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## Original Article

# Effects of supplementation of vitamin E and selenium during late gestation on milk somatic cells count and incidence of retained placenta in dairy cows

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**Summary**

The somatic cell count (SCC) is widely used to predict the health of cow's mammary gland and the suitability of milk for human consumption. The main aim of this study was to evaluate the effects of additional supplementation of vitamin E and selenium at two intervals during late gestation on SCC and retained placenta (RP) in dairy cows. The 138 multiparous Holstein dairy cows were selected, which were randomly divided into two treatment and control groups. The cows in the control group (n=66) received a single injection of vitamin E and selenium at one dose (20 cc/cow) on day 250 of pregnancy, and in the treatment group (n=72) received two injections of vitamin E and selenium on days 250 and 270 of gestation. The SCC, milk production, RP percentage, and reproductive indices were calculated in all cows. The results showed that the SCC, milk production, RP percentage, and reproductive indices of cows in the treatment group have no significant difference from the control group. However, the calving to the first insemination interval and RP percentage in the treatment group was reduced. According to the results of the present study, it seems that more detailed studies are needed to find the effects of additional supplementation of vitamin E and selenium on the incidence of RP, milk, and SCC.

**Keywords:** dairy cows, retained placenta, SCC, selenium, vitamin E

## Introduction

Importance effects of vitamin E and Se in reproductive factors are well known. Deficiencies of this element have increased the incidence of mastitis in dairy cows (Smith et al., 1997). In human and animal health, vitamin E affected several functions. For example, vitamin E is protected

polyunsaturated fatty acids (PUFAs) against auto-oxidation. Vitamin E is required 0.4–0.8 mg/gr PUFAs. PUFA of cell membranes are particularly attacked by ROS (reactive oxygen species), so ROS destroys the cell membrane, which initiates a chain reaction of lipid destruction. Vitamin E has numerous important roles within the body because of its antioxidant activity. The most important antioxidant located in cell membranes, where Vitamin E is reduced peroxidation reactions in membranes (Putnam, 1987). Oxidation is linked to numerous pathologic conditions and diseases, so vitamin E is effective against cancer, aging, arthritis, and cataracts.

The high somatic cell count of milk is a concern for human consumer's health. Milk Somatic cells are derived from the blood leukocytes, as well as mammary epithelial cells. Milk leukocytes are consisting of neutrophils, macrophages, and lymphocytes (li et al., 2014).

It is suggested that 250 000≤ cells/ mL be used as an indicator of infection. High SCC has been known as a reliable index of subclinical mastitis in dairy cows (Smith, 1996; van den Borne et al., 2010). The use of SCC as an indicator has been related to the potential for human consumer's health risk (Heeschen, 2005).

Retained placenta (RP) is one of the reproductive disorders in cows, defined as when the animal is unable to expel fetal membranes within 12 hours after parturition. The etiology of retained placenta is complicated and not yet understood. Some parameters like age, breed, hormonal status, heredity, nutrition, environment, and immunity can contribute to the development of the retained placenta. Defense mechanisms in the transitional period due to pregnancy, calving, and early lactation, are weak (Mallard et al., 1998). During parturition, the fetal membranes detach from the maternal endometrium because of two immune mechanisms, so that blood supply to the placenta decrease and the placenta becomes a "foreign body" that the maternal immune system must attack and expel it. Pregnancy in dairy cows induced oxidative stress, which can be due to dysfunction of the immune system and inflammatory responses that increased the periparturient disorders (Sordillo and Aitken, 2009). RP is developed with a disbalance of the antioxidant capacity, reduce estrogen, and increase PGF2α production, accumulation of linoleic and arachidonic acids in the placental tissue (Wischral et al., 2001). Selenium is one of the important elements in the antioxidative defense. The active form of Selenium is incorporated in glutathione peroxidases (GPx) (Reeves and Hoffmann, 2009). In cows, the GPx activity rises several days before parturition and gradually decreases afterward.

Vitamin E reacted with the peroxyl radicals (Bernabucci et al., 2005). Vitamin E produces hydroperoxides that must be removed by GPx because of its toxic effect (Gutteridge and Halliwell, 1990)

The main aim of this study was to evaluate the effects of additional supplementation of vitamin E and selenium during the late gestation on SCC and RP in dairy cows.

**Materials and methods**

The present study was performed in one of the industrial herds of dairy cows in Esfahan province. All cows were kept in an open shed system, were milked three times a day, and artificially inseminated after a voluntary waiting period of about 55 days after calving. Also, all cows were under the same environmental and climatic conditions, and feeding was done by TMR. Cows were examined for reproduction and inspection weekly. For each cow, lactation period, calving status, lactation diseases, and milk production were extracted and recorded. At the time of treatment, the body condition score (based on grades 1 to 5) of studied cows was recorded. Heat detection was based on the observation of standing estrus. Total numbers of 138 multiparous dairy cows with the appropriate nutritional conditions during late gestation were selected. Before starting treatment, all cows were thoroughly examined to ensure their clinical health and proper reproductive function. The concentration of selenium (300µg/kg D.M per day) in the daily diet was following the dietary requirements of dairy cows (Mehdi and Dufrasne, 2016). In this study, cows were randomly divided into two experimental groups. The first group (control) consisted of 66 dairy cows that received a single subcutaneous injection of vitamin E and selenium at a dose of 20cc (1gr Vit E, 10mg Se) per cow (made by Nasr Fariman Company) on day 250 of gestation. The second group (treatment group) consisted of 72 dairy cows that received two subcutaneous injections of vitamin E and selenium at a dose of 20cc (1gr Vit E, 10mg Se) per cow at 250 and 270 days of gestation. Parameters such as calving to first estrus interval, calving to first insemination interval, calving to conception interval (days open), service per conception, the incidence of RP, calving interval, milk somatic cells, and milk production in the first 120 days after calving and monthly between the two groups were compared and analyzed.

Statistical analysis of the data was performed using SPSS statistical software (Version 22.0, SPSS Inc., Chicago, Illinois). Statistical analysis of conception rate in the first insemination and incidence rate of RP were performed by chi-square test. The Means of reproductive indices (calving to first estrus

interval, calving interval, days open), somatic cells count, and average milk production in the first, second, third and fourth months, and during 120 days postpartum were compared between groups using t-test. The results were presented as mean ± SE. Values of $P < 0.05$ were considered as statistically significant.

**Results**

In the present study, 138 cows were examined, which results of some factors related to reproductive performance, SCC, milk production, and RP are presented in figures 1 to 4.

Figure 1 shows the reproductive indices of the studied cows in the treatment and control groups. The results showed that the reproductive indices of the treatment group were not significantly different from the control group. However, the calving to the first insemination interval in the treatment group was reduced ($P> 0.05$).

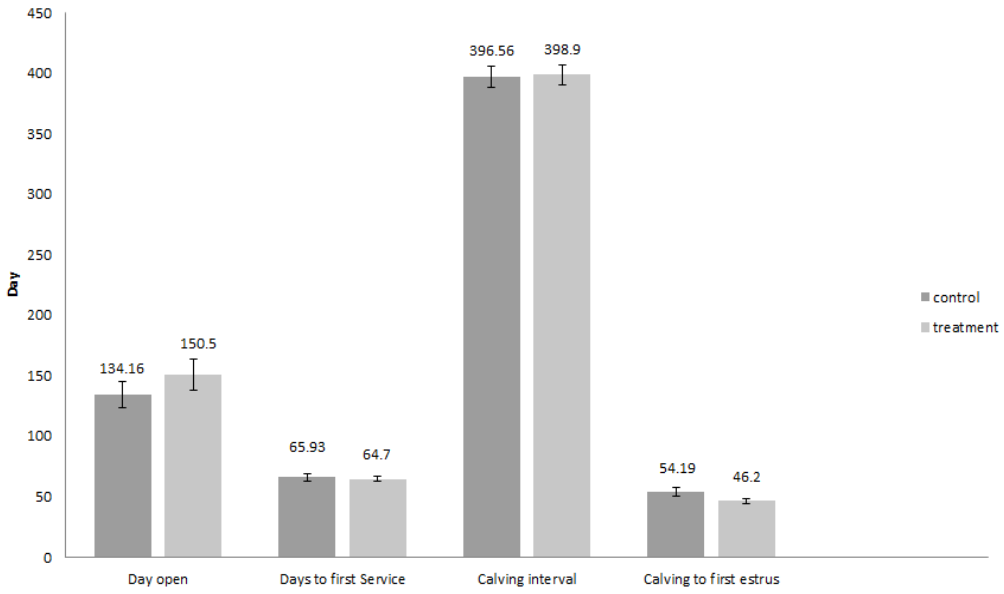


**Fig. 1.** Reproductive performance (mean ± SE) of cows in different treated groups

The service per conception rate of the studied cows was 3.1 ± 0.3 and 3.7 ± 0.4 in the control and treatment groups, respectively that were not significantly different between studied groups.

Injections of vitamin E and selenium did not increase milk production in different months (first, second, third, fourth, and mean for the first 120 days) after calving compared to the control group.

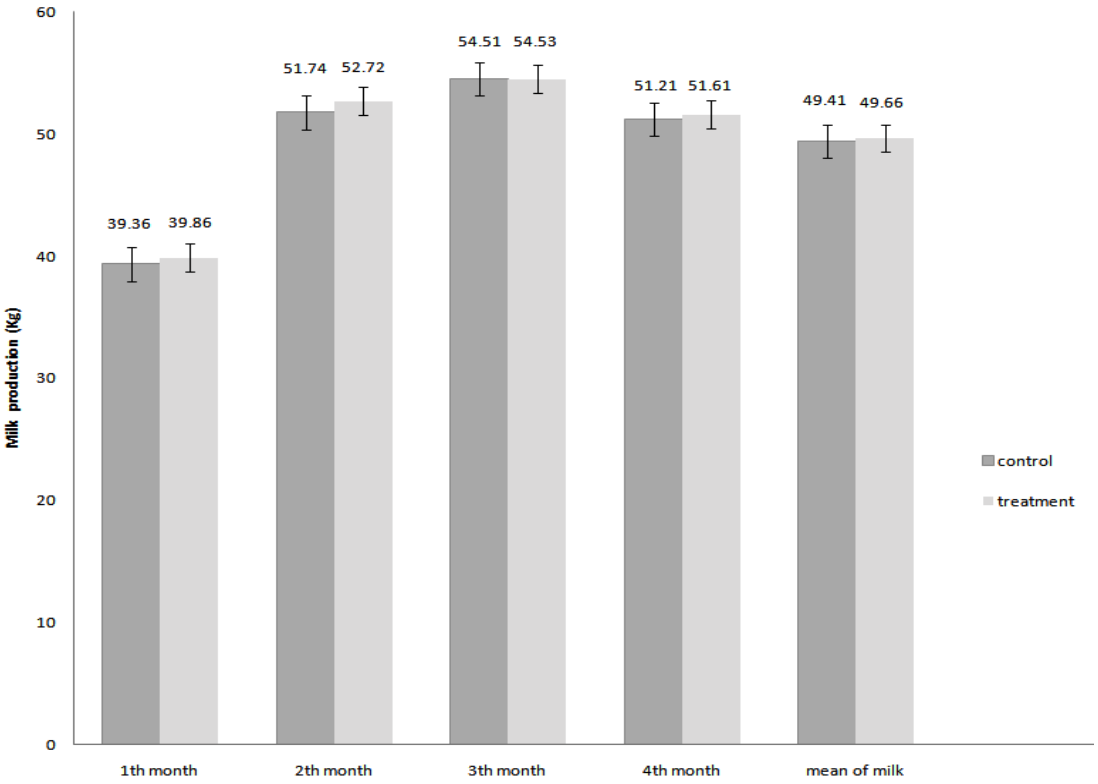


**Fig. 2.** Milk production (mean ± SE) of cows in different treated groups

The SCC in the treatment group increased compared to the control group, although this increase was not statistically significant (P > 0.05) (figure 3).

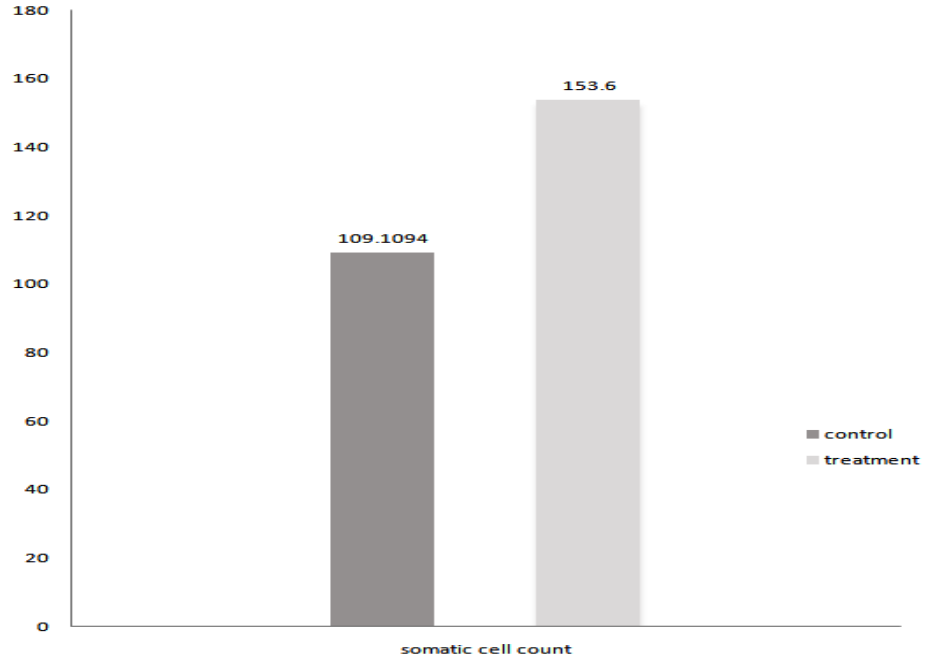

**Fig. 3.** The SCC of cows in different treated groups

In figure 4, the results of this study showed that the incidence of RP in the treatment group was not significantly different from the control group. In this study, two injections of vitamin E and selenium compared to one injection decreased the incidence of RP after calving, but this effect Se was not statistically significant ($p > 0.05$).

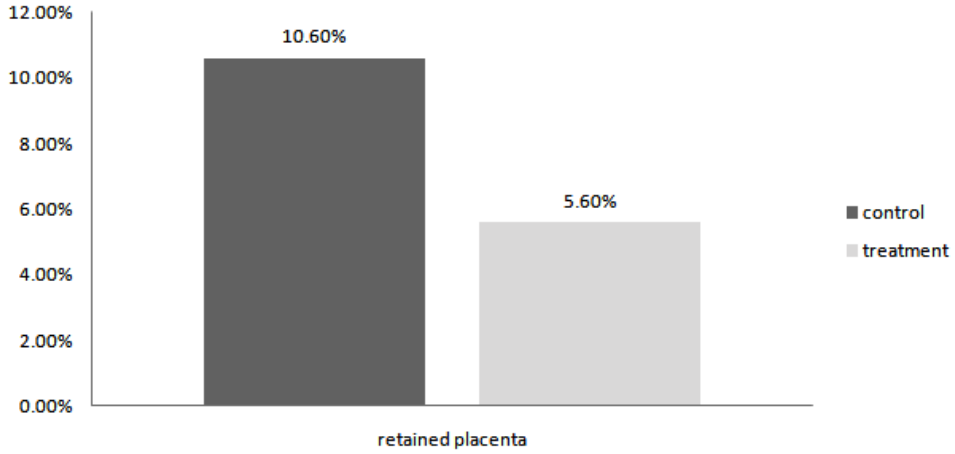


**Fig. 4.** RP percent of cows in different treated groups

## Discussion

RP is a common problem in dairy cows, and it can influence the fertility of dairy cows (Dubuc et al., 2010). The causes of RP have not been completely explained. An important factor in reducing the incidence of RP is the maternal immune and antioxidant statuses before calving. During the transition period, from 14 days before to 21 days after calving, a reduction of immune function is happening in dairy cows, especially on the day of calving when neutrophil and lymphocyte functions are disordered (Goff and Horst, 1997). Increasing the risk of RP has been linked to reducing neutrophil function (Kimura et al., 2002). Increasing neutrophil function has been supposed to be important in detachment of the feto-maternal tissues (Gunnink, 1984); when the concentration of α-tocopherol in blood decreased, so the risk of RP increased (Miller et al., 1993). According to Miller et al. (1993) administration of 50 mg of sodium selenite to gather with 680 IU of *α*-tocopherol acetate decreased the incidence of RP from 51.2% in the control group of cows to 8.8% in the treated group. Besides, it was previously reported that selenium alone was at least as effective as a combination of selenium with vitamin E

(Julien et al., 1976). Poor reproductive performance is economically deleterious in dairy cows because it reduces milk production and increases the cost of feeding, treatment, and artificial insemination (Eger et al., 1985). Administration of selenium and glutathione peroxidase (selenium metabolite) has good effects on the immune system and fertility, increasing health and weight of calves, reducing the incidence of retained placenta, and reducing the time of days open. Therefore its consumption in the diet of livestock is necessary (Deori et al., 2014). According to the results of the present study, the interval between calving to the first estrus in the treatment group, which received two injections of vitamin E and selenium during the transition period, was significantly lower than the control group. The results of this study showed that there was no statistical difference in reproductive indices of cows in the treatment group compared to the control group. In agreement with the results of this study, some researchers reported that injection of vitamin E and selenium late gestation in cattle did not improve postpartum reproductive performance (Kafilzadeh et al., 2014). Contrary to the results of this study, some researchers reported that injections of vitamin E and selenium late gestation in cows improve postpartum reproductive performance (Moeini et al., 2009). Some studies have shown that pregnant animals are more sensitive to selenium deficiency than non-pregnant animals. Vitamin E and selenium deficiency will increase the prevalence of reproductive disorders during pregnancy and postpartum. Cows receiving vitamin E and Se supplementation have been reported to increase the expulsion of the placenta and uterine contractions (Sattar, 2003). The effects of Se and vitamin E supplementation on ovulation in cows have been studied. For example, it has been found that there is a significant increase in ovulation rate after the addition of vitamin E and selenium or selenium alone (Castro et al., 2009). This discrepancy in the results of various studies on reproductive factors may be due to differences in the selenium status of animals before treatment, the frequency and duration of treatment, or the dose of Se and vitamin E (Amer and Badr, 2008).

The results of the present study showed that two injections of vitamin E and selenium in the late gestation did not increase milk production in different months after calving compared to one injection of the supplement. In agreement with our study, some studies have shown that the use of vitamin E and selenium supplements in late gestation has no effect on milk production and the quality and quantity of milk (Khorsandi et al., 2013). Contrary to these results, some studies have shown that

consumption of vitamin E and selenium increases milk production in cows (Sattar et al., 2003). Selenium supplementation, irrespective of source, tended to reduce the prevalence of intramammary infection (IMI) and decrease the prevalence of quarters with high SCC at calving (Ceballos-Marquez et al., 2010). The use of bovine milk SCC as an indicator has been related to the potential for human health risk (Heeschen, 2005). The SCC data correlate well with data for clinical mastitis. Bulk tank SCC values are indicative of the prevalence of intra-mammary infection. Both SCC and the rate of clinical mastitis were correlated negatively with the concentration of Se in plasma. Plasma activity of GSH-PX was not associated with rate of clinical mastitis but was negatively correlated with SCC (Hogan et al., 1989). The Reasons for these contradictory results between studies may include the milk production level of each cow, their access to the diet produced in different regions and conditions, other nutritional factors in the diet, the intake of vitamin E and selenium, the periods of consumption and the time of its consumption or the season in which the research was conducted (Arechiga et al., 1994). The SCC is a primary indicator for the diagnosis of subclinical mastitis and milk quality. In a study, it was shown that in the eighth week of lactation, the group injected with more vitamin E and selenium had a significant reduction in somatic cells compared to the control group. But, in the 12th week of lactation, there was no difference between treatment and control groups (Niwińska and Andrzejewski, 2017). Some studies have reported that consumption of selenium in the diet of dairy cows increases the concentration of selenium in the blood, and increased selenium levels improve glutathione peroxidase activity, reduce the number of somatic cells, reduce the incidence of mastitis and increase the functional efficiency of neutrophils. It can have a positively affect on milk production and mammary gland health (Mehdi and Dufrasne, 2016). The results of the present study showed that two injections of vitamin E and selenium in the pre-calving period did not have a significant effect on the number of milk somatic cells in the treatment group compared to the control group. In agreement with the present study, some researchers reported that injections of vitamin E and selenium in the precalving period have no effect on the somatic cell count and sometimes increase it in the treatment groups (Bouwstra et al., 2010). Some believe that taking vitamin E supplements sometimes, contrary to expectations, has negative effects on livestock production and health (Bouwstra et al., 2010). Due to high production and oxidative stress, vitamin E free radicals are formed during high production conditions,

which must be neutralized by a network of other antioxidants called the vitamin E reduction system (Bouwstra et al., 2010). This may justify some of the results of the present experiment.

## Conclusions

Based on the results of the present study, it was found that additional administration of vitamin E and selenium in the precalving period has reducing effects on calving to the first estrous interval but has no beneficial effects on other reproductive factors as well as milk production and Somatic cell count. According to the results of the present study and some other studies, it seems that more detailed studies are needed to find the effects of additional administration of vitamin E and selenium on the incidence of RP, SCC, and other parameters.

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## Conflicts of interest

The author declared no potential conflicts of interest for publication of this paper.

## Ethical approval

Not applicable

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