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Selenium and Vitamin E on Reproductive Health of Dairy Cattle: An Overview

Souvik Dhara[1*], Swati Thakur[2], Sk Md Sadique Anwar[3], Abhinaba Maiti[4], Tanay Ghosh[5] and Ruokuobeinuo Houzha[4]

[1]*Department of Animal Reproduction, Gynaecology and Obstetrics, Assam Agricultural University, Khanapara, Guwahati- 781022, Assam, India*
[2]*Department of Veterinary Physiology, LUVAS, Hisar, Haryana (124004), India*
[3]*Department of Livestock Products Technology, West Bengal University of Animal and Fishery Sciences, 68, K.B. Sarani Kolkata-37, West Bengal, India*
[4]*Department of Veterinary Physiology, G. B. Pant University of Agriculture & Technology, Pantnagar-263145, Uttarakhand, India*
[5]*Department of Animal Nutrition, Assam Agricultural University, Khanapara, Guwahati- 781022, Assam, India*

**Corresponding author*

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Production of higher level of reactive oxygen species (ROS) than normal level causes oxidative stress to the cells, leading to lipid peroxidation (LPO) and ultimate tissue damage. The endogenous antioxidant system present in the body neutralizes ROS produced in the cells. Selenium (Se) and vitamin E play an antioxidant role and help to scavenge the ROS in the body system. The normal dietary recommendation of Se is approximately 0.1-0.3 ppm of DM intake in dairy cattle. The recommended requirement of vitamin E is about 80 IU/kg DMI in the dry and post-partum period; and about 20 IU/kg during lactation in case of dairy cattle. Both the selenium and vitamin E separately and in combination have potential effects on reproductive health in dairy cattle, especially during the transition period. Deficiency of these may cause free radical accumulation and damage to cell membranes which further disrupt several processes including steroid hormone and prostaglandin synthesis, impaired immune system, lower reproductive performance and reproductive disorders. Normally animals get these two important antioxidants from their natural diet. But if the diet is deficient, then, supplementation is recommended. Supplementation of Se and vitamin E during pre partum period decreases the occurrence of post partum complications in dairy cattle.

Introduction

Trace minerals (cobalt, copper, iodine, iron, manganese, selenium and zinc) and fat-soluble vitamins (A, D, E and K) play an important role in structural components of animal body fluids and tissues (Mee, 2004). They also have significant role in functioning of intracellular detoxification of free radicals, synthesis of reproductive steroid hormones, enzyme activities and regulators of cell replication and differentiation (Spears and Weiss, 2008). Micronutrients in small quantities work indispensable for normal cellular metabolism, growth and maintenance of healthy reproductive life (Thakur *et al.,* 2020). Deficiency of these micronutrients as well as their excess may cause reproductive disorders in animals such as impaired spermatogenesis, decreased libido in the male, whereas in dairy cows it affects embryonic development and survival, post-partum recovery, milk production and immunity in farm animals (Sharma *et al.,* 2007). Animals generally obtain these nutrients through available lush green grasses and fodders. The necessity of dietary supplementation of micronutrients occurs during lean period or deficiency. The recommended dietary supplementation along with normal daily feed requirement is essential in order to prevent reproductive disorders and for continuous production (William, 2004). During transition period and heat stress, reactive oxygen species (ROS) are produced in the cells (Keshri *et al.,* 2021). The endogenous antioxidant system present in the body neutralizes ROS produced in the cells (Castillo *et al.,* 2003). Increased production of ROS than normal level causes oxidative stress leading to lipid peroxidation (LPO) and tissue damage (Sharma *et al.,* 2011). Selenium and vitamin E act as antioxidant help to scavenge ROS in the body system. Several studies have proven antioxidative properties of selenium (Se) and vitamin E in cattle and other animals. Vitamin E and selenium have common properties in antioxidant system and immune response in reproduction of dairy animal (Xiao *et al.,* 2021). Their deficiency and toxicity directly interfere with the reproductive health of the dairy animals, especially during the transition period. Therefore, proper balancing of these two antioxidants in the diet of the dairy animal is required. This review aims to discuss in detail about the role of Selenium and vitamin E in reproductive life of dairy cattle.

Selenium (Se)

Background and functions

Selenium (atomic number 34) categorized as non-metal micronutrient, located at the fourth period of periodic table (Perrone *et al.,* 2015). Se is an important trace element acting as antioxidant. Its deficiency is associated with poor growth, health and fertility disorders in dairy animals (Logan *et al.,* 1990) Deficiency results into many complications, however, excess may causes lameness, sore feet, deformed claws and alopecia of tail in affected animals. Se plays a role in defence against accumulation of hydroperoxides during cellular metabolism (Rutigliano *et al.,* 2008**)**. This biological function is accomplished through the selenoproteins such as glutathione peroxidase family (GPx), iodothyronine de-iodinases and thioredoxin reductases, where Se is an integral structural component. These proteins have seleno-cysteineaminoacids (Se-Cys) at critical positions in the active centre of the enzyme (Youcef and Isabelle, 2016). These free radicals include superoxide anions (O^2), hydrogen peroxide (H_2O_2) and hydroxyl redicals(OH^-). These products are extremely reactive and can disrupt the functions of normal proteins and lipids in the cells. GPx catalyses the reduction of hydrogen peroxide and hydroperoxides formed from fatty acids and other substances (Spears and Weiss, 2008).

The organic Se is found in the form of selenate and selenite. Se content in soils varies with the soil type, texture, organic matter content and precipitation. The assimilation of the Se in the plant is influenced by the physico-chemical factors of the soil, such as the redox status, pH and microbiological activity (Harrison *et al.,* 1984).Plants absorb selenate tenfold better than selenite. In plants, Se is richly present in stems, leaves, seeds rather than in roots(Hidiroglou, 1979). In the biological system, Se is mostly of plant origin and is present in the form of seleno-amino acid which includes seleno-methionine and seleno-cysteine (Gergely *et al.,* 2006; Ullah *et al.,* 2020). Se content in forages varies with the type, soil and region. Forage having sulphur amino acids stores more Se than fodder. Sulphur (S) present in the methionine and cysteine is substituted by selenium to form seleno-methionine (Se-Met) and seleno-cysteine (Se-Cys), absorbable forms in the animal body and accumulate in tissues (Wichtel *et al.,* 1996). Selenized yeast has been reported as more bioavailable source of selenium as compared to Se selenite (Juniper *et al.,* 2006).

Sources and requirement

Adequate Se intake in animals can be achieved through dietary supplementation, by adding into drinking water, along with mineral salts and also as Se enriched yeast or by other routes of administration (injections, implants).

Dietary supplementation of Se should be based on the efficacy range to avoid deficiency or toxicity. The safety margin of selenium is low, so that its deficiency events are low than toxicity (Yasothai, 2014). The normal dietary requirement of Se is approximately 0.1-0.3 ppm of DM intake in animals (Ahuja and Parmar, 2017). Acute selenium toxicity signs start to appear at 5-8 mg/kg DM (Arshad *et al.,* 2021).

The most common forms of selenosis are chronic selenosis, referred to as alkali disease and acute selenosis, popularly known as blind staggers. Such animals will show sloughing of hair, hoofs and lameness. Marked deficiency of Se will occur when dietary content is less than 0.05 mg/kg DM and maximum standard of Se content in foods is set at 300 µg/kg DM/day in beef cattle and 100 µg/kg DM/day in dairy cattle (Mehdi and Dufrasne, 2016; Qazi *et al.,* 2018). Plants growing in soil containing high levels of Se are hazardous to animals.

Significant effects of Selenium on reproduction

In the body, absorbed Se is accumulated in the reproductive organs and endocrine glands like ovary, adrenal and pituitary glands (Buck *et al.,* 1981). Normal Se concentration in plasma of dairy cattle varies from 0.09 to 0.30 mg/L. When the concentration of Se becomes lesser than recommended value, animal shows lower reproductive performance and when concentration is high then animal might be shows reproductive disorders (Jukola *et al.,* 1996). Low level of Se results into many reproductive disorders in cattle such as in chronic selenosis where it lowers fertility by supporting the growth of ovarian cysts and prolonging anoestrus (Goff, 2005). Other reproductive problems include silent or irregular oestrus, early embryonic death, poor uterine involution, cystic ovaries and metritis (Randhawa and Randhawa, 1994). In sub-clinical Se deficiency, reproductive performance may be reduced per conception, high incidence of mastitis and retained placenta due to impaired functioning of neutrophils (Goff, 2005).Furthermore, Mohammed *et al.* (1991) pointed that high selenium concentrations in blood have been a potential risk of ovarian cysts in dairy cattle.Se toxicity in pregnant animal results into abortions, still birth, weak and lethargic calves as Se accumulate in the foetus at expense of the cow (Patterson *et al.,* 2003; Hosnedlova *et al.,* 2017).

Supplementation of Se may improve conception rate at first service (Ganie *et al.,* 2014; Ullah *et al.,* 2020), reduce the incidence of metritis and ovarian cyst during postpartum period (Wilde, 2006), postpartum uterine involution (Amin *et al.,* 2016) and increase fertility by reducing early embryonic mortality. Se supplementation during milking and transition period improves reproductive performance (Ullah *et al.,* 2020). Indeed, there is increased expression of the selenoprotein gene (GPx-1) in granulosa cells of large healthy follicles which have an antioxidant role during follicular development (Ceko *et al.,* 2015).It has been also found that nanoselenium supplementation could promote the development of secondary follicles(Wu *et al.,* 1979).

Injection of Se after parturition stimulates the growth of ovulatory follicles, stabilizes hormonal balance and growth of uterus, thus, days in open period decreased (Ahn *et al.,* 2021). During pregnancy, animal are subjected to oxidative stress which can be managed by supplementing Se as an antioxidant (Dimri *et al.,* 2010). Harrison *et al.,* (1984) reported that the incidence of cystic ovarian disease was reduced by 19% at 14 days of post-partum cow supplemented diet with extra selenium.

Vitamin E (Tocopherol)

Background and functions

Vitamin E is a fat-soluble vitamin, its activity can be found in the naturally occurring four tocopherol (α, β, γ and δ) and four tocotrienol (α, β, γ and δ) forms (Regina and Traber, 1999). These forms are important fat-soluble primary antioxidants of cell membranes and function as an intra-cellular antioxidant scavenger for free reactive oxygen and lipid hydro-peroxidase. It converts them into non-reactive forms which are essential for body functions like growth, reproduction, prevention of diseases and maintains the integrity of tissues. Among these forms α–tocopherol has highest biological activity and most abundant in nature (Sheppard *et al.,* 1993). For the first time, essential role of vitamin E in the reproduction was reported in rat (Evans and Bishop, 1922). Vitamin E is not synthesized in the rumen. Absorption of vitamin E is closely related to digestion and absorption of fat in the diet, thus, fat mal-absorption syndrome can cause vitamin E deficiency in the body (Bramley *et al.,* 2000). Choline containing materials improve the absorption of fat and vitamin E from diet in the gut (Pinotti *et al.,* 2003). Vitamin E binding protein, known as tocopherol binding protein (TAP), a 46 kD protein expressed mostly in liver, brain and prostate, and helps in transportation of vitamin E in various intracellular compartment (Zimmer *et al.,* 2000).

Vitamin E is a potent lipid-soluble, chain breaking antioxidant that prevents the propagation of free radial reaction (Packer, 1994). This fat-soluble vitamin also has a crucial role in immune response of the body (Baldi, 2005). Supplementation of vitamin E can improve the killing potentiality of the circulating neutrophils and enhances the function of macrophages in cattle (Franklin *et al.,* 2005; Xiao *et al.,* 2021). In cells, vitamin E functions as an antioxidant that terminates the chain of oxidative processes by donation of its phenolic hydrogen to chain propagating lipid peroxyl radicals, leadings to enhanced formation of the less reactive α-tocopheroxyl radical (Zhang and Omaye, 2001). Under normal conditions, every cell produces ROS, but excess ROS production causes lipid peroxidation and cell death. α-tocopherol potentially inhibits ROS production and protects the cells from oxidative stress related damages especially during transition period of cow (Baldi, 2005; Xiao *et al.,* 2021).

Sources and requirement

In nature, plants synthesise vitamin E and is richly present in their products. Whole cereal grains, green forage and others including good quality hay, alfalfa are very good sources and wheat-germ oil is the most concentrated natural source (Sapkota, 2020). The feedstuffs contain vitamin E in highly variable amounts for fresh and stored forages. Vitamin E concentration in hay and silage is about 20 to 80% lower than fresh green forages (Zust *et al.,* 1996). Processing of the feed ingredients with heat treatment and grinding significantly reduces the vitamin E concentration (Weiss, 1998).In rumen, portion of ingested vitamin E is destroyed and destruction rate increased with increment of concentrate in the diet(Alderson *et al.,* 1971).

National Research Council (NRC) recommended 15 to 60IU as the daily nutritional need of vitamin E in adult cattle, 40–60 IU daily for nursing calves. Vitamin E does not cross the placenta; however, it is concentrated in colostrum. Vitamin E supplemention can greatly increase colostral tocopherol (Carter *et al.,* 2005). The recommended requirement of vitamin E is about 80 IU/kg DMI in the dry and post-partum period, and about 20 IU/kg during lactation (NRC, 2001). The diets containing 75- 190

IU of vitamin E/kg for dry cows and between 25 and 50 IU/kg for lactating cows are beneficial (Weiss, 1998). Supplementation of vitamin E up to 500 IU to calves improves the immune response (Reddy *et al.*, 1987).

Significant role of Vitamin E on Reproduction

In vitamin E deficiency, the accumulated free radicals not only damage cell membrane, but also disrupt several processes like synthesis of steroid hormones (LH, FSH and ACTH) (Garnsworthy *et al.*, 2008) and prostaglandins, sperm motility and development of embryo. Supplementation of vitamin E significantly increase the progesterone level during diestrus period (Khan *et al*., 2016) which suggests that vitamin E has a role in luteal cell functions (Shehab-El-Deen *et al.*, 2010). Khan *et al.*, (2016) reported that supplementation of 1000 IU vitamin E with basal diet for 40 days during diestrus period has significant positive effect on stress related markers and follicular wave. Therefore, negative impact of vitamin E deficiency has been observed on various components of the reproductive events including ovulation rate, uterine motility, conception rate and post-partum activities like expulsion of fetal membrane, embryo survival, milk production, and post natal growth. It is also observed that supplementation of vitamin E during the pre-partum period appears to be important factor in the efficiency of selenium treatment for prevention of retention of placenta in dairy cow (Panda *et al.*, 2006). Weiss (1998) reported that supplementation of vitamin E to dairy cows during the peripartum period has improved the function of circulating neutrophils and enhances the immune response. Vitamin E deficiency primarily affects fetal membrane (Allison and Laven, 2000). During parturition, the plasma concentration of α–tocopherol decreases, which increases the requirement of vitamin E at post-parturient period (Goff *et al.*, 2002) and incidence of retained fetal membrane (RFM) (Miller and Brzezinska-Slebodzinska, 1993). At periparturient period the supplementation of vitamin E is very important because inadequate dietary intake of vitamin E rapidly reduced plasma α–tocopherol level and increased the occurrence of RFM, leading to subsequent degradation of uterine health and reproductive performances (Dubuc *et al.*, 2010). Pontes *et al.*, (2015) conducted an experiment in dairy cow with three preterm injection of vitamin E (1000 IU) at 258 ± 3, 265 ± 3, and 272 ± 3 days of gestation with basal diet containing vitamin E supplementation 50% lesser than recommendation of NRC. Results revealed there was reduction of incidence of RFM from 20.1 to 13.5%, marked reduction of pregnancy loss from 21.1 to 14.5%, still birth 14.9 to 6.8% and first postpartum insemination from 64.3 ± 1.2 to 62.1 ± 1.3 days. The actual mechanism of vitamin E in improvement of incidence of RFM is still unclear. Supplementation of vitamin E during preterm increases the concentration of α–tocopherol in tissue including circulating lymphocytes (Weiss *et al.*, 1992), which may increase the availability and activity of polymorphonuclear cells (Hogan *et al.*, 1992).Vitamin E supplementation also improves total antioxidant status of the body and subsequently improves the immune response of the body, which causes shedding of placenta from maternal uterine bed immediately after parturition (Kimura *et al.*, 2002). Vitamin E deficiency during prepartum period might have negatively affected the maternal immune response after parturition leading to delay of maternal immune mediated rejection of the fetal membrane (Pontes *et al.*, 2015). Baldi *et al.*, (2000) provided extra 1000 IU/day/cow vitamin E along with basal diet containing 1000 IU vitamin E (total 2000 IU/day/animal) to dairy cow during a period of 7 days before calving to 14 days after calving. It was recorded that supplementation of extra vitamin E reduced the number of insemination (1.32 vs. 2.17), conception days (83.8 vs. 111.3) and placental removal time (9.7 h vs. 11.7 h) than animal fed basal diet without extra vitamin E.

Table.1 Effect of selenium supplementation of Reproductive performance in Cattle

Prameter	Form of Se	Dose	Effects	Reference
Plasma Progesterone	Inorganic	0.5 mg/kg DMI	Increased	Kamada and Hodate,1998
Uterine health	Organic	0.3 mg/kg DMI	Improved	Silvestre *et al.*, 2007
Uterine involution	Inorganic	0.1 mg/kg DMI	Earlier	Harrison *et al.*, 1986
Superovulation	Inorganic	0.5 mg/kg DMI	Increased number of follicles	Ratto *et al.*, 2008
Second service pregnancy rate	Organic	0.3 mg/kg DMI	Increased	Silvestre *et al.*, 2007
Retained placenta Puerperal metritis	Organic	0.3 mg/kg DMI	Reduced incidence	Cerri *et al.*, 2009
Conception rate in first insemination	Organic	0.5 ppm	Increased	Khalili *et al.*, 2019
Days open	Organic	0.5 ppm	Decreased	Khalili *et al.*, 2019
Service per conception and days open	Inorganic (Injectable)	40 mg/animal	Lower service per conception and decreased days open	Ahn *et al.*, 2021

Table.2 Effect of Vitamin E supplementation of Reproductive performance in Cattle

Prameter	Period of treatment	Dose	Effects	Reference
Retained fetal membrane	Prepartum	1000 IU	Decreased incidence	Pontes *et al.*, 2015
Stillbirth	Prepartum	1000 IU	Decreased incidence	Pontes *et al.*, 2015
Days to first observed estrus	Prepartum	1000 IU	Decreased the days	Campbell and Miller, 1998
Retained fetal membrane	60 days prepartum to 30 days postpartum	2000 IU	12% reduction of incidence	Panda *et al.*, 2006
Postpartum estrus interval	30 to 60 days postpartum	1000 IU	Decreased	Panda *et al.*, 2006
Days open and service per conception	30 to 60 days postpartum	1500 IU	Reduced	Panda *et al.*, 2006
Pregnancy at first service	21 days prepartum	500 mg	Increased	Arechiga*et al.*, 1994
Plasma progesterone (P4) level	40 days during diestrus phase	1000 IU	Increase the level of P4	Khan *etal.*, 2016

Table.3 Effect of Vitamin E along with se supplementation of Reproductive performance in Cattle

Period of treatment	Results	Reference
14 days before calving	Reduced the incidence of RFM, lower days open and service per conception	Moeini *et al.*, 2009
21 days before calving and on the day of calving	Decreased the incidence of metritis, number of services per conception and the service period, but had no effects on the incidence of RFM	Bayril *et al.*, 2015
40- 60 days before calving	Placental expulsion period, uterine involution period, postpartum insemination interval, service period and number of services per conception in the cows of treated group were significantly ($p<0.05$) lesser compared with those of the control group.	Sattar *et al.*, 2007
Dry period	Improved fertilization rate	Segerson *et al.*, 1981
14 and 7 days prepartum	Significant reduction of incidence of RFM (8.3 vs 50%) and mastitis (8.3 vs 41.6%). Decreased the postpartum uterine involution period, calving to first estrus interval, calving to first service interval, calving to conception interval and service per conception compared to untreated group.	Damarany, 2021
250 and 270 days of gestation	Reduced RFM and calving to first insemination interval	Hajibemani *et al.*, 2020
Late gestation period	Reduced incidence of mastitis, calving to conception interval and increased estrus detection and conception rate.	Hoque *et al.*, 2016
Prepartum period	Reduced the incidence of difficult birth	Hidiroglou *et al.*, 1987
21 days of prepartum	Reduced incidence of RFM but non-significant	Gupta *et al.*, 2005

Significant effects of Vitamin E together with Selenium on reproduction

Vitamin E is one of the factors influencing dietary Se consumption (Kim *et al.*, 1997) and their antioxidant functions are interdependent. A diet low in vitamin E may increase the need of Se or selenium deficiency may partially compensated by an adequate intake of vitamin E. Both deficiencies may results into malfunction of thyroid metabolism.

This decreases growth rate and milk production, wide range of reproductive problems and altered phagocytic responses to fight against diseases. Deficiencies may cause free radicals to accumulate and damage the cell membranes which further disrupt several processes including steroid hormone synthesis and prostaglandins. Combined effect of vitamin E with selenium prevents the cellular oxidative stress and lipid peroxidation of cell membrane by suppressing hydroperoxide formation (Putnam and Comben, 1987). In Se deficient dairy cows, provision of more vitamin E (680 IU) and Se (50mg) in diet during the pre-partum period appears to reduce reproductive disorders such as retained placenta. During prepartum period, parenteral supplementation of vitamin E and selenium show positive effects on improvement of conception rate, reduction of days open and incidence of RFM (Harrison *et al.*, 1984; Vanegas *et al.*, 2004), might be due to improved uterine muscular function (Youssef *et al.*, 1985). Supplementation of Vitamin E-Se during dry period in dairy cow improves humeral immune responses to bacterial and viral antigens (Larsen, 1988), and reduces post-partum reproductive disorders (Cortese, 1988) like RFM (Moeini *et al.,* 2009), uterine involution (Sattar *et al.,* 2003), number of service per conception (Arechiga *et al.,* 1998). In a study Kim *et al.,* (1997) treated 120 dairy cows by dividing in four groups: control, only vitamin E (500 IU) injection, injection of selenium (40 mg) only and combination of vitamin E (500 IU) and selenium (40 mg) intramuscularly, 20 days before calving. Results revealed that combination of treatment significantly reduced the incidence of RFM compared to selenium and vitamin E alone.

Another study conducted Brozos *et al.* (2009) on 456 dairy cows, treated with vitamin E (1000 IU), Selenium (0.05 ppm) and ammonium chloride (60 g) along with basal diet containing vitamin E (80 IU/Kg) and selenium (0.2 ppm). The study revealed reduced incidence of RFM in treated group (10.6%) compared to control (17.8%). Arechiga *et al.* (1994) reported that supplementation of vitamin E and selenium (10 ml Inj. MU-SE®, intramuscularly) 21 days before parturition decreased the incidence of RFM (3.0 vs 10.1%), number of service per conception (2.3 vs 2.8), calving conception interval (121 vs 141 days) and increased pregnancy rate (41.2 vs 25.3%).

Selenium and vitamin E both act as potent cellular antioxidant and save the cells from free radicals generated during oxidative stress. They have potential role on humeral immunity and provide defence in the body.

This trace mineral and lipid soluble vitamin potentially maintains the reproductive performance of the dairy cattle especially during post-partum and transition period. The serum concentration of Se and vitamin E is very important to maintain, as very low, as well as high concentration might cause low reproductive performance and post-partum reproductive disorders. Animal naturally gets Se and vitamin E from feed stuff but if the basal diets are deficient of these, then supplementation as oral or parenteral forms are recommended.

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