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# Invited review: impact of specific nutrient interventions during mid-to-late gestation on physiological traits important for survival of multiple-born lambs

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*To improve production efficiency, the sheep meat industry has increased flock prolificacy. However, multiple-born lambs have lower birth weights, increased mortality and reduced growth rate compared with single-born lambs. Lamb mortality is a major issue for livestock farming globally and solutions are required to increase survival to realise the value of increased flock fecundity. Nutrition during gestation can influence maternal–foetal placental nutrient transfer and thus foetal growth and organ/tissue development, as well as improve postnatal productivity. This review covers the challenges and opportunities associated with increased prolificacy, highlights gaps in our knowledge and identifies some opportunities for how targeted intervention with specific nutrients during mid-to-late pregnancy may influence lamb survival and productivity with a specific focus on pasture-based systems. This time frame was selected as intervention strategies in short-time windows post-pregnancy scanning and before lambing to improve lamb survival in high-risk groups (e.g. triplets) are likely to be the most practical and economically feasible options for pasture-based extensive farming systems.*

**Keywords:** sheep, nutrients, survival, production, foetus

## Implications

Improving lamb survival and performance is key to enhancing productivity of sheep farming enterprises worldwide. Pastoral-based production systems often present a challenging environment to manipulate nutrition due to difficult terrain, vast land masses and remote locations. The potential for targeted dietary interventions to influence a range of production phenotypes including survival, growth and meat production offers exciting opportunities to realise the value of increased ewe fecundity. Targeted nutritional interventions in critical developmental time windows may offer potential tools for farmers to improve lamb survival and production performance especially in multiple-born lambs.

## Introduction

Perinatal lamb mortality is a major welfare and production issue for sheep farming systems worldwide. In Australia alone, the production costs are estimated at AU$ 450M with prevention costs estimated at AU$ 100M (Lane *et al.*, 2015). Perinatal mortality is a complex problem involving the interaction of nutrition, environmental factors, sheep genotype and management.

Pasture-based sheep production is a relatively low cost, efficient and sustainable system that enable countries like New Zealand to compete as a global exporter of food and fibre (Morris and Kenyon, 2014). In New Zealand, >95% of the sheep diet is provided through grazed pasture and forage crops (Hodgson *et al.*, 2005) or even higher in the hill country environments where topographical challenges limit the ability to feed supplements. With expansion of the dairy industry, sheep farming is now located in these more challenging hill country environments which is often of lower fertility and subject to climatic extremes (Morris and Kenyon, 2014). These changes in the farming system pose additional changes to identifying intervention strategies to improve lamb survival.

As there is minimal genetic control over litter survival, with the main source of variation being temporary environmental effects (Everett-Hincks *et al.*, 2005), nutrition is probably one of the most important environmental effects that influences

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lamb survival and performance. As such, feeding management and/or strategic feeding systems may produce tools for farmers to improve lamb survival. Prior reviews have described the effect of nutrition during the peri-conceptional period on foetal programming and health (Oliver *et al*., 2007; Fleming *et al*., 2012), the role of the plane of maternal nutrition and foetal programming on production (Symonds *et al*., 2010; Kenyon and Blair, 2014), vascularity of nutrient transferring issues including the placenta (Vonnahme *et al*., 2015), the effect of maternal trace element and vitamin supplementation on the lamb (Rooke *et al*., 2008), the impact of amino acids (AA) in sheep production (McCoard *et al*., 2016) and the potential reasons for the lack of transfer of scientific knowledge into practice to improve neonatal survival in small ruminants (Dwyer *et al*., 2016). This review focusses on the impact of maternal supplementation with specific nutrients in the mid-to-late gestation period, the potential underpinning mechanisms involved and the potential opportunities to increase survival of multiple-born lambs in pasture-based grazing systems with a particular focus on placental nutrient transfer, birth weight, viability and thermoregulation.

## Production impact of multiple births

Implementation of nutritional, genetic, management and health strategies by New Zealand sheep farmers has resulted in a 24% increase in the number of lambs born per ewe mated in the last 18 years (increase of 100% to 124% from 1990; Morel *et al*., 2008). However, lambing percentages >200% have been described (Shorten *et al*., 2013), which are associated with a greater proportion of twin- and triplet-born lambs (Amer *et al*., 1999).

It is well accepted that twins and triplets have reduced birth weight which leads to higher mortality rates at lambing compared with singletons (Scales *et al*., 1986; Gootwine *et al*., 2007). An average mortality rate of 15% to 20% in twins and 25% to 40% in triplets has been reported in New Zealand (West *et al*., 2008; Stafford, 2013), with similar rates observed in Australia (Hinch and Brien, 2014) and other areas of the globe (Rowland *et al*., 1992; Dwyer, 2007). The first 24 h of life is critical for lamb survival, with nearly 50% of all mortalities occurring during this time frame (Dwyer, 2007). The negative relationship between lamb survival and number of lambs born highlights the importance of identifying strategies to increase lamb survival in multiple-born lambs to realise the value of the improvements in ewe fecundity.

## Intra-uterine growth restriction

Intra-uterine growth restriction (IUGR) is common in multiple-born lambs and is a significant problem for agricultural animal production. The term IUGR is often used to describe a wide range of phenotypic outcomes in offspring that have experienced a restricted intra-uterine environment, and is defined as decreased intra-uterine growth velocity (Ergaz *et al*., 2005) and thus reduced foetal growth potential. Most studies have focussed on the target outcome of human health where IUGR is often a condition resulting from drastically reduced conceptus nutrient and oxygen supply in late gestation mainly resulting from placental insufficiency. The consequences of IUGR in multiple-born lambs include reduced foetal growth and thus birth weight, higher mortality rates (see review by Kenyon, 2008), reduced neonatal growth rate and lower muscle mass (McCoard *et al*., 1997). In addition, IUGR can result in permanent negative effects on growth, feed efficiency, body composition and thus poor finishing, meat quality and long-term health, thereby decreasing farmer profits (Wu *et al*., 2006; Kenyon and Blair, 2014).

## Placental development and nutrient transfer

The primary determinant of foetal growth, and thus lamb birth weight, is the supply of nutrients which depends on placental transport as illustrated by the positive correlation between placental and foetal weight (Mellor, 1983). Sheep have a cotyledonary placentation where the exchange of nutrients and waste products happens at discrete sites called placentomes (Ford, 2000). These discrete units of foetal–maternal exchange are composed of foetal (cotyledon) and maternal (caruncle) components (Ford, 2000) which can be classified based on their shape (type A to D) and may differ in their maternal–foetal exchange area, oxygen exchange efficiency and glucose transport (Fowden *et al*., 2006). Transport of nutrients across the placenta is determined by a range of factors including the concentration gradient between maternal and foetal blood, placental blood flow and metabolism, and specific membrane-bound transporter expression and activity.

In sheep, uterine capacity is a key factor limiting foetal survival and growth, especially when ewes are carrying multiples (Gootwine *et al*., 2007). Lower birth weights in multiple litters are associated with smaller placentae with reduced placentome number and weight per foetus in twin compared to singleton foetuses (McCoard *et al*., 2001; Rumball *et al*., 2008; van der Linden *et al*., 2013) and decreased total placental vascularity (Vonnahme *et al*., 2008), suggesting reduced placental nutrient transport. However, the smaller placentas associated with twins have been shown to be more efficient (van der Linden *et al*., 2013) which may be a function of compensatory changes in placental structure and function to deliver an adequate nutrient supply to the foetus to support growth (Rumball *et al*., 2008). The role of changes in placentome morphology on nutrient transport has not been evaluated directly and warrants further investigation.

Developmental changes occur in maternal and foetal plasma AA concentrations during pregnancy in sheep. Factors that influence maternal and foetal AA profiles include breed (Ashworth *et al*., 2011), the stage of pregnancy

(Kwon *et al.*, 2003) and maternal nutrient status (Kwon *et al.*, 2004). We have reported that increased foetal growth in response to maternal arginine supplementation from 100 days of pregnancy to term (McCoard *et al.*, 2013) is associated with improved placental growth, development and function (van der Linden *et al.*, 2015). Twins have reduced plasma arginine, leucine, histidine and glutamine compared with singletons (van der Linden *et al.*, 2013), which indicates that AA transport may differ between twin and single placentae and/or differential metabolism of the foetoplacental unit. The mechanism responsible for these differences is unclear however leucine, arginine and glutamine are known activators of the mechanistic target of rapamycin (mTOR) signalling pathway which is a placental nutrient sensor (Roos *et al.*, 2007) that coordinates maternal nutrient availability and foetal nutrient supply (Jansson and Powell, 2006). In early pregnancy, this pathway plays an important role in the survival and development of the ovine conceptus (Kim *et al.*, 2011) and in humans; placental mTOR signalling is markedly down-regulated during IUGR (Roos *et al.*, 2007). Hyperthermia-induced growth restriction in sheep is also associated with perturbations in placental mTOR signalling (Arroyo *et al.*, 2009). In other species, mTOR signalling controls placental AA transport by regulating the expression of specific AA transporters (Roos *et al.*, 2005 and 2007). Thus, the activation of placental mTOR signalling and AA transporter expression leading to increased foetal growth is a potential mechanism underpinning the effect of maternal arginine supplementation on ovine foetal growth.

Another potential mechanism mediating the effect of maternal arginine supplementation on foetal growth is placental metabolism of arginine into nitric oxide (NO) which is a major mediator of ovine placental–foetal blood flow during pregnancy (Rosenfeld *et al.*, 1996). Arginine is the major substrate used for NO production and the two enzymes responsible for this process are inducible (iNOS) and constitutive (cNOS) nitric oxide synthase. Kwon *et al.* (2004) report that the activities of iNOS and cNOS and levels of NO peak in the intercotyledonary placenta, placentome and intercaruncular endometrium during mid-to-late gestation of Columbia crossbred ewes – a period of rapid foetal growth. In addition, it has been reported that treatment of Suffolk ewes, restricted to 50% NRC requirements with sildenafil citrate, dose-dependently increased total AA's and polyamines in amniotic fluid, allantoic fluid and foetal serum without affecting values in maternal serum, and foetal weight in nutrient-restricted ewes (Satterfield *et al.*, 2010). Sildenafil citrate works by inhibiting the enzyme that breaks down cyclic guanosine monophosphate, the metabolite produced by NO stimulation of guanylate cyclase, and which is responsible for tissue vasodilation. Interestingly, it has recently been shown that NO synthesis stimulates mTOR activation (Ito *et al.*, 2013; Capobianco *et al.*, 2015), suggesting that either of these pathways maybe involved in regulating ovine placental AA transport, and potentially contribute to the differences in placental efficiency between twins and singletons. A greater functional understanding of the foetoplacental unit in relation to nutrient transfer in multiple-born lambs is required to develop nutritional intervention strategies to improve foetal outcome in sheep.

## Birth weight

Birth weight is a key contributing factor to lamb mortality with low birth weight increasing a lamb's risk for starvation and exposure (Dwyer and Morgan, 2006). The birth weight of triplet-born lambs was reported to be 19% to 24% lower than twin-lamb birth weights (Morris and Kenyon, 2004; Everett-Hincks *et al.*, 2005; Everett-Hincks and Dodds, 2008), and 36% to 40% lower than singles (Scales *et al.*, 1986). Lower birth weight is associated with increased surface area to body–mass ratio and lower body energy reserves (Alexander, 1978) which can increase mortality when exposured to cold conditions (Dwyer and Morgan, 2006). The optimal birth weight range for lamb survival is 4 to 6 kg (Dalton *et al.*, 1980; Morel *et al.*, 2008).

Specific AA supplementation during pregnancy has been shown to enhance foetal growth, and thus birth weight in sheep. Notably, intravenous bolus injection with 155 μmol arginine–HCl/kg BW three times daily between 60 days gestation and birth increases birth weight in single and twin lambs from under-fed ewes (Lassala *et al.*, 2010). However, while the birth weight of quadruplet lambs was increased when well-fed Booroola Rambouillet ewes were injected with an intravenous bolus of 345 μmol arginine–HCl/kg BW, three times daily, from 100- to 21 days of gestation, the birth weight of triplets, twins or singletons was unchanged (Lassala *et al.*, 2011). In contrast, when twin-bearing Romney ewes were given an intravenous bolus injection of 345 μmol arginine–HCl/kg bodyweight, three times a day, from 100 days gestation to birth, the birth weight of female but not male lambs was increased (McCoard *et al.*, 2013) suggesting supplementation during the last 2 weeks of gestation may have the potential to influence the birth weight of twin-born lambs. Alternatively, the differences between these studies may reflect breed differences in their response to AA supplementation, or potentially an influence of the nutritional value of the basal diet despite both being formulated to meet or exceed National Research Council (NRC) requirements. Overall, these studies highlight the potential for specific AA supplementation during key developmental time windows (late gestation) to influence lamb birth weight which may have important consequences for survival, especially in lower birth weight lambs. The effect of supplementation with other AA beyond arginine on lamb birth weight has yet to be evaluated. Furthermore, delivery methods that enable delivery of AA via the diet to avoid rumen degradation such as rumen-protected formulations or AA analogues (McCoard *et al.*, 2016) are required before practical evaluation of the impact of AA supplementation can be evaluated in pasture-fed multiple-bearing ewes on farm.

Iodine deficiency can lead to lamb mortality (Sargison *et al.*, 1998). Lamb birth weight is negatively affected by grazing ewes on kale crops during gestation, an effect which is reversed with maternal iodine supplementation during pregnancy (i.m.: iodised arachis oil; high = 400 mg, medium = 300 mg; Knowles and Grace, 2015). Kale is a complementary forage crop that has low iodine concentrations and contains glucosinolates that inhibit thyroid utilisation of iodine through releasing thiocyanate goitrogens (Stoewsand, 1995), therefore in this case, maternal iodine supplementation was correcting for iodine deficiency. However, provided dietary intake of >0.2 to 0.30 mg I/kg dry matter is obtained (Grace and Knowles, 2010), dietary intake of iodine is usually adequate. Consistent with this notion, birth weight is unaffected by maternal iodine supplementation (26.6 mg/day in the diet) from 119 day gestation to term in ewes fed fresh silage (McGovern *et al.*, 2015). Similarly, lamb birth weight was unaffected in twin- or triplet-born lambs from pasture-fed ewes supplemented with iodine (i.m. injection of 1.5 ml iodised peanut oil 35 days *postpartum*) despite elevated maternal iodine levels throughout gestation (Kerslake *et al.*, 2010). These studies suggest that provided ewes are not iodine deficient, supplementation with iodine during mid-to-late gestation is likely to have limited impact on survival of multiple-born lambs.

Maternal supplementation with polyunsaturated fatty acid (PUFA) increases gestation length in several species (reviewed by Capper *et al.*, 2006) resulting in a more physiologically mature foetus at birth. However, supplementation with 12 g/ewe per day algae-derived PUFA DHA in twin-bearing Targhee ewes in the last 30 days of gestation and early lactation had no effect on lamb birth weight (Keithly *et al.*, 2011). Other studies have also demonstrated variable effects of trace elements and vitamins throughout gestation on lamb birth weight (reviewed by Rooke *et al.*, 2008). However, studies where ewes are supplemented in the last trimester of gestation are scarce. Capper *et al.* (2005) reported that vitamin E supplementation (500 mg/kg; 6 weeks *prepartum*) of twin- and triplet-bearing ewes increased lamb birth weight. Supplementation of pasture grazing ewes deficient in cobalt with 0.03 or 0.06 mg cobalt/day via weekly drenching throughout gestation also increased lamb birth weight (Quirk and Norton, 1987). However, maternal supplementation with selenium, an antioxidant, had inconsistent effects (Hammer *et al.*, 2011). More research is required to establish whether maternal supplementation strategies in mid-to-late gestation can benefit lamb birth weight in pasture-fed ewes where trace element and mineral status of the ewes is adequate. The practical considerations for maternal trace element supplementation to ewes in pasture-based systems has been reviewed elsewhere (Grace and Knowles, 2012).

## Neonatal vitality

Lighter birth weight, newborn lambs or lambs with lower rectal temperatures exhibit reduced vigour (Dwyer and Morgan, 2006), and less drive to suckle (Alexander and Williams, 1968), which increased their risk of hypothermia (Dalton *et al.*, 1980). Increased mortality and morbidity of multiple-born lambs has been linked to compromised immune function (Dønnema *et al.*, 2015). Vitamin E is one of the micronutrients that may have an impact on immune functions and health. It protects biological membranes from oxidative damage by acting as scavengers of reactive oxygen species and is linked to IgG production (Huber, 1988). A number of vitamin E supplementation studies have been conducted during mid-to-late pregnancy to assess the production performance of multiple-born lambs. Dønnema *et al.* (2015) have shown that oral vitamin E supplemented (360 IU/ewe per day; 6 to 7 weeks *prepartum*) Norwegian White Sheep with ⩾3 lambs have a significantly lower rate of stillbirths compared with control ewes. However, this was not observed in ewes with ⩽2 lambs, which is in agreement with a previous study conducted in twin-bearing Hardy Speckled Faces ewes orally supplemented with 200 IU vitamin E per ewe per day for the last 8 weeks of gestation (Merrell, 1998). The mechanism of action is currently not known, however it could be linked to reduced oxidative stress or lack of IgG stimulation in twin-bearing ewes (Daniels *et al.*, 2000).

Long-chain PUFAs have also been used to assess their effect on lamb viability, as they are known to influence neuronal division, synaptic transmission and retinal development potentially improving early neonatal behaviour. Several studies have shown that supplementation of twin- and triplet-bearing ewes with PUFAs in the last 4 to 9 weeks of gestation improved lamb vigour (Capper *et al.*, 2005 and 2006; Pickard *et al.*, 2005 and 2008). For example, inclusion of 6 or 12 g of DHA from 9 weeks before lambing improved measures of lamb vigour including time to suckle and time to stand (Pickard *et al.*, 2005 and 2008).

## Thermoregulation

Brown adipose tissue (BAT) is a specialised fat store that is used by the newborn lamb to generate about 50% of the total heat produced (Symonds and Lomax, 1992; Satterfield and Wu, 2011), facilitating an effective adaptation to the cold challenge of the extra-uterine environment and preventing hypothermia (Alexander and Williams, 1968). Hypothermia is a major cause of on-farm lamb losses in the first few days of life (Everett-Hincks and Dodds, 2008). Low birth weight lambs exhibited lower rectal temperatures (Dwyer and Morgan, 2006), greater lactate concentrations (Stafford *et al.*, 2007) and lower plasma thyroid hormone concentrations (Kerslake *et al.*, 2010). These factors are known to negatively impact on the ability of a newborn lamb to maintain body temperature after birth and likely contribute to mortality (Kerslake *et al.*, 2010). We have shown that during cold exposure there was a rapid decrease in heat loss in the newborn lamb (McCoard *et al.*, 2014b). Therefore, increasing BAT stores and/or the activity of BAT has the potential to improve survival.

Rooke *et al.* (2008) reviewed the role of trace elements and vitamin supplementation of the ewe on various traits in the lamb including thermoregulatory capacity. Of the micronutrients evaluated (Cobalt, Copper, Iodine, Iron, Manganese, Selenium (Se), Zinc, vitamins A and E and n-3 fatty acids), Se, vitamin E and fatty acids were identified as the most likely candidates to improve lamb survival. Many of the studies undertaken have evaluated supplementation throughout pregnancy and/or have studied the responses in ewes fed a concentrate or a conserved forage-based diet, rather than within a pasture-based feeding system. As some diets are deficient in some micronutrients, for example lower vitamin E levels in dry stored feeds compared with spring fresh forage (Kivimae and Carpena, 1973), many of the studies reported in the literature may have limited application to a pasture-based system. Trace element supplementation in pasture-fed ewes can improve lamb performance (Grace and Knowles, 2012) however specific evaluation of thermoregulatory capacity of the neonates following maternal supplementation has not been directly evaluated.

Specific PUFA such as linoleic acid are a key energy source for BAT in lambs (Lammoglia *et al*., 1999). However, twin-bearing Taghee ewes supplemented with 12 g/ewe per day of algae-derived DHA during the last 30 days of gestation had no effect on lamb thermogenesis (Keithly *et al*., 2011). In contrast, twin-bearing ewes fed rumen-protected fat which was high in saturated and monounsaturated fatty acids or high in n-6- and n-3-PUFAs at a level of 2% or 4% for the last 40 days of gestation may improve cold tolerance in newborn lambs (Chen *et al*., 2007). It is important to note however, that when fed at 8% of the ewe diet, cold tolerance was markedly reduced coupled with reduced palmitate oxidation from BAT indicating decreased ability to oxidise fatty acids, independently of cytochrome c oxidase activity, GDP binding or uncoupling protein 1 (UCP-1) gene expression. These observations highlight the potential for PUFAs to increase thermogenesis but in a dose-dependent manner.

Iodine supplementation of the ewe can elevate thyroid hormone level in the ewe and newborn lamb (Andrewartha *et al*., 1980; Rose *et al*., 2007) which may increase rectal temperatures in the lamb compared with supplemented lambs (Donald *et al*., 1994). Negative relationship between maternal iodine supplementation in late gestation and immunoglobulin G (IgG) levels in the newborn lamb have been reported (Boland *et al*., 2006; Rose *et al*., 2007; Boland *et al*., 2008). Recently, McGovern *et al*. (2015) reported that ewes supplemented with 26.6 mg/day iodine (either as calcium iodate or potassium iodide) mixed in concentrate feed as a carrier, from 119 days gestation to term, was linked to a failure of IgG absorption and thus passive transfer which may have been the result of suppressed thyroid hormone status. These results imply there are negative effects of maternal iodine supplementation in late gestation, however, the direct effect on lamb survival and subsequent impact on lamb survival and immune function later in life remain to be established. In ewes fed a 100% pasture diet, Kerslake *et al*. (2010) reported no difference in lamb heat production following maternal iodine supplementation (i.m. injection of 1.5 ml iodised peanut oil; Flexidine 26% w/w iodine bound to ethyl esters of unsaturated fatty acids in oil 35 days before mating) despite elevating maternal iodine levels throughout gestation.

Parenteral arginine supplementation of well-fed twin-bearing ewes from 100 to 140 days of gestation has been shown to increase brown fat stores in the foetuses at 140 days gestation by about 15% (McCoard *et al*., 2013) through increased fat cell hypertrophy, resulting in a 0.6°C increase in core-body temperature of twin-born lambs within 2 h of birth (McCoard *et al*., 2014a). Increased BAT deposition in foetuses from under-fed ewes and diet-induced obese sheep at 125 days of gestation has also been reported in response to maternal arginine supplementation (Satterfield and Wu, 2011). These studies highlight the benefits of maternal arginine supplementation to increase thermoregulatory capacity and potential survival. However, validation of these findings in the field, and quantification of the impacts on lamb survival are required.

The development of BAT and onset of BAT thermogenesis is mediated by rapid up-regulation of genes including UCP-1 around birth (Symonds *et al*., 2011). Expression of UCP-1 is a marker of BAT thermogenesis and several factors and cofactors influence UCP-1 expression including PPAR$\gamma$-co-activator-1$\alpha$ (PGC-1$\alpha$) which regulates mitochondrial biogenesis and oxidative metabolism and PRD1-BF-1-RIZ1 homologous domain containing protein-16 (PRDM-16) which is responsible for BAT lineage determination (Kajimura *et al*., 2010). We have shown that increased BAT mass in late gestation foetuses in response to maternal arginine supplementation is associated with increased expression of UCP-1 and PRDM-16, and that plasma cortisol may up-regulate UCP-1 expression in the near-term ovine foetus (McCoard *et al*., 2014a). Up-regulation of PRDM16 indicates that arginine may signal the commitment of precursor cells to the BAT lineage which in turn may have important implications maintaining neonatal core-body temperature, as well as mediating whole body metabolism, adipocyte-muscle cross-talk and energy partitioning (Satterfield and Wu, 2011, Tan *et al*., 2012). Nitrous oxide and mTOR signalling have been implicated in the arginine-induced changes in mitochondrial biogenesis and thus BAT (Tan *et al*., 2012). In the ovine neonate mTOR signalling may play a greater role (McCoard *et al*., 2014a); however, this remains to be evaluated directly.

Although non-shivering thermogenesis is the first line of defence against cold exposure in the newborn lamb, the second line of defence is shivering thermogenesis which is initiated only after body temperatures fall significantly (Alexander and Williams, 1968). Shivering thermogenesis can provide up to 50% of maximal heat production during cold exposure in the newborn lamb. Shivering and non-shivering thermogenesis to facilitate heat production during cold exposure in the newborn lamb are equally important, with shivering thermogenesis becoming the primary source of heat production after the first few days of life (Alexander and Williams, 1968). It has been postulated that adaptation to the extra-uterine environment post-birth may involve cross-talk between different muscle and fat deposits and their interaction with other organs involved in BAT function (Symonds, 2013), however these interactions remain to be elucidated. Skeletal muscle and BAT may have a common origin (Seale *et al*., 2008) and in humans a link between muscle volume and functional BAT has been suggested in children and adolescents (Gilsanz *et al*., 2011), highlighting the potential importance of skeletal muscle growth during gestation and its contribution to BW thermoregulatory capacity and thus survivability at birth.

Twin-born lambs have reduced muscle mass compared with singletons (McCoard *et al*., 1997), with the divergence in muscle mass appearing after 100 days gestation (McCoard *et al*., 2001) contributing to the lower birth weight of twins. During foetal development, skeletal muscle has lower priority, in terms of nutrient partitioning, compared with other tissues such as brain, heart and liver, resulting in muscle being more vulnerable to nutrient deficiency (Zhu *et al*., 2006). Newborn lambs also exhibit high rates of AA oxidation supporting the notion that low-birth weight lambs at birth are less mature compared with high birth weight lambs in some aspects of metabolic and endocrine development (Greenwood *et al*., 2002).

Amino acid availability regulates skeletal muscle mass by stimulating protein synthesis and reducing protein degradation. Amino acids act as precursors of nitrogenous substances, such as polyamines and NO which likely mediate growth and development of muscle fibres (Wu *et al*., 2010). In addition, AA exert a signalling effect on the regulating factors controlling myogenesis (Yoon and Chen, 2013). In the later stages of pregnancy, skeletal muscle growth increases rapidly and the foetus responds to infusion of specific (e.g. arginine) or a mix of AA by increasing protein synthesis (Liechty *et al*., 1999; de Boo *et al*., 2005). This response during foetal life appears to be associated with the plasma level of insulin in the foetus (Brown and Hay 2006). However, in IUGR sheep models, when AA are infused directly into the foetus, net foetal protein accretion increases independently of insulin changes (Brown *et al*., 2012).

**Table 1** *Summary of the observed effects on foetal-neonatal growth and development when supra-nutritional levels of specific nutrients are supplied to multiple-bearing ewes during mid-to-late pregnancy*

| Interventions | Protocol | Phenotypic effect | Reference |
|---|---|---|---|
| Arginine | 345 μmol Arg–HCl/kg BW 3 times daily to from 100 days of gestation to term | Improved placental growth, development and function | van der Linden *et al.* (2015) |
| | 155 μmol Arg–HCl/kg BW 3 times daily between 60 days gestation and birth | Increased birth weight in single and twin lambs | Lassala *et al.* (2010) |
| | 345 μmol Arg–HCl/kg BW 3 times daily to from 100 to 121 days of gestation | Increased birth weight of quadruplet lambs | Lassala *et al.* (2011) |
| | 345 μmol Arg–HCl/kg BW 3 times a day from 100 days gestation to birth | Increase in birth weight of female but not male lambs<br>Increased brown fat stores in the foetuses | McCoard *et al.* (2013) |
| | 345 μmol Arg–HCl/kg BW 3 times a day from 100 to 140 days gestation | Increase in core-body temperature | McCoard *et al.* (2014a) |
| | 345 μmol Arg–HCl/kg BW 3 times a day from 100 days gestation to birth | Increase in the capacity for protein synthesis in foetal muscle | Sales *et al.* (2014) |
| Iodine | 100 mg potassium iodide/2 weeks; 90 days *prepartum* | Increased rectal temperatures | Donald *et al.* (1994) |
| | 200 IU/ewe per day in the last 8 weeks of gestation | No effect on birth weight | Merrell (1998) |
| | 26.6 mg/day as calcium iodate or potassium iodide from 119 days gestation to term | No effect on birth weight<br>Failure of IgG absorption | McGovern *et al.* (2015) |
| | I.m injection of 1.5 ml iodised peanut oil 35 days pre-mating (long-acting depot) | No effect on birth weight<br>No effect on heat production | Kerslake *et al.* (2010) |
| Fat | 2%, 4% or 8% (of total fat intake) rumen-protected fat (high in saturated or monounsaturated fatty acids); 40 days *prepartum* | Increased cold tolerance in newborn lambs in the 2% and 4% groups and reduced cold tolerance in the 8% group | Chen *et al.* (2007) |
| | PUFA 45 g/kg concentrate | Increased lamb vigour | Capper *et al.* (2006) |
| | DHA; 12 g/ewe per day 9 weeks *prepartum* for varying durations | Trend for increased gestation length<br>Increased lamb vigour | Pickard *et al.* (2005 and 2008) |
| | DHA; 12 g/ewe per day DHA in the last 30 days of gestation | No effect on birth weight | Keithly *et al.* (2011) |
| Vitamin E | 500 mg/kg; 6 weeks *prepartum* | Increased lamb birth weight of twin and triplet | Capper *et al.* (2005) |
| | 360 IU/ewe per day; 6 to 7 weeks *prepartum* | Decreased rate of stillbirths | Dønnema *et al.* (2015) |

IgG = immunoglobulin G; PUFA = polyunsaturated fatty acid.

During late gestation, changes in specific rather than total intracellular muscle AA concentrations are associated with lower muscle mass in twins (Pacheco *et al*., 2010). Notably, arginine and glutamine appeared to be closely related to foetal mass and the mass of the semitendinosus muscle (Pacheco *et al*., 2010). Further, a reduction in the concentration of specific intracellular free AA such as arginine, leucine, valine and glutamine which play important roles in muscle growth, may be limiting for skeletal muscle hypertrophy in twins (Sales *et al*., 2013), consistent with the correlation between the weight of the foetal semitendinosus muscle in twins with intracellular concentrations of free arginine ($r = 0.66$, $P < 0.01$) and glutamine ($r = 0.49$, $P < 0.01$) in late gestation (Sales *et al*., 2014).

Compared with singletons, twin foetal sheep have down-regulated mTOR signalling in late gestation which may be related to long-term restricted nutrient availability leading to reduced ribosome number and abundance of the translational machinery per ribosome (Sciascia *et al*., 2010). These results may explain, at least in part, the restricted myofibre hypertrophy and reduced muscle mass observed in twins relative to singletons. Activation of mTOR signalling in skeletal muscle is under the control of the arginine-family of AA (e.g. arginine and glutamine) and leucine (Meijer and Dubbelhuis, 2004). Consistent with this notion, maternal intravenous bolus injection of arginine three times daily from 100 days gestation to birth increased the capacity for protein synthesis in foetal muscle which is associated with increased abundance of mTOR near birth (Sales, 2014). In the same experiment, we reported that maternal arginine administration increased core-body temperatures of the lambs within 2 h of birth (McCoard *et al*., 2014a). Although arginine increased the capacity for skeletal muscle growth in lambs, the potential cross-talk between skeletal muscle growth and thermoregulatory capacity of the lambs remains to be elucidated. Furthermore, the effect of maternal arginine supplementation on lamb survival in pasture-fed ewes in a commercial farm system also remains to be determined.

## Future prospects

Improved productivity and profitability of the sheep meat industry has been made possible by increasing lambing percentages. However, multiple-birth lambs suffer from IUGR which negatively impacts early-life development and growth. The application of specific nutritional components such as AA, vitamins, trace elements and PUFAs in mid-to-late gestation (summarised in Table 1) have the potential to influence traits associated with lamb survival including placental nutrient transfer and thus foetal growth, birth weight, lamb vigour and thermoregulatory capacity. Undoubtedly, further research into the utility of macro and micronutrients in pasture-fed ewes on foetal growth, lamb survival and postnatal performance, critical intervention time windows and identification of delivery routes and stages of growth that are both cost-effective and practical to implement in pasture-based grazing systems should be the focus of future research activities. Further, discovery of the role other nutrients play in regulating foetal growth and survival is required to increase our knowledge of the potential for nutraceuticals to decrease lamb mortality and morbidity. We hope the animal field will grasp this line of research and continue to expand this knowledge base and the potential it has in improving sheep production.

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