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Prepared by: Shimin Liu [a]
David Masters [a]
Mark Ferguson [bcd]
Andrew Thompson [bc]

UWA Institute of Agriculture, University of Western Australia [a]
Department of Agriculture and Food of Western Australia [b]
School of Veterinary and Biomedical Sciences, Murdoch University [c]
The New Zealand Merino Company Ltd [d]

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A literature review on the roles of vitamin E in the reproduction of sheep

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Executive summary

Improving lamb, weaner and ewe survival are a high priority for the sheep industry. Several projects submitted to the National RD&E Plan for Sheep Reproduction (ISBN: 9781741919943) related to developing nutritional and breeding strategies to modulate immune competency of ewes and their offspring to enhance survival were recommended for further development. There is emerging evidence that the activity of the immune system could play an important role in determining the fitness of animals and their ability to cope with environmental stressors. The two most 'at risk' phases of reduced innate immunity in most mammals are in the infant and elderly (Kollmann *et al*. 2012), and these two periods of reduced immune function coincide with periods of increased mortality in sheep. There is evidence of genetic variation in the development of acquired immunity (Berggren-Thomas *et al.* 1987), and that selection for higher production results in a reduction in immune response across a range of species (Rauw *et al*. 1998). Berggren-Thomas *et al.* (1987) demonstrated that in ewes that had been inoculated with a foreign antibody, the titres of antibodies in their new born lambs was a heritable trait and was positively associated with their likelihood of survival. There is some evidence that higher genetic potential for wool production is associated with lower lamb survival and weaner survival, although it is not known conclusively whether the reduction in survival is a trait of the lamb or of the ewe or both. Ferguson *et al.* (2007) reported lower lamb survival from high fleece weight ewes, and recent analysis indicates lambs from high fleece weight sires or high growth sires have lower survival at the same birth weight both pre- and post weaning (Thompson *et al*, in preparation). Immune capacity can be altered by nutrition, and supplementing animals with vitamin E and selenium (Se) or sulphur amino acids is known to improve immune responses (Morris 2002; Rooke *et al*. 2008). Several project concepts submitted to the National RDE Plan for Sheep Reproduction proposed further R&D to better understand the potential link between the immune competence of the lamb and its likelihood of survival.

Prior to funding this R&D, MLA commissioned a literature review of '*Vitamin E status and reproduction in sheep: Potential implications for Australian sheep production systems'*. This review has been completed and will also be submitted for publishing in a Special Edition of Animal Production Science following MLA approval. The review focuses specifically on the role of Vitamin E on reproduction of sheep, and how responses to vitamin E could be influenced by other nutrients involved in the management of oxidative stress and immune function such as Se and sulphur amino acids. The main conclusions are that further investigation is justified to quantify the impacts of vitamin E supplementation, in combination with other natural antioxidants lacking in dry pasture, on the quality and quantity of sperm produced and especially the potential to improve lamb survival through boosting maternal innate immunity and vitamin E reserves in the newborn. The justified areas for further research are listed in Appendix 1 (Literature Review).

1. Background

Several related projects were submitted to the National Reproduction RDE strategy, including 'Defining the response in lamb, weaner and ewe survival vitamin E supplementation' (RIST, Victoria), 'Defining the role of the immune system in Merino lamb and weaner survival' (Murdoch University, WA), and 'Longer living ewes – using immune function and biological type to increase reproductive efficiency' (DPI, Victoria). A workshop involving the proponents of the above concepts and MLA was held in Perth on 14th December 2012. The workshop with MLA involved brief discussions of the project concepts and focused mainly on how these concepts could be integrated into a RDE program and the potential structure of that program. An outcome from the workshop was to split the overall program into four phases. Phase 1, a literature review on the roles of vitamin E in reproductive performances of ewes and rams, and lamb survival, is the focus of this Final Report.

2. Project objectives

1. Draft literature review on the potential effects of vitamin E on the reproductive performance of ruminants, with a particular focus on sheep production in Australian farming systems. Possible roles of supplementing vitamin E, together with other nutrients involved in the management of oxidative stress, in the modulation of immune competency of ewes and lambs will also be discussed.
2. Identify potential research areas in manipulating vitamin E status for enhancing immune competency and improving the reproduction efficiency of sheep in Australian environment.

3. References

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Abstract

Vitamin E concentration in pastures and feeds varies substantially. Concentration in dried pastures, stubble and most grains are below the recommended requirement of 10-25 mg/kg dry matter (DM). Sheep grazing in an environment when dry pastures and stubble are their primary source of nutrients for a few months have a high risk of developing vitamin E deficiency. If the low vitamin E status coincides with late gestation, the neonate is likely subject to deficiency of vitamin E. Some of the consequences of this are well known with nutritional myopathy (with high mortality) a risk in young growing sheep unless vitamin E supplements are provided.

Vitamin E deficiency plays a major role in the management of oxidative stress within the body. The high metabolic rate in sperm and high concentration of polyunsaturated fatty acids (PUFA) in sperm membranes make sperm subject to oxidative damage. Oxidative stress may also compromise follicular development and ovarian activity. Vitamin E, most likely through its role in managing the consequences of lipid peroxidation is also involved with an improvement in immune response. For these reasons vitamin E status is important for reproductive efficiency in both the male and female and in the survival of lambs and weaners.

The low vitamin E status in grazing sheep is potentially exacerbated by a lack of other nutrients involved in the management of oxidative stress and immune function such as selenium and sulphur amino acids (Cysteine & Methionine). Selenium is the best known of these and selenium deficiency is a characteristic of many high rainfall grazing areas. Dry pastures are also lacking in sulphur amino acids.

In considering possible consequences for reproduction further investigation is justified into:

- Low vitamin E, in combination with low levels of other natural antioxidants on the quality and quantity of sperm produced prior to and during mating;
- Follicle development, fertilisation and embryonic mortality in Se supplemented ewes;
- Low vitamin E reserves in the newborn – consequences of large dosages to ewes prior to parturition to boost lamb reserves, and economic assessment;
- Potential benefits in lamb survival through boosting maternal innate immunity with vitamin E supplements;
- Options for boosting antioxidant and immune function in ewes and lambs through “immune pack” nutrient options that may target nutrients lacking in dry pastures (e.g. vitamin E, sulphur amino acids, Zn, Se). Consideration should be given to the inclusion of an immunological challenge such as a parenteral antigen or a biological inert stimulant within these experiments.

1. Introduction

There is a large volume of literature describing the biological roles of vitamin E and its application to preventing disease and improving health and productive performances of farm animals. The chemistry and biological functions of vitamin E have been well reviewed (Burton and Traber 1990; Bramley *et al.* 2000; Debier 2005) as have the effects of supplementing ruminants with vitamin E (McDowell *et al.* 1996). The role of vitamin E on immune status of ewes and lambs has also been recently reviewed (Rooke *et al.* 2004). This current review will focus on the effects and potential effects of vitamin E deficiency on the reproductive performance of ruminants, with a particular focus on sheep production in Australian farming systems. Possible roles of supplementing vitamin E in the modulation of immune competency of ewes and lambs are also discussed. Because vitamin E and selenium (Se) function synergistically in maintaining the antioxidant status in the body, supplementation of vitamin E with Se is also briefly addressed.

Within this review, vitamin E refers to all tocol and tocotriennol derivatives with similar biological activity (Agricultural Research Council 1980). The biopotency of these isomers varies widely. dl-α-tocopherol has the highest biopotency (Hidiroglou *et al.* 1992). One International Unit of vitamin E is defined as the biopotency of 1 mg dl-α-tocopherol acetate, and 1 mg d-α-tocopherol has the equivalent of 1.5 IU vitamin E activity (Agricultural Research Council 1980). The acetate ester is air-stable, has no activity as an *in vitro* antioxidant but is readily hydrolyzed in the animal gut to non-esterified or free tocopherol. This is the potent *in vivo* antioxidant (Burton and Traber 1990; McDowell *et al.* 1996) and a commonly used commercial product.

In this review, the term of vitamin E is used when biological roles and status in the body are discussed, and α-tocopherol is used when concentration in samples and requirements by animals are referred to.

2. Background

2.1 Basic functions of vitamin E and selenium in the body

Biological membranes are composed largely of phospholipid molecules, cholesterol, and membrane-bound proteins. These must be protected against peroxidation. Peroxidation of lipids in the membrane results in disruption of the membrane (Hoskins *et al.* 2012). It is well recognized that vitamin E is the major chain-breaking, lipid-soluble antioxidant that prevents lipid peroxidation in mammalian tissues (Burton and Ingold 1986). Vitamin E is oriented with its chromanol head group towards the surface and with the hydrophobic phytyl tail buried within the hydrocarbon region of the membrane (Burton and Ingold 1986). This structural location of vitamin E enables protection of the membrane from attack by the peroxyl radicals. The metabolites of vitamin E in the body may also play roles, for example, it has been proposed that α-tocopherol quinone is an essential enzyme cofactor of carnitine-dependent, channelled mitochondrial fatty acid desaturases (Infante 1999). Because there are many types of fatty acids susceptible to peroxidation, lipid peroxidation generates complex products including hydroperoxides, aldehydes, and polymeric materials. These products of peroxidation are believed to be pathogenic and contribute to the aetiology of various diseases (Niki *et al.* 2005; Niki 2009).

Se exists mainly as selenocysteine in mammalian tissues and is a highly potent biological catalyst in redox reactions. It has been suggested that there are up to 100 selenoproteins (Brown and Arthur 2001), and about 25 of these mammalian selenoproteins have been functionally characterized (Papp *et al.* 2007). Mostly they are involved in redox regulation of the intracellular signalling, redox homeostasis and thyroid hormone metabolism. Four of them are glutathione peroxidases (GPX). These enzymes reduce lipid hydroperoxides to their corresponding alcohols and reduce conversion of free hydrogen peroxide to water (Meister and Anderson 1983; Barceloux 1999). One type of GPX, GPX-4, is a phospholipid hydroperoxidase that protects cells against membrane lipid peroxidation (Arthur 2001). Another selenoprotein, sperm mitochondrial capsule selenoprotein, has been found in spermatozoa (Behne *et al.* 1988; Brown and Arthur 2001). This protein is localized in the middle section the spermatozoon and it is necessary for the integrity of the sperm flagella (Holben and Smith 1999). Spermatozoa contain the highest concentration Se of any mammalian tissues (Brown and Arthur 2001) and this makes sperm sensitive to the Se changes in the body. Se deficiency impairs sperm mitochondrial capsule synthesis and this affects sperm motility (Brown and Arthur 2001).

Vitamin E and selenium therefore act synergistically in the protection of cell membranes. Vitamin E prevents lipid peroxidation as an integrated component of cell membranes, while GPX functions to reduce peroxides mainly in the cytosol of the cell (Burton and Traber 1990; McDowell *et al.* 1996). Deficiency of vitamin E and Se results in an oxidative stress which leads to the damage of cell membranes and nutritional myopathy (NM).

2.2 Vitamin E requirements of sheep

The requirement of α-tocopherol in ruminants is currently assessed as the amount required to prevent the development of clinical or subclinical myopathies. Agricultural Research Council (1980) and Freer *et al.* (2007) suggested that the minimum requirement of α-tocopherol in the diet of growing or pregnant sheep lies between 10 and 20 mg/kg dietary DM. This recommendation may increase to 15-28 mg/kg, if the dietary Se content is low (Agricultural Research Council 1980). Considering Se concentrations in many Australia soils, pastures and feeds are low (Tinggi 2003), the higher range of α-tocopherol requirement may be more appropriate for Australian conditions, at least for some times of the year. Most importantly requirement may be reduced if conditions are poor and animals are maintaining or losing weight, conversely, strategies to increase growth rates in sheep grazing dry pastures will need to consider a potential for increased requirements of vitamin E.

2.3 Indicators of vitamin E deficiency in grazing sheep

The concentration of α-tocopherol in plasma is most commonly used to indicate vitamin E status in the body. There are significant correlations between α-tocopherol concentration in plasma and α-tocopherol concentrations in many tissues in the body (Fry *et al.* 1993; Hidiroglou *et al.* 1994). When Merino wether lambs were fed a vitamin E deficient diet (α-tocopherol concentration <1 mg/kg), the α-tocopherol concentration in plasma fell at the same rate as the α-tocopherol concentrations in skeletal muscles, heart muscle, adrenal and adipose tissue, while the depletion rates in the liver and erythrocytes were even faster than in plasma (Fry *et al.* 1993). The correlation coefficients between the plasma concentrations and the concentrations in these tissues ranged from 0.80 to 0.97 (Fry *et al.* 1993).

The critical concentration of α-tocopherol in muscles of sheep for the development of NM has not been established. In the study by Fry *et al.* (1993), 4 month old Merino wethers had α-tocopherol concentrations ranging from 1.57 to 3.15 mg/kg tissue in three muscles (*M. tensor fascia lata*, *M. vastus lateralis*, and *M. vastus intermedius*) at the beginning of the experiment in an animal house. After 14 weeks of consuming a vitamin E deficient diet the concentrations declined to 0.29 to 0.54 mg/kg across the same muscles. Different muscles had significant differences in α-tocopherol concentrations (Fry *et al.* 1993). By the end of the experiment, these sheep had become vitamin E deficient so these concentrations of α-tocopherol in muscle provide a reference to define vitamin E deficiency.

Because of the existence of the close relationships between plasma α-tocopherol concentration and the concentrations in other tissues, plasma α-tocopherol has also been linked to the development of NM. The critical threshold of 0.7 mg/L has been defined for sheep (White and Rewell 2007). This was derived from an elegant field study that used indicators of muscle damage (creatine kinase [CK] and aspartate aminotransferase [AST]) to identify subclinical or approaching vitamin E deficiency. Sheep with CK activity of 400-1200 U/L and AST activity of 150-270 U/L were classified as having mild to moderate muscle damage (White and Rewell 2007). Others have also used these enzymes in conjunction with clinical symptoms to diagnose vitamin E deficiency (Fry *et al.* 1994; Smith *et al.* 1994).

2.4 Vitamin E concentrations in feedstuffs and pastures

The *α*-tocopherol content in feeds and pastures commonly used in Australia is summarized in Table 1. This table also includes some data from the USA. The table clearly shows that α-tocopherol concentrations in grains, dried pastures and crop stubbles are usually lower than the 10 mg/kg DM minimum requirement for growing and reproducing sheep.

2.5 Seasonal variation of vitamin E status in sheep and concentrations in pastures in the Mediterranean environment

Given the low concentration of α-tocopherol in dry fed and gains, low vitamin E status and vitamin E deficiency would be expected during the seasonal drought that is characteristic of the Mediterranean environment (Fry *et al.* 1996a). White and Rewell (2007) sampled weaners and ewes from 38 farms in autumn across the southern agricultural region of Western Australia, and analysed the vitamin E and Se status of sheep. They reported that 58% of weaner flocks and 16% of ewe flocks had low vitamin E status suggesting vitamin E deficiency is widely spread in this environment in autumn, particularly in weaner flocks. Others have also reported that ewes consuming dry feed and crop stubbles have low α-tocopherol concentrations in plasma and tissues, and their vitamin E status remains low until green feed become available after rain (Kumagai and White 1995).

While the dead, dry residues of crops and pastures have low vitamin E content, some perennial plants that are able to remain green during the summer drought act as a growing vitamin E supplement throughout the year. For example halophytic perennials such as saltbush (Atriplex spp) contain high concentration of vitamin E (Pearce *et al.* 2005), similar to green pastures (Table 1). Vitamin E also functions as an antioxidant in plant tissues, and the high concentrations of α-tocopherol in these plants are most likely a result of plant adaption to the high salt and low water

environment.

While there is a strong seasonal pattern in vitamin E status, NM occurrence is irregular and is also related to growth and production of the sheep and to the depletion of body reserves. Fry et al (1993) fed Merino wether lambs with a vitamin E deficient diet (α-tocopherol concentration <1 mg/kg) for 14 weeks and calculated that the plasma α-tocopherol concentration declined rapidly at the start of depletion (0.064 mg/L per week) and gradually slowed down to 0.01 mg/L per week by week 14. The mean of the depletion rates over the period of 14 weeks was 0.03 mg/L per week. In another animal house experiment with Merino weaners fed a vitamin E deficient diet, the depletion rate averaged 0.038-0.04 mg/L per week (Fry *et al.* 1996b). The approximated mean of these rates, 0.04 mg/L per week could be used to estimate the depletion of vitamin E body reserve. At this rate it would take about 8 weeks for plasma α-tocopherol concentration to reduce from 1 mg/L to the 0.7 mg/L critical threshold for the development of NM (White and Rewell 2007). The depletion rate could change depending on contents of vitamin E and polyunsaturated fatty acids in feeds and pastures and Se status in the body.

Table 1. *α*-tocopherol concentrations in feedstuffs and pastures commonly used in sheep production systems

Feedstuffs	Territories/regions	*α*-tocopherol content (mg/kg DM)	References
Corn	USA	1.4-14.7	Cort *et al.* 1983
Barley	USA	7.9-9.6	Cort *et al.* 1983
	Australia	0.6-6.9	Doncon and Steele 1988; Smith *et al.* 1994; Pearce *et al.* 2005
	Sweden	11.7	Hakkarainen and Pehrson 1987
Lupin seeds	Australia	3.5	Doncon and Steele 1988
Oats	USA	4.4-7.9	Cort *et al.* 1983
	Victoria, Australia	1.0	Ponnampalam 2012
			Estimated from the figure
	Sweden	9.2	Hakkarainen and Pehrson 1987
Peas	Undefined	3.0	Bramley *et al.* 2000
Wheat	USA	8.4-12.1	Cort *et al.* 1983
Canola meal	Undefined	14.5	Downey and Bell 1990
Cottonseed meal	USA	1.1-18.7	Cort *et al.* 1983
Soybean meal	USA	0.9-2.9	Cort *et al.* 1983
Alfalfa dehydrated, or meal	USA	27.5-83.6	Cort *et al.* 1983
Barley chaff	Australia	2.5-12.6	Doncon and Steele, 1988; Smith et al., 1994
Oat chaff	Australia	0.19	Doncon and Steele 1988
Lupin stubble	Australia	0.33	Doncon and Steele 1988
River saltbush,	Western Australia	116	Pearce *et al.* 2005
Oldman saltbush		139	
Perennial pasture	Victoria, Australia	44	Ponnampalam 2012
Annual pasture		24	Estimated from the figure
Grass hay		4	
Lucence hay		23	
Rhodes pasture (Aug-Nov)	Queensland, Australia	116-270	Lanari *et al.* 2002
Pasture	South Australia		Baumgurtel *et al.* 1994
(March)		2-18	
(August)		26-166	

3. Vitamin E status and reproduction in males

3.1 Susceptibility of spermatogenesis and semen quality to vitamin E deficiency

Research on the effects of vitamin E on reproduction of male animals has focused on spermatogenesis and semen quality. There is little data on the effects of vitamin E on the onset of puberty and libido of males. Brown (1994) reviewed the effects of nutrition on male reproduction and recognized that the reproductive organs in immature animals are more sensitive to dietary nutrition than adult bulls, rams and boar but that nutritional-induced changes in reproductive functions are temporary. Given reproduction is a high priority to ensure species survival in most animals, temporary sensitivity to changes in dietary nutrition make biological sense.

There are two characteristics of spermatozoa that make them potentially susceptible to vitamin E and/or selenium deficiency:

- Spermatozoa have a relatively high requirement for energy for capacitation, hyperactivation, motility, acrosome reaction, and normal fertilization.
- Spermatozoa have a high content of polyunsaturated fatty acids (PUFA).

Production of the required energy generates high levels of reactive oxygen species (ROS). ROS leak out during the respiratory process from mitochondria in the spermatozoa or can be generated from leukocytes in the semen and from defective spermatozoa (Aitken 1995). ROS are pro-oxidants and recent studies in humans have found that an excess of ROS can cause damage to sperm DNA and cell membranes leading to elevated apoptosis of spermatids, defective sperm and infertility (Aitken 1995; Rolf *et al.* 1999; Blount *et al.* 2001; Agarwal *et al.* 2006; Desai *et al.* 2010). Low sperm motility has been attributed to damage of the mitochondrial membrane leading to a reduction of energy supply (Zorn *et al.* 2012). Antioxidants can detoxify these free radicals and improve semen quality (Rolf *et al.* 1999; Tunc *et al.* 2009).

The possible mechanisms of DNA damage caused by ROS have also been recently reviewed (Sakkas and Alvarez 2010). The authors pointed out that immature sperm produce high levels of ROS and this can cause damage during the processes of spermatogenesis and post-testicular transport through the seminiferous tubules to the epididymis. The ROS-induced DNA damage can be prevented by using antioxidants (Sakkas and Alvarez 2010).

PUFA are preferentially incorporated into the plasmalogen fraction of phospholipids (Alvarez and Storey 1995) with spermatozoa containing high levels of PUFA, particularly docosahexanoic acid (DHA) in the cell membrane (Jones 1979; Aitken 1995; Lenzi 2000; Tavilani *et al.* 2008). The high PUFA in spermatozoa gives the plasma membrane the fluidity needed for membrane fusion during fertilization. Free ROS will cause lipid peroxidation of polyunsaturated fatty acids (PUFA) in sperm. This lipid peroxidation results in a loss of the fluidity and integrity of the plasma membrane and spermatozoa lose their function (Aitken 1995). The damage of the plasma membrane also leads to disruption of the enzymes in the membrane, for example, Ca^{2+}-Mg^{2+} ATPase, and spermatozoa lose their capacity to regulate the intracellular ions that are involved in the control of sperm motility (Aitken 1995).

Increased lipid peroxidation in spermatozoa from male subjects with poor quality of semen has been reported in humans. Tavilani *et al.* (2008) measured malondialdehyde (MDA, the end product of catabolism of lipid peroxides) concentration in spermatozoa from asthenozoospermic patients and found it was significantly higher than that in normozoospermic males. In other research, free 8-isoprostane concentration (the product of arachidonic acid peroxidation) was 7 times higher in patients with seminal abnormalities than in healthy males (Khosrowbeygi 2007). Jones (1979) reported that lipid peroxides applied exogenously (*in vitro*) at concentrations as low as 30 ng/ml were powerfully spermicidal and caused sperm to become immotile within a few minutes.

3.2 The role of vitamin E for long-chain PUFA synthesis in the testicular tissue

The very high concentrations of DHA and other long-china PUFA in spermatozoa in humans and rats indicate that there is *in situ* synthesis of these fatty acids in the testicular tissue (Infante and Huszagh 1998; Lenzi 2000). The synthesis, DHA in

particular, has been confirmed in the mitochondria (Infante 1999). Infante (1999) proposed that during the *in situ* synthesis α-tocopherol quinone (a derived metabolite of α-tocopherol in mammalian tissues) is an essential enzyme cofactor of carnitine-dependent, channelled mitochondrial fatty acid desaturases. α-tocopherol quinone can serve as hydrogen acceptor during the reaction of the fatty acid desaturation (Infante 1999). If this hypothesis is confirmed then the roles of vitamin E on reproduction in the male are not restricted to the prevention of lipid peroxidation. Vitamin E could play a critical role in the modulating long-chain PUFA synthesis in mitochondria. This is required for fully functional spermatozoa membranes.

3.3 Supplementation of vitamin E on reproduction of male farm animals

Research on the roles of ROS and lipid peroxidation on semen quality is mainly on human males diagnosed with poor quality of semen, leading to infertility. There have been some studies on supplementation with exogenous vitamin E in farm animals. The results from these recent publications are summarized in Table 2. Although this review aims to address the reproductive issues in rams and bulls, the data are scarce. So the data from boars and rabbits are also collected. In some of those reports, Se was supplemented.

Table 2. The effects of supplementation of vitamin E and Se on reproductive performances of male farm animals.

Animals	Treatments	Vit E and Se concentrations	Reproductive performances	References
30 Aohan fine-wool sheep, aged 5 months	Pen trial, fed a basal diet containing 3.42 IU/kg Vit E; Dosed 20, 200, 1000, and 2000 IU/d Vit E for 12 months.		200 IU Vit E dose maximized ejaculate volume, sperm concentration, total sperm output, and sperm motility; no effects on sperm count.	Yue *et al.* 2010
14 Ossimi rams, mature	Injection of 2.3 mg Se and 450 mg Vit E twice per week for 1 month.		Increased ejaculate volume, sperm motility and concentration, reduced dead and abnormal sperm counts.	Mahmoud *et al.* 2012
8 Chios rams, 3 year old	Orally dosed 1mg/kg.d α-tocopherol for 40 days, respectively in autumn (estrous period) and spring (anestrous period).	Plasma: 0.85-1.34 mg/L in control and 1.0-4.9 mg/L in treatment	No effects on testosterone in plasma, plasminogen activator activity, plasminogen activator inhibitor and plasmin inhibitors in seminal plasma in either season; Increased PAA and t-PAI in spermatozoa in spring.	Rekkas *et al.* 2000
20 boars	Fed 1000 mg/d α-tocopherol orally up to 7 weeks.		Increased spermatozoa counts; Reduced TBARS.	Brzezimska-Slebodzinska *et al.* 1995
192 boars from weaning to 145 kg BW in Expt 1; 12 boars, 8 month old in Expt 2, and 44 boars of 18 month old	Fed diets : (1) 0.5 mg/kg Se; (2) 220 mg/kg α-tocopherol; (3) 0.5 mg/kg Se + 220 mg/kg α-tocopherol; Semen measured from 9 months of life. Control diet: 0.063 mg/kg Se and 3.46 mg/kg α-tocopherol.	Serum (mg/L, IU/L): Se: 0.033-0.052 in control and 0.17-0.27 in supplementation; Vit E: 0.28-0.48 in control and 1.71-2.41 in Vit E treatment. Testis (mg/kg, IU/kg): Se: 0.28-0.38 in control, 0.70-0.80 in Se treatment; Vit E: 0.74-0.95 in control and 0.87-1.28 in Vit E treatment.	No effects on testis size and weight; No effects on ejaculate volume, sperm concentration and total sperm count. Se: lowered fertilization rate of oocytes, after 12-24 hours of insemination to gilts.	Marin-Guzman *et al.* 1997

Animals	Treatments	Vit E and Se concentrations	Reproductive performances	References
10 boars, >18 month old in 3 experiments	Fed diets: (1) 0.5 mg/kg Se; (2) 220 mg/kg α-tocopherol; (3) 0.5 mg/kg Se + 220 mg/kg α-tocopherol; Conducted from 12-18 months of life		Vit E: no effects on structural abnormalities of spermatozoa, ATP concentration in spermatozoa; Se: reduced abnormal spermatozoal mitochondria, increased ATP concentration of spermatozoa.	Marin-Guzman *et al.* 2000
16 boars	Fed diets: (1) 0.5 mg/kg Se; (2) 250 IU/kg Vit E; (3) 0.5 mg/kg Se + 250 IU/kg Vit E; Conducted in fresh and warm seasons		Enhanced sperm motility, normal sperm, head abnormalities and retention of cytoplasmic droplets; Vit E, or Vit E + Se increased sperm concentration in both seasons, and total sperm output; Se and Vit E reduced tail abnormalities only in fresh season.	Echeverria-Alonzo *et al.* 2009
40 boars	Fed from 70 to 180 days of age; Control diet contained 0.2 mg/kg Se and 30 mg/kg Vit E, and supplementation diet contained 0.5 mg/kg Se + 60 mg Vit E.	Se concentration: Plasma: 0.26-0.28 mg/L; Seminal plasma: 0.043-0.051 mg/L.	No effects on testes volume, ejaculate volume, sperm motility, Elevated sperm concentration, and total sperm count; Lowered sperm with defects, and defective acrosomes.	Kolodziej and Jacyno 2005
60 boars, aged 6-10 months	Fed diets for 33 weeks: (1)Vit C; (2) fat soluble vitamins (VA, VD and Vit E); (3) water soluble vitamins.	Vit E: undetectable in seminal plasma.	No effects on boar libido, total sperm number per ejaculate, cumulative sperm production, sperm motility, abnormal sperm count.	Audet *et al.* 2004
60 boars, aged 70-180 days	Fed diets containing: (1)0.2 mg/kg inorganic Se + 30 mg/kg Vit E; (2) 0.2 mg/kg organic Se + 60 mg/kg Vit E; (3) 0.4 mg/kg organic Se + 60 mg/kg Vit E. Semen collected from 180 days.		High Se and Vit E increased testes volume, sperm concentration, and total sperm count, and reduced defect sperm; No effects on ejaculate volume and sperm motility.	Jacyno *et al.* 2005

Animals	Treatments	Vit E and Se concentrations	Reproductive performances	References
42 rabbits, 5 month old	Control diet: 8 mg/kg Vit E; (1) 200 mg/kg Vit E; (2) 1.5% fish oil: (3) fish oil + 200 mg/kg Vit E; For 19 weeks, and measured conducted weeks 7-8 and 18-19.	No difference in α-tocopherol in semen.	No effects on ejaculate volume, total sperm output, motility, and viability; Vit E increased sperm concentration.	Gliozzi *et al.* 2009
40 New Zealand White rabbits	Control diet: 65 mg/kg α-tocopherol and 0.1 mg/kg Se; (1) 200 mg/kg α-tocopherol; (2) 0.5 mg/kg Se; (3) 200 mg/kg α-tocopherol + 0.5 mg/kg Se For 9 weeks	Vit E concentrations: Plasma: 2.5 – 7.3 mg/L; Semen: 0.41 -0.75 mg/L	No effects on live spermatozoa, acrosome reacted, spermatozoal mobility, and fertility rate and litter size	Castellini *et al.* 2002
40 New Zealand White rabbits, 5 month old	Control diet: 65 mg/kg α-tocopherol; supplements in drinking water: (1) ascorbic acid 1.5 g/L; (2) Vit E 1.0 g/L; (3) Vit C+ Vit E; For 12 weeks		Enhanced ejaculate volume, pH of semen, sperm concentration, total sperm output, sperm motility index, packed sperm volume, while reduced dead and abnormal sperm counts. Vit E had more profound effects than ascorbic acid; No additive effects between Vit E and ascorbic acids.	Yousef *et al.* 2003

Supplementation of vitamin E, either orally dosed or injected, to rams in two experiments resulted in improved semen quality, including increased ejaculate volume, sperm concentration and motility (Yue *et al.* 2010; Mahmoud *et al.* 2012); the number of dead and abnormal sperm was reduced in the experiment where sperm morphology was examined (Mahmoud *et al.,* 2012). However it should be noted that in the experiment by Mahmoud *et al.* (2012), vitamin E and Se were injected simultaneously. The third ram experiment did not examine semen quality, but reported that the oral dose of α-tocopherol acetate enhanced the levels of plasminogen activators, plasminogen activator inhibitors, and plasmin inhibitors, in spermatozoa in spring (ie, anestrous period). An improvement of these parameters is consistent with potential benefits to fertilization (Rekkas *et al.* 2000). Yue *et al.* (2010) offered rams a basal diet containing 3.42 IU vitamin E /kg, with a series of vitamin E supplements ranging from 20 up to 2000 IU/d. The best quality semen was achieved with a dose of 200 IU/d. The vitamin E concentration in the basal diet was lower than the requirement of 10-25 mg/kg DM recommended for reproductive ewes (Agricultural Research Council 1980), and could be considered as a vitamin E-deficient diet. Compared with rams fed the basal diet only, supplementation of 200 IU/d vitamin E significantly increased ejaculate volume, sperm concentration and motility and the total sperm output, but there were no differences in normal and abnormal sperm counts. If the feed intake was assumed to be about 1.5 kg/d DM (the actual intake was not reported), a dose of 200 IU/d plus the vitamin E in the basal diet is equivalent to 135 mg/kg DM, five times the top of the range of the recommended intake (Yue *et al.* 2010). Comparing the results between the treatments of 20 IU/d and 200 IU/d, the only significant difference was found in the total sperm output, with no differences in all the other parameters. The results suggested that the dose of 20 IU/d vitamin E is adequate with little additional benefit at higher doses.

In seven experiments with boars, as shown in Table 2, vitamin E supplementation ranged from 30 mg to 250 mg/kg DM, or orally dosed 1000 mg/d. Most of these experiments included Se supplementation from 0.2 to 0.5 mg/kg DM. Four out of the seven experiments showed increased sperm concentration and motility, and reduced defective sperm counts, without any influence in ejaculate volume. Three experiments did not show any significant improvement in semen quality. In the experiment by Marin-Guzman *et al.* (1997), supplementation with 0.5 mg/kg Se lowered fertilization rate of oocytes. Most of these experiments did not report vitamin E concentration in the basal diets, so it is not clear whether the basal diets provided adequate vitamin E to the boars. Overall, dietary supplementation of vitamin E up to 250 mg/kg DM appears to improve semen quality. Again, the effects of Se cannot be ruled out.

Dietary supplementation of vitamin E to male rabbits did not result in any benefit in semen quality regardless of vitamin E concentrations (8 and 65 mg/kg DM) in the basal diets (Castellini *et al.* 2002; Gliozzi *et al.* 2009), unless the supplement was in drinking water (Yousef *et al.* 2003).

3.4 Implications for grazing rams

The aim of any mating program is to maximise the number of ewes that can be mated and successfully fertilized by rams over the joining period, which typically lasts for 5 to 7 weeks. Semen quality is therefore important because of the high costs of producing sires with high breeding values.

The number of ewes that any ram can successfully inseminate is in part dependant on sperm concentration, total sperm output (a product of ejaculate volume and sperm

concentration), sperm motility and count of abnormal sperm. The review of biochemical processes required for production of high quality sperm indicates that there is an essential requirement for anti-oxidants including vitamin E and selenium. There are no controlled studies that have compared the relative requirements for reproduction compared to muscle integrity. Currently it is assumed that if an animal has sufficient vitamin E to prevent NM, it has enough for all other processes within the body. This has been demonstrated for wool growth and liveweight gain (Fry *et al.* 1996a; Fry *et al.* 1996b) but not reproduction. The limited results available from studies on vitamin E on sperm production and quality indicate that it is possible the requirement to support male reproduction is higher than required for liveweight gain and wool growth.

What is clear is that vitamin E supply and vitamin E status of grazing sheep are at their lowest when the requirement for high quality sperm is at its highest during mating (summer/autumn). It is also known that during this time testicular volume is also at its lowest (Masters and Fels 1984). The reduction in testicular volume is usually attributed to protein and energy supply (Martin *et al.* 1987; Murray *et al.* 1990), however this is also a period of low availability of most natural antioxidants and a time when there is an increased development of the germ cell to develop into fully mature spermatozoa in mature rams (Brown 1994). Supplements used during this time of the year are usually hay or grains. Neither of these contains useful quantities of vitamin E and may exacerbate a deficiency in this nutrient.

4. Vitamin E status and reproduction in the female

4.1 Susceptibility of follicular development and ovarian activity to vitamin E deficiency

Maintaining normal follicular development and ovarian activity is critical for reproduction of the female. A recent review proposed roles for ROS and antioxidants in ovarian activity (Devine 2012). The review suggested that the primordial and primary follicles are less sensitive to oxidative stress, whereas apoptosis of antral follicles and granulosa cells, and oocytes are sensitive to the change of antioxidant status, particularly to glutathione (GSH) concentration. Reduced GSH concentration prevents the formation of the male pronucleus, increased atretic antral follicles and apoptosis of antral follicles (Devine 2012). GSH influence is most likely to be attributable to its function in modulating the cellular redox state and quenching free radicals in reactions catalyzed by GPX and GSH reductase. Se concentration alters GPX activity, and would have a potential effect on follicular development for this reason. The function of vitamin E in the development of follicles and ovarian activity are unknown, but ovulation-induced oxidative base damage to the ovarian epithelium of ewes can be prevented by supplementation of vitamin E (Murdoch and Martinchict 2004). Research in this area is scarce, particularly in ruminants. Effects of nutrition (mainly protein and energy) on reproduction of ruminants has been reviewed recently (Robinson *et al.* 2006) and also reported by Lassoued *et al.* (2004).

The effects of supplementation of Se and vitamin E on uterine motility and fertility of ova in 60 ewes maintained on either an adequate, or inadequate plane of nutrition have been reported. Intramuscular injections of 10 mg Se and 136 mg α-tocopherol acetate was at 21-day intervals and after 150 days of the supplementation, the Se and vitamin E treatment enhanced fertility and uterine contraction only when ewes were maintained on an adequate plane of nutrition (Segerson and Ganapathy 1981).

The effects of Se and vitamin E supplements on fertilization of ova and number of sperm per fertilized ovum have been studied in Charolais cows (Segerson and Libby 1982). Cows with a low Se status received 40 mg of Se and 544 mg α-tocopherol acetate by intramuscular injection at 14-day intervals. After 75 days of treatment, the ova with spermatozoal numbers increased from 13.5 in control to 36.4 in supplemented cows, indicating increased sperm transport (Segerson and Libby 1982). Since Se and vitamin E were supplemented together in these two experiments it is not known if the responses were to Se, vitamin E or a combination of both of these nutrients.

When gilts were supplemented with vitamin E, the number of mature follicles present on the ovaries was not affected, but more follicles were counted (Akomas *et al.* 2011).

There have been only a couple of experiments where ewes were supplemented with Se and Se plus vitamin E before mating. An intra-muscular injection of 2.1 mg Se to Karacabey Merino ewes, or Se plus 250 mg vitamin E significantly enhanced oestrus response by 10-13%, and fecundity rate was also increased (Koayuncu and Yerlikaya 2007). However, compared the effect of Se, no extra benefit was observed in the Se plus vitamin E treatment in this experiment. In experiments by Segerson *et al.* (1986), Suffolk crossbred ewes were injected intra-muscularly with 15 mg Se plus 204 mg α-tocopherol acetate monthly from 90 days before mating until weaning, or 30 days before mating and then 15 days before lambing, there were no significant changes in lambing rate, and number of lambs born alive per ewe exposed. (Scales 1974) summarised a series of 5 trials where Se supplementation was provided to Merino ewes in New Zealand, and reported that barren ewes was reduced by about 12%.

From these few experiments, there is no convincing evidence that vitamin E, rather than Se, supplementation is beneficial for oocyte and follicle development, oestrus, or for fertility, however, as with sperm production, a lack of antioxidant capacity is likely to compromise follicle development and fertilisation.

4.2 Embryonic mortality and foetal death

There is no evidence that vitamin E deficiency causes embryonic mortality and foetal death in ruminants even though these appear to be the most important causes of reproductive losses in commercial animal production (Vanroose *et al.* 2000). In the review by Vanroose *et al.* (2000), most of the embryonic death occurs during the first days after fertilization and during the process of implantation. Mortality was estimated to be 10-30% in goats and 10-40% in cows with early foetal death about 5-10% (no data was cited for sheep). Important causes of the losses included infection and specific nutrient deficiencies or malnutrition, including vitamin A, Cu, Zn, and I, particularly in the very early stages of gestation (Vanroose *et al.*, 2000).

5. Vitamin E status and lamb survival

Neonatal lamb mortality is a significant contributor to reproductive wastage between conception and weaning in Australia and other countries (reviewed by (Hatfield *et al.* 2000; Pollard 2006; Hinch and Brien 2013). Mortality rates vary significantly between farms, regions and sheep breeds and mortality of twin born lambs is typically about double the mortality of single lambs. Fowler (2007) reported that mortality rates from commercial flocks with nearly 100,000 ewes was on average around 30%, and mortality rates are even greater if ewes have been under fed during pregnancy or the

climatic condition at lambing are poor (Behrendt *et al.* 2011; Oldham *et al.* 2011). Most lamb deaths occur within the first three days after birth and dystokia and starvation, mismothering and exposure and the main causes of death. A recent analysis of 26,630 birth records from the Sheep CRC Information Nucleus Flock, which involved 8 flocks across Australia over four years and multiple ewe and sire breeds, indicated that more than half the lambs that died were either dead at birth or died as the result of dystokia, and more than 30% died of starvation, mismothering and exposure (Jones *et al.* 2013). Wiener (1983) also examined the results of 586 post-mortems, and found that 11% of dead lambs were stillbirths or delayed births, 11% died from dystokia, 10% died from congenital defects of various types, 25% were weak lambs, 14% to infectious diseases and 16% to non-infectious diseases.

5.1 Vitamin E status of ewes and lamb mortality

The effects of supplementation of vitamin E, and in some cases with Se, during gestation in order to improve ewe's performance and reduce lamb mortality have been investigated in a number of experiments. The results are summarized in Table 3. Among these 12 reports, only three showed reduced lamb mortality (Segerson *et al.* 1986; Kott *et al.* 1998; Yaprak *et al.* 2004), two reported increased lamb birth weight (Segerson *et al.* 1986; Yaprak *et al.* 2004), six demonstrated increased weaning weight (Kott *et al.* 1983; Segerson *et al.* 1986; Kott *et al.* 1998; Gabryszuk and Klewiec 2002; Ali *et al.* 2004; Koyuncu and Yerlikaya 2007), whereas the other four papers reported no effects of supplementation of vitamin E on lamb mortality and weaning weight (Kumagai and White 1995; Daniels *et al.* 2000; Dafoe *et al.* 2008; Rooke *et al.* 2009). Although Koyuncu and Yerlikaya (2007) reported vitamin E supplementation increased the incidence of oestrus and fertility of ewes, four other reports (Kott *et al.* 1983; Kumagai and White 1995; Gabryszuk and Klewiec 2002; Yaprak *et al.* 2004) showed no effects on ewe oestrus, fertility, and prolificacy, neither on liveweight changes and wool production (Fry *et al.* 1996b; Daniels *et al.* 2000).

The inconsistency of these results is most likely due to variation in vitamin E and Se composition of the control diets and the low number of animals used in most studies. For example, the concentrations of vitamin E in plasma of untreated ewes were >0.9 mg/L in the experiments of Kumagai and White (1995), >1.24 mg/L in experiment of Daniels *et al.* (2000), >1.5 mg/L in ewes used by Gabryszuk *et al.* (2002), and >0.9 mg/L in experiment by Rooke *et al.* (2009). All higher than the critical threshold of 0.7 mg/L for preventing NM (White *et al.* 2007). Based on analysis of our own lamb survival data, only two or three of the studies that have investigated the effects of Vitamin E supplementation on lamb survival used sufficient animals to detect effects on survival of 10% or less. Studies are required with larger number of ewes grazing dry pastures during summer and autumn.

It could be cautiously summarized that supplementation of Se, rather than vitamin E, before mating may increase the incidence of oestrus and reduce embryonic mortality, and that supplementation of vitamin E late in gestation had little influence on fertility and prolificacy or on ewe productive performance (weight gain and wool production). As for lambs the supplementation had the least effect on the number of lambs born and birth weight but tended to enhance live weight gain before weaning.

5.2 Vitamin E status in new-born lambs (placental transfer)

Prepartum supplementation of vitamin E to ewes is not an efficient method of improving vitamin E status in neonates before suckling (McDowell *et al.* 1996; Rooke *et al.* 2004). Vitamin E does not cross the placenta in any appreciable amounts

(Kumagai and White 1995; McDowell *et al.* 1996). An *in vitro* model (isolated placental cotyledon system) showed vitamin E was transferred at a rate only 10% that of L-glucose (Schenker *et al.* 1998). Oral supplementation of vitamin E (approximately 250-280 mg/d α-tocopherol acetate per ewe) to pregnant ewes 5 weeks before joining until 1 week prior to the start of lambing increased α-tocopherol concentrations in both plasma (0.38 vs 0.25 mg/L) and liver (3.08 vs 2.11 mg/kg) of the lambs from supplemented ewes compared with those from unsupplemented ewes, but these plasma concentrations were still <15% of those in their mothers (approximately 3 vs 1.6 mg/L), regardless of treatment (Kumagai and White 1995). In an experiment by Capper *et al.* (2005) crossbred ewes were fed either a basal diet that contained 62 mg/kg or 522 mg/kg α-tocopherol acetate from 6 weeks prepartum until 4 weeks postpartum. The plasma α-tocopherol concentration for ewes fed the low vitamin E diet declined with time from the initial value of about 2 mg/L, whereas the concentration for ewes fed the high vitamin E diet increased. Plasma α-tocopherol even for lambs born to ewes fed the high vitamin E diet was undetectable in 9 out of 12 neonatal plasma samples (<0.043 mg/L), however α-tocopherol concentrations in brain tissue and *semimembranosis* muscle were elevated to about 2-2.8 mg/kg and 0.8-1.1 m/kg respectively (Capper *et al.* 2005).

Cuesta *et al.* (1995) injected 4.5 and 9.0 mg α-tocopherol per kg live weight, in two equal doses at two week of intervals, to pregnant ewes 4 weeks prepartum. This resulted in serum α-tocopherol concentrations of 1.4 and 4.3 mg/L respectively for lambs at birth.

When vitamin E supplementation was provided in gelatin capsules at three doses of 0, 15, 30 and 60 mg/d α-tocopherol acetate per ewe, to crossbred ewes from day 28 prepartum until day 28 postpartum, serum α-tocopherol concentrations in ewes after 28 days of the supplementation increased from 0.66 mg/L to 0.93, 1.94, 2.63 and 4.07 mg/L across the four treatment. In neonatal lambs the concentration was 0.4 mg/L with no significant effects of prepartum vitamin E supplementation to ewes (Njeru *et al.* 1994). It should be noted that the α-tocopherol concentration in unsupplemented control in this experiment also increased to 0.93 mg/L during the experiment, indicating that there was an adequate level of vitamin E even in the basal diet.

The above reports indicate that:

- Neonates have very low vitamin E status before suckling;
- Vitamin E status in tissues of the neonate may be improved slightly by supplementing ewes prepartum with high levels of vitamin E.

Since colostrum provides very high levels of vitamin E (see below), implication of the low vitamin E status in the foetus for mortality at birth may be minor and dependant on lamb vigour and suckling within 24 hours of birth.

5.3 Vitamin E in colostrum

Vitamin E is readily transferred into the mammary gland and into colostrum and milk. Supplementation to ewes to elevate vitamin E concentration in colostrum is an effective method of improving the vitamin E status in the neonates (Pehrson *et al.* 1990; Njeru *et al.* 1994; Kumagai and White 1995; McDowell *et al.* 1996; Capper *et al.* 2005). Njeru *et al.* (1994) reported α-tocopherol concentration in colostrum of 3.3, 6.8, 8.0 and 9.6 mg/L in response to supplementations of 0, 15, 30 and 60 mg/d α-tocopherol acetate 28 days prepartum and 28 days postpartum to ewes. The corresponding serum α-tocopherol concentration of the lambs 3 days after birth

increased from 0.23-0.4 mg/L up to 1.41, 1.84, 2.43 and 4.46 mg/L with the four levels of supplementation. Injection of 4.5 and 9.0 mg α-tocopherol per kg live weight to pregnant ewes 4 weeks prepartum resulted in colostrum α-tocopherol concentrations of 7.0 and 9.9 mg/L (Cuesta *et al.* 1995). A single intramuscular injection of 500 mg α-tocopherol acetate 2 weeks before lambing or oral supplementation of 150 mg/d α-tocopherol acetate during 3-4 weeks before lambing doubled α-tocopherol concentrations in ewes at lambing and in colostrum (Pehrson *et al.* 1990). In an experiment by Kumagai and White (1995), α-tocopherol concentration in colostrum was 299 mg/L after supplementation of about 250-280 mg/d α-tocopherol acetate per ewe 5 weeks before joining until 1 week prior to the start of lambing, compared with α-tocopherol concentration of 193 mg/L in control sheep.

These results clearly indicate that high vitamin E concentration in colostrum can rapidly alter vitamin E status of the neonates after suckling. Considering the efficiency of supplementation to boost colostrum and milk vitamin E concentrations a large dose of vitamin E administrated to ewes in the late stage of pregnancy and the early stage of lactation can be justified to ensure adequate vitamin E status in young lambs. Even so, cost effective studies need to be carried out to assess the cost of the supplementation, benefits from potentially increased lamb survival and growth performance, and economical return".

5.4 Vitamin E and nutritional myopathy (NM)

In experiments where there is a clear deficiency of vitamin E in the diet, the contribution of vitamin E deficiency in lamb and weaner mortality is well known. Nutritional myopathy caused by vitamin E efficiency has been reported as the cause of lamb and weaner mortality in Mediterranean climates during summer and autumn (Steele *et al.* 1980; Caple and McDonald 1983) and has also been reported in lambs born to ewes fed roughage and grain with low levels of vitamin E (Watson *et al.* 1988). Susceptibility appears to be decreased by feed deprivation (Allen *et al.* 1986) meaning that animals that are well fed with a diet containing low levels of vitamin E are more susceptible to NM. In some instances Se supplements have been partially effective in treating the myopathy but vitamin E was completely effective (Allen *et al.* 1986).

Lambs and weaners will therefore be predisposed to NM if born during summer and autumn and will be dependent on vitamin E stores in the body, vitamin E supplied from milk and colostrum or vitamin E in supplements prior to growth of green feed in winter.

Table 3. The effects of supplementation of vitamin E on productive performance of pregnant ewes and weaners, and lamb survival.

Animals	Treatments	Performances of ewes	Performance of lambs	References
175 Suffolk crossbred ewes in 2 experiments	Grazing + supplementation feeds; Expt 1: injection of i.m. 15 mg Se + 204 mg α-tocopherol acetate monthly from 90 days before mating until weaning; Expt 2: injection of i.m. 15 mg Se + 204 α-tocopherol acetate 30 days before mating and then 15 days before lambing. Lambs were injected with 2 mg Se + 136 α-tocopherol acetate monthly from day 1 until weaning.	Expt 1: lesser number of ewes lambing (0.82 vs 0.96)*. Expt 2: no difference in number of ewes lambing. Serum Se concentration at lambing: 0.04-0.155 mg/L. *significant	Expt 1: heavier birth weight*, higher numbers of lambs born alive (1.81vs 1.61); lesser stillborns per ewe lambing (0.05 vs 0.34); lower lamb survival. Heavier weaning weight per ewe lambing; Expt 2: heavier birth weight*. Serum Se concentration at weaning: 0.015-0.065 mg/L.	Segerson *et al.* 1986
125 medium wool ewes	Injected s.c. 272 mg α-tocopherol acetate and/or 4 mg Se monthly during pregnancy.	No effects on fertility (number of ewes lambing of ewes bred), prolificacy (number of lambs born/ewe), and lamb sex ratio. Blood Se concentration: 0.068-0.242 mg/L.	No effects on number of lambs born, and number of lambs weaned/ewe bred; Vit E or Se increased preweaning survival and weaning weight/ewe by 8-10%.	Kott *et al.* 1983
1302 mature Rambouillet and Targhee ewes	Fed alfalfa-grass hay/barley diet, supplemented 330 mg/d α-tocopherol acetate 3 weeks prepartum.	No effects on ewe weight, body conditions score, fertility or prolificacy.	Reduced lamb mortality from 17% to 12% only in ewes lambed in the early part of the lambing season, and increased total weaning weight per ewe; The effects not exist in lambs born in the late part of lambing season.	Kott *et al.* 1998
420 Merino pregnant ewes, 3-6 year old	Grazing for 10 months; (1) vitamins (6 mg/d retinol + 300 mg/d α-tocopherol); (2) minerals mix at 25 g/d; (3) vitamins + minerals; Treatments applied 5 weeks before joining until 1 week prepartum.	Minerals increased ewe body weight by 3%, wool growth by 4.4%, Vitamins increased their plasma and liver concentrations of ewes, but had no effect on ewe reproductive performance; Plasma Vit E concentration: 0.9-3.5 mg/L.	Vitamins increased their concentrations in liver and foetus. No effects on lamb body weights or wool growth. Minerals increased birth and weaning weights of single lambs by 7.3% and 3.7%.	Kumagai and White 1995

Animals	Treatments	Performances of ewes	Performance of lambs	References
160 Merino weaner	Animal house experiment, fed a Vit E deficiency diet, 18 weeks; (1) α-tocopherol acetate in aqueous dispersion, oral drench at 120 mg/kg followed by 1000 mg per fortnight; (2) α-tocopherol acetate in arachis oil, 400 mg/mL, 2000 mg i.m. in neck; (3) α-tocopherol acetate in spray dried formulation 100 mg/kg feed.	No influences on liveweight gain and wool growth rate; Control weaner: plasma α-tocopherol acetate remained below 0.7 mg/L over 18 weeks.		Fry *et al.* 1996b
250 Merino wether in 3 experiments, 5 month old	Grazing in dry season for 5-6 month; (1) α-tocopherol acetate in aqueous dispersion, oral drench at 120 mg/kg followed by 1000 mg per fortnight; (2) α-tocopherol acetate in arachis oil, 400 mg/mL, 2000 mg i.m. in neck; (3) α-tocopherol acetate in an emulsion (200 g/L), 2000 mg injected s.c. in the groin region; (4) α-tocopherol acetate in an emulsion (200 g/L), injected i.m. 5 mL each leg; (5) α-tocopherol acetate + Se injected s.c.	No influences on liveweight gain and wool growth rates; Control wether: plasma α-tocopherol acetate declined from 0.95 to 0.22 mg/L by week 18, remained under 0.7 mg/L until season break, a few sheep developed myopathy; Plasma concentration declined at 0.038-0.04 mg/L.week.		
52 Targhee twin-bearing ewes	Fed in a research ranch, orally dosed 400 mg/d α-tocopherol daily 32 days before lambing; challenged with parainfluenza type 3 vaccination (PI3)	No effects on ewe body weight and body condition score; No effects on serum IgG, anti-PI3 titer and colostral IgG. Serum Vit E concentration: 1.24-1.87 mg/L.	No effects on birth weight, 30 day weight postpartum and lamb survival; No effects on serum IgG and anti-PI3 titer. Serum Vit E concentration: 1.0-1.5 mg/L.	Daniels *et al.* 2000
150 Polish Merino ewes, 2 and 3 year old	Grazing + feeds; Injected i.m. (1) 2.1 mg Se; (2) 2.1 mg Se + 250 mg Vit E 4 weeks before the mating and then 4 weeks before lambing. Pasture + feeds provided Se 0.1-0.15 mg/kg DM.	No effects of Vit E+Se supplementation on oestrus, fertility (% ewe lambing), and prolificacy (number of lambs born of ewes lambing); Plasma Vit E concentration 1.5-2.5 mg/L, Se concentration 0.09-0.13 mg/L.	Se, Vit E+Se supplementation reduced lamb birth weight; whereas Se supplementation increased weight gain of lambs to 28 day of life.	Gabryszuk and Klewiec 2002

Animals	Treatments	Performances of ewes	Performance of lambs	References
About 350 crossbred meat type ewes	Pen trial, treatments applied 4 weeks before lambing; (1) injection i.m. 86 mg α-tocopherol/d; (2) 10 mg/kg Se in supplemented minerals; (2) 90 mg/kg Se in supplemented minerals.		No effects of Vit E on lamb birth weight; Se increased birth weight; Vit E enhanced weaning weight of lambs; No effects on lamb survival in year 1. In year 2, Vit E supplementation had a greater survival rate for twin lambs only, but no for single and triple lambs; No effect of Se on lamb survival.	Ali *et al.* 2004
52 Awassi ewes	Fed dry grass/barley/supplement; Supplementing 400 mg α-tocopherol acetate/d 3 weeks prepartum	Increased ewe body weight at lambing and turnout (30 days post lambing); No effects on ewe fertility and prolificacy.	Heavier birth weight, reduced lamb mortality (26 vs 5%).	Yaprak *et al.* 2004
90 Karacabey Merino ewes, 2-3 year old	Grazing + feed (1) 5 mL 0.1% Na_2O_4Se (2.1 mg Se); 2) 5 mL 0.1% Na_2O_4Se + 250 mg Vit E; Injected i.m. before mating and lambing season.	Se and/or Vit E increased the incidence of oestrus by >10%, and fertility by 16-31%.	Se enhanced oestrus rate by 10-13%. Born more lambs (8-10 more, not significant), no difference in birth weight; Increased daily weight gain and body weight to day 60 postpartum.	Koyuncu and Yerlikaya 2007
1182 Targhee ewes in 2 year Expt	Group fed, supplemented in the last 40 days of gestation; (1) 226 g/d safflower seeds; (2) 226 g/d safflower seeds + 350 IU Vit E; (3) 340 g/d barley; (4) 226 g/d barley + 350 IU Vit E.		No effects on birth weight, weaning (120 days) weight, number of lamb born, and weaning survival.	Dafoe *et al.* 2008
80 crossbred twin bearing ewes, 21 month old	Pen trial, fed diets contained extra 50, 100, 150, and 200 IU Vit E/d, 7 weeks before lambing.	Ewe plasma Vit E concentration 0.9-2.5 mg/L, colostrum Vit E 4.0-17.9 mg/L; No effect on birth difficult score.	No effects on lamb birth weight and weaning weight, vigour and sucking assistance scores.	Rooke *et al.* 2009
18 ewes, aged 1–9 year	Pen trail, fed diet contained 53 mg/kg Vit E and 0.2 mg/kg SE. Injection i.m. 30 IU Vit E every fortnight, 7 weeks prepartum for totally 24 weeks.	Ewe plasma Vit E concentration: 2.1 mg/L for control, 3.45 mg/L for supplement, Se concentration: 0.20-0.21 mg/L.	Increased IgG in late pregnancy; No effect on serum lysozyme activity.	Anugu *et al.* 2013

Abbreviations: i.m.: intramuscular; s.c.: subcutaneous; Controls were included in all the experiments, but is not listed out in Table.

6. Vitamin E and immunity of ewes and lambs

6.1 Assessment of the immune system

The immune system is a complex network with many cell types and accessory proteins including the antibodies. Function of the immune system can vary widely depending on characteristics of the infection and antigen. Such variations in the immune response make it a real challenge for nutritionists to define the requirement of nutrients for optimal immune function. Stimulating discussions on the generic relationship between nutrition and immunity can be found in a review by Klasing (2007).

The immune system may be divided into innate and adaptive immune systems. The innate immune system provides the first line of defence against invading microorganism, and consists of cell types of phagocytes (neutrophils and macrophages), natural killer (NK) cells, master cells and dendritic cells, and some molecules like acute phase proteins, complements and some cytokines. The invaded bacteria are killed by the phagocytic process. Nature killer cells kill self cells containing viruses and some tumor cells. Master cells and basophils, after being activated, release pharmacological mediators which cause vasodilation, increase vascular permeability, and attract leukocytes into the site of degranulation. The roles of dendritic cells are to recognise microbial antigens through innate receptors and process and present them to T lymphocytes (T-cells). The complement compromises a large number of interdependent proteins with a series of functions including initiating inflammation process, enhancing the engagement of the microbes to various types of the cells and killing the microbes. Acute phase proteins maximize activation of the complements and opsonization of invading microbes (Lydyard *et al.* 2000).

The adaptive immune system consists of B lymphocytes (B-cells, produced in the thymus) and T-cells (produced in the bone marrows), and antibodies and cytokines generated from these cells. The surfaces of B- and T-cells bear receptors specific to antigens, so the cells have memories of the antigens and expand very rapidly when the antigen enters again. Cytokines in both the systems are small molecules secreted by various immune cells in response to stimuli with complex functions including mediating among the cells to regulate immunity (Lydyard *et al.* 2000). Although the immune system is divided into innate and adaptive systems, both of these systems work together (Lydyard *et al.* 2000).

Measuring responses of such a complex immune system to nutritional interventions is an interesting challenge. It is unlikely measures can cover every aspect of the immune systems. A basic question will be what measures can represent immune competency? A review by Cunninghan-Rundles (2002) discusses how to evaluate the effects of nutrients on immune function. Others (Stephensen 2001; Walrand 2001) proposed that the responses of the selected effectors from both the innate (eg, neutrophil count in blood and functions) and adaptive immune (eg, various types of lymphocytes, and antibodies) systems should be monitored.

6.2 Vitamin E and Se status and immune competency

Roles of vitamin E on immunity in mammals has been reviewed thoroughly (Tengerdy 1989; McDowell *et al.* 1996; Meydan and Beharka 1996; Moriguchi and Muraga 2000; Hughes 2002; Rooke *et al.* 2004; Rooke 2008). The basic mechanism of vitamin E on the immune system relies on its functions as an antioxidant to lipid

peroxidation, then probably ROS (Meydan and Beharka 1996). These functions are linked to the innate immune system and the cells involved in the adaptive immunity. Another possible mechanism is that high levels of vitamin E intake can reduce prostaglandin E_2 (PGE_2) which inhibits lymphocyte proliferation and NK cell activity (Hughes 2002). A reduction of glucocorticoids caused by high intakes of vitamin E could also play a role, as glucocorticoids function in immunosuppression (McDowell *et al.* 1996).

Vitamin E deficiency has been shown to impair immune response and supplementation with higher than recommended dietary levels enhancing humoral and cell-mediated immunity. Hughes (2002) reported that vitamin E deficiency (low serum concentration) in 3-year-old humans was associated with lowered lymphocyte proliferation and serum IgM concentration, and increased susceptibility to infectious diseases; while supplementation of vitamin E to premature infants enhanced neutrophil phagocytes but decreased the ability of neutrophils to kill bacteria, and increased antibody production, lymphocytes proliferation, NK cell activity, and macrophage phagocytosis (Hughes 2002). Enhanced serum IgG concentration, lymphocyte stimulation, phagocytosis and humoral immune responses in calves supplemented with vitamin E were also summarized in a review by McDowell *et al.* (1996). In rat, mouse and chicken models, the dietary vitamin E level required to optimize immune responsiveness (lymphocyte proliferation, T cell activities, NK cell activity, phagocytic activity) is much higher than that required for preventing myopathy (Meydan and Beharka 1996).

The effects of vitamin E and Se on the immune status of farm animals have been reviewed by Finch and Turner (1996), and on ewes and lambs were reviewed by Rooke *et al.* (2004). According to Rooke *et al.* (2004) supplementation of vitamin E (300-400 mg/d or 1000-3000 mg per administration) to ewes tended to increase IgG, but not necessarily the antibody titre against the administrated antigens (unless 3000 mg vitamin E was supplemented). In response to Se supplementation (from 0.1 to 1 mg/kg DM) to ewes, the antibody titre was enhanced, IgG in lambs was increased, but not IgG in ewes; when vitamin E and Se were both supplemented, the antibody titre and IgM were increased, but not IgG (Rooke *et al.* 2004). Reduced lymphocyte reactivity and neutrophil activity were found in sheep, cattle, goat, and pigs, fed on diets low in Se (<0.04 mg/kg DM) and vitamin E (<11.4 mg per kg feed respectively) (Turner and Finch 1990). Se deficiency certainly reduces neutrophil functions in goats and cattle and the functions were enhanced by Se, vitamin E, or combined supplements (Finch and Turner 1996). Supplementation of Se to elevate the Se concentration of 0.037 mg/kg DM in the basal diet to 0.16 mg/kg DM fed to ewes throughout the pregnant and lactation increased serum IgG concentration in ewes only in the last third stage of gestation, and IgG concentration in lambs up to 60 day old. The supplements did not influence birth weight of lambs (Rodinova 2008). In a recent report where ewes were given 30 IU α-tocopherol/kg BW at 2 week intervals staring 7 weeks prepartum for 24 weeks, and vaccinated with *Clostridium tetani* and *Clostridium perfringens*, the supplementation increased serum IgG concentration after the vaccination, but had no effects on serum lysozyme (an indicate of neutrophil activity) and serum anti-tetanus toxoid IgG (Anugu *et al.* 2013). It should be noted that the basal diet (16% concentrate pellet plus mixed grass hay) in this report contained vitamin E of 53 IU/kg DM and Se 0.2 mg/kg DM, with corresponding serum vitamin E concentrations >2mg/L (Anugu *et al.* 2013), indicating that the ewes were not vitamin E deficient state according to the criteria discussed previously in this review.

To analyse the possible relationships between dietary vitamin E status, and the effects of supplementation of vitamin E and Se on the innate immune systems, we

pooled the data cited by Finch and Turner (1996), Rooke *et al.* (2004) and some of recent reports in Table 4. The immune responses in this table refer to lymphocyte and neutrophil activities and serum immunoglobulins concentrations (not the antibodies to administered mitogens) measured in the reports. We grouped the responses into no response or positive response to supplementation, and then listed vitamin E and Se concentrations in the corresponding basal diets. Across different species, the positive immune responses to supplementation were generated from the basal diets with Se concentrations <0.08 mg/kgDM with only one exception (0.13 mg/kg DM), whereas when the basal diets contained Se ≥0.08 mg/kg DM no significant immune responses to the supplementation were observed with one exception 0.068 mg/kg DM).

In the no response category, vitamin E concentrations in the diets ranged from 0.012 to 48 mg/kg DM; while in the positive response group, vitamin E concentrations varied from 0.03 to 44 mg/kg DM. These data suggest that dietary Se level is more critical than vitamin E concentration to the innate immune response, and the effects of supplementing vitamin E appear to depend on Se status, not vice versa. Since Se is involved in the redox reactions to scavenge ROS and many types of selenoproteins in redox regulation, it may be extrapolated that the function of Se on the innate immune system may be associated more with the redox state than with lipid peroxidation.

Considering the response in the innate immune system to Se concentrations at <0.08 mg/kg DM, we propose Se concentration of 0.1 mg/kg DM in diet as the minimum concentration to support the immune system. This is in line with the recommendation by Agricultural Research Council (1980).

The data presented in Table 4 does not provide a clear indication of the dietary vitamin E level required for immune competency but it does provide evidence of responses to vitamin E supplements. Avoiding deficiency is certainly necessary. Dietary supplementation of 250-400 IU/kg DM, or intramuscular injections, may be considered to provide benefits to reproductive performance of ewes. These may also ensure no impairment of immune function at the same time.

Table 4. Se and vitamin E concentrations in basal diets in experiments where lymphocyte and neutrophil activities and serum immunoglobulins were measured in responses to supplementation of Se, vitamin E or both.

	No response to supplementation		Positive response to supplementation		References
	Se	**Vitamin E**	**Se**	**Vitamin E**	
Chicken	0.068	11	0.02	ns	
			ns	43	Finch and Turner 1996
Cattle	0.3	0.012	0.05	0.032	Finch and Turner 1996
	0.3	ns	0.01	ns	
			0.016	ns	
			0.06	44	
			0.076	ns	
			0.02	ns	
Pig	0.1	39	0.02	7	Finch and Turner 1996
	0.089	0.29			
	0.15	45			
	0.2	11			
	0.1	22			
Ewe/lamb	0.13	Ns	0.04	ns	Finch and Turner 1996
			0.13	ns	
			0.06	inadequate	
			0.05	ns	
	0.08	5	0.04	ns	Rooke *et al.* 2004
			0.037	ns	Rodinova 2008
	0.2	48			Anugu *et al.* 2013
Goat	0.08	adequate	0.05	0.164	Finch and Turner 1996
			0.05	ns	
			0.08	adequate	

ns: not specified in the report.

6.3 Association of immune status between ewes and their offspring

Considering the weak immune capacity of the neonate, the question arises as to whether immune competency of the neonate and lamb can be enhanced through modulating immune status of their mothers during gestation and lactation. If this is the case, Se and vitamin E deficiency in the mother may compromise acquisition of immune competency in the neonate.

No specific studies on this topic are available however, the neonate gets immunoglobulins from colostrum, and approximately 90% of immunoglobulin is IgG (Hatfield *et al.* 2000). In ruminants, transfer of maternal antibodies to the foetus is prevented by the placenta barriers (Campbell *et al.* 1977). At birth, neutrophils are the predominant lymphocytes in the blood, and complements in the colostrum are

important to enhance uptake of bacteria by neutrophils and monocytes in calves and lambs; the numbers of circulating monocytes and NK cells in newborns is similar to those of adults and phagocytosis is enhanced by ingestion of colostrum (Firth *et al.* 2005). The intestine of the ruminant neonate is permeable to macromolecules during the first 24 to 48 hours, which allows immunoglobulins to be absorbed intact during this period to protect animals (Gilbert *et al.* 1988). Hence ingestion of colostrum is critical for the function of the innate immunity and survival in the neonate.
It would be expected therefore that any reduction in immunoglobulins caused by a deficiency of Se and/or vitamin E could reduce the supply of these immunoglogulins in colostrum to the lamb, and therefore increase the susceptibility of the lamb to infectious diseases causing death.

7. Interactions between vitamin E and other nutrients

Interactions between vitamin E and other nutrients are highly relevant. Many nutrients play a role in as part of the antioxidant system. Others may act as pro-oxidants. The nutritional balance is typically much more relevant during periods of nutritional or physiological stress.

7.1 Polyunsaturated fatty acids

PUFA are a substrate for peroxidation and therefore the concentrations of PUFA in pastures and feedstuffs influences both vitamin status and vitamin E requirements of sheep. Lipid peroxidation increases dramatically when the number of unsaturated groups (double bonds) increases. For example, oxidation of linoleate (C18:2) is 10 times faster than that of oleate (C18:1), and oxidation of linolenate (C18:3) is 20 to 30 times faster than that of oleate (Labuza and Minnesota 1971; Richard 2006). In short, PUFA with more double bonds is more vulnerable to oxidation (Schafer *et al.* 2003). Therefore, high levels of PUFA in feeds and pastures are usually associated with increased PUFA concentrations in cell membranes and the tissues, which raises a risk of increasing lipid peroxidation and resulting in oxidative stress and instability of cell membranes. In such cases more vitamin E is required to counteract the potentially detrimental effects of PUFA peroxidation.
Green pastures contain high levels of PUFA, particularly α-linolenic acid (Dierking *et al.* 2010), but also have high levels of α tocopherol and other antioxidants (Wood *et al.* 2003). Hakkarainen and Pehrson (1987) measured total tocopherol and PUFA (linoleic and linolenic acids only) concentrations in fresh grass, grass silage, hay, barley and oats collected in southwest Sweden. Tocopherol (equivalent to dl-α-tocopherol) concentrations were 200, 176, 19, 33 and 21 mg/kg DM, and linolenic acid was 9.5, 8.8, 2.3, 1.5 and 1.0 g/kg DM; the ratios of vitamin E to linolenic acid were 21.1, 20.1, 8.1 22.3 and 22.5 (mg/g) respectively. These results show that green pastures not only have high levels of linolenic acid (C18:3), but also very high levels of vitamin E. The almost constant ratio of vitamin E to linolenic acids probably means that plants may also use vitamin E to protect their own tissues from oxidative stress. Processing hay results in a loss of vitamin E activity, but has a smaller effect on PUFA concentrations, resulting in high ratio of PUFA to vitamin E. This indicates that feeding hay and crop stubbles may pose a higher risk of creating a vitamin E deficiency than green feed or grains.

7.2 Sulphur amino acid metabolism

The metabolism of sulphur-containing amino acids (SAA, methionine and cysteine) is directly linked to the redox modulation in the body. Relevant activities include:

- Cysteine *per se* is a potent reducing agent, and with cystine forms a redox couple in tissues;
- Cysteine is a substrate for *de novo* GSH synthesis. GSH is the predominant small molecular antioxidant in mammalian tissues;
- A form of selenocysteine, functions as the redox moiety in selenoproteins. This involves redox regulation of intracellular signalling, redox homeostasis and thyroid hormone metabolism (Brown and Arthur 2001). A shift of the redox status in the tissue will alter generation of ROS and the process of lipid peroxidation.

All these functions have an indirect influence on vitamin E requirements and status.

The demand for cysteine in wool-producing sheep, particularly Merinos, adds an extreme burden to the cysteine status and this may compromise its roles in the redox modulation. Cysteine/cystine accounts for about 10% (8.6-13.1%) of the amino acids in wool protein, this is approximately 8 times higher than the 1.3% in the whole body. Therefore higher wool growth in wool-producing sheep compared to meat breeds, will irreversibly draw a large quantity of cysteine from the metabolic pool.

SAA also have a role in immune function. Supplementing SAA to broilers (above requirements for growth) resulted in a significant dose-related increases in total antibody, IgG, and response to the mitogen phytohemagglutinin-P, but not in IgM, suggesting more SAA are required for optimal antibody response (Tsiagbe *et al.* 1987). An abomasal infusion of 2 g/d cysteine to sheep challenged by intestinal parasites showed that cysteine infusion tended to increase peripheral eosinophilia and globular leukocytes in the abomasum, and anti-ovalbumin IgG response, whereas there was no effect on IgG responses to tetanus toxin (Miller *et al.* 2000).

The major source of protein in the small intestines of sheep is derived from ruminal microorganisms. The protein from the microorganisms contains approximately 43 g SAA/kg, similar to the amino acid profiles in tissues, but much lower than that required to support wool growth (Liu and Masters 2003). For this reason, SAA are the primary limiting nutrients for wool growth in Merinos (Reis 1967).

Most importantly, SAA are limiting production when sheep pastures are dead and dry and intake of both protein and energy are low (Mata *et al.* 1997). This means that sheep grazing in a Mediterranean climate have a combined low intake of both SAA and vitamin E during summer and autumn.

7.3 Other nutrients

Other nutrients may also interact with vitamin E in the management of lipid peroxidation within the body. Copper, zinc, manganese, iron or nickel are cofactors on the superoxide dismutase enzymes that catalyses the reaction to convert free superoxide radicals into hydrogen peroxide. Iron is also a cofactor in the enzymes catalase that catalyses the reaction to convert hydrogen peroxide to water and oxygen. Interactions between these elements in the lipid peroxidation process have been demonstrated in laboratory, but not farm animals (Paynter 1980).

8. Conclusions and proposed research priorities

Mediterranean environments have a long term dry season each year. Dry pastures have a very low vitamin E concentration, which produces a high risk of vitamin E deficiency to sheep. Based on the depletion rate of vitamin E in plasma and tissues

as described above, the vitamin E status could change from an adequate state (eg, plasma vitamin E concentration >1 mg/L) to a deficient state (<0.7 mg/L in plasma) over a period of about 2 months. As indicated by White and Rewell (2007) a high proportion of the grazing sheep flock has a low vitamin E status by the end of the dry season.

The supply of vitamin E alone is not the only consideration; other nutrients that work synergistically with vitamin E in the management of oxidative stress and immune function are also in short supply in summer and autumn, particularly the sulphur containing amino acids. Se is also naturally deficient in many high rainfall sheep growing areas. Sheep grazing at this time may therefore be compromised in their capacity to manage oxidative stress and challenge infection.

In considering possible consequences for reproduction further investigation is justified into:

- Low vitamin E, in combination with low levels of other natural antioxidants on the quality and quantity of sperm produced prior to and during mating;
- Follicle development, fertilisation and embryonic mortality in Se supplemented ewes;
- Low vitamin E reserves in the newborn – consequences of large dosages to ewes prior to parturition to boost lamb reserves, and economic assessment;
- Potential benefits in lamb survival through boosting maternal innate immunity with vitamin E supplements;
- Options for boosting antioxidant and immune function in ewes and lambs through “immune pack” nutrient options that may target nutrients lacking in dry pastures (eg vitamin E, SAA, Zn). Consideration should be given to the inclusion of an immunological challenge (eg a parenteral antigen or a biological inert stimulant) within these experiments.

Requirements for vitamin E to prevent NM and for wool growth and liveweight gain appear to be established. In some experiments responses in reproduction and immune function have been reported at higher intakes of this vitamin. This should be investigated where multiple treatment levels are an option.

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