



Effect of Prepartum Vitamin E and Selenium on Antibody Transfer in Colostrum and Cattle Calves

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ABSTRACT

Present study was intended to evaluate the effect of vitamin E and selenium supplementation on passive transfer of antibodies in colostrum and calves against FMD and HS vaccine. Thirty-five pregnant cattle were selected and seven groups were made consisting five cattle. Group A was controls: Animals in Group B and C were at 45 days prepartum with and without VES: animals in D and E were at day 60 prepartum with and without VES: animals in F and G were at 75 days prepartum stage with and without VES: respectively. At day 0 of experimental trial VES was given to animals in group C, E and G. All groups were given oil based HS vaccine at day 15 and alum based FMD trivalent vaccine at day 30 of experimental trial except Group A. Blood samples from adult cattle were collected at day 0, 15 and 30 after parturition and in cattle calves at age of 0, 14, 28 and 42. Colostrum sample was collected at 0, 12 and 24 h after parturition. Antibody titer was determined by Complement fixation test. Results showed that highest antibody titer was developed in cattle, colostrum and their calves in group G in which treatment was started 75 days prepartum and VESS was given. It was concluded that vitamin E and selenium supplementation improves both active and passive immune system if given before 75 days of parturition.

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Authors' Contribution

SK, MI and AAA designed the project. KP conducted the study and wrote the paper. AP helped in analysis of data. MIK and NUK helped in preparation of manuscript.

Key words

Selenium, Vitamin E, Cattle, Calves, Colostrum.

INTRODUCTION

Period before and after parturition is stressful for cattle and calves and it is associated with increased incidences of the disease due to immune suppression and increased susceptibility to disease (Mallard *et al.*, 1998; Waller, 2000). Suppression of immune system is probably due to hormonal changes and deficiency of certain nutrients (Goff and Horst, 1997). Selenium is important micromineral for normal functioning of various systems of an animal as it is important component of glutathione peroxidase (Arthur, 2000). It is important component of immune system and deficiency of selenium is associated with impaired immune response (McKenzie *et al.*, 1998). It is also reported that selenium deficiency decreases resistance of the animal against various diseases (Huang and Yang, 2002).

Vitamin E is important antioxidant fat soluble vitamin which must be included in animal diet because animal body cannot synthesize it. Though, it is present in the grass

but its level is variable in it and should be added as supplement in the feed (Persson-Waller *et al.*, 2007). Both Selenium and vitamin E have synergistic role because both are antioxidant (Bendich, 1990; Hogan *et al.*, 1996). Level of vitamin E and selenium is important factor for normal immune function in cow due to their antioxidant properties (Hogan *et al.*, 1993; Politis *et al.*, 1996; Sordillo, 2016), same is the case with young calves (Cipriano *et al.*, 1982; Eicher-Pruett *et al.*, 1992).

Pregnancy is critical stage for calves and need of antioxidants during pregnancy increases many times (Goff and Stabel, 1990; Weiss *et al.*, 1990; Meglia *et al.*, 2006). Similarly, during early lactation animal is in more oxidative stress (Gong and Xiao, 2016). Immune system is not developed in newborn calves due to which they cannot fight with the infectious agents. So, passive transfer of antibodies in compulsory for the survival of neonates which come to them through colostrum (Niewiesk, 2014). Increased incidence of diseases in calves is associated to the impaired passive transfer to the antibodies (McEwan *et al.*, 1970). It is also reported that IgG concentration of pregnant animals drops near parturition (Kehrli and Goff, 1989; Detilleux *et al.*, 1995).

Foot and mouth disease (FMD) is infectious,

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contagious and acute disease of sheep, goat, cattle, buffalo and wild animals. It is caused by the *Aphthovirus* of *Picornaviridae* family which is characterized by low mortality but high mortality (Parida, 2009; Jamal *et al.*, 2010). Due to this property this disease is very concerning in the endemic areas because large number of animals and different type of animals are affected in each outbreak causing ongoing losses (Onono *et al.*, 2013). Similarly Hemorrhagic septicemia (HS) is infectious, contagious and acute disease which, unlike FMD, is highly fatal and affect mainly cattle and water buffalo in tropical regions especially Asia and Africa (De Alwis, 1999; Shivachandra *et al.*, 2011). It is bacterial disease caused by gram negative bacteria *Pasteurella multocida* (Horadagoda *et al.*, 2001; Hodgson *et al.*, 2005; Vannie *et al.*, 2007). Both of the disease are economically very important and annually lot of money is wasted in prevention and treatment of FMD and HS (Hodgson *et al.*, 2005; Knight-Jones and Rushton, 2013). Vaccination is a common practice performed in the endemic areas against FMD and HS to prevent animals along with other practices like culling the effected animal, restriction of movement of animals and animal products (Verma and Jaiswal, 1998; Vannie *et al.*, 2007).

Keeping in view the importance of the disease, importance of prevention and crucial stage for dam and calf this study was designed to evaluate the effect of vitamin E and Selenium supplementation along with HS and FMD vaccination at various days before parturition on antibody production in cattle and passive transfer of antibodies in colostrum and calves.

MATERIALS AND METHODS

Selection and grouping to the of the animals

Thirty-five adult, healthy and pregnant with no history of HS and FMD were selected in experimental trial. Animal included were at the stage of 45 to 75 days prepartum based on insemination records. Selected animals were randomly divided into seven groups each containing five animals. Groups were made based on days remaining in the parturition. Group A was negative control and no treatment was given to that group. Animals in: Group B and C were at stage 45-day pre-partum with and without VES, respectively: Group D and E were 60 days prepartum with and without VES: Group F and G were 75 days prepartum with or without VES, respectively.

Treatment of groups

At day 0 of experimental trial groups C, E and G were given vitamin E and selenium supplementation in the form of injection (1 ml = 25 mg tocopherol acetate + 2.2 mg

sodium selenate). At day 15 of experimental trial oil based HS vaccine was administered and at day 30 of experimental trial alum based FMD trivalent vaccine was administered to all groups except Group A *i.e.*, negative control.

Sample collection

Two types of samples were collected *viz.*, blood and colostrum. Blood samples were collected from both dams and calves. Blood samples from dams were collected at day 0, 15 and 30 post-partum. And from calves at day 0, 14, 28 and 42 of age. Colostrum samples were collected at 0, 12 and 24 h after parturition. Blood was allowed to clot for 30 min and serum was separated by centrifugation following Tuck *et al.* (2009). Colostrum samples were centrifuged at 10000 ×g at 4 °C for 30 min. Fat layer was removed and clear supernatant was used for antibody titration (Cook *et al.*, 1978).

Antibody titration

Antibody titer of the sample, for both *Pasteurella multocida* and FMD virus, was determined by Complement Fixation Test (CFT). For HS Standardization of sheep red blood cell (RBC), complement and amboceptors was performed according to Kent and Fife (1963), and for FMD Antibody titer was estimated following OIE (2012). For CFT, amboceptors (antispecies antibodies) were raised in rabbit by continuous challenge with washed sheep RBC. Rabbit serum containing amboceptors was titrated with 1.5% suspension of washed sheep RBC. After titration sub agglutination level was used to sensitize sheep RBC for further use. Sensitization of sheep RBC was done at 37°C with continuous stirring for 60 min. Guinea pig complement was used as hemolytic agent. It was titrated with sensitized RBC and diluted in phosphate buffer saline (PBS) to make 3 hemolytic units (3HU) of complement. Antigen for HS was prepared and standardized according to Boulanger and Gwatkin (1955) and Tanaka (1926) with little modification as mentioned below. *Pasteurella multocida* was grown on Mueller-Hinton broth and broth was centrifuged 3000 ×g for 30 min and supernatant was discarded. The resulting pellet was washed three times with 30 mM tris-HCl, suspended in 0.5 ml 20% sucrose in 30 mM tris-HCl. Then 50 micro liter lysozyme (10 mg/ml) was added and mixture was kept at freezing point for 30 min and sonicated at 200 watts for 10 min on ice. After sonication, the solution was again centrifuged and clear supernatant was used as antigen. Before use, antigen was titrated for anti-complementary activity with 3HU of complement. The highest dilution showing slight anti complementary activity was selected and half of its value was used for complement fixation test.

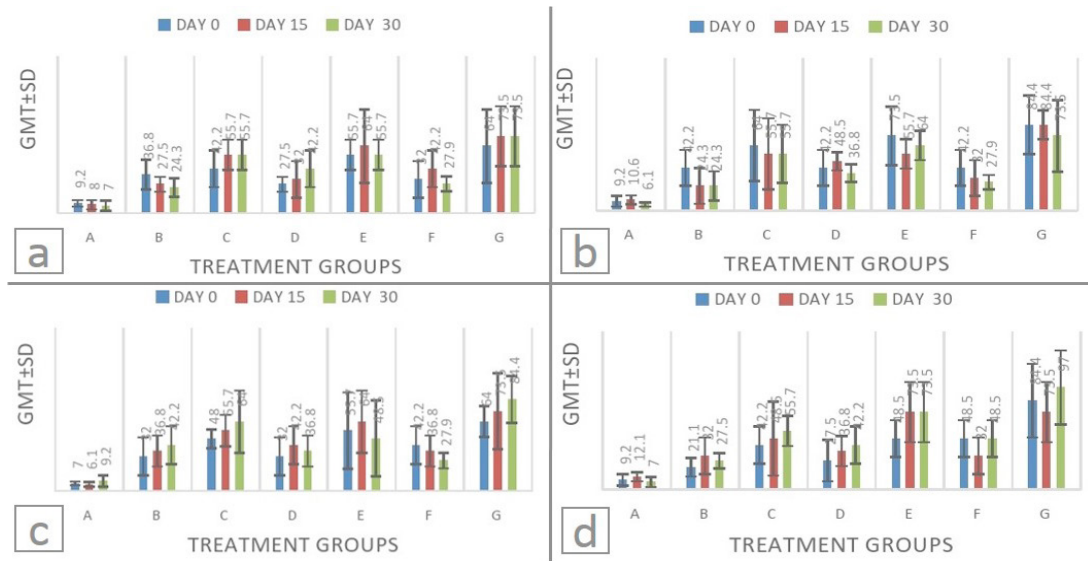


Fig. 1. Geometric mean titer and standard deviation of FMD virus strains O, A and Asia 1 of adult cattle at day 0, 15 and 30 of parturitions (a, b and c) and *Pasteurella multocida* (d). Study includes seven groups: A, control; B, 45 days prepartum group without vitamin E and selenium supplementation; C, 45 days prepartum group with vitamin E and selenium supplementation; D, 60 days prepartum group without vitamin E and selenium supplementation; E, 60 days prepartum group with vitamin E and selenium supplementation; F, 75 days prepartum group without vitamin E and selenium supplementation; G, 75 days prepartum group with vitamin E and selenium supplementation. Highest antibody titer ($p < 0.05$) was shown in group G in which VES was given along with vaccination 75 days before parturition. Very minimal antibodies were seen in control.

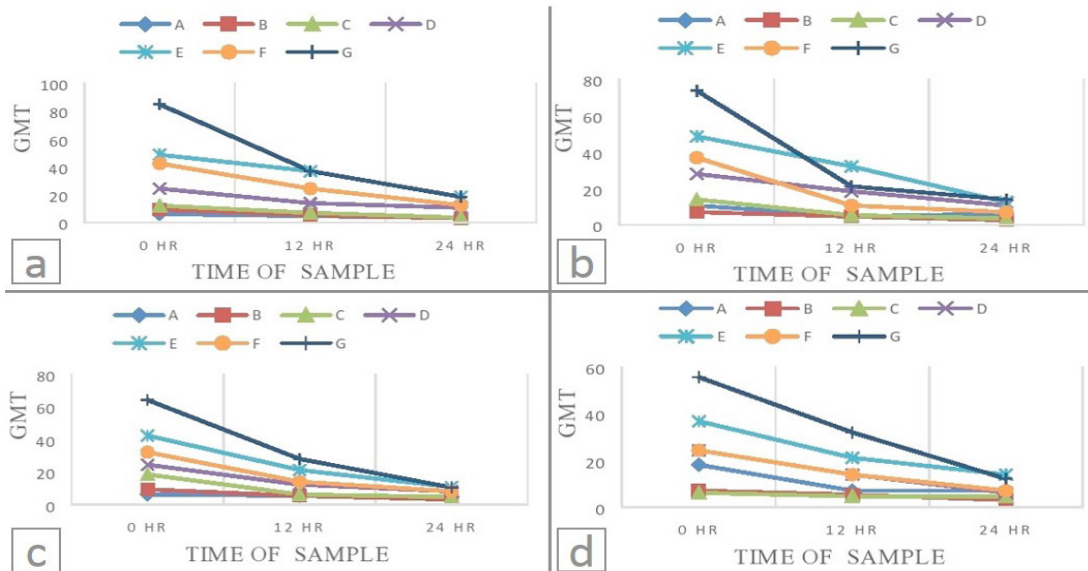


Fig. 2. Comparison of geometric mean titer of FMD virus strains O, A and Asia 1 (a, b and c) and *Pasteurella multocida* (d). Study includes seven groups: A, control; B, 45 days prepartum group without vitamin E and selenium supplementation; C, 45 days prepartum group with vitamin E and selenium supplementation; D, 60 days prepartum group without vitamin E and selenium supplementation; E, 60 days prepartum group with vitamin E and selenium supplementation; F, 75 days prepartum group without vitamin E and selenium supplementation; G, 75 days prepartum group with vitamin E and selenium supplementation. Highest antibody titer was shown at 0 and it keeps on falling till 24 h for each group. Among groups highest ($p < 0.05$) antibody titer was observed in group G at all stages of sampling.

Statistical analysis

Data was collected at Microsoft Excel 2016 and results of the treatment groups were compared at day various sampling days of the presented study with one-way analysis of variance on IBM Statistical Package for the Social Sciences (SPSS) 20.0. LSD and Tuckey's post hoc test was used to compare the treatment groups with 0.05 level of significance.

RESULTS

Antibody titer in adult cattle

Geometric mean titer (GMT) and standard deviation (SD) of treated groups is shown in Figure 1 for *Pasteurella multocida* and three strains of FMD virus *i.e.* O, A and Asia 1. Results showed that highest ($p < 0.05$) antibody titer was observed in Group G for all strains of FMD and HS, in which selenium supplementation was given along with vaccination and treatment was started 75 days before parturition. Group C also shown development of antibodies but significantly lower than group D. No significant antibody titer was developed in the control group and Group B in which treatment was started 45 days before parturition.

Antibody titer in colostrum

Trend in geometric mean titer of colostrum at 0, 12 and 24 h of experimental trial against *Pasteurella multocida* and three strains of FMD virus strains *i.e.* O, A and Asia 1 is shown in Figure 2. Highest ($p < 0.05$) antibody titer was observed in group G for each strain, followed by group E. No significant development of the antibody titer was observed in group A and B. antibody titer was maximum in the colostrum which was taken immediately after the parturition. A sharp fall of antibody titer was observed in all groups at 12 and 24 h but the fall in Group E and Group G was less pronounced.

Antibody titer in calves

Antibody titer of the calves at day 0, 14, 28 and 42 against three strains of FMD virus and against *Pasteurella multocida* is shown in Figure 3. Results show that highest ($p < 0.05$) antibody titer was observed in group G at day 14 after the parturition followed by Group F at the same day. After that it starts decrease and minimal concentration is observed at day 42 of experimental trial. All others groups show minimal (0.05) antibody titer as compared to the Group G.

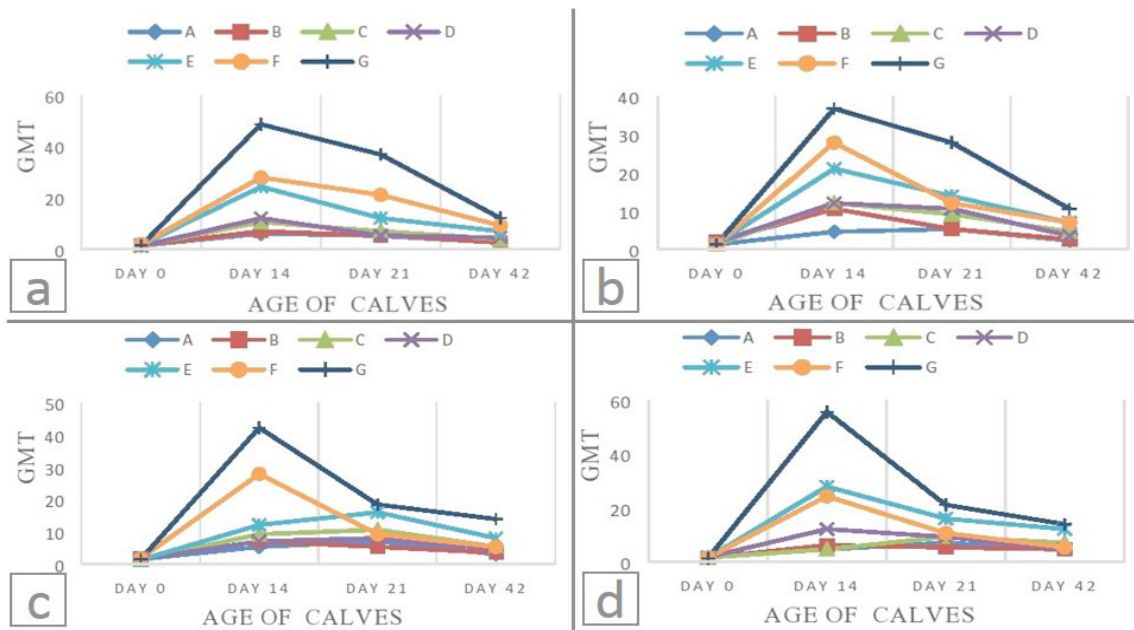


Fig. 3. Comparison of geometric mean titer of treatment groups against FMD virus strains O, A and Asia 1 (a, b and c) and *Pasteurella multocida* (d). Study includes seven groups: A, control; B, 45 days prepartum group without vitamin E and selenium supplementation; C, 45 days prepartum group with vitamin E and selenium supplementation; D, 60 days prepartum group without vitamin E and selenium supplementation; E, 60 days prepartum group with vitamin E and selenium supplementation; F, 45 days prepartum group with vitamin E and selenium supplementation; G, 75 days prepartum group with vitamin E and selenium supplementation. At day 0 minimal number of antibodies was observed which increases suddenly and with the passage of time it starts dropping again. Highest antibody ($p < 0.05$) titer was observed in group G at any time.

DISCUSSION

Highest antibody titer against *Pasteurella multocida* and FMD virus in adult cattle was observed in group G in which vitamin E and selenium supplementation was given at the 75 days before parturition. There was very low antibody titer in groups A showing that there is no involvement of field agent and there was no outbreak of FMD and HS during experimental trial. There was significantly lower antibody titer in group F in which treatment was started at 75 days prepartum. The results of present study are in agreement with previous researches which claim vitamin E and selenium supplementation improve the immune response of chicken (Marsh *et al.*, 1981), pig (Peplowski *et al.*, 1980; Blodgett *et al.*, 1986; Hayek *et al.*, 1989) lamb (Larsen *et al.*, 1988; Reffett *et al.*, 1988), cattle (Droke and Loerch, 1989) and horse (Baalsrud and Overnes, 1986). Group E showed produced a bit lower level of antibodies but there was significant difference because peak production of FMD antibodies is obtained at 7 days post vaccination (Golde *et al.*, 2005) and for HS vaccination take lot more time to reach at maximum level but protective level is obtained earlier (Sabia and Hari, 2014).

Highest colostral antibody titer was obtained against all strains of FMD virus and *Pasteurella multocida* in group G in which vitamin E and selenium was given at the 75 days prepartum confirming that vitamin E and selenium improves passive transfer of antibodies. Antibody titer at 45 and 60 day prepartum group was higher in selenium and vitamin E supplemented groups.

Highest antibody titer was observed in selenium supplemented groups as compared to non-supplemented groups. Results of our study are in agreement to previous researchers. Swecker *et al.* (1995) evaluated the effect selenium supplementation on specific antibodies against lysozyme, results showed that colostral antibodies were higher in selenium supplemented groups. Similar results were obtained by Awadeh *et al.* (1998) who evaluated the effect of selenium supplementation on humoral immune response in ewes, highest antibody in colostrum was observed in selenium supplemented groups. Same results were obtained by Stewart *et al.* (2013) and Hall *et al.* (2014) both of them worked on ewes to evaluate effect of selenium supplementation on colostral antibodies. Highest antibody titer was observed in which treatment was started day 75 pre-partum in present study. FMD vaccine need at least 7 days to reach maximum production of antibodies (Golde *et al.*, 2005) and for HS vaccination take even more time (Sabia and Hari, 2014). For 75-day group, there was lot of time for efficient production and transfer of antibodies.

Lactation is always a stressful period for the animal

because animals produce milk in lot of quantity and must recover parenchyma. There is increased requirement of oxygen producing higher quantities of reactive oxygen species (Gitto *et al.*, 2002). Normally animal body is at equilibrium and ROS production and destruction continues but at periparturient period animal has to face the increased oxidative stress due to decreased capacity of the antioxidant system to remove ROS (Bernabucci *et al.*, 2005; Castillo *et al.*, 2005; Sordillo *et al.*, 2007). This oxidative stress during the periparturient period also affect the immunity (Sordillo and Aitken, 2009). Oxidative stress means the higher level of ROS in the body which is very toxic to lipids, protein and DNA cause damage to them (Schieber and Chandel, 2014). No doubt the ROS are beneficial for message transduction in innate and adaptive immune responses (West *et al.*, 2011; Kamiński *et al.*, 2013), excess of ROS causes damage to the immune responses (Mittal *et al.*, 2014). Selenium is important component of antioxidant defense system in both animals and humans because it is the integral part of glutathione peroxidase (Stadtman, 1996; Schwarz and Foltz, 1999) and other similar selenoproteins which perform antioxidant function (Gladyshev, 2001). Similarly vitamin E is also important antioxidant, its deficiency causes impairment of immune system and its supplementation improves it (Tengerdy and Brown, 1977). Selenium is needed for normal functions of immune system both innate and adaptive (Hall *et al.*, 2011; Hujeriletu *et al.*, 2013; Stewart *et al.*, 2013). Basically, there are two type of immune responses active and passive. Active immune response means development of antibodies by using antigen as a signal to produce antibodies in the body. While passive immunity means taking already prepared antibodies from some other source and the natural example for this is the production of antibodies in mothers and transfer of it through colostrum. Though the active immune response is long lasting the passive transfer of antibodies is important in initial days of life. Main type of antibody which is transferred through colostrum in the ruminants is IgG which comes from mother serum (Baumrucker *et al.*, 2010). Above account explain possible mechanism to improve the immune status at prepartum stage by the vitamin and selenium supplementation. Meanwhile some researches says there is no effect of vitamin E and selenium supplementation on passive transfer of antibodies to colostrum and offspring (Lacetera *et al.*, 1996).

Highest antibody titer in cattle calves was observed in group G in which selenium and vitamin supplementation was given along with vaccination. Results of the study are in agreement with previous researchers. Awadeh *et al.* (1998) and Swecker *et al.* (1995) concluded selenium supplementation improves the passive transfer of IgM

and IgG respectively in cattle calves. Increase in passive transfer of antibodies to the lambs was also observed by Rock *et al.* (2001). But it is useless to use it above certain level after which there is no significant improvement was observed (Koenig and Beauchemin, 2009).

This increased level of antibodies in calves may be associated with the higher level of antibodies in colostrum and there may be the effect of selenium in improvement of efficiency of antibody absorption from small intestine because it is proved earlier that higher content of selenium in colostrum improves the absorption of the immunoglobulins by the small intestine (Kamada *et al.*, 2007) and Selenium supplementation during prepartum period improves the selenium and antibodies content of serum and colostrum (Hall *et al.*, 2014). Similarly, supplementation of selenium and vitamin E to the mother is thought to be very important method to supplement the newborn calves and preventing them from selenium deficiency because both can cross placental and mammary gland barriers and can enter to fetal tissues and milk (Ghany-Hefnawy *et al.*, 2007; Hidiroglou *et al.*, 1995; Rock *et al.*, 2001; Stewart *et al.*, 2013).

In present study colostrum offered the calves was taken by their own mothers and selenium supplemented groups had higher antibody titer at any stage at which the calves were given colostrum. We did not check the selenium level but it is understood that selenium and vitamin supplemented groups has higher level of vitamin E and selenium in there colostrum and mother which do not receive these in diet do not have sufficient quantity to deliver their calves (Quigley and Drewry, 1998; Hall *et al.*, 2014).

Exact mechanism how selenium improves absorption of antibodies is still unknown but it may be associated with reduction in proteolytic activity in stomach of calves by reducing the efficiency of trypsin which increases chances of survival of the antibodies and chances of uptake by small intestine increases. Absorption period for antibodies varies but it is considered that absorption of the antibodies is reduced after 6 h which keep on reducing till 24 h after which there is very minute or no absorption of antibodies (Quigley and Drewry, 1998). Increased uptake could also be associated with the increased numbers of transporter or there may be the growth and vascularization of mammary tissue (Meyer *et al.*, 2012). It is also a hypothesis that selenium acts directly on the intestinal epithelium and improves pinocytosis (Kamada *et al.*, 2007).

CONCLUSION

Present study concluded that prepartum supplementation of cattle with vitamin E and Selenium

along with vaccination improves the antibody titer in animal and improves colostral content of antibodies and passive transfer of antibodies.

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Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article

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