0.73 $\pm$ 0.008 <sup>a</sup>
7	0.031 $\pm$ 0.008 <sup>b</sup>	0.069 $\pm$ 0.009 <sup>a</sup>
9	0.029 $\pm$ 0.005 <sup>b</sup>	0.071 $\pm$ 0.008 <sup>a</sup>
11	0.028 $\pm$ 0.006 <sup>b</sup>	0.072 $\pm$ 0.007 <sup>a</sup>
13	0.029 $\pm$ 0.007 <sup>b</sup>	0.075 $\pm$ 0.006 <sup>a</sup>
15	0.031 $\pm$ 0.004 <sup>b</sup>	0.072 $\pm$ 0.007 <sup>a</sup>
<b>Calves:</b>		
0 (at birth)	0.021 $\pm$ 0.004 <sup>b</sup>	0.043 $\pm$ 0.007 <sup>a</sup>
1	0.021 $\pm$ 0.005 <sup>b</sup>	0.040 $\pm$ 0.008 <sup>a</sup>
3	0.023 $\pm$ 0.003 <sup>b</sup>	0.036 $\pm$ 0.009 <sup>a</sup>
5	0.022 $\pm$ 0.007 <sup>b</sup>	0.034 $\pm$ 0.006 <sup>a</sup>
7	0.020 $\pm$ 0.006 <sup>b</sup>	0.033 $\pm$ 0.007 <sup>a</sup>
9	0.024 $\pm$ 0.005 <sup>b</sup>	0.032 $\pm$ 0.005 <sup>a</sup>
11	0.022 $\pm$ 0.004 <sup>b</sup>	0.032 $\pm$ 0.007 <sup>a</sup>
13	0.021 $\pm$ 0.005 <sup>b</sup>	0.032 $\pm$ 0.008 <sup>a</sup>
15	0.024 $\pm$ 0.007 <sup>b</sup>	0.031 $\pm$ 0.007 <sup>a</sup>

values with different superscripts in the same row are significantly different at ( $P < 0.05$ ).

Selenium levels in blood serum of calves at birth, 6 weeks after initiation of selenium treatment of dams, was significantly ( $P < 0.05$ ) higher than those of control. These results indicated that pre-partum supplemented selenium for cows was able to cross the placental membrane. Perry et al., (1978) showed significant differences ( $P < 0.05$  or  $P < 0.01$ ) between selenium concentrations in serum of calves from dams that received 2 or 5 mg selenium / day than those of control dams.

### **Reproductive Performance:**

The reproductive performance of cows as affected by pre-partum treatment with vitamin E and selenium is presented in table 3. Results indicated that the pre-partum treatment with vit. E. and Se. reduced significantly ( $P < 0.05$ ) the duration of udder edema and made calving significantly easier.

The incidences of retained placenta and metritis in treated cows were zero and 10%, respectively, whereas, in control group, they were 30 and 40%, respectively. The differences were statistically significant ( $P < 0.05$ ). The present results are in harmony with those found by Segerson et al., (1981), Harrison et al., (1986), Erskine et al., (1988) and Kim et al., (1997). They found that the effectiveness of supplemental selenium injections with vitamin E decreased the incidence of retained placenta and metritis in dairy cattle.

The interval from calving to complete uterine involution in treated Balady cows was 12 days sig-

nificantly shorter ( $P < 0.05$ ) than that in control group. This is in accordance with the results of Harrison et al., (1986) and Kolchina (1991), who found that pre-partum treatment of dairy cattle with vit. E. and Se. decreased uterine size per unit time and days to minimum uterine size. This suggested an improvement of uterine muscular function, due to a decrease in the time from calving to complete uterine involution.

Treated cows showed significantly shorter ( $P < 0.05$ ) interval from calving to first estrus compared to control group. It reduced the period to first detected estrus from  $41.8 \pm 8.3$  to  $33.5 \pm 3.51$  days. Julien et al., (1976) emphasized the beneficial influence of selenium injection on ovarian activity and uterine muscular function. It should be noted, however, that one cow in control group did not show signs of the first estrus (silent heat), so the next estrus was recorded as first estrus. When this was considered, average of interval from calving to the first detected estrus was longer than the average to the first ovulation.

The present results showed the benefits of vitamin E and selenium on the average interval from parturition to conception (days open) in Balady cows. Treated cows conceived 19.4 days earlier than the control group ( $P < 0.05$ ). These results are in agreement with those reported by Kolchina et al., (1991) and Archiga et al., (1994). A significant decrease ( $P < 0.05$ ) in number of services per conception was recorded for treated cases (1.8)

compared to control (2.4) (Table 3). Awad et al., (1985) reported that pre-partum selenium treatment decreased number of services per conception, while Kim et al., (1997) indicated that there was no differences between treated and control groups in postpartum number of services per conception.

Table (3): Effects of selenium and vitamin E administration during late stage of pregnancy on reproductive performance and incidence of reproductive disorders in Balady cows (mean  $\pm$  S.E).

Traits	Control group	Treated group
Udder edems (day)	20.3 $\pm$ 1.33 <sup>b</sup>	15.3 $\pm$ 0.91 <sup>a</sup>
Calving ease (score)	2.3 $\pm$ 0.07 <sup>b</sup>	1.6 $\pm$ 0.09 <sup>a</sup>
Retained placenta (%)	10 <sup>b</sup>	zero <sup>a</sup>
Uterine involution (day)	34.1 $\pm$ 4.6 <sup>b</sup>	22.1 $\pm$ 3.1 <sup>a</sup>
Metritis (%)	40	30
First ovulation (day)	40.7 $\pm$ 13.7 <sup>b</sup>	34.1 $\pm$ 2.63 <sup>a</sup>
First estrus (day)	41.8 $\pm$ 8.3 <sup>b</sup>	33.5 $\pm$ 3.51 <sup>a</sup>
Service period (day)	78.5 $\pm$ 11.4 <sup>b</sup>	66.2 $\pm$ 7.62 <sup>a</sup>
Days open (day)	120.6 $\pm$ 11.8 <sup>b</sup>	101.2 $\pm$ 13.5 <sup>a</sup>
Number of services per conception	2.4 $\pm$ 0.7 <sup>b</sup>	1.8 $\pm$ 0.3 <sup>a</sup>

#### Body Weight and Daily Gain of Calves:

Body weight and daily gain of calves from treated and control dams are presented in table 4. Increase in weight tended to be higher in calves from treated dams than those of the control. The difference in body weight increase was statistically significant ( $P < 0.05$ ). Such effect on weight of calves was described by Perry et al., (1978), who recorded a beneficial effect of selenium on born calves when it was treated to their dams.

Table (4): Effects of selenium and vitamin E treatment of pregnant Balady cows on body weight changes of their newborn calves (mean  $\pm$  S.E).

Traits	Control group	Treated group
Initial body weight (Kg)	24.1 $\pm$ 3.1	123.9 $\pm$ 1.2
Weaning weight (Kg)	70.3 $\pm$ 8.5 <sup>b</sup>	79.1 $\pm$ 9.3 <sup>a</sup>
Total weight gain (Kg)	46.2 $\pm$ 4.6 <sup>b</sup>	55.2 $\pm$ 7.1 <sup>a</sup>
Daily weight gain (Kg)	0.44 $\pm$ 0.09 <sup>b</sup>	0.53 $\pm$ 0.13 <sup>a</sup>

The present result is in agreement with those of Ikeuchi et al., (1997) who indicated that calves from dams fed on a diet supplemented with Se and Vit. E. showed greater tendency in daily weight gain. Results of previous studies (Nebbia, 1982) have indicated that pregnant cows fed a diet severely deficient in Se, vit E., or both deliver calves with white muscle disease, general unthriftiness and poor growth performance. It was described previously (El-Gaafarawy et al., 2000) that vit E (Lipophilic antioxidant vitamin) and selenium (antioxidant minerals) may have a beneficial effects as an immunocompetance antioxidant on the immune status of neonatal Balady calves as non-specific humeral immunostimulant and on improving their growth rate.

#### Concentration of Immunoglobulin Fractions (IgG and IgM) in Serum of Calves:

It has been mentioned that bovine milk contains up to one million lymphocytes per milliliter, about half of which are T cells. These milk lymphocytes may survive for 36 hours in the intestine of the newborn calves and have the ability to penetrate the intestinal wall and reach the liver. This

was taken as evidence that cell mediated immunity may be transferred to bovine neonates by this way (Tizard, 1996). It is relevant to note that bovine colostrum stimulates phagocyte activity and oxidative metabolism of neonates (Leblanc and Pritchard, 1988).

The changes and mean values of immunoglobulin fractions in serum of calves from treated and non-treated dams are presented in table 5. Concentration of immunoglobulin fractions IgG and IgM in sera of normal healthy control calves at pre-colostral time indicated hypo-immunoglobulinaemia (9.87 and 1.83 mg/ml, respectively).

Concentrations of both IgG and IgM at 1st week of calves' life from treated dams were highly elevated ( $P < 0.05$ ) when it was compared to those from non-treated cows. This increase could be credited to B-lymphocytes stimulation by an antioxidant, which consequently, elevated the level of immunoglobulins (Hughes, 1999).

Values of IgG declined to reach a minimum level at 7th and 11th weeks in calves from control and treated group, respectively, while IgM concentration decreased by the 7th week. Generally IgM values of calves from treated cows were significantly decreased than those of control except at the first week. This may be due to fundamental differences in bovine immune system including development of B cell lineage, humoral response and the cytokine control of antibody production in cattle, which may differ from the regulatory pathways that have been described in mouse and human (Estes, 1996). Cytokines are known to regulate Ig class switching by positively or negatively regulating germ line CH transcription (Tizard, 1996). The present work may suggest that Vitamin E and Se play a role in cytokines or class switching, the process by which B- lymphocyte can alter expression in Ig heavy chain isotype and expanding the capabilities of the humoral immune response of neonatal calves.

Table (5): Effect of Vit E and Se treatment of Balady cow on the level of serum immunoglobulins Mean  $\pm$  S.E.

Age (week)	Serum IgG of calves (mg/ml)							
	Precolostrum	1 W	3W	5 W	7 W	9 W	11 W	Weaning
Control	9.8 $\pm$ 0.8	54.2 $\pm$ 6.4	53.6 $\pm$ 3.2	43.5 $\pm$ 7.6	40.6 $\pm$ 3.7	42.5 $\pm$ 4.8	44.5 $\pm$ 5.3	42.4 $\pm$ 2.7
Treated	9.8 $\pm$ 0.9	62.8* $\pm$ 6.1	62.6* $\pm$ 7.5	55.8* $\pm$ 4.5	50.8* $\pm$ 8.9	46.3* $\pm$ 7.8	35.2* $\pm$ 8.7	48.8* $\pm$ 2.5
Age (week)	Serum IgM of calves (mg/ml)							
	Precolostrum	1 W	3W	5 W	7 W	9 W	11 W	Weaning
Control	1.83 $\pm$ 0.3	2.9 $\pm$ 0.5	2.6 $\pm$ 0.4	2.5 $\pm$ 0.3	2.3 $\pm$ 0.2	2.6 $\pm$ 0.4	2.8 $\pm$ 0.3	3.0 $\pm$ 0.2
Treated	1.83 $\pm$ 0.3	3.2* $\pm$ 0.4	1.95 $\pm$ 0.4	1.8 $\pm$ 0.3	1.7 $\pm$ 0.7	1.8 $\pm$ 0.9	2.1 $\pm$ 0.9	2.0 $\pm$ 0.7

\* Significantly different ( $P < 0.05$ ) from control.

In conclusion, this study indicated that vitamin E and selenium, given to late pregnant Balady cows, might have an effect on their reproductive performance. Calves born to dams given Se and vitamin E in late pregnancy have higher immunoglobulins at 1st week of age and higher growth rate between birth and weaning. This will explore the possible effect of vitamin E and Se on late pregnant Balady cows and on the growth and immune response of their neonates.

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