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Influence of antepartum administration of immunopotentiators on reproductive efficacy of buffalo and viability of their newborn

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Summary

The authors determine the efficacy of prepartum immunopotentiators administered during late gestation on postpartum fertility, IgG levels and calf viability. Fifty buffalo were divided into five groups (10 animals in each group). Group I was the control group. Each animal in Group II received 30 ml intramuscularly of viteselen (1.7 mg sodium selenium and 150 mg vitamin E/ml). Each animal in Group III received a subcutaneous injection of bacillus Calmette-Guérin (BCG) at 0.5 ml. In Group IV, each animal received 12.5 ml of levamisole hydrochloride intramuscularly, while those in Group V received 10 ml of ultra-corn subcutaneously. The immunopotentiators were administered according to each group 60 days prior to the anticipated date of parturition. Postpartum fertility was assessed by close observation and rectal examination after parturition. Colostrum from the dams and sera from the newborn were collected to estimate the level of immunoglobulin (IgG). Body weight, growth rate and viability of the calves were recorded after parturition. Prepartum treatment with viteselen reduced the period of foetal membrane expulsion by 2 h compared to the other groups. Concomitantly, the uterine involution period was significantly shorter in animals treated with viteselen and ultra-corn than in the other groups. Injection of viteselen, BCG or ultra-corn significantly reduced the calving to the first oestrus interval and length of postpartum service period (by 57, 54, 48 days and 67, 57, 44 days, respectively) than the levamisole group. The IgG level was significantly higher in both the colostrum of the dam and in newborn serum after administration of immunopotentiating agents. Furthermore, the viteselen injection resulted in a significantly higher level of IgG in both dam colostrum (at parturition) and calf serum in comparison to the other groups. The calves from viteselen and ultra-corn treated dams showed a higher growth rate and better health condition than the controls. Prepartum administration of immunopotentiators appears to be beneficial, promising and offer improvements to postpartum reproductive performance and calf viability in Egyptian buffalo. Finally, additional work involving a larger number of animals is suggested.

Keywords

Buffalo, Calf, Colostrum, Egypt, Immunoglobulin, Immunopotentiator, Prepartum, Viability.

Somministrazione di immunopotenziatori nel periodo preparto: valutazione dell'efficacia riproduttiva nei bufali e della vitalità perinatale nei vitelli bufalini

Riassunto

Gli autori valutano l'efficacia degli immunopotenziatori somministrati durante l'ultima fase di

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gestazione sulla fertilità postpartum, sui livelli di IgG e sulla vitalità perinatale dei vitelli bufalini. Cinquanta bufali sono stati divisi in 5 gruppi (10 animali per ogni gruppo). Il Gruppo I è stato il gruppo di controllo. Ad ogni animale del Gruppo II sono stati somministrati per via intramuscolare 30 ml di viteselen (1,7 sodio-selenio e 150 ml di vitamina E/ ml). Agli animali del Gruppo III sono stati somministrati per via sottocutanea 0,5 ml di bacillus Calmette-Guérin (BCG). Agli animali del Guppo IV sono stati somministrati per via intramuscolare 12,5 ml di levamisole cloridrato, mentre agli animali del Gruppo V, per via sottocutanea, 10 ml di Ultracorn. Gli immunopotenziatori sono stati somministrati ai quattro gruppi 60 giorni prima del parto. La fertilità postparto è stata valutata attraverso osservazione e ispezione rettale dopo il parto. Sono stati prelevati il colostro dalle fattrici e campioni di sangue dai vitelli per valutare il livello di immunoglobuline (IgG). Dopo il parto sono stati registrati il peso corporeo, l'indice di accrescimento e la vitalità dei vitelli. Il trattamento preparto con viteselen (Gruppo II) ha ridotto il periodo di espulsione degli invogli fetali di due ore rispetto agli altri gruppi. Inoltre il periodo di involuzione uterina si è significativamente ridotto negli animali trattati con viteselen e Ultracorn. La somministrazione di viteselen, BCG o Ultracorn ha considerevolmente ridotto l'intervallo tra il parto e la comparsa del primo estro e il periodo di interparto (rispettivamente a 57, 54, 48 giorni e 67, 57, 44 giorni) rispetto al gruppo trattato con levamisole. Il livello di IgG era significativamente più alto nel colostro delle fattrici e nel sangue dei vitelli dopo somministrazione di agenti immunopotenziatori. Tuttavia la somministrazione di viteselen fa aumentare significativamente il livello di IgG sia nel colostro che nel siero dei vitelli. I vitelli trattati con viteselen e Ultracorn hanno mostrato un indice di accrescimento più alto e un migliore stato sanitario rispetto al gruppo di controllo. La somministrazione di immunopotenziatori in preparto sembra essere vantaggiosa, garantendo e migliorando le performance riproduttive postparto e la vitalità perinatale nel bufalo egiziano. Si suggerisce un approfondimento della ricerca attraverso l'utilizzo di un maggior numero di animali.

Parole chiave

Bufalo, Colostro, Egitto, Immunopotenziatori, Immunoglobulina, Preparto, Vitalità.

Introduction

A wide variety of antibacterial agents, most of which are antibiotics, are employed for the prevention and treatment of infection in livestock. These antibiotics can cause various problems, such as residues, the emergence of bacterial resistance as well as suppression to the host resistance. A depressed immune function causes a marked increase in the incidence of opportunistic infection (29). Furthermore, stress of pregnancy, parturition and lactation cause suppression of the host defence mechanism and increase susceptibility of the animal to infection during pregnancy, parturition and lactation (27).

Many drugs have been used recently to increase the resistance of animals by improving the humoral and cell mediate immune response, such as dihydroheptaprenol (28), bacillus Calmette Guérin (BCG) (40), vitamin B2 (33), selenium (6), vitamin E (35), vitamin E and selenium (14), levamisole hydrochloride (1) and ultra-corn (41). Non-specific immunostimulants have received considerable attention in the veterinary field. They appear to provide an efficient way of stimulating the immune system in a non-specific manner with few adverse side effects.

Much interest has developed in implementing biosecurity programmes 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 (Ig) from dams to the calves through the ingestion of colostrum (32) and its subsequent absorption from the small intestine. Their net effect may therefore be to improve weight gain and to lower mortality and morbidity rates among animals. The principal immunostimulants are usually microbial preparations that are rapidly taken up by macrophages (17). Successful attempts to improve reproductive efficiency by administering immunopotentiators during late gestation in cows (15) and sows (36) have been reported previously. However, little

information is available for buffalo. The present investigation aimed at evaluating the efficacy of some immunopotentiators in improving the postpartum reproductive performance, IgG level and calf viability in Egyptian buffalo.

Materials and methods

Animals

This investigation was conducted on fifty buffalo during late gestation that were held on a private farm in the Sharkia Governorate. The animals were 4 to 7 years old and were apparently healthy and free from common infectious and contagious diseases as verified by the Veterinary Services. All experimental animals received prophylactic routine treatment against internal, external and blood parasites. The prophylactic immunisation programme included biannual vaccination against foot and mouth disease, Rift Valley fever and haemorrhagic septicaemia. The last vaccination was administered four months prior to the commencement of the experiment. All animals were dried off for 75 days prior to parturition. Each animal was supplied with a daily allowance of 15 kg corn silage, 5 kg vicia hay, 2 kg wheat straw, 2 kg wheat hay, 0.5 kg soybean meal, 1 kg concentrate barley grain and vitamins+minerals. Globally, the ration includes 15.7 kg dry matter, 11.5% crude protein and 22% crude fibre, in addition to green fodder.

Treatment schedule

The animals were randomly divided into one of five treatment groups (with ten animals in each group). The animals in Group I were kept as the untreated controls. Each animal in Group II was injected intramuscularly in the thigh 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 Group III was given 0.5 ml subcutaneously of BCG (Pasteur Mérieux, Lyons). Buffalo in Group IV were injected intramuscularly with 12.5 ml of levamisole hydrochloride (Chemical Industries Development, CID-Giza, Egypt). Those in Group V received 10 ml of ultra-corn subcutaneously (2 ml/100 kg body weight) (Virbac Co., France). The immunopotentiators were given two doses, with treatments one week apart, 45 days prior to the expected date of parturition.

Analysis of immunoglobulin G levels

Colostral samples were collected within 1 h of parturition (first milking) from buffalo (at parturition) and on day 4 postpartum in all five groups. Blood samples were collected from the jugular vein of newborn calves (aged 4 and 14 days). Colostrum and serum samples were frozen at –20ºC until the IgG level was determined. An analysis was performed according to Godden *et al.* (18). Frozen colostrum was submitted for the determination of the IgG level by using the bovine IgG Vet radial immunodiffusion (RID) kit (Bethyl Laboratories, Inc., Montgomery, Texas). Due to the very high levels of IgG, colostrum samples were first diluted (1:10) with distilled water and then 5 µl of the diluted sample was tested. This initial tenfold dilution was taken into account when back calculating the 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 serum.

The samples were placed on the plates and left at room temperature for a minimum of 18 h, after which the precipitation ring diameters were measured and IgG values calculated. Three standards with known values (625, 2 500 and 5 000 mg/dl) were also tested for each run. The diameters of the known standards were then used to calculate the serum samples tested.

Reproductive performance

All the buffalo investigated were kept under close observation during parturition and throughout the postpartum period until they were confirmed as pregnant again by rectal palpation. After birth, the animals were kept individually in open pens and the period prior to the expulsion of the foetal membranes was recorded. Rectal palpation was performed

twice a week to monitor uterine involution. Thereafter, the animals were let loose with vasectomised marker bulls. Oestrus was confirmed in marked buffalo by rectal palpation and close observation of the animals and these animals were inseminated using thawed semen.

Viability of newborn calves

Calves born to the control and treated groups were weighed and kept under close observation. Their viability (examination of the health condition), health problems and growth rate (kg/day) were recorded.

Statistical analysis

Data were analysed using statistical applications (SAS) analysis system package (26). Significant differences between each two means were evaluated utilising Duncan's multiple rang test (DMRT) (11).

Results

The mean values (±SE or 'standard error') of various parameters of postpartum reproductive performance of the buffalo in the five groups are given in Table I. The immunopotentiators appeared to improve reproductive performance. Prepartum treatment with viteselen reduced the period of foetal membrane expulsion compared to the other groups. Both viteselen and ultra-corn treatments had a significantly (p<0.05) shorter uterine involution period compared to the animals in the control group. Additionally, the groups treated with viteselen, BCG and ultra-corn had a significantly (p<0.05) shorter calving to oestrus interval and first service period compared to the control group. Levamisole hydrochloride appeared to improve reproductive performance.

The IgG levels in the colostrum of dams and in the serum of newborn calves were quantified (Table II). In general, the concentration of IgG was significantly higher (p<0.05) in the groups injected with immunopotentiators than in the controls. At parturition, the level of colostral IgG was significantly higher (p<0.05) in the group injected with viteselen than in the other groups. On day 4 postpartum, the IgG level was not significantly different between the treated groups; however the colostral IgG was significantly higher than in the controls.

The sera of newborn calves revealed a significantly higher (p<0.05) level of IgG in the group that had received viteselen than the other substances or control groups aged 4 days and also revealed significant higher level (p<0.05) in groups injected with viteselen, levamisole and ultra-corn than the BCG or control group at 14 days of age.

The control dams delivered only eight calves because there was one case of abortion and one case of stillbirth, while the group that received levamisole had one case of abortion. Meanwhile, viteselen, BCG and ultra-corn treated dams delivered 10 healthy calves (in

Table I
Mean values of various parameters of the postpartum reproductive performance of buffalo treated with immunopotentiating agents

Reproductive parameters	Group				
	Control (n=10)	Viteselen (n=10)	BCG (n=10)	Levamisole (n=10)	Ultra-corn (n=10)
Foetal membrane expulsion period (h)	9.83 ± 1.57	6.51 ± 0.81*	8.73 ± 1.43	8.72 ± 1.03	8.57 ± 1.03
Uterine involution period (days)	48.91 ± 4.17	27.12 ± 2.29*	37.22 ± 2.39	34.23 ± 7.29	30.91 ± 3.22*
Calving to first oestrus interval (days)	185.11 ± 28.17*	110.72 ± 15.45*	113.11 ± 17.01*	167.50 ± 22.90*	119.70 ± 17.55*
Service period (days)	225.31 ± 33.03	143.71 ± 16.49*	153.76 ± 13.08*	210.79 ± 42.60*	166.29 ± 18.49*
Services required per conception	2.36 ± 0.39	1.62 ± 0.19	1.81 ± 0.15	2.00 ± 0.17	1.60 ± 0.15

BCG bacillus Calmette-Guérin

* values in the same row are significantly different at p<0.05

Table II
Mean levels (mg/ml) of immunoglobulin in the colostrum of dams and the serum of newborn calves

Animal	Group				
	Control	Viteselen	BCG	Levamisole	Ultra-corn
Dams	n=10	n=10	n=10	n=9	n=10
At parturition	17.30 ± 0.33	23.80 ± 0.79*	21.27 ± 0.88	20.55 ± 0.83	20.75 ± 0.91
Day 4 postpartum	13.60 ± 0.87	20.14 ± 1.42*	17.93 ± 0.87	17.79 ± 0.62	18.01 ± 0.81
Newborn calves	n=8	n=10	n=10	n=9	n=10
Aged 4 days	6.36 ± 0.63	13.18 ± 0.83*	9.22 ± 0.44	9.12 ± 0.67	10.11 ± 0.72
Aged 14 days	4.18 ± 0.57	10.59 ± 0.87*	6.28 ± 0.75	7.58 ± 0.87*	7.43 ± 0.72*

BCG bacillus Calmette-Guérin

* values in the same row are significantly different at $p<0.05$

each group). The calves from viteselen and ultra-corn treated dams showed higher body weight, growth rate and better viability in comparison with the controls (Table III). Moreover, the control calves showed more severe pneumonia and enteritis as a result of which two calves died within one month of birth. In contrast, the calves born to the prepartum-treated dams showed mild disease symptoms (BCG and levamisole groups) and only one calf (11.1%) from the levamisole group died due to mild enteritis.

Discussion

The intake of proper nutrients during prepartum and early postpartum helps to maintain the animal in good condition and avoids many problems. Vitamin E and selenium are essential nutrients for the proper function of various reproductive characteristics of mammalian females (3, 13). Moreover, pregnant animals are more susceptible to selenium deficiency than non-pregnant animals which, in turn, increases the incidence of prepartum and postpartum reproductive disorders (47). The significantly shorter placental expulsion period in the viteselen-treated buffalo in this study may be due to an improved uterine muscular function. Both vitamin E and selenium have antioxidant functions that protect biological systems from oxidative degradation (10, 16, 47). In addition

Table III
Health status of calves delivered from dams treated prepartum with immunopotentiating agents

Parameter	Group				
	Control (n=10)	Viteselen (n=10)	BCG(n=10)	Levamisole(n=9)	Ultra-corn(n=10)
No. of foetuses born	8	10	10	9	10
Abortion	1	0	0	1	0
Stillbirths	1	0	0	0	0
Average body weight at birth (kg)	34.7	38.3	34.5	35.9	39.3
Average body weight at 3 months postpartum (kg)	63.5	83.8	72.4	74.9	80.5
Growth rate (kg/day)	0.320	0.505	0.421	0.433	0.457
Diseases and clinical signs	Severe pneumo-enteritis (2 calves)	(Non) diseased calves	Mild enteritis (1 calf)	Mild enteritis (1 calf)	(Non) diseased calves
Mortality at first month	2/8 (25%)	0/10 (0%)	0/10 (0%)	1/9 (11.1%)	0/10 (0%)
Survival rate	6/8 (75%)	10/10 (100%)	10/10 (100%)	8/9 (88.9%)	10/10 (100%)

BCG bacillus Calmette-Guérin

to their general antioxidant roles, selenium and vitamin E may be involved indirectly in prostaglandin synthesis where peroxy radicals are a normal part of the metabolic pathways (10). Vitamin E has been implicated in the control of phospholipase A2 activity (34) which is responsible for cleaving arachidonic acid from membrane phospholipids. Arachidonic acid is the common precursor for all prostaglandins and related compounds.

The IgG concentration was significantly higher in the groups (dams and newborn calves) injected with immunopotentiators than in the control group. The level of colostral IgG was significantly higher at parturition for the viteselen injected group compared to the levamisole and control groups, and the levels remained higher in the injected group than in the controls. The sera of newborn calves revealed significantly higher IgG levels in the group that received viteselen than the other substances or control groups either at day 4 or day 14 of age, with no significant decrease in the IgG level when the age of the calves increased. 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 (32) and its subsequent absorption from the small intestine. When the mean colostrum-serum IgG levels were evaluated immediately after birth and on day 4, they were found to be higher in the experimental group than in the control group. When blood-serum IgG levels of the calves were measured, they were found to be higher after suckling colostrum (0-4 days after birth) than at 14 days after birth in the experimental and control groups (2). 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 h 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 (43). Subsequently, the amount of IgG in the colostrum of the dam depended mainly upon prepartum administration of immuno-potentiators, and in calves depended mainly upon consumption of colostrum directly after parturition.

The control dams delivered only eight calves due to one abortion and one stillbirth. Meanwhile, viteselen-treated dams delivered 10 normal calves, with the exception of one case of abortion in the levamisole group. The calves from viteselen-treated dams showed higher body weight, growth rate and better vitality compared to the levamisole treated and control groups. Moreover, the control calves showed more pneumonia and enteritis, as a result of which two calves (25%) died within one month of birth. In contrast, the calves born to the prepartum-treated dams showed mild disease symptoms and only one calf (11.1%) died from the levamisole-treated dams. The newly born calves had a heavier body weight, a better healthy status and higher resistance to pneumonia and enteritis. Additionally, there was a significant reduction in the incidence of foetal death (from 24.8% to 4.8%) following the injection of levamisole to cows during the dry period (15). Consequently, a high IgG concentration was observed in calves supplemented with vitamin E/selenium (24, 43). Circulating IgG has been related to preweaning growth (30) as well as long-term performance of calves (45). Thus, some commercial calf raisers will pay dairy producers a premium for providing calves with serum total protein that exceeds some critical thresholds (usually >5.2 g to 5.5 g of total protein/dl of serum). Another critical control point during the live of the calf is the consumption of colostrums during the first 24 h which is essential to provide animals with the antibodies and other proteins that calves require to stay healthy. The amount of colostrum (IgG) consumed determines the level of passive immunity and resistance to disease. When calves consume insufficient amounts of IgG from colostrum within the first 24 h of life, they are much more susceptible to developing diseases 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 (37). Measuring a calf's level of passive immunity within the first week of life enables

the producer to know the effectiveness of colostrum management and calf feeding programme. However, the importance of achieving adequate levels of colostral immunoglobulins to protect the neonate from enteric disease and septicaemia has long been recognised (39, 46). It would be anticipated that as a result of better health, the newly born calves would show enhanced weight gain. The effects on newborn calves are somewhat more difficult to understand (39).

The significant reduction in calving to first oestrus and the shorter service period in the buffalo treated with viteselen compared with the control animals in this study supported a previous study (4) that reported that calving to the first oestrus and the length of the service period were significantly reduced in cows treated with prepartum injection of vitamin E/selenium. On the contrary, after prepartum vitamin E/selenium injection, there was no improvement on the subsequent postpartum reproductive performance of dairy cows (19). This discrepancy might be attributed to differences in the prepartum selenium status of the animals and the frequency of injections (3). A significant decrease in the number of services per conception was obtained in our investigation following prepartum viteselen injection. These findings confirmed those obtained by others (9, 21). Additional studies have demonstrated that supplementation with selenium and vitamin E reduces the incidence of retained placenta (12, 21, 23), metritis, cystic ovaries (21), clinical signs of mastitis (8) and the time of uterine involution in cows with metritis (22). Selenium preferentially accumulates in the placenta, ovary, pituitary and adrenal glands, suggesting specific requirements for selenium in those tissues (7, 20). Several studies indicated that both humoral and cellular immune responses are enhanced by vitamin E/ selenium treatment (4, 31). However, a highly significant elevation of gamma globulins in the vitamin E/selenium supplemented buffalo compared to the control was recorded (24, 25).

Levamisole hydrochloride also appeared to improve reproductive performance. Since the mechanism by which levamisole modifies the biological response is not known, a standard dose and schedule of drug administration was not established. However, levamisole is a chemical preparation that was originally applied as an anthelmintic drug against adult and larval stages of gastrointestinal nematodes and lung worm in cattle, sheep, pigs, dogs and cats (42). Beside the anthelmintic activity of levamisole, it has a broad range of effects on the immune system by stimulating cell-mediated immunity through potentiating the rate of T-lymphocyte differentiation, the responsiveness to antigen and mitogens and the activity of effector lymphocytes. Furthermore, levamisole stimulate phagocytosis and chemotaxis for neutrophils and monocytes (5, 44). The most important enhancement of its activity occurs in cells, the function of which is inefficient or suppressed (38). Moreover, 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 (15). The reproductive performance of cattle was thought to improve following injection of levamisole. The response to levamisole differs from species to species given the differences in physiological mechanisms (36). The different dose and frequency of administration of levamisole, along with the different experimental species, may account for the reduced effect of levamisole in the present investigation.

In conclusion, although the buffalo have been criticised for poor reproductive performance, it appears that positive effects could be obtained by prepartum immunopotentiation using viteselen, BCG, levamisole hydrochloride or ultra-corn. Additional work involving a larger number of animals is suggested.

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