

Supplemental Antioxidants to Enhance Fertility in Dairy Cattle

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Introduction

It is one of the paradoxes of biology that oxygen, which is required for energy production in aerobic organisms, is also very toxic. Many biological reactions generate reactive oxygen species (ROS) that strip electrons from biological molecules such as fatty acids, proteins and DNA. Exposure of cells to ROS can lead to disruptions in membrane structure, reduced activity of enzymes and other proteins, and genetic mutations. Given their potential harm, it is no surprise that a variety of systems exist to limit the consequences of ROS production. Antioxidants eliminate ROS through direct or enzymatically-catalyzed reactions while other molecules reverse oxidative damage caused by ROS. The magnitude of adverse changes in physiology caused by an increase in ROS generation depend not simply on the amount of ROS produced but rather on the balance between concentrations of ROS and antioxidant systems.

This paper will provide a brief background regarding oxygen metabolism and ROS generation, review briefly some of the biochemical systems for protection from free radicals and then describe some of the experiments to evaluate whether reproduction of lactating dairy cows can be enhanced by changing antioxidant status. The take-home message is that there are opportunities for using antioxidant supplementation to reduce incidence of retained placenta, enhance uterine health and improve fertility, particularly during heat stress. There is, however, variability in the magnitude of the response to antioxidant treatment. Differences in antioxidant, dosages, routes of administration and antioxidant status of control cows are probably responsible for some of the differences between studies.

Production of Reactive Oxygen Species

During oxidative phosphorylation, molecular oxygen is usually reduced by the addition of four electrons to produce two molecules of water (Figure 1). However, about 1 to 2% of the oxygen consumed is not completely reduced and ROS such as superoxide (formed by reduction of oxygen with one electron), hydrogen peroxide (two electrons) and hydroxyl radical (three electrons) are formed. Other chemical reactions also result in generation of reactive oxygen species. Reactive oxygen species are also generated during prostaglandin synthesis and by polymorphonuclear leukocytes (i.e., PMNL, also called neutrophils) to kill ingested microorganisms. In PMNL, oxygen is converted to superoxide by NADPH oxidase. This ROS is in turn reduced to hydrogen

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peroxide by superoxide dismutase and then converted to hypochlorite free radical (HOCl) by myeloperoxidase.

For unsaturated fatty acids, oxidation by ROS in the presence of iron generates additional ROS through the Fenton reaction that leads to the formation of lipid peroxides. A chain reaction consuming unsaturated membrane fatty acids can ensue. ROS status in animals is often determined using the TBARS assay (thiobarbitric acid reactive substances) that measures acetaldehydes generated from products of lipid peroxidation.

Given the relationship between oxygen consumption and ROS generation, one would expect that physiological states that are associated with increases in metabolism, such as lactation, growth, exercise, and heat stress, would also be associated with increased ROS generation. Indeed, there is experimental evidence for a relationship between metabolism and ROS in lactating dairy cows. In one study, cows with higher milk yields also had higher concentrations of lipid hydroperoxides in serum (Löhrke et al., 2005). Production of ROS was also increased in cows with higher body condition score (BCS) before calving and with greater losses in body condition (BCS) after calving (Bernabucci et al., 2005). In another study, the amount of TBARS in the blood was higher in summer than winter (Bernabucci et al. (2002) (Figure 2).

Generation of ROS can increase during inflammation probably because of the involvement of prostaglandins and PMNL in inflammatory processes. Thus, for example, TBARS in blood increase after surgery for displaced abomasum (Mudron et al., 2007). As shown in Figure 2, parturition is also sometimes associated with an increase in ROS generation.

Antioxidant Systems

Antioxidants can be categorized based on their mode of action as well as the cellular compartment (cytoplasmic vs. membrane) in which they reside. Some antioxidants act as free radical sinks that inactivate ROS through donation of an electron. The major antioxidant in the cytoplasmic compartment of the cell is the tripeptide glutathione (GSH; γ -glu-cys-gly) which can donate the hydrogen on the sulphydryl group of its cysteine directly to ROS. Other antioxidants that act as free radical sinks include ascorbic acid (vitamin C), also in the cytoplasm, and the membrane antioxidants α -tocopherol (vitamin E), β -carotene and ubiquinol (coenzyme Q). Dietary antioxidants that react directly with ROS in water-soluble compartments include various polyphenols and flavonoids.

In addition to free radical sinks, there are a group of enzymes that reduce free radicals, including CuZn superoxide dismutase and Mn superoxide dismutase that convert superoxide to hydrogen peroxide and catalase; glutathione peroxidase (which uses GSH as an electron donor); and peroxiredoxin that convert hydrogen peroxide to water. Other enzymes repair damage to oxidized molecules including glutathione transferase (reduction of oxidized lipids), thioredoxin reductase (which uses an electron

from thioredoxin to reduce oxidized proteins) and glutathione reductase (converts oxidized glutathione to the reduced state). As is evident from their name, these enzymes often require metal cofactors such as copper, zinc, manganese, and for glutathione peroxidase, selenium. While these metals are not strictly antioxidants, their concentration and availability can affect redox status.

Production of molecules involved in limiting actions of ROS is under physiological control. For example, amounts of glutathione peroxidase in serum (Bernabucci et al., 2002) and glutathione peroxidase mRNA in mammary tissue (Aitken et al., 2009) increase after the onset of lactation. Heat stress is also associated with an increase in concentrations of superoxide dismutase and intracellular thiols in circulating erythrocytes (Bernabucci et al., 2002).

One would expect that manipulation of antioxidant status would improve reproductive function if 1) production of ROS in reproductive tissues was in excess to what could be neutralized by endogenous antioxidant systems and 2) antioxidants were administered in a manner that would improve the redox state in reproductive tissues. A summary of key experiments designed to test effects of antioxidant supplementation on reproductive function follows.

Antioxidant Supplementation and Retained Placenta

It has been known since 1969 that prepartum administration of vitamin E and selenium can reduce the incidence of retained placenta (Trinder et al., 1969). In some cases, retained placenta was reduced in frequency by administration of either vitamin E or selenium alone (Eger et al., 1985; Erskine et al., 1997). Dietary supplementation with β -carotene has also been shown to reduce incidence of retained placenta (Michal et al., 1994).

The beneficial effects of antioxidant supplementation depend upon the adequacy of vitamin E and selenium in the diet. Prepartum treatment with vitamin E tended to reduce incidence of retained placenta in cows with low ratios of α -tocopherol:cholesterol but did not affect incidence in cows with higher ratios (Le Blanc et al., 2002). Similarly, prepartum supplementation with selenium did not affect incidence of retained placenta in a herd of cows receiving adequate amounts of selenium in the diet (Hidiroglou et al., 1987). Available evidence suggests that both organic and inorganic sources of selenium are effective in preventing retained placenta (Cerri et al., 2009).

It is likely that the beneficial effect of antioxidant supplementation on incidence of retained placenta involves improved immune function. Indeed, neutrophil function is enhanced by vitamin E and selenium supplementation prepartum (Spears and Weiss, 2008) and β -carotene can enhance lymphocyte capacity for proliferation (Michal et al., 1994). Occurrence of retained placenta has been associated with defects in function of neutrophils (Kimura et al., 2002) and macrophages (Miyoshi et al., 2002).

Use of Antioxidants to Improve Uterine Health

Given the effectiveness of antioxidants in altering immune function (Spears and Weiss, 2008), it is reasonable to hypothesize that antioxidant supplementation would reduce the incidence of infectious disease in the uterus. Reducing incidence of retained placenta should also reduce uterine infections because occurrence of retained placenta predisposes cows to uterine infections (Le Blanc et al., 2008). In fact, feeding 300 or 600 mg/d β -carotene for 8 wk beginning 4 wk before expected calving reduced the proportion of cows diagnosed with metritis from 18% in controls to 7% (300 mg) or 8% (600 mg) (Michal et al., 1994). Similarly, intramuscular injection of 3,000 mg vitamin E at 8 to 14 d before calving reduced the incidence of metritis from 9% to 4% (Erskine et al., 1997). In contrast, incidence of uterine infections was not reduced by two injections of vitamin E and selenium at 2 wk before expected calving and at within 24 h of parturition (Bourne et al. 2008). Also, a single injection of 3,000 IU vitamin E at 1 wk before expected calving did not reduce the incidence of metritis or endometritis (Le Blanc et al., 2002).

Effects of Antioxidant Supplementation on Fertility

Administration of antioxidants could affect fertility in two ways. A reduction in incidence of retained placenta or uterine infections caused by prepartum administration of antioxidants could increase fertility because pregnancy success after insemination is reduced in cows with these disorders (Kim and Kang, 2003; López-Gatius et al., 2006; McDougall et al., 2007). Secondly, the oocyte and pre-implantation embryo are susceptible to damage by ROS (Schwarz et al., 2008; Favetta et al., 2008; Moss et al., 2009) and increasing the antioxidant status of the reproductive tract in the postpartum period might improve competence of the oocyte or embryo for development.

Despite the potential for improving fertility through antioxidant administration, results have been inconsistent. In a study in Mexico, a single injection of vitamin E and selenium at 21 d before expected calving increased the proportion of cows inseminated at first service that became pregnant (Arechiga et al., 1994) (Table 1). In another Mexican study (Arechiga et al., 1998a), injection of vitamin E and selenium at Day 30 after calving did not affect first service conception rate but tended ($P=0.07$) to increase conception rate at second service (69.8 vs. 52.1%) and reduced services per conception (1.7 vs. 2.0). One interpretation of these results is that the vitamin E and selenium was affecting uterine health and cows having a second service were more likely to have an infectious uterine disease and to be responsive to antioxidant treatment.

In contrast to these positive results, fertility was not improved by two intramuscular injections of vitamin E and selenium at 2 wk before expected calving and within 24 h of parturition (Bourne et al., 2008) or by daily feeding of 1610 mg vitamin E from 4 wk before expected calving to 2 wk after calving (Persson Waller et al., 2007). Similarly, there was no benefit of multiple injections of β -carotene on fertility of lactating cows (Gossen et al., 2004).

Antioxidant Supplementation During Heat Stress

Heat stress can have catastrophic effects on fertility of lactating dairy cows (Hansen, 2007a). There is evidence to indicate that one of the causes for embryonic mortality in heat-stressed cows is production of ROS by embryos developing at elevated body temperatures (Hansen, 2007b). In mice, administration of melatonin, an indoleamine with antioxidant properties, reduced effects of heat stress on embryonic survival (Matsuzuka et al., 2004). In lactating dairy cows, however, most antioxidant treatments used have been ineffective in improving fertility of cows subjected to heat stress. Unsuccessful treatments include administration of vitamin E at insemination (Ealy et al., 1994), injections of β -carotene at -6, -3 and 0 d relative to insemination (Arechiga et al., 1998a) and multiple injections of vitamin E and selenium before and after calving (Paula-Lopes et al., 2003).

There is, however, one study reporting beneficial effects of antioxidant feeding on reproductive function of lactating cows during heat stress (Arechiga et al., 1998b). Cows that received a diet formulated to contain an additional 400 mg/d β -carotene for at least 90 days beginning at ~15 days postpartum were more likely to be pregnant at 120 days postpartum (35%) than cows not receiving supplemental β -carotene (21%). There was also a non-significant tendency for the proportion of cows pregnant to first service to be higher cows receiving supplemental β -carotene (14.6 vs. 9.3%). While not conclusive, additional experimentation involving long-term feeding of antioxidants to improve fertility during heat stress is warranted.

Where Do We Go From Here?

As is apparent from this short review, antioxidant treatments have not consistently improved reproductive function. Although various treatments have been shown to prevent retained placenta, uterine infectious disease and infertility, similar treatments applied to other groups of cows have been without effect. Some of this variation in response, which is a general characteristic of experiments with dairy cattle, reflects variation in experimental design and limited experimental power caused by using insufficient numbers of animals per treatment. In addition, there is considerable variation between herds in antioxidant status. Treatments that improve reproductive function in a herd experiencing a nutrient deficiency may be without effect in another herd fed adequately. Physiological factors that affect ROS production, for example milk yield and occurrence of heat stress, may also influence redox status of the cow and the magnitude of an antioxidant effect.

A successful antioxidant treatment is one that reaches reproductive tissues in high enough concentrations to reduce damage caused by ROS. The effective concentration will depend upon the reducing capacity of the antioxidant as well as its solubility in cytoplasmatic and membrane compartments of the cell. Success at achieving effective concentrations will depend on the route of administration, the degree to which the molecule escapes ruminal degradation, and clearance rate from tissues and blood. It is not clear whether the antioxidants tested to date (vitamin E, selenium,

and β -carotene) are optimal in terms of distribution to reproductive tissues and in reactivity towards ROS. New candidates for antioxidant therapy are continually emerging including the polyamine resveratrol found in grape skin that has been reported to increase sperm output in rats (Juan et al., 2005). Several molecules have been identified that may reduce actions of ROS induced by heat stress. Anthocyanins found in purple sweet potato have been reported to reduce effects of elevated temperature on embryo survival in vitro (Sakatani et al., 2007), and epigallocatechin gallate, a flavonoid in green tea, was reported to protect oocytes from heat stress in vivo in mice (Roth et al., 2008). All of these antioxidants are natural products found in plants. It may be that feedstuffs for dairy cattle can be identified that contain antioxidants with the potential for counteracting effects of ROS on reproductive function.

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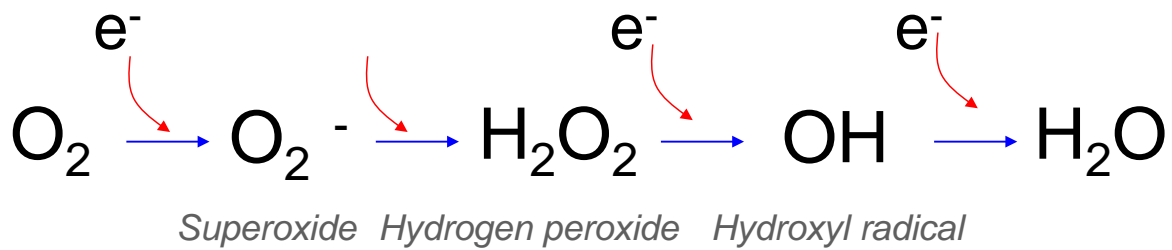


Figure 1. Reduction of molecular oxygen to water.

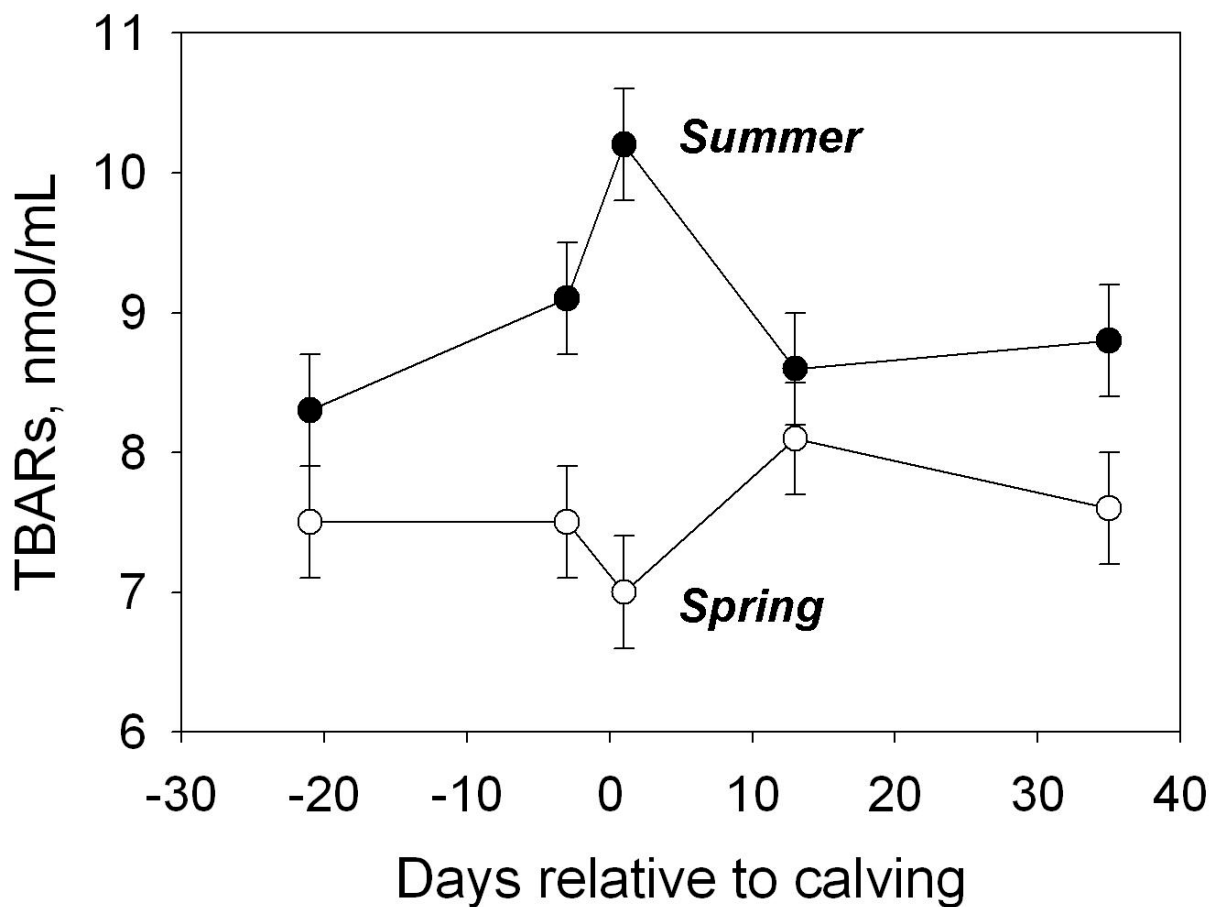


Figure 2. Changes in concentrations of thiobarbituric reactive products (TBARs) in erythrocytes during the transition period in cows calving in summer and spring in Italy (Bernabucci et al., 2002).

Table 1. Effect of prepartum injection of vitamin E and selenium on postpartum reproductive function of lactating dairy cows ^{a,b, c}

	Control	Vitamin E/selenium	P
Incidence of retained fetal membranes	10/99 (10.1%)	3/99 (3.0%)	0.06
Interval to first insemination, d	67 ± 2.6	67 ± 2.6	>0.10
Pregnant at first service	24/95 (25.3%)	40/97 (41.2%)	0.02
Services per conception	2.8 ± 0.17	2.3 ± 0.17	0.03
Interval to conception, d	141 ± 7.7	121 ± 7.6	0.06

^a Treatment was 500 mg vitamin E and 109.5g sodium selenite, i.m., at 21 d before expected calving.

^b Data represent fractions of cows and percent (incidence of retained fetal membranes and pregnant at first service) or least-squares means ± SEM (other traits).

^c Data are from Arechiga et al. (1994).

SESSION NOTES