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Influence of Prepartum Administration of Viteselen and/or Ultra-Corn on Reproductive Performance and Calves Viability in Buffaloes

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Abstract: This study aimed to determine the efficacy of prepartum administration of Viteselen (VS) and Ultra-corn (UC) given at the late gestation on the IgG level, postpartum fertility and viability of newborn calves. Forty five buffaloes were divided into 4 groups. First group served as control (n=9), while the others served as experimental groups (n=12/group). Second group received 30 ml of viteselen (1.7 mg sodium selenium and 150 mg vitamin E/ml). Third group was injected with ultra-corn (2 ml/100 Kg body weight). Fourth group was injected with viteselen and ultra-corn. The drugs were injected in 2 doses prior to anticipate date of calving (60 days). The postpartum fertility was assessed. Colostrum from dams (at calving and day-4 postpartum) and serum from newborns (at day-4 and day-14 old) were collected to estimate the level of immunoglobulin (IgG) by using Vet-RID kit. Body weight, growth rate and viability of newborn calves were recorded. The level of IgG was significantly higher ($P<0.05$) in both dam colostrum and newborn serum after administration of VS and/or UC. Moreover, VS injection resulted in a significant ($P<0.05$) higher level of IgG in both dam colostrum (at calving) and calves serum (at day-4 and -14 old) than the other groups. The periods required for fetal membrane expulsion and uterine involution were significantly ($P<0.05$) reduced in VS group when compared to UC or control groups. Concomitantly, injection of VS or UC significantly ($P<0.05$) reduced the calving to first estrus interval and length of postpartum service period than the control buffaloes. The number of services required per conception decreased with VS+UC but insignificant difference resulted between the groups. Only one case of abortion was received from VS+UC treated dams, while VS or UC treated dams delivered 100% healthy calves. The calves from treated dams (especially VS) showed higher body weight, growth rate/day and better vitality than the control. No mortality in the 1st month occurred between newborns from VS and UC treated dams compared to other groups. *In conclusion*, prepartum administration of VS and/or UC appear to be beneficial, promising and improve the reproductive performance and calf viability in buffaloes and additional work involving a larger number of animals is suggested.

Key words: Prepartum · Viteselen · Ultra-corn · IgG · Postpartum · Calves · Viability

INTRODUCTION

A wide variety of antibacterial agents mainly antibiotics are employed for the prevention and treatment of infection in livestock, these antibiotics cause various problems such as residue and the emergence of bacterial resistance as well as suppression to the host resistance. Depressed immune function causes a marked increase in the incidence of opportunistic infection [1]. Moreover, stress of pregnancy, parturition and lactation cause suppression of the host defense mechanism and increase susceptibility of the animal to infection during pregnancy, parturition and lactation [2].

Recently, there are many drugs used to increase the resistance of the animal by improving the humeral and cell mediate immune response such as Dihydroheptaprenol [3], vitamin B2 [4,5], selenium [6], vitamin-E [7], vitamin-E and selenium [8,9] and ultra-corn [10]. Non-specific immunostimulants have been received considerable attention in the vet-field. They appear to provide an efficient method of stimulating the immune system in a non-specific manner with few adverse side effects. Immunostimulating compounds have the potential to counteract the effect of environmental or microbial immuno-suppressive factors. They may thus reduce morbidity and economic losses resulting from sub clinical

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or mild infectious diseases [11]. Because they may also act as adjuvant, they can also potentiate immune responses to applied vaccine.

A great deal of interest has developed in implementing biosecurity programs to prevent the transmission of infectious disease to dairy replacement calves. One potential method of transmission of infectious diseases to dairy calves is through feeding infective colostrum and milk. The immune status of the newborn calves is dependent upon the passage of immunoglobulins from dams to the calves through the ingestion of colostrum [12] and its subsequent absorption from small intestine. Their net effect may therefore be to improve weight gain and to lower mortality and morbidity rates among animals. The major immunostimulants are usually microbial preparations that are rapidly taken up by macrophages [13]. Successful attempts to improve reproductive efficiency by administering immunopotentiators during late gestation in cows [14] and sows [15] have been reported previously. However, little information is available for buffaloes. Thus, the present investigation aimed to evaluate the efficacy of some immunopotentiators in improving the IgG-level, postpartum reproductive performance, as well as, viability of newborn calves in the buffaloes under the Egyptian conditions.

MATERIAL AND METHODS

Animals: This investigation was conducted on 45 buffaloes in the last trimester of gestation and belonging to a private farm at Sharkia Governorate, Egypt. The experiments were performed in the period from May/2006 until December/2007. The animals were 5.47±1.14 years old, apparently healthy (weighted 485.92±36.74) and free from common infectious and contagious diseases as proved by Veterinary Services Authorities. All the animals were received prophylactic routine treatment against internal, external and blood parasites. The prophylactic immunization program included biannual vaccination against FMD, Rift Vally Fever and Hemorrhagic Septicemia. The last vaccination was given 4 months before the beginning of the experiment. All animals were dried off for 75 days prior to parturition. Each animal was supplied daily with 6 Kg concentrates, in addition to 25-30 Kg Barseem clover during the green season or darawa (green maize) during the dry season with suitable amount of rice straw.

Treatment schedule: The animals were randomly divided into one of four treatment groups. The first group served as the untreated controls (n=9). Another three groups served as experimental animals (each group included 12 buffaloes). Those in second group were injected i.m. with 30 ml of viteselen (1.7 mg sodium selenium and 150 mg vitamin E/ml) (Egyptian Co. for Chemical and Pharmaceutical-ADWIA, 10th of Ramadan City). Each buffalo in third group received 10 ml s.c. (2 ml/100 Kg body weight) ultra-corn which is a complete lysate of bacterial extract produced by ultrasound treatment of *Corynebacterium cutis*. (Virbac Co., France). In fourth group, the animals were injected with viteselen and ultra-corn. The immunopotentiators were given 2 doses one week apart, the first injected on day 60 prior to the expected date of calving [41].

Analysis of IgG level: Colostral samples were collected within one hour of calving (first milking) from buffaloes (at calving) and at day-4 pp in all 5 groups. Blood samples were collected from newborn calves (at day-4 and day-14 old). Colostrum and serum samples were frozen at –20°C until determination of IgG. Frozen colostrum was submitted to determination of IgG level by using IgG Vet-RID (Radial Immunodiffusion) kit (Bethyl Laboratories, Inc., Montgomery, TX) Analysis was performed as previously reported [16]. Due to the very high levels of IgG, colostrum samples were first diluted x10 with distilled water and then 5 µl of the diluted sample was tested. This initial 10-fold dilution was taken into account when back-calculating colostrum IgG level for each sample. Serum IgG concentrations were determined using the same test kit and using the same general testing process. Serum IgG concentrations were determined according to kit instructions and using 5 µl of serum.

After the samples were placed on the plates, they were left at room temperature for a minimum of 18 hrs and then the precipitation ring diameters measured and IgG values calculated. Three standards with known values (625, 2500 and 5000 mg/dl) were also tested for each run. The diameters of the known standards were then used to calculate the tested serum samples.

Reproductive performance: All the investigated buffaloes were kept under close observation during parturition and in the postpartum period until they were confirmed as pregnant again. After calving, the animals were kept individually in open pens and the period prior to the expulsion of the fetal membranes was recorded in hours.

Rectal palpation was performed twice a week to examine the time of uterine involution (return to the non-pregnant size). Thereafter, estrus was confirmed in buffaloes by rectal palpation and they were inseminated using thawed frozen semen from a fertile bull.

Viability of the newborn calves: Calves born to the control and treated groups were weighed and kept under close observation. Their viability and health problems were examined clinically and growth rate (Kg/day) was also recorded: (wt. at 3 months – wt. at calving)/90 days.

Statistical analysis: Data were analyzed using SAS analysis system package [17]. Significant differences between each two means were evaluated utilizing Duncan's Multiple Range Test (DMRT) [18].

RESULTS

The IgG level in colostrum of dams and serum of newborn calves were estimated (Table 1). Generally, the concentration of IgG was significantly ($P<0.05$) higher in the groups injected with immunopotentiators than control one. At calving, the level of colostral IgG was significantly ($P<0.05$) higher in the group injected with VS than the other test or control groups. At day-4 postpartum, the level of IgG was non-significant between the groups received immunostimulants but significant compared to the control one. Regarding the sera of newborn calves, they revealed significantly ($P<0.05$) higher level of IgG in the group administered VS than test or control group at day-4 old and revealed a significant ($P<0.05$) higher level with VS and VS+UC injected groups than UC injected group at day-14 old.

Table 1: Mean (±S.E.) level (mg/ml) of immunoglobulin in colostrum of dams and serum of newborn calves after viteselen and/or ultra-corn antepartum administration

		Experimental groups		
Parameters	Control group	VS	UC	VS+UC
Parturient dams	N=9	N=12	N=12	N=12
At parturition	17.30±0.33 ^{c}	23.80±0.79 ^{a}	21.27±0.88 ^{b}	20.55±0.83 ^{b}
Day-4 postpartum	13.60±0.87 ^{b}	20.14±1.42 ^{a}	17.93±0.87 ^{a}	17.79±0.62 ^{a}
Newborn calves	N=7	N=12	N=12	N=11
Day-4 old	6.36±0.63 ^{c}	13.18±0.83 ^{a}	9.22±0.44 ^{b}	9.12±0.67 ^{b}
Day-14 old	4.18±0.57 ^{c}	10.59±0.87 ^{a}	6.28±0.75 ^{bc}	7.58±0.87 ^{b}

Means with different superscripts in each row are significantly different at level ($P<0.05$). VS=Viteselen; UC=Ultra-corn

Table 2: Mean (±S.E.) values of postpartum reproductive performance of buffaloes treated with viteselen and ultra-corn and health status of their newborns

		Experimental groups		
Items	Control group (n=9)	VS (n=12)	UC (n=12)	VS+UC (n=12)
Postpartum reproductive parameters				
Placental expulsion (hr)	9.83±1.57 ^{b}	6.51±0.81 ^{a}	8.73±1.43 ^{b}	8.72±1.03 ^{b}
Uterine involution (days)	48.91±4.17 ^{a}	27.12±2.29 ^{b}	37.22±2.39 ^{ab}	34.23±7.29 ^{ab}
Calving to 1stestrus (days)	185.11±28.17 ^{a}	110.72±15.45 ^{b}	113.11±17.01 ^{b}	167.50±22.90 ^{ab}
Service period (days)	225.31±33.03 ^{a}	143.71±16.49 ^{b}	153.76±13.08 ^{b}	210.79±42.60 ^{ab}
Services per conception	2.36±0.39 ^{a}	1.62±0.19 ^{a}	1.81±0.15 ^{a}	2.00±0.17 ^{a}
Health status of delivered calves				
Aborted feti	1	0	0	1
Still birth	1	0	0	0
BW at birth (kg)	34.7	38.3	34.5	35.9
BW 3 months pp (kg)	63.5	83.8	72.4	74.9
Growth rate (kg/day)	0.320	0.505	0.421	0.433
Mortality at 1st month	2/9 (22.2%)	0/12 (0%)	0/12 (0%)	1/11 (9.1%)
Survival rate	7/9 (77.8%)	12/12 (100%)	12/12 (100%)	10/11 (90.9%)

Means with different superscripts in each row are significantly different at level ($P<0.05$).

VS=Viteselen; UC=Ultra-corn; BW=Body Weight; pp=Postpartum

The mean values (±S.E.) of various parameters of postpartum reproductive performance of the buffaloes in four groups are illustrated in Table 2. The immunopotentiators appeared to improve the reproductive performance. Prepartum treatment with VS reduced the period of fetal membrane expulsion compared to the other groups. Both of VS and VS+UC treatments had a significantly ($P<0.05$) shorter uterine involution period compared to the animals in the UC or control groups. Additionally, the VS and UC treated groups had a significantly ($P<0.05$) shorter calving to 1st estrus interval and first service period compared to the control groups. There was insignificant number of services required per conception between the control and experimental groups.

The control dams delivered only 7 calves because there was one case of abortion and one case of stillbirth, while the group received VS+UC has one case of abortion. Meanwhile, VS and UC treated dams delivered 12 healthy calves. The calves from treated dams showed higher body weight, growth rate/day and better vitality in comparison with the control. Likewise, body weight and growth rate/day were more in newly born calves from VS than UC groups. Moreover, no mortality in the 1st month were occurred between the newborn calves resulted from VS and UC treated buffaloes compared to the other groups.

DISCUSSION

Prepartum and early postpartum are critical periods for dairy animals. Proper nutrients intake in these periods helps in keeping the animal in a good condition and avoiding many problems. Vitamin-E and selenium [19] and ultra-corn [10,43,44] are essential nutrients for proper function of various reproductive characteristics of mammalian female. Moreover, pregnant animals are more susceptible to selenium deficiency than non-pregnant animals, which in turn increase the incidence of prepartum and postpartum reproductive disorders [20].

The level of IgG was significantly higher in both dam colostrum and newborn serum after administration of immunopotentiating agents than the control group. Moreover, VS injection resulted in a significant higher level of IgG in both dam colostrum and calves serum in comparison to the other test or control groups. However, the immune status of the newborn calves is dependent upon the passage of immunoglobulins from dams to the calves through the ingestion of colostrum [12,21] and its subsequent absorption from small intestine. When the mean colostrum-serum IgG levels immediately after birth and on Day 4 were evaluated, they were found to be higher in the experimental group than in the control group. On Day 4 and Day 14 after birth, IgG levels were found to be higher in the experimental group compared to the control group. Moreover, an important reason that calves have variable blood IgG at 24 hours of age is due to variation in colostrum IgG content. Colostrum composition is remarkably variable, as colostral IgG can range from a low to high content [22]. Subsequently, the amount of IgG in dam's colostrum depended mainly upon prepartum administration of immunopotentiators and in calves depended mainly upon consumption of colostrum directly after parturition.

The significant shorter placental expulsion period in VS treated buffaloes in the present study may be due to improved uterine muscular function. Both vitamin-E and selenium have antioxidant functions that protect biological systems from oxidative degradation [23-25]. In addition to their general antioxidant roles, selenium and vitamin-E may be involved indirectly in prostaglandin synthesis where proxy radicals are a normal part of the metabolic pathways [24]. Vitamin-E has been implicated in the control of Phospholipase-A2 activity [26], which is responsible for cleaving arachidonic acid from membrane phospholipids.

The significant reduction in calving to first estrus and shorter service period in the buffaloes treated with VS compared with the control animals in the present study supported a previous study [27], who reported that the calving to the first estrus and the length of the service period were significantly reduced in cows treated with prepartum injection of vitamin-E and selenium. Contradirectorily, after prepartum vitamin-E/selenium injection, there was no improvement on the subsequent postpartum reproductive performance of dairy cows [28]. This discrepancy might be attributed to differences in the prepartum Se status of the animals and frequency of the injections [19]. A significant decrease in the number of service per conception was obtained in the present investigation following prepartum VS injection. These findings reinforced those obtained by others [29,30]. Additional studies have shown that supplementation with Se and vitamin-E reduces the incidence of retained placenta [29,31,32], metritis, cystic ovaries [29], clinical signs of mastitis [33] and time of uterine involution in cows with metritis [34]. Selenium preferentially accumulates in the placentoms, ovary, pituitary and adrenal glands, suggesting specific requirements for Se in those tissues [23,35]. Several studies indicated

that both humeral and cellular immune response are enhanced by vitamin-E/selenium treatment [27,36,37]. Additionally, cows given vitamin-E and selenium in late pregnancy produce large quantities of colostrum and milk and have less difficulty in drop of placenta [38].

Regarding preparatum ultra-corn (UC) treated group, a significant decrease was observed concerning uterine involution period, calving to first estrus interval as well as the service period, but still less than VS treated buffaloes. No available literature for the effect of prepartum treatment with UC on postpartum reproductive performance. However, ultra-corn is a complete lysate of bacterial extract produced by ultrasound treatment of *corynebacterium cutis*. Administration of UC induced marked immunopotentiating effect in cattle, poultry and buffaloes via increasing phagocytosis, lymphocyte transformation index, total leukocytic count, lymphocytes, monocytes, antibody titre, immunoglobulin and gave protection against mortality [10,39,40]. Some of these results are readily explicable on the basis of the expected effects of these immunostimulating corynbacteria. Thus killed corynbacteria (ultra-corn) are readily phagocytosed by macrophages and stimulate the release of tumor necrosis factor and inter-leukin I. These monokines may have a secondary effect on lymphocyte function and so stimulate the immune system non-specifically [40]. Concomitantly, UC has several beneficial effects [41], where it stimulated phagocytosis; cell-mediated immunity; antibodies production and also it stimulated anti-virus defenses in increasing interferone production.

The control dams delivered only 9 calves because there was one case of abortion and one case of stillbirth. Meanwhile, VS and UC treated dams delivered 12 normal calves (in each group), except a case of abortion in VS+UC group. The calves from VS and treated dams showed higher body weight, growth rate/day and better vitality in comparison with the UC or control groups. Moreover, the control calves showed more severe pneumonia and enteritis as a result of which 2 (22.2%) calves died within one month of birth. In contrast, the calves born to the prepartum treated dams showed mild disease symptoms and only one calf (9.1%) from UC treated dams died. The present results support the other views [10,40] who found that, UC treatment to late pregnant dams induced better state of delivery with no retained placenta or stillbirth in comparison with the control group. The newly born calves were of heavier body weight, better healthy status and highly resistance to disease. The pre and postpartum supplementation with vitamin-E/selenium combination improved the reproductive efficiency and immune status of Egyptian buffaloes and resulted in a significant elevation of gamma globulins [37]. Consequently, a high immunoglobulin-G concentration was observed in calves supplemented with vit.E/Se [42]. Circulating IgG has been related to preweaning growth [43] as well as long term performance of calves [44], thus some commercial calf raisers will pay dairy producers a premium for providing calves with serum total protein that exceeds some critical threshold (usually >5.2 to 5.5 g of total protein/dl of serum). Others will reduce the amount they pay to the producer if total protein is too low. Although passive immunity has an important effect on calf health, there are a number of other factors that influence the overall cost of morbidity and mortality on a calf raising operation. These other factors include the level of exposure of calves and level of stress to which calves are exposed. Another critical control point during the calf's life is the first 24 hours. Consumption of colostrum is essential to provide animals with the antibodies and other proteins that calves need to stay healthy. The amount of colostrum (and immunoglobulin, or IgG) consumed determines amount of passive immunity and resistance to disease. When calves consume insufficient amounts of IgG from colostrum within the first 24 hours of life, they are much more susceptible to developing disease and possibly dying. A major reason that preweaning mortality is higher than optimum (defined as less than 5% of calves born alive) is due to inadequate IgG intake [22]. Measuring a calf's level of passive immunity within the first week of life allows the producer to know the effectiveness of the colostrum management and calf feeding program. Because this is so important to the health and survival of the calf, it is an essential part of monitoring the overall heifer operation. However, the importance of achieving adequate levels of colostral immunoglobulins to protect the neonate from enteric disease and septicemia has long been recognized [45].

It would be anticipated that as a result of increased health, the newly born calves would show enhanced weight gain. The effect on newborn calves is somewhat more difficult to understand. Clearly, the killed bacteria given to pregnant cows will effectively stimulate their macrophage function and the release of cytokines. Proteins of this size may readily cross the placenta. Thus, any immunostimulating effect on the dam should also be effective in the fetus. Calves born to treated buffaloes may thus be at a significant advantage over

untreated calves in the face of microbial challenge (in ultra-corn group). Stimulation of non-specific defense mechanisms has the potential to counteract at types of immunosuppressive effects in animals. This is especially true in a country such as Egypt, where there is a high level of subclinical diseases in reared animals. Subclinical virus infections, suboptimal nutrition, intestinal parasitism and so on may all result in mild immunosuppression.

In conclusion, prepartum supplementation of buffaloes with immunopotentiators such as viteselen, ultra-corn or a combination improved postpartum reproductive efficacy and viability of their newborn calves. Thus, additional work involving a larger number of animals is suggested to reach appropriate results.

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