Exposure of ewes to stressors in mid- and late-pregnancy: Postnatal effects on the ewe and lamb

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ABSTRACT

This thesis set out to examine the effect of maternal undernutrition and exposure to stressors between pregnancy day 50 to 100 and 100 to 147 of pregnancy on the ewe and her lamb. The long-term effects of these stressors during pregnancy on lamb growth, plasma cortisol response to a stressor, metabolism, behaviour and future reproductive success were examined.

Mid-pregnancy shearing

The component of mid-pregnancy shearing that causes the increase in lamb birth weight is unknown. It was hypothesised that the increase in lamb birth weight was due to the stress response of the ewe to shearing. This work examined the effect of a range of stressors at approximately day 80 of pregnancy. These stressors included yarding, crutching and sham-shearing that may be components of the shearing procedure that produce a stress response. In addition, repeated stressors between day 74 and 106 of pregnancy including isolation, sham-shearing and exogenous cortisol injection were used to examine the role of a longer-term stress response on lamb birth weight.

Mid-pregnancy shearing has consistently resulted in an increase in lamb birth weight, however all the other stressors investigated had no effect. Therefore, the hormonal stress response of ewes to shearing was unlikely to be the cause of the increase in lamb birth weight. Mid-pregnancy shearing also resulted in minor changes in ewe and lamb behaviour 12 to 24 h after birth. Shearing during pregnancy had no effect on the cortisol response of ram lambs to handling or castration however differences were observed between singleton- and twin-born lambs.

Ewe nutrition during pregnancy

Ewes mildly undernourished in between days 70 and 107 of pregnancy that were then provided with adequate nutrition between days 108-145 of pregnancy gave birth to lambs with similar birth weights as ewes well-fed during both periods. Therefore the effects of undernutrition on lamb birth weight can be minimised if undernutrition can be limited to the earlier period (day 70-107 and 108-147 of pregnancy). Mild undernutrition in both periods (day 70 – 145 of pregnancy), resulted in lambs that were lighter and that exhibited behaviours associated with a greater ‘drive’ to maintain contact with their dam than lambs born to ewes well-fed during the same period. This suggests that even mild undernutrition in both mid- and late- pregnancy should be avoided.

The effect of maternal nutrition and lamb litter size on the behaviour of female offspring was examined at 1 and 2 years of age. Litter size had no effect on ewe behaviour at 1 year of age however at 2 years of age twin-born ewes had higher maternal behaviour scores than triplet-born ewe lambs. Nutrition of the maternal grand dam during pregnancy had only a minor effect on the behaviour of female offspring at 1 year of age and no effect on maternal behaviour at 2 years of age.

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INTRODUCTION

The management of ewes during pregnancy can have implications for the survival and later performance of offspring. During pregnancy the exposure of ewes to stressors (shearing, isolation and transport), inadequate nutrition and increased litter size not only alter lamb birth weight and survival but may also result in long term changes in the behaviour, metabolism and stress response of offspring. The management issues of greatest interest in New Zealand are the use of mid-pregnancy shearing, level of nutrition during pregnancy and the increase in litter size resulting from a push towards increasing reproductive performance.

Mid-pregnancy shearing (approximately day 70 of pregnancy) causes an increase in birth weight of between 6 and 16% in singleton lambs and 5-26% in twins (Morris and McCutcheon, 1997; Smeaton et al., 2000; Revell et al., 2002; Kenyon et al., 2004). Pregnancy-shearing (commonly occurring between day 50 and 100 of pregnancy) has other advantages including improved wool quality, enhanced shelter seeking behaviour of ewes at parturition and a reduction in the number of ewes becoming recumbent (Husain et al., 1997; Morris et al., 1999; Kenyon et al., 2003). However, shearing during pregnancy exposes the ewe to cold, wet and windy winter weather conditions that can result in hypothermia and death (Dabiri et al., 1995a; Dabiri et al., 1995b; Husain et al., 1997).

Ewes underfed during pregnancy have lambs that are lighter at birth than well-fed ewes (Mellor, 1983; Robinson et al., 1999). Feeding guidelines to achieve optimal lamb birth weights for singleton, twin and triplet bearing ewes have been identified (Morris et al., 1993; Morris and Kenyon, 2004). Some regions within New Zealand, however, do not have sufficient herbage growth during the winter months to maintain ewes at the prescribed levels (Matthews et al., 2000) thereby resulting in ewes fed at sub-optimal levels during pregnancy.

In New Zealand, ewe lambing percentages have increased from 100% in 1990 to 128% in 2005 (M&WNZ - Economic Service, 2006a) which has resulted in an increase the proportion of ewes bearing twin and triplet fetuses (Amer et al., 1999). Twin and triplet lambs have greater mortality rates in the first 3 days of life than singleton lambs (Dalton et al., 1980) which is due in large part to their lighter birth weights (Hinch et al., 1983). It is hypothesised that an increase the birth weight of twin and triplet lambs should increase their survival rates to weaning.

This thesis will investigate each of these issues mostly at a practical level with some supporting data on metabolic and hormonal principals. The general hypothesis investigated is that the “management of ewes during pregnancy can alter lamb birth weight and the performance of offspring later in life”. The thesis firstly addresses the mid-pregnancy shearing by investigating what component of shearing in mid-pregnancy (on approximately day 70 of pregnancy) increases lamb birth weight and whether shearing results in long-term changes in the behaviour and stress response of offspring (Chapters 2, 3a, 3b and 4). Attention then turned to the effects of differing ewe nutrition during pregnancy on offspring at birth (Chapter 5), at 1 year of age (Chapters 6a and 6b) and at 2 years of age (Chapter 7). Finally the effect of both shearing and differing nutrition during pregnancy on the stress response of ram lambs was considered in Chapter 8. The majority of chapters in this thesis have been submitted to journals for publication, therefore there are commonalities within the introductions to chapters. Two papers have been published from Chapters 3 and 7 therefore the chapters have been divided into sections (a) and (b).

CHAPTER 1

Literature review

Lamb survival: the New Zealand Perspective

Lamb survival is affected by lamb birth weight and size and the behaviour of both the ewe and the lamb after birth. Lamb birth weight has a curvilinear relationship with survival, whereby both the lightest and heaviest lambs have the highest mortality rates (Dalton et al., 1980; Nowak and Poindron, 2006). The birth weight range for optimal lamb survival is 3.0 to 5.5 kg (Dalton et al., 1980; Alexander, 1984). Lambs that weigh less than 3.0 kg are at increased risk of starvation/exposure due to insufficient body reserves and reduced vigour and lambs that weigh more than 5.5 kg have a greater incidence of birth injury and dystocia (Dalton et al., 1980; Nowak and Poindron, 2006). Kerslake et al. (2005) noted that the average weight of twin and triplet lambs was lower for those that died in the first 3 days of life than their counterparts that survived the same period.

The behaviour of the ewe and lamb plays a vital role in lamb survival. The behaviour of the ewe also facilitates the development of an exclusive ewe-lamb bond. Within minutes of birth the ewe begins to lick the lamb which stimulates and dries the neonate and results in maternal recognition of the lamb (Lynch et al., 1992b). The behaviour of the lamb also plays an important role in establishing a ewe lamb bond. Lambs are more likely to bond with their dam if they stand soon after birth, suck soon after standing and maintain close contact with their dam (Alexander, 1988). Failure to develop a bond may result in the ewe rejecting her lamb (Lynch et al., 1992b), thereby increasing the risk of lamb death due to starvation and exposure (McCutcheon et al., 1981).

An increase in lamb birth weight, particularly of light-weight lambs, has the potential to increase lamb survival, increase farm profits and reduce the animal welfare implications of lamb mortality. Two practical ‘on-farm’ methods of increasing lamb birth weights are mid-pregnancy shearing (Kenyon et al., 2003) and the provision of adequate nutrition to the pregnant ewe (Robinson, 1996; Kenyon and Webby, 2007).

This review is divided into 2 parts; the first addresses the effects of mid-pregnancy shearing on the ewe and the lamb and the second part examines the effects of ewe nutrition during pregnancy.

Mid-pregnancy shearing

Shearing produces both acute and chronic stress responses in sheep. The components of shearing that elicit a stress response include exposure to dogs and vehicles during mustering, fasting for up to 24 h, handling by the shearer, sound of shearing machinery and finally fleece removal (Hargreaves and Hutson, 1990c, d, b).

Stress and the stress response

The definition of stress has been debated at length but there is no widely accepted definition. For the purpose of this review, Moberg's (2000) definition will be used. Moberg (2000) states that stress is "the biological response elicited when an individual perceives a threat to its homeostasis". Stressors are the perceived threat and can originate from within the animal or the environment (Friend, 1991). Stressors can produce acute and chronic physiological responses. Chronic stressors can be either a continuous stressor or several stressors that are present at the same time (Dwyer and Bornett, 2004)

An animal's response to a stressor is divided into three parts: recognition of a stressor, the biological defence against the stressor and the consequences of the stress response (Moberg, 2000). The central nervous system perceives a threat to the individual's homeostasis and develops a biological response or defence which can contain a combination of behavioural, autonomic and neuroendocrine responses (Moberg, 2000).

A change in behaviour is usually the first and least biologically expensive response to a stressor (von Holst, 1998; Moberg, 2000). Stressors produce an acute response, often referred to as the fight or flight response (Cannon, 1963). Molony and Kent (1997) noted that behavioural responses to pain could protect the animal, minimise pain, assist in healing, elicit help, prevent infliction of additional pain and induce learning to avoid recurrence of the event.

The initial response to stress is produced by the autonomic nervous system (Moberg, 2000). The autonomic nervous system includes both the sympathetic and parasympathetic nervous systems (von Holst, 1998). The sympathetic nervous system produces the catecholamines, adrenaline and to a lesser extent noradrenaline (Moberg, 1985; Tortora and Grabowski, 1996). These catecholamines produce an increase in heart rate, blood pressure and respiration rate (Tortora and Grabowski, 1996). In addition, they produce an increase in blood flow to the brain, heart and skeletal muscles and a decrease in clotting time (von Holst, 1998). The metabolic effects of adrenaline and nor-adrenaline result in an increase in plasma concentrations of glucose, free fatty acids and lactate (von Holst, 1998). Collectively, these changes allow the animal to react quickly to a stressor (Tortora and Grabowski, 1996).

If a challenge persists the body can adapt to tolerable stressors (von Holst, 1998). This adaptation is produced by the neuroendocrine system. The most commonly researched neuroendocrine response to a stressor is the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Matteri et al., 2000). Figure 1 demonstrates the activation of the HPA axis in response to a stressor. Neurones in the paraventricular nucleus secrete corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) which stimulate the anterior pituitary to produce adrenocorticotropic hormone (ACTH; (von Borell, 1995; Tilbrook et al., 2000). Under the control of ACTH, glucocorticoids are produced by the adrenal cortex (von Holst, 1998; Ferguson and Hoenig, 2001). The release of glucocorticoids acts in a negative feedback system whereby glucocorticoids inhibit the release of CRH from the hypothalamus which decreases corticotrophin secretion by the pituitary gland (von Holst, 1998; Cunningham, 2002). There is some evidence that glucocorticoids have a negative feedback effect at the level of the pituitary (Cunningham, 2002).

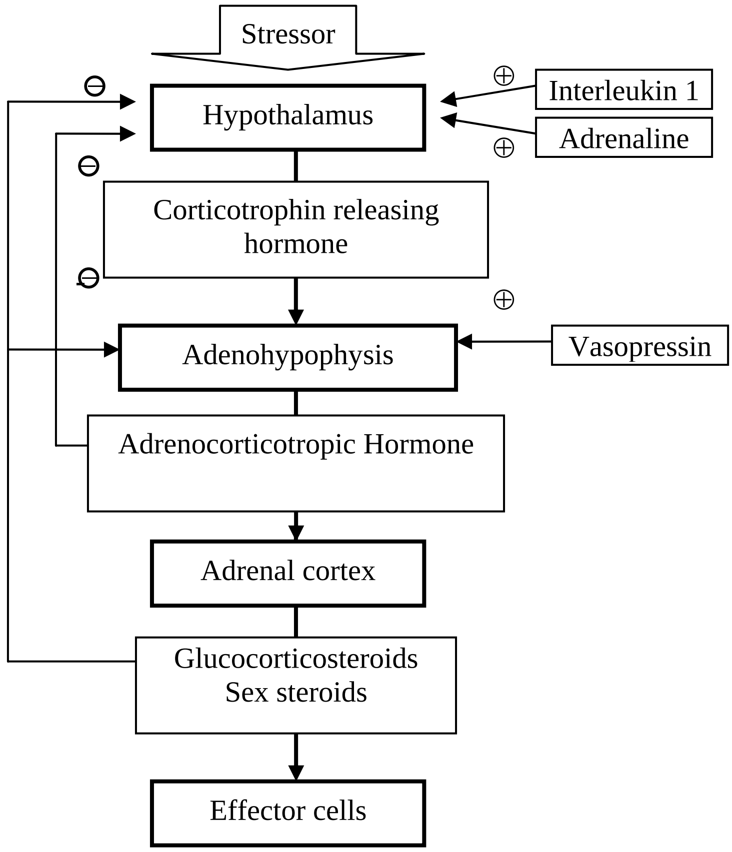


Figure 1.The hypothalamic-pituitary-adrenal axis indicating stimulating (+) and inhibiting (–) influences (reproduced from (von Holst, 1998)

The neuroendocrine system is involved in the function of almost all physiological systems (Moberg, 1985, 2000). Therefore the HPA response to a stressor causes wide-ranging physiological changes. Elevated levels of corticosteroids increase the production of glucose from protein resources, increase glycogen deposition in the liver and the inhibit glycogenolysis and the conversion of amino acids to proteins and fatty acids to triglycerides (Bentley, 1998; von Holst, 1998). The activity of the HPA system increases in a graded way in response to stressors, therefore plasma cortisol has been measured extensively to assess the effect of stressors (Mellor et al., 2000).

Prolonged or severe stress can incur a significant biological cost to the individual (Moberg, 2000). In humans and animals, chronic elevation of glucocorticoids can result in loss of muscle mass, hypertension, osteoporosis, gastric ulceration, increase disease susceptibility decreased sexual activity and psychoses (von Holst, 1998; Moberg, 2000). In sheep, chronic stressors can retard the growth of young animals and impair function of the immune and reproductive systems (Dwyer and Bornett, 2004).

Stress during pregnancy

Prenatal stress has been defined by Braastad (1998) as “stress experienced by the pregnant mother which affects the development of the offspring”. In farm animals, stress during pregnancy can occur as a result of transport (Lay et al., 1997a; Lay et al., 1997b; Roussel et al., 2006), shearing (Kenyon et al., 2003), undernutrition (Bell, 2006), hyperthermia (Yeates, 1956; Collier et al., 1982; McCrabb et al., 1993; Mellado et al., 2000) and isolation (Roussel et al., 2004). Prenatal stressors can alter fetal growth and development resulting in reduced (Lay et al., 1997b; Mellado et al., 2000) or increased live weight at birth (Morris et al., 1999; Roussel et al., 2004) and abnormal postnatal endocrine and behavioural responses to a stressor (Lay et al., 1997b; Roussel et al., 2004; Roussel et al., 2005).

Effect of prenatal stress on the fetal growth and development

Prenatal stress can alter fetal growth and development through the movement of glucocorticoids from maternal circulation to the uteroplacental tissues (Braastad, 1998; McMillen and Robinson, 2005). The movement of maternal glucocorticoids through the placenta is regulated by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) which converts cortisol into the physiologically inactive form, cortisone (Klemcke, 1995; Seckl, 1998). The activity of 11β-HSD-2, however, provides an incomplete barrier allowing the passage of 10-20% of active maternal glucocorticoid (Seckl and Meaney, 2006). In addition, maternal undernutrition and glucocorticoid exposure can reduce placental activity of 11β-HSD-2. Therefore changes in maternal cortisol concentrations can result in altered fetal plasma cortisol concentrations (Otten et al., 2004; Seckl and Meaney, 2006).

Fetal cortisol concentrations gradually increase from 10 to 15 days before term with a final rapid increase in the last 3-5 days before birth (Silver and Fowden, 1988). This increase in cortisol inhibits fetal growth and aids in the differentiation and maturation of fetal tissues (Fowden, 1995). The effect of elevated maternal glucocorticoid concentrations on fetal development has been examined by injecting ewes with hydrocortisone (cortisol) and synthetic glucocorticoids (betamethasone and dexamethasone). A chronic low dose infusion of hydrocortisone (80 mg/day) into the pregnant ewe for 10 days from day 119 of pregnancy resulted in a reduction in lamb growth, during the infusion period, but had no effect on fetal weight in late-pregnancy (Jensen et al., 2002). This finding is in agreement with Jobe et al. (2003), who reported that repeated maternal doses of hydrocortisone (6 mg/kg) on day 117 of pregnancy had no effect on fetal weight a week after treatment ceased. However, single and repeated maternal injections of the synthetic glucocorticoid betamethasone (0.5 mg/kg) beginning on day 104 of pregnancy resulted in reduced fetal weight in both late gestation and at term (Ikegami et al., 1997; Jobe et al., 1998; Sloboda et al., 2000). In addition, dexamethasone infusion (1 mg/kg) between day 25 and 45 of pregnancy reduced the fetal weight of lambs on day 30 of gestation (Moritz et al., 2002).

Abnormally high concentrations of maternal glucocorticoids crossing the placenta can influence the development of the offspring's HPA axis (Zarrow et al., 1970; Barbazanges et al., 1996). Lambs born to ewes exposed to exogenous glucocorticoids during pregnancy have elevated basal plasma cortisol concentrations (Matthews, 2000; Sloboda et al., 2000; Sloboda et al., 2002) and altered postnatal adrenal responses to acute stressors compared to offspring of control ewes (Fowden et al., 1998; Matthews, 2000). At 25 days of age, lambs born to ewes that were repeatedly isolated from day 112 of pregnancy had higher basal cortisol concentrations than control lambs (Roussel et al., 2004). In cattle, calves born to cows repeatedly transported on days 60, 80, 100, 120 and 140 of a 281 day pregnancy had a slower cortisol clearance rate in response to a stressor than control calves, at both 10 and 150 days of age (Lay et al., 1997b).

Stressors in the sheep husbandry

During their lifetime sheep may be exposed to a number of stressors including injury, disease, shearing, hyperthermia, hypothermia and poor nutrition (Dwyer and Bornett, 2004). In New Zealand's pastoral production system, ewes are commonly exposed to shearing and undernutrition during pregnancy.

The effect of shearing during pregnancy on the ewe and lamb

Winter shearing (during pregnancy) has been used by New Zealand farmers, as part of a twice-yearly shearing management strategy, since the mid 1940's (Coop and Drake, 1949). Shearing ewes during pregnancy, as part of a twice yearly shearing policy, provides advantages over shearing once a year. Benefits include improved wool quality, greater spread of seasonal labour requirements, more regular cash-flow, elimination of the costs of pre-lamb crutching and a reduction in the incidence of ewe deaths from recumbency (Frengley, 1964; Livingston and Parker, 1985; Morris et al., 1999).

Shearing ewes during pregnancy, however, may also result in hypothermia and if the weather conditions are cold, wet or windy, ewe deaths (Hutchinson, 1966; Dabiri et al., 1995a; Morris et al., 1999).

Shearing includes a number of stressors: fasting, exposure to humans and dogs, social gathering and isolation, loud noises and cold stress as a result of fleece removal. When compared with transport, isolation and up-ending, wool removal produces the greatest increase in plasma cortisol concentrations (Pierzchala et al., 1983; Hargreaves and Hutson, 1990b).

Effect of shearing on the ewe

Cold stress

In New Zealand ewes are generally mated in autumn and give birth in spring therefore mid-pregnancy shearing occurs during the winter months thus exposing ewes to cold stress. Cold stress is indicated by a reduction in rectal temperature (Dabiri et al., 1995b) and an increase in heat production (Dabiri et al., 1995a). After shearing, pregnant ewes have lower rectal temperatures of between 0.2 to 0.5 °C compared to unshorn ewes (Nedkvitne, 1972; Vipond et al., 1987; Dabiri et al., 1995b). This decrease in rectal temperature can persist for up to 20 days after shearing (Husain et al., 1997). To compensate for the loss in insulation and decrease in body temperature after shearing, ewes increase their heat production by approximately 25-30% (Davey and Holmes, 1977; Symonds et al., 1986; Symonds et al., 1988b).

Cold, wet or windy weather conditions at the time of shearing can result in ewe deaths if the ewe is unable to produce enough heat to compensate for the decrease in insulation (Gregory, 1995). The use of a “cover” or “snow” comb can be used to reduce ewe mortality rates. The cover comb leaves a residual fleece depth of 5 mm compared to 3 mm using the standard comb (Dabiri et al., 1995b). This increase in the residual fleece depth results in greater insulation (Hutchinson et al., 1960). Dabiri et al. (1995b) reported ewe mortality rates of 3% in ewes shorn with a cover comb and 14% in ewes shorn with a standard comb when minimum temperatures averaged 0.5 °C for 2 days in the 4 days after shearing in late-pregnancy (P114).

Behaviour

Newly shorn sheep show altered grazing and shelter seeking behaviour. Newly shorn sheep are more likely to graze during the day than at night and spend more time standing and less time lying down during the night than unshorn sheep (Hutchinson and McRae, 1969). Ewes shorn in mid- and late-pregnancy are also more likely to find shelter than unshorn sheep and at lambing a greater proportion of ewes give birth in sheltered areas increasing the lambs chance of survival (Done-Currie, 1980; Gregory, 1995).

Voluntary feed intake and metabolism

Shearing has been associated with an increased feed intake of non-pregnant sheep (Wodzicka-Tomaszewska, 1964). The effect of shearing on the feed intake of pregnant ewes is variable. Shorn ewes maintained on pasture either show no effect of shearing on intake (Parker et al., 1991; Husain et al., 1997; Kenyon et al., 2002b; Revell et al., 2002) or increased intakes compared to unshorn ewes (Dabiri et al., 1996; Kenyon et al., 2002c).

The acute metabolic response to shearing includes increases in the plasma concentrations of non esterified fatty acids (NEFA), glucose, β-hydroxybutyrate (β-OHB) and glycerol (Aulie et al., 1971; Thompson et al., 1982; Russel et al., 1985; Astrup and Nedkvitne, 1988; Symonds et al., 1988a). These substrates are mobilised to provide additional energy required for the increase in heat production. Symonds et al. (1986; 1989) stated that increased heat production resulting from shearing, was almost totally accounted for by the increased oxidation of maternal fat reserves.

Cold exposure, due to shearing, causes long-term changes to the metabolism of the pregnant ewe. Shearing during pregnancy results in lower insulin concentrations, increased sensitivity to insulin, increased glucose entry rates and increased NEFA entry and oxidation rates than unshorn ewes (Symonds et al., 1986, 1988a; Symonds et al., 1988b; Symonds et al., 1989). These metabolic changes result in increased maternal (Symonds et al., 1988a; Clarke et al., 1997a; Morris et al., 2000) and fetal plasma glucose concentrations (Thompson et al., 1982).

Live weight and body condition score

The effect of pregnancy shearing on ewe live weight is inconsistent. The majority of studies have reported no difference in the live weight of shorn and unshorn ewes during pregnancy (Morris and McCutcheon, 1997; Morris et al., 2000; Kenyon et al., 2002b, c; Revell et al., 2002). Several studies, however, reported that shorn ewes had lower live weights than unshorn ewes (Morris and McCutcheon, 1997; Smeaton et al., 2000; Kenyon et al., 2005b) and one study reported that shorn ewes were heavier than unshorn ewes (Kenyon et al., 2006a).

Ewe condition scores have received much less attention. However, four studies reported no effect of shearing on the condition of pregnant ewes (Dabiri et al., 1996; Clarke et al., 1997a; Kenyon et al., 2002b; Revell et al., 2002). Indeed, two studies reported that shorn ewes were in better condition than unshorn ewes (Symonds et al., 1988a; Kenyon et al., 2002c).

Effect of shearing during pregnancy on placental development

The effect of pregnancy shearing on placental development has received little attention to date. Symonds et al. (1988b) examined the effect of shearing on the metabolism of uterine tissues in late pregnancy in response to shearing ewes around day 90 of pregnancy. They found that shearing had no effect on blood flow across uterine tissues or on the extraction or utilisation of glucose. However, in that study there were no differences in the weights of the placenta or fetuses of lambs born to shorn and unshorn ewes. Revell et al. (2002), however, reported that although lamb birth weights were increased by shearing on day 70 of pregnancy (0.5 kg), placental weights on day 140 of pregnancy were similar for shorn and unshorn ewes. They concluded that the increase in lamb birth weight resulting from mid-pregnancy shearing may be due to enhanced glucose transport across the placenta. An increase in glucose supply may not require an increase in placental weight rather than an increase in the activity of glucose transporters.

Effect of shearing on the lamb

Birth weight

Shearing ewes during pregnancy was first reported to increase lamb birth weights in housed ewes the early 1970’s by Rutter et al. (1971), Adalsteinsson (1972) and Nedkvitne (1972). In New Zealand, the effect of shearing ewes maintained on pasture during pregnancy was not examined until the mid 1990’s. Both Morris and McCutcheon (1997) and Husain et al. (1997) reported that lamb birth weights were increased after shearing ewes during pregnancy.

Shearing during pregnancy has had inconsistent effects on lamb birth weight (Kenyon et al., 2003). In early pregnancy (between days 5 and 30) shearing had no effect on lamb birth weight (Kenyon et al., 2005a). During mid-pregnancy (days 50 to 100) shearing generally resulted in increased lamb birth weights in either singletons or twins but rarely in both singletons and twins or twins and triplets (Table 1). Shearing ewes in late-pregnancy (day 101 to parturition; Table 1) resulted in the increased birth weight of lambs in two (Husain et al., 1997; Morris and McCutcheon, 1997) of five studies (Dabiri et al., 1994; Dabiri et al., 1996; Kenyon et al., 2006b). The increase in birth weight ranged from 6 to 16% in singleton and 5 to 26% in twin lambs (Table 1). The inconsistency of these findings may be due to the weight of the lambs born to unshorn ewes, as light-weight lambs may have a greater capacity to respond than heavier lambs (Kenyon et al., 1999).

Two studies, to date, have examined the effect of mid-pregnancy shearing on lamb body size. Kenyon et al. (2005b) and Sherlock et al. (2003) reported that although lambs born to shorn ewes were heavier, there was no difference in crown rump or thoracic girth lengths of lambs born to shorn and unshorn ewes.

Kenyon et al. (2002b; 2003) concluded that in order for pregnancy shearing to increase lamb birth weight the ewe required the potential and the means to respond. That is, the ewe must be destined to give birth to lambs with a low birth weight, have adequate body reserves and be provided with adequate nutrition during pregnancy.

The inconsistent nature of the effects of shearing during pregnancy on lamb birth weight and size provides an opportunity for greater understanding of the mechanisms that alter lamb birth weight. Additional studies to examine lamb birth weight and size may also allow for the identification of the factors important for the increase in birth weight and size. In addition, the effect of shearing during pregnancy on lamb survival can be more thoroughly examined.

Table 1. The effect of shearing ewes maintained on pasture in early- mid- and late-pregnancy on lamb live weight(kg) at birth and during the postnatal period

Reference	Day shorn	Litter size	Birth weight (kg) [1]	Age (days)	Weight (kg) [1,2]	Age (days)	Weight (kg) [1,3]
			Unshorn vs. shorn		Unshorn vs. shorn		Unshorn vs. shorn
(Kenyon et al., 2005a)	5	1, 2 or 3	4.4 vs. 4.5	27	10.4 vs. 10.1	93	18.9 vs. 17.6
	30		4.4 vs. 4.6	27	10.4 vs. 10.6	93	18.9 vs. 18.0
(Morris et al., 2000)	50	1	5.1^{a} vs. 5.8^{b}	45	15.6 vs. 14.6	95	26.1 vs. 26.2
		2	4.5 vs. 4.4	45	11.4 vs. 10.8	95	20.8 vs. 19.9
(Revell et al., 2000)	69	1	5.8 vs. 5.7				
		2	4.3^{a} vs. 5.4^{b}				
(Kenyon et al., 2002b)	70	1	4.9^{a} vs. 5.6^{b}	38	15.2 vs. 16.0	84	25.5 vs. 25.6
		2	4.2 vs. 4.4	38	12.0 vs. 12.3	84	21.6 vs. 21.8
(Kenyon et al., 2002c)	70	1 + 2	5.1^{a} vs. 5.4^{b}	38	14.1 vs. 14.0	76	21.9 vs. 21.8
(Revell et al., 2002)	70	1	4.6^{a} vs. 5.4^{b}	42	17.4 vs. 16.6	95	34.2 vs. 31.3
		2	4.2 vs. 4.4	42	13.3 vs. 13.3	95	27.3 vs. 27.2
(Morris et al., 2000)	70	1	5.1^{a} vs. 5.9^{b}	45	15.6 vs. 15.9	95	26.1 vs. 27.4
		2	4.5 vs. 4.2	45	11.4 vs. 9.9	95	20.8 vs. 18.7
(Morris and McCutcheon, 1997)	70	1	6.2 vs. 5.8	44	16.2^{a} vs. 14.0^{b}	100	25.6 vs. 24.3
		2	4.3^{a} vs. 5.0^{b}	44	10.1 vs. 10.7	100	18.3 vs. 19.2
(Cloete et al., 1994)	70	2	4.2 vs. 4.3	56	14.4^{a} vs. 15.3^{b}		
(Kenyon et al., 2002a)	70	1	6.0 vs. 6.2				
		2	4.7^{a} vs. 5.1^{b}				
		3	3.9 vs. 4.2				
(Smeaton et al., 2000)	70	1 (1997)	5.2 vs. 5.2			~100	29.0 vs. 29.3
		2 (1997)	3.9^{a} vs. 4.1^{b}			~100	19.8 vs. 21.2
		1 (1998)	4.6 vs. 4.8			~100	26.9^{b} vs. 28.4^{a}
		2 (1998)	3.9^{a} vs. 4.1^{b}			~100	19.1 vs. 20.0
(Kenyon et al., 2005b)	70	2	4.8 vs. 5.2			~100	25.5 vs. 24.8
(Kenyon et al., 2006a)	70	2 + 3 (farm 1)	4.3^{a} vs. 4.7^{b}			86	25.6^{a} vs. 26.7^{b}
		2 + 3 (farm 2)	4.3^{a} vs. 4.4^{b}			86	22.5 vs. 22.6
(Kenyon et al., 2006b)	79	1	4.8^{a} vs. 5.1^{b}	55	20.9 vs. 21.3	96	25.9 vs. 26.4
		2	3.5 vs. 3.9	55	15.3 vs. 16.9	96	20.1 vs. 21.5

Reference	Day shorn	Litter size	Birth weight (kg)[1]	Age(days)	Weight (kg)[1,2]	Age (days)	Weight (kg)[1,3]
			Unshorn vs. shorn		Unshorn vs. shorn		Unshorn vs. shorn
(Parker et al., 1991)	91	1 + 2	5.0 vs. 4.9			101	22.9 vs. 23.4
(Cam and Kuran, 2004)	100	1	3.2^{a} vs. 3.7^{b}			75	20.2^{a} vs. 24.6^{b}
		2	3.1 vs. 3.5			75	16.7^{a} vs. 20.1^{b}
(Morris et al., 2000)	100	1	5.1^{a} vs. 5.9^{b}	45	15.6 vs. 15.2	95	26.1 vs. 26.3
		2	4.5 vs. 4.8	45	11.4 vs. 10.8	95	20.8 vs. 20.9
(Morris and McCutcheon, 1997)	100	1	6.2 vs. 6.0	44	16.2^{b} vs.14.3^{a}	100	25.6^{b} vs. 23.9^{a}
		2	4.3^{a} vs. 4.7^{b}	44	10.1 vs. 11.0	100	18.3 vs. 19.7
(Husain et al., 1997)	115	1 +2	4.3^{a} vs. 4.9^{b}			80	20.4 vs. 21.3
(Dabiri et al., 1996)	118	1 +2	4.5 vs. 4.4	33	11.1 vs. 10.7	84	18.4 vs. 17.6
(Kenyon et al., 2006b)	119	1	4.8 vs. 4.7	55	20.9 vs. 20.3	96	25.9 vs. 25.3
(Morris and McCutcheon, 1997)	130	1	6.2 vs. 5.6	44	16.2^{b} vs. 14.3^{a}	100	25.6^{b} vs. 23.8^{a}
		2	4.3^{a} vs. 4.6^{b}	44	10.1 vs.10.6	100	18.3 vs. 18.7

[1] Means are presented as unshorn then shorn, means with different superscripts are significantly different (P<0.05)

[2] Live weights of lambs recorded in the early postnatal period (between days 20-60 after the mid-point of the parturition period)

[3] Live weights of lambs recorded in the late postnatal period (between days 70 and 100 after the mid-point of the parturition period)

Possible causes for the increase in lamb birth weight from mid-pregnancy shearing

The component of mid-pregnancy shearing that produces the increase lamb birth weight is unclear. Initially, it was believed that increased ewe feed intakes after shearing resulted in a greater supply of nutrient to the fetus (Rutter et al., 1971; Russel et al., 1985). However, an increase in lamb birth weight has been observed in the absence of increased ewe feed intake (Husain et al., 1997; Kenyon et al., 2002b; Revell et al., 2002). Revell et al. (2000) reported that an increase in voluntary feed intake was observed in twin- but not singleton-bearing ewes and that an increase in birth weight was observed in twin- but not singleton-born lambs. Therefore, it is unlikely that ewe intake alone, is the cause of the increase in lamb birth weight however the influence of ewe intake on the movement of nutrient across the placenta and maternal nutrient partitioning is unclear. Therefore, no definitive conclusion can be made regarding the role of ewe intake on lamb birth weight response to shearing.

Shearing ewes during pregnancy can result in a 0.5 to 1.5 day increase in gestation length (Kenyon et al., 2002b; Revell et al., 2002; Cam and Kuran, 2004). In the final 25 days of gestation, fetal growth rates are 150-200 g/day (Rattray et al., 1974) therefore the observed increases in gestation length would account for only part of the total increase in birth weight. Furthermore, increases in lamb birth weight have been observed in lambs of similar gestational age (Kenyon et al., 2002c).

Maternal thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) can be elevated for up to 7 weeks after shearing (Westra and Christopherson, 1976; Symonds et al., 1988a; Morris et al., 2000). T_3 and T_4, in conjunction with catecholamines, have a stimulatory effect on lipolysis and heat production (Cunningham, 2002). Therefore elevated plasma levels of T_3 and T_4 may result in increased rates of lipolysis and NEFA oxidation (Symonds et al., 1989). T_3 and T_4 have also been linked with increased fetal growth

(Bell et al., 1989), however, a study that artificially elevated ewe plasma T_3 concentrations did not alter lamb birth weight (Kenyon et al., 2005b). This suggests that elevated thyroid hormones alone were not the cause of the increase in lamb birth weight however they may play an integral role in the metabolic response of the ewe to shearing.

Maternal adaptation to shearing during pregnancy is geared towards maintaining the supply of glucose to the fetus (Symonds et al., 1988a; Clarke et al., 1997a). In late-pregnancy, housed shorn ewes offered either maintenance (Symonds et al., 1988a) or sub-maintenance rations (Clarke et al., 1997a) had elevated plasma glucose concentrations compared to unshorn ewes. Under pastoral conditions, Morris et al. (2000) reported that shorn ewes had elevated plasma glucose concentrations compared to unshorn ewes. Maternal glucose diffusion across the placenta is driven by the concentration gradient therefore elevated maternal glucose produces an increase in fetal glucose concentrations (Symonds et al., 1986). Stevens et al. (1990) demonstrated that fetuses infused with glucose in late-pregnancy were 18% heavier at birth than saline infused fetuses. Therefore the increase in maternal glucose resulting from shearing may be the mechanism by which birth weights are increased. However, ewe plasma glucose concentrations are not always elevated (Sherlock et al., 2003). Symonds et al. (1988b) reported a 26% increase in whole-body glucose entry rate of shorn than unshorn but no elevation in maternal plasma glucose concentrations. Therefore placental glucose transfer may be more informative than maternal plasma glucose concentrations in examining the role of glucose in the increase in lamb birth weight after mid-pregnancy shearing.

The effect of shearing during pregnancy on ewe glucose metabolism may also be influenced by maternal insulin. Revell et al. (2000) observed a decrease in the insulin response of shorn ewes to a glucose challenge on days 109 and 131 of pregnancy. They concluded that this reduced insulin response may be due to either a reduced capacity to release insulin or an increase in the sensitivity of maternal tissues to insulin of shorn compared to controls ewes. In addition, Symonds et al. (1986) reported that shorn ewes showed long-term inhibition of insulin secretion and increased insulin sensitivity compared to unshorn ewes (Symonds et al., 1988b; Revell et al., 2000). These changes in insulin sensitivity and secretion may contribute to the increase in lamb birth weight.

An alternative hypothesis is that the ewes' stress response to shearing may cause the increase in lamb birth weight. Exposure to prenatal stressors, including transport and social isolation in ruminants, have resulted in increased fetal and birth weights (Lay et al., 1997a; Roussel et al., 2004).

Postnatal effects of shearing during pregnancy on the ewe and lamb

Ewe live weight

Ewe live weight during lactation is generally unaffected by shearing during pregnancy (Symonds et al., 1990; Parker et al., 1991; Dabiri et al., 1996; Kenyon et al., 2002b). However, Morris et al. (2000), reported that ewes shorn on days 50, 100 or 130 of pregnancy had lower live weights than unshorn ewes on day 45 of lactation. On the contrary, Kenyon et al. (2006b) and Smeaton et al. (2000) reported that shearing resulted in an increase in the live weight of adolescent and mature ewes during lactation, respectively.

Lamb live weight

Shearing ewes during pregnancy had mixed results on lamb weights after birth. Results of shearing during pregnancy were divided evenly between no difference in postnatal weight (Dabiri et al., 1994; Morris et al., 2000; Kenyon et al., 2002c; Kenyon et al., 2006b) or an increase in postnatal live weights of lambs born to shorn ewes (Cloete et al., 1994; Morris and McCutcheon, 1997; Morris et al., 1999; Kenyon et al., 2004). In two studies, there was a difference in birth weight but no difference in weaning weights of lambs born to shorn compared to unshorn ewes (Morris et al., 2000; Kenyon et al., 2002c). The results of these trials indicate that although the difference in birth weight is statistically significant, birth weight difference may not be biologically significant or birth weight may not alter lamb growth rates to weaning. It appears unlikely that the difference in lamb birth weight was not biologically significant. Kenyon et al. (2004) concluded that heavy lambs had greater growth rates to weaning and therefore greater weaning weights than lighter lambs. Therefore, the effect of the increase in lamb birth weight on postnatal growth rates remains unclear.

Lamb heat production

The ability of the lamb to generate heat to a level equal to, or greater than, heat lost to the environment is critical for survival (McCutcheon et al., 1981; Haughey, 1993). In housed sheep, lambs born to shorn ewes had enhanced cold-resistance and were more likely to responded to cold without shivering than lambs born to unshorn ewes (Symonds et al., 1992). Under pastoral conditions, results are not as clear. Revell et al. (2002) reported that twin lambs born to shorn ewes had a 16% greater summit metabolic rate (SMR) but that singleton lambs had a 26% lower SMR than their counterparts born to control ewes. The summit metabolic rate is defined as the “highest metabolic rate that can be induced in a resting animal in any cold environment” (Anon., 2003b). Stott and Slee (1985) reported that ewes that were shorn and kept at 6°C for 2 weeks before shearing gave birth to lambs that had a greater capacity for non-shivering thermogenesis. However, two studies by Kenyon et al. (2002b; 2002c) reported no effect of ewe shearing treatment on the proportion of lambs to reach summit metabolism or on their summit metabolic rate when expose to cold water and air flow (draught).

Kenyon et al. (2002b) concluded that pregnancy shearing may have no effect on the thermoregulatory capability of the newborn lamb and that any increase in survival is largely due to an increase in lamb birth weight.

Lamb survival

Ewes shorn in late-pregnancy are more likely to seek shelter at lambing (Done-Currie, 1980; Gregory, 1995), have easier access to the udder for the lamb (Kenyon et al., 2003) and give birth to heavier lambs which may result in an increase in lamb survival. The majority of studies, however, reported no effect of shearing during pregnancy on lamb survival rates to weaning (Cloete et al., 1994; Morris et al., 1999; Smeaton et al., 2000; Kenyon et al., 2002a; Kenyon et al., 2006b). In one trial involving 1002 twin-bearing ewes, lambs born to ewes shorn on day 67, but not 130, of pregnancy had a 3% greater survival rate than lambs born to unshorn ewes (Morris et al., 1999). It is unclear what role the increase in lamb birth weight resulting from pregnancy shearing plays in survival. Kenyon et al. (2006a) reported that in a study conducted concurrently on two farms, the increase in survival was only observed on the farm that also had an increase in birth weight. However, in another study, Kenyon et al. (2002a) found that twin lambs born to ewes shorn on day 70 of pregnancy were 0.4 kg heavier than lambs born to unshorn ewes but had similar survival rates to weaning. The benefits of an increase in lamb birth weight may be reduced if shearing negatively affects ewe and lamb behaviour. To date the effect of shearing during pregnancy on ewe and lamb behaviour has not be examined.

Postnatal behaviour and stress response

The effect of pregnancy shearing on ewe and lamb behaviour at birth has not been examined. Furthermore, the postnatal consequence of pregnancy shearing on the lamb’s response to stressors has not been examined. It has been reported that during pregnancy, exposure to stressors such as isolation, transport and undernutrition and elevated glucocorticoid concentrations alter lamb birth weight, the lamb's HPA axis function and offspring behaviour after birth (Avishai-Eliner et al., 2002; Dwyer et al., 2003; Roussel et al., 2004). Therefore shearing ewes during pregnancy may have a negative impact on lamb behaviour and response to stress which may decrease lamb survival and productivity.

Summary of the effects of mid-pregnancy shearing

Shearing ewes in mid-pregnancy commonly results in an increase in lamb birth weight, however, the underlying mechanism that produces the increase in birth weight remains elusive. Stressors during pregnancy, such as isolation and transport, have been shown to result in an increase in lamb birth weight. Therefore, it is possible that the component of shearing that alters fetal growth is stress.

The effect of ewe nutrition during pregnancy on the lamb

Ewe nutrition and body condition prior to breeding and throughout pregnancy has significant effects on the birth weight of lambs. In a pastoral-based production system chronic mild undernutrition is more likely to be encountered than acute severe undernutrition or starvation (Mellor, 1983). During the first two-thirds of pregnancy, ewe feeding requirements for maintenance are similar to levels required for non-pregnant ewes but in the third trimester, metabolisable energy (ME) requirements increase by 50-100% in order to support the exponential growth of the fetus (Geenty and Rattray, 1987). The feeding requirements of ewes during pregnancy depend upon the ewe’s live weight and the number of fetuses. For example, 1 month prior to lambing ewes bearing singleton, twin and triplet fetuses require an additional 2.6, 4.6 and 6.5 MJ ME per day, respectively (Geenty and Rattray, 1987). Morris and Kenyon (2004) recommend that during mid- and late-pregnancy twin- and triplet-bearing ewes be offered pastures with a minimum sward height of 4 cm (~1200 kg DM) to achieve optimal lamb birth weight.

This section is not intended to provide an exhaustive discussion on ewe undernutrition during pregnancy, for more in depth reviews see reviews by Mellor (1983) and Robinson et al. (1977; 1999). The emphasis of this section will be on the postnatal effects of maternal nutrition on the lamb.

Ewe metabolism

In the pregnant ewe, undernutrition can result in altered plasma concentrations of metabolites such as glucose, β-OHB and NEFA (Chandler et al., 1985; O'Doherty and Crosby, 1998). In well-fed ewes maternal glucose is the primary source of fuel for fetal and placental tissues (Bell and Bauman, 1997). The growing conceptus makes substantial demands on the maternal glucose supply and can account for 30-50% of whole body glucose utilisation in well-fed ewes (Oddy et al., 1985). The long-held belief was that the demands of the conceptus took priority over maternal tissues (Barcroft, 1946; Oddy et al., 1985). However, Hay et al. (1983b; 1984) and stated that the fractional distribution of glucose by the gravid uterus is unchanged in hypoglycaemic and normoglycaemic ewes between days 130 and 140 of pregnancy. Leury et al. (1990) offered singleton-bearing ewes 0.3-0.4 of their predicted energy requirements for 7 to 21 days between days 123 and 138 of gestation. Additional responses to maternal undernutrition in the ewe include maternal hypoglycaemia, reduced uterine and umbilical uptakes of glucose, reduced placental glucose transfer capacity and a decreased demand for maternal glucose by fetal, placental and uterine tissues (Bauman and Currie, 1980; Leury et al., 1990).

In a number of mammalian species lipids are accumulated in adipose tissue during the first two-thirds of pregnancy, after which time, there is an increase in fat mobilisation (Guesnet et al., 1991). When comparing non-pregnant and pregnant ewes (between day 115 and 122 of pregnancy) fed to achieve a zero energy balance for non-uterine tissues, pregnant ewes had elevated plasma concentrations of NEFA indicating mobilisation of body fat (Petterson et al., 1994).

Insulin concentrations decrease during pregnancy (P30 to P130) and to a larger extent in twin- than singleton-bearing ewes (Sigurdsson, 1988). In addition, maternal tissues (muscle, adipose tissue and liver) become resistant to the effects of insulin in late pregnancy thereby increasing the availability of maternal glucose to the less insulin dependent conceptus (Petterson et al., 1994). Placental transport of glucose is regulated by glucose transporter 1 (GLUT 1) and therefore is independent of insulin concentrations.

Effects of prenatal undernutrition

Placental size

Ewe nutrition and placental size are two major factors that affect lamb birth weights (Reynolds and Redmer, 1995; Redmer et al., 2004). In sheep, placental growth proceeds rapidly between day 40 and day 75-80 of gestation when maximum placental size is attained (Figure 2) (Kelly, 1992; Ehrhardt and Bell, 1995; Gootwine et al., 2007). During this period, day 40 to 80 of gestation, there is rapid proliferative growth (Ehrhardt and Bell, 1995).

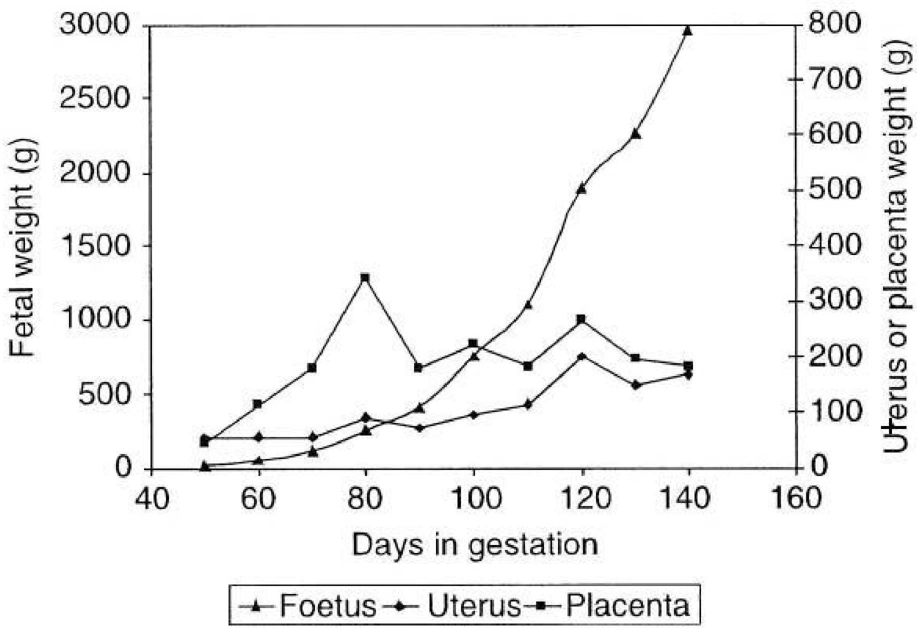


Figure 2. Increase in weight of the fetus (▲), placenta (■) and uterus (◆) during gestation in sheep (reproduced from (Gootwine et al., 2007))

The placenta delivers nutrients to the fetus and removes waste products from the fetal circulation (Kelly, 1992). The delivery of nutrients to the fetus is determined by placental size, functional capacity, rate of blood flow and the concentration gradient between maternal and fetal circulation (Kelly, 1992). Placental size limits birth weight therefore poor placental development will result in small lambs. Mellor (1983) reported that almost two-thirds of the variation in lamb birth weight is attributable to variation in placental weight. The critical period of sensitivity of maternal nutrition on placental growth occurs between days 40 and 80 of gestation (Robinson et al., 1999). The effects of maternal nutrition on placental size varies according to the live weight and condition of the ewe at breeding; ewes in good condition may have enhanced placental growth in response to nutrient restriction while light ewes show decreased placental size (Kelly, 1992; McCrabb et al., 1992a; Robinson et al., 1999).

Fetal growth

Maternal glucose and amino acids supply the fetus with most of the carbon and nitrogen required for growth (Bell, 1995). In response to maternal undernutrition both maternal and fetal glucose concentrations are decreased (Bauer et al., 1995; Osgerby et al., 2002). In the fetus there is a switch to endogenous glucose production in response to maternal fasting (Hay et al., 1984) and severe underfeeding (Leury et al., 1990). In contrast, a normoglycaemic late-pregnancy fetus liver produces little glucose (Dalinghaus et al., 1991).

After glucose, lactate is the major carbohydrate consumed by the fetus (Battaglia and Meschia, 1986). In sheep, lactate is produced by the uterus and released into both maternal and fetal circulation (Battaglia and Meschia, 1986). In the fetus, the oxidation rates of glucose and lactose are relative to their concentrations (Hay et al., 1983a). Therefore as concentrations increase so does the fraction that is oxidised thus sparing the oxidation of other substrates such as amino acids. Conversely, when concentrations fall the oxidation of other substrates increases (Battaglia and Meschia, 1986).

The fetus adapts to the inadequate substrate supply through the slowing or cessation of fetal growth (Harding and Johnston, 1995), thus reducing glucose utilisation and inhibiting protein synthesis (Bell, 1995; Bloomfield and Harding, 1998). Exogenous and endogenous amino acids become the major oxidative fuel substrate for the fetus during ewe fasting as indicated by a 50-70% increase in fetal urea production (Lemons and Schreiner, 1983; Bloomfield and Harding, 1998).

Fetal growth follows an exponential pattern (Figure 2 and Figure 3), with small increases in fetal weight in early gestation which become more rapid in mid- and late-gestation (Ferrell, 1991). Fetal growth is limited by placental size, litter size and maternal nutrition. Maternal uterine space is limited therefore as litter sizes increase and individual lamb birth weights decrease (Gardner et al., 2007). When the placenta reaches its maximum weight (around day 90 of pregnancy) the fetus has gained only 10% of its potential birth weight, therefore 90% of fetal growth occurs after this time (Rattray et al., 1974; Redmer et al., 2004).

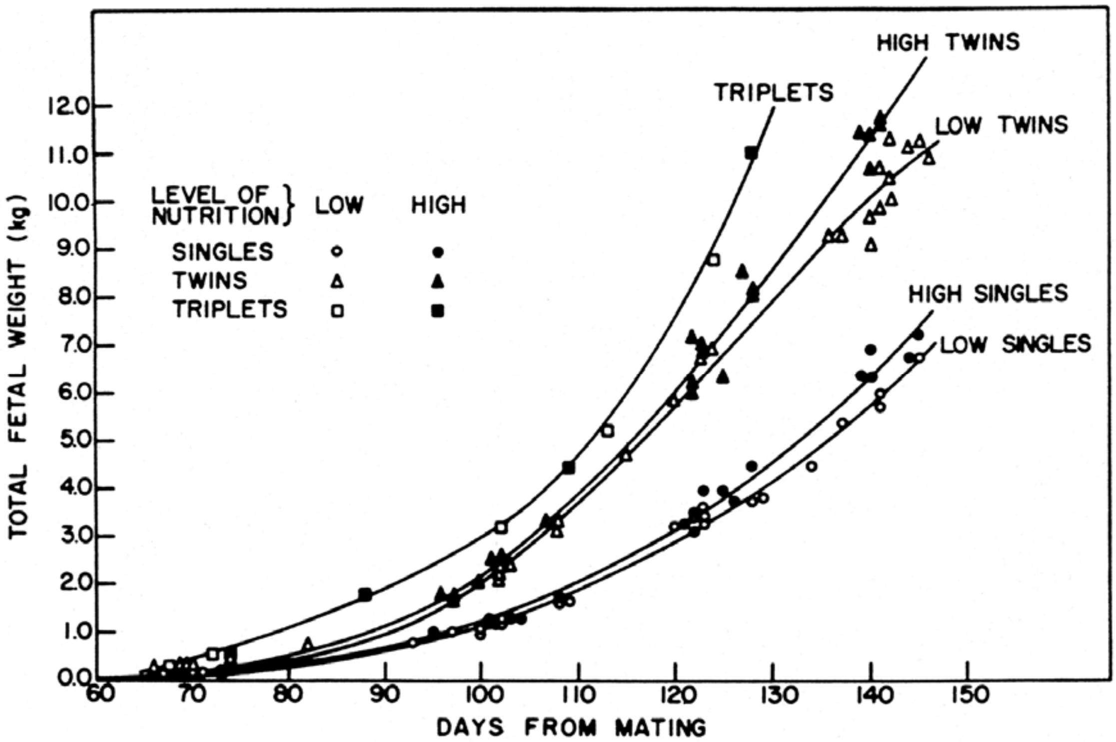


Figure 3. The relationship between day of gestation and fetal weight of singleton (•), twin (■) and triplet (▲) lambs whose dam were offered a maintenance ration (open symbols) or fed ad libitum (closed symbols) (reproduced from (Rattray et al., 1974)

In early pregnancy (from breeding to day 50 of gestation) the increase in fetal weight is small and the energy requirements of the conceptus are low (Robinson, 1977). In most studies, ewe undernutrition during this period had little effect on mid- or late-pregnancy fetal weights (Table 2). Everitt (1964) reported that fetuses of ewes bearing singleton fetuses fed to lose 25% of their body weight during pregnancy were lighter and smaller on day 90 of gestation than fetuses of ewes fed to gain 25% of their body weight.

Table 2.The effect of maternal undernutrition in early to mid-pregnancy on fetal growth

Author	Period of pregnancy intake was restricted (days)	Litter size	Outcome measured (days)	Nutritional treatments [1]	Fetal growth reduced
(Oliver et al., 2005)	-30 to 60	1	145	Lose (L) or gain (H) 15% LW	No
(Everitt, 1964)	0 to 90	1	90	Lose (L) or gain (H) 12% LW	Yes, L<H
(Heasman et al., 1998)	28 to 77	1	145	0.5 M (L) or 2M (H)	No
(Heasman et al., 2000)	28 to 77 re-fed M	1	145	0.5 M (L) or 1.2 M (H)	No
(Clarke et al., 1998)	30 to 80	1	80	0.6 M (L) or 1.35 M (H)	No
(McCrabb et al., 1992b)	30 to 96	1	96+140	Lose (L) or gain (H) LW	No
(Newnham et al., 1991)	30 to 96 re-fed M	2	142	0.3 M (L) or 1 M (H)	Yes L<H
(Newnham et al., 1991)	30 to 96 re-fed 2M	2	142	0.3 M (L) or 1 M (H)	Yes L<H
(Davis et al., 1981)	40 to 95	1+2	137	Low or High pasture	No
(McCrabb et al., 1992b)	50 to 96	1	96+140	Lose (L) or gain (H) LW	No

[1] M indicates the proportion of the requirements for the maintenance of ewe conceptus free live weight, L indicates ewes in the low feed group and H indicates ewes in the high feed group

In late-pregnancy (between day 90 of pregnancy and parturition), placental development is complete but fetal growth is rapid (Figure 2). Severe maternal undernutrition in the final 40 to 50 days of pregnancy can reduce fetal growth by between 30 to 70% and in some cases cause a cessation in fetal growth (Mellor, 1983). Mellor and Murray (1982) devised a system to measure fetal changes in crown-rump length (CRL) in utero as an indicator of fetal growth. They reported that between days 90 and 111 of gestation the rate of fetal growth was constant and then gradually decreased to day 142 of pregnancy.

In mid- to late-pregnancy (commencing on day 64 to 115 of gestation), maternal undernutrition produces inconsistent effects on fetal growth (Table 3). The effects of maternal undernutrition may vary due to level and length of restriction and stage of gestation the restriction is imposed (Redmer et al., 2004). The live weight and condition of ewes at breeding may also affect fetal weights. Ewes that weighed less than 55 kg at breeding and were fed a maintenance ration from day 30 of pregnancy to parturition had fetuses that were lighter than ewes offered the same ration but that weighed more than 60 kg at breeding (Clarke et al., 1997b). However, no differences were observed in the weight and size of fetuses (at day 146 of gestation) of ewes that maintained medium (body condition score of 2.9 and live weight of 46.6 kg) or lean body condition (body condition score of 2.0 and live weight of 40.6 kg) during pregnancy (McNeill et al., 1997).

The majority of studies that examined the effects of maternal nutrition on fetal body dimensions (thoracic girth and CRL) identified no difference between fetuses of well-nourished and undernourished ewes (McCrabb et al., 1992a; Clarke et al., 1998; Hawkins et al., 1999). In studies that identified differences in CRL, differences were generally observed in the absence of birth weight differences (McCrabb et al., 1992b; Heasman et al., 1998; Heasman et al., 1999). Few studies have examined the effect of ewe nutrition on thoracic girth. Morris and Kenyon (2004) reported that lambs born to ewes offered pasture with an average height of 2 cm were lighter and had smaller girths than ewes offered 4, 6 or 8 cm, but CRL did not differ.

Table 3.The effect of maternal undernutrition in mid- to late-pregnancy on lamb growth

Author	Restriction Period (days of pregnancy)	Litter size	Outcome measured (days)	Nutritional treatments	Fetal growth reduced
(Morris and Kenyon, 2004)	64-term	2+3	Birth	1.7, 3.7, 5.6, 6.8 cm pasture height	Yes, 1.7<3.7, 5.6 and 6.8
(Rattray et al., 1974)	70-140	1+2	141	1.5M (L) or 2 M (H)	Yes L<H
(McCrabb et al., 1992b)	75-96	1	96+140	Lose (L) or gain (H) LW	No
(Oddy and Holst, 1991)	87-108	1	Birth	Restricted (L) or *ad libitum* (H)	No
(Oddy and Holst, 1991)	87-115	1	Birth	Restricted (L) or *ad libitum* (H)	Yes L<H
(Rattray, 1979)	90-140	1	140	1 M, 1.1 M, 1.3 M, 1.5 M or 1.7 M	Yes 1<1.1<1.3<1.5<1.7
(Davis et al., 1981)	95-140	1+2	137	Low or High pasture allowance	No
(Oddy and Holst, 1991)	95-116	1	Birth	Restricted (L) or *ad libitum* (H)	No
(Oddy and Holst, 1991)	95-123	1	123	Restricted (L) or *ad libitum* (H)	P123 Yes L<H, Birth No
(Morris et al., 1993)	115-140	1+2	Birth	2.8, 4.9, 7.1 or 8.5 cm pasture height	No
(Mellor and Murray, 1981)	112-142	1+2	142	Glucose 1-1.4 (L) or 2.6-3.2 (H) mmol	Yes L<H
(Mellor and Murray, 1981)	112-131 then H	1+2	142	Glucose 1-1.4 (L) or 2.6-3.2 (H) mmol	Yes L<H

[1] M indicates the proportion of the requirements for the maintenance of ewe conceptus free live weight, L indicates ewes in the low feed group and H indicates ewes in the high feed group

Fetal HPA axis

Fetal HPA axis function is influenced by maternal nutrition during pregnancy. Any early insult is likely to have the most severe impact if it occurs early in gestation as changes as brain developmental events such as neurogenesis, gliogenesis, cell migration and differentiation may be altered (Lesage et al., 2006). A great deal of work has focussed on early gestation. The effect of maternal feed restriction in early pregnancy (day 0 to 70 of pregnancy) had inconsistent effects on fetal HPA axis function. Hawkins et al. (1999; 2000; 2001) conducted a series of studies that examined the effect of providing ewes with 85 or 100% of their maintenance requirements from conception until day 70 of pregnancy. In two studies, they noted that fetuses of undernourished ewes had suppressed plasma cortisol concentrations in response to a corticotrophin releasing hormone plus arginine vasopressin (CRH+AVP) challenge between days 113 and 116 and between days 125 and 127 of gestation (Hawkins et al., 1999; Hawkins et al., 2000). In addition, one study reported elevated ACTH concentrations between days 113 to 116 of pregnancy (Hawkins et al., 1999). Fetuses of undernourished ewes had reduced expression of glucocorticoid receptor mRNA in the anterior pituitary (Hawkins et al., 2001). The effects of early pregnancy (60 days prior to conception to between 7 and 30 days of gestation) nutrient restriction on basal cortisol and ACTH concentrations in late gestation are mixed (Edwards and McMillen, 2002; Bloomfield et al., 2004; Jaquiery et al., 2006). Jaquiery et al. (2006) concluded that during undernutrition it was unlikely that fetuses would be exposed to excess maternal cortisol during periconceptional undernutrition but that it may occur later in gestation if maternal plasma cortisol concentrations return to normal when placental 11β-HSD-2 remained low. In addition, Bloomfield et al (2003b) reported that periconceptional undernutrition (from 60 days prior to and 30 days after breeding) resulted in premature delivery of the fetus as a result of accelerated maturation of the fetal adrenal gland.

Postnatal effects of maternal nutrition

Ewe and lamb behaviour

The expression of appropriate maternal behaviour by the ewe is vital for lamb survival (Figure 4). Appropriate maternal behaviours include grooming and licking of the lamb, low-pitched bleating, maintenance of close contact with the lamb, cooperation with the lamb's sucking attempts and lamb recognition (Lynch et al., 1992b; Dwyer and Lawrence, 2005). Lamb behaviour including bleating, successful attempts to suck, maintenance of close contact with dam and recognition of dam are also associated with lamb survival (Nowak, 1996; Dwyer and Lawrence, 2005).

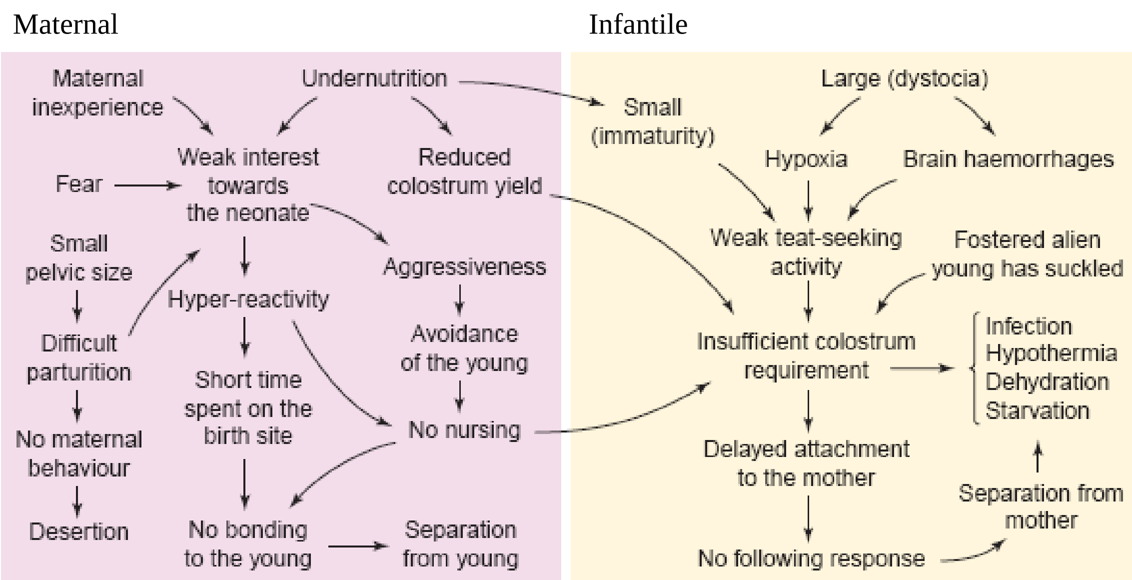


Figure 4. Summary of maternal and infantile factors leading to inadequate mother-young interactions during the neonatal period (reproduced from (Nowak et al., 2000)

The ewe's intense attraction to amniotic fluid results in the ewe beginning to lick the lamb within minutes of giving birth (Levy and Poindron, 1987; Lynch et al., 1992b). Grooming is important for lamb survival, grooming dries the lamb, stimulates thermoregulation and respiration and results in maternal recognition of the lamb through the ingestion of fluids and membrane (Alexander, 1988; Nowak et al., 2000). Litter size can affect the amount of time that a lamb receives attention after birth. Lynch et al., (1992b) reported that the last born twin or triplet receives more attention than the other lambs for the first 30 min after birth. However, twin and triplet-born lambs receive less total attention than singleton lambs. Maternal undernutrition during pregnancy can also alter ewe behaviour in the days after birth Ewes underfed from day 28 of pregnancy until parturition, had a greater motivation to graze during the first 3 days after parturition than well fed ewes, spent less time grooming their lambs and expressed more aggressive behaviours toward their lamb (Dwyer et al., 2003). In addition, ewes underfed during the final 6 weeks of pregnancy were more likely to desert their lambs than well-fed ewes (Putu et al., 1988).

The expression of ewe and lamb behaviour, immediately after birth, appears to be primarily to assist the lamb to suck. In order for the lamb to successfully suck it must be able to stand and move to the udder (Dwyer and Lawrence, 2005). Through grooming the ewe stimulates and orientates the lamb (Alexander and Williams, 1964). When examining the effects of ewe nutrition during pregnancy, Dwyer et al. (2003) found no effect of maternal feeding level on lamb behaviours. They reported that increased lamb birth weights resulted in a more rapid progression in the expression of neonatal behaviours. In addition, a decrease in lamb birth weight was associated with reduced lamb vigour and activity (Moore et al., 1986; Dwyer, 2003). Undernutrition of triplet bearing ewes resulted in lambs that were less likely to stand, locate their dam's udder and maintain contact with their dam 12 h after birth than lambs of well-fed ewes (Everett-Hincks et al., 2005a).

In the immediate postnatal period, the ewe's vocalisation changes from a high-pitched or 'protest' bleat to a low-pitched rumble, that is considered to be a 'care-giver' bleat (Dwyer et al., 1998). The low-pitched bleat is important for the formation and maintenance of the ewe lamb bond (Nowak and Lindsay, 1990; Dwyer et al., 1998). Ewes and lambs respond to each others bleats (Shillito Walser et al., 1982). The lamb's vocalisations are a continual behaviour that is related to the lambs ability to recognise its dam (Nowak et al., 1989; Lindsay, 1996). The effect of ewe nutrition during pregnancy on ewe and lamb bleating behaviours is inconsistent. Dwyer et al. (2003) found no effect of ewe nutrition on ewe or lamb behaviour, but Everett-Hincks et al. (2005a) reported that the bleating behaviour of ewes and their lambs 12-24 h after birth was affected by maternal nutrition. Twin-bearing ewes offered pasture with a 2 cm sward height had a higher rate of low-pitched bleating than ewes offered swards of 6 cm (Everett-Hincks et al., 2005a). In the same study, twin and triplet lambs born to ewes offered pasture with an average sward height of 6 or 8 cm were less likely to bleat after tagging than lambs born to ewes on lower allowances (2 or 4 cm swards). These results indicate that poor maternal nutrition can impair lamb bleating behaviour, particularly of triplet lambs, which may result in poor bonding with their dam.

Ewes develop a selective bond with their lambs that ensures they will only allow their own lambs to suck (Poindron et al., 1984). Failure to develop an exclusive bond can result in the death of the lamb as a result of starvation and exposure. In the hours prior to and after parturition, ewes become attracted to the birth site and their lamb however this attraction rapidly fades if they are separated (Poindron and Le Neindre, 1980). A 4 h separation after birth resulted in half the ewes rejecting their lamb which increased to 80% after a separation of 24 h (Poindron and Le Neindre, 1980). Ewes recognise their lambs within 30 to 120 minutes after birth, however, lambs are initially attracted to any large object (Smith et al., 1966). After 12 h a lamb can recognise its dam at close quarters (Nowak et al., 1987) and after 3 days from a distance of several meters (Nowak, 1991). The development of the ewe-lamb bond is dependent upon the time a ewe remains at the birth site (Nowak and Poindron, 2006). The longer a ewe and her lambs remain at the birth site, the lower the chance they will be temporarily or permanently separated (Alexander et al., 1983). Murphy et al. (1994) concluded that ewes should remain at the birth site undisturbed for 6 h. Ewe nutrition greatly influences the time ewes spend at the birth site. Hungry ewes are tempted to move away from the birth site in order to graze which leads to more frequent separation (Lynch et al., 1992b; Nowak, 1996).

The ewe’s maternal behaviour 1-2 days after birth can be assessed using the maternal behaviour score (MBS) that was developed by O’Connor et al. (1985). The MBS is a rank from 1 to 5 which assesses the ewe’s reaction to her lambs being handled by a shepherd (Table 4). The ewe’s desire to return to her lambs is tested against the aversive presence of the person handling her lambs. High maternal behaviour scores have been linked with increased survival (O'Connor et al., 1985; Everett-Hincks et al., 2005b) and increased weaning weights (O'Connor, 1996). Ewe nutrition during pregnancy has been reported to influence MBS with low intake ewes being more likely to receive low scores than well-fed ewes (Dwyer et al., 2003). Everett-Hincks et al. (2005a), however, found no effect of ewe nutrition on MBS.

Table 4. Description of the Maternal Behaviour Score (MBS)(O'Connor et al., 1985)

Score	Description of maternal behaviour
1	Ewe flees at the approach of the shepherd, shows no interest in lambs and does not return
2	Ewe retreats further than 10 m but comes back to her lambs as shepherd leaves them
3	Ewe retreats to such a distance that tag identification is difficult (5 to 10 m)
4	Ewe retreats but stays within 5 meters
5	Ewe stays close to the shepherd during handling of her lambs

In the weeks after birth the behaviour of the ewe toward her lamb changes. Ewes gradually restrict the frequency and duration of suckling by walking away from the lamb (Lynch et al., 1992b). Hinch et al. (1987) observed that in the first month of life the ewe actively seeks out the lamb but after this time the ewe stops prior to reaching the lamb and bleats loudly before walking slowly away from the lambs and towards the flock.

Lamb growth

Lamb postnatal growth rates accelerate after birth and peak after approximately 50 days (Fitzhugh, 1976). The lamb’s postnatal growth has a positive relationship with birth weight (Greenwood et al., 1998). Small newborn lambs have a prolonged period after birth before they begin to gain weight. Greenwood et al. (1998) concluded that this was the sole reason for the reduced weaning weight. It would be expected that ewe condition at parturition and nutrition during lactation would alter lamb weaning weights, but this does not appear to be the case. Parker and McCutcheon (1992) concluded that ewes could buffer the effects of poor nutrition during lactation by mobilising body reserves. In that study, however, the minimum pasture sward height provided was 3.5 cm which resulted in ewes losing 0.4 of a body condition score during lactation. The majority of studies that examined the effects of ewe nutrition during pregnancy found no effect of nutrition on lamb postnatal growth rates or weaning weights (Holst et al., 1986; Scales et al., 1986; Smeaton et al., 1999). Supplementing ewe intakes with concentrates in late-pregnancy and higher pasture allowances in lactation (Rattray et al., 1982) have been reported to increase lamb growth rates to weaning (Dawson et al., 2005). Morris and Kenyon (2004) concluded that differences in lamb live weight on day 37 of lactation, but not day 87, was due to a carryover effect of birth weight.

Hypothalamic-pituitary-adrenal axis function

The postnatal effect of maternal undernutrition on lamb HPA function has received growing interest. Alterations in the activity of the HPA axis resulting from maternal undernutrition during pregnancy can result in offspring with a disposition for metabolic, endocrine and cardiovascular diseases in adult life (Lesage et al., 2006). Ewe undernutrition during pregnancy results in an increase in HPA responsiveness in the lamb. Hawkins et al. (2000) reported that lambs born to ewes offered 0.85 of their maintenance requirements in early pregnancy had elevated basal plasma cortisol and ACTH concentrations at 85 days of age. Borwick et al. (2003), however, reported there was no effect on basal cortisol and ACTH concentrations of ewe lambs at 26 or 31 weeks of age after restricting their dam to 0.7 of their maintenance requirements between day 100 of pregnancy and parturition. This may reflect different effects of the timing of the feed restriction. Nutrient restriction in late pregnancy (day 100 to 145 of pregnancy) may have a lesser effect on the lamb's stress response as the fetal HPA axis is functional in the second half of gestation (Lesage et al., 2006).

The effect of maternal nutrient restriction on lamb plasma cortisol and ACTH concentrations is inconsistent and altered with lamb age. Chadio et al. (2007) reported that at 2 months of age but not at 5.5 or 10 months, lambs born to ewes offered 0.5 of their maintenance requirements in early pregnancy had greater plasma cortisol and

ACTH responses to a CRH challenge than lambs born to ewes offered maintenance requirements. In addition, at 12, but not 4, months of age ewe lambs born to ewes offered half the requirements to maintain maternal live weight in early pregnancy had elevated basal cortisol concentrations than ewe lambs born to ewes offered maintenance requirements.

Summary of the effect of maternal undernutrition

Mild chronic maternal undernutrition during pregnancy is common in an extensive production system. Regions of New Zealand have limited pasture growth during winter months and are unable to provide ewes, particularly ewes bearing twin and triplet fetuses, with optimal nutrition throughout pregnancy. Poor nutrition during pregnancy can impact not only on lamb birth weight and survival but also on ewe and lamb behaviour after birth and the lamb's HPA axis function. The implications for mild chronic undernutrition on the long-term behaviour, response to stressors and reproductive success of offspring is unknown.

Purpose and scope of the investigation

Lamb mortality in New Zealand ranges between 5 and 25% and it is believed that this can be reduced through achieving optimal lamb birth weights. In particular, the increase in the birth weight of light-weight lambs may reduce mortality. In addition, the behaviour of the ewe and lamb after birth plays an important role in lamb survival. Two possible approaches to increasing lamb birth weight in an extensive pastoral production system are through shearing at approximately day 80 of pregnancy and altering ewe nutrition between days 70 of pregnancy and parturition. In addition the effects of these manipulations on ewe and lamb behaviour and the stress response to lambs require research.

The aims of this thesis were to:

- Identify an alternative to mid-pregnancy shearing to increase lamb birth weights without placing the ewe at risk of hypothermia.
- Determine if stress produced by shearing is the component of mid-pregnancy shearing that produces the increase in lamb birth weights.
- Determine the effect of stressors in mid-pregnancy (at approximately day 80 of pregnancy) on the behaviour of the ewe and lamb 12 to 24 h after birth.
- Establish the effects of shearing on approximately day 80 of pregnancy on the cortisol response of ram lambs to a painful stimulus.
- Identify the critical period for ewe nutrition during mid (between days 70 and 107 of pregnancy) and late-pregnancy (day 108 to 145 of pregnancy) that influences lamb birth weight.
- Determine the effect of ewe nutrition in mid (day 70 to 107 of pregnancy) and late-pregnancy (day 108 to 145 of pregnancy) on the behaviour of the ewe and lamb 12 to 24 h after birth.
- Examine the effect of ewe nutrition between day 64 and 132 of pregnancy on the behaviour of female progeny at 8 months of age and their reproductive success and maternal behaviour at 2 yr of age.
- Determine the effect of ewe undernutrition between days 70 and 145 of pregnancy on the cortisol response of lambs to a painful stimulus.

CHAPTER 2

The effect of mid-pregnancy shearing or yarding stress on ewe post-natal behaviour and the birth weight and post-natal behaviour of their lambs.

Publications:

Corner R.A., Kenyon P.R., Stafford K.J., West D.M., Oliver M.H. The effect of mid-pregnancy shearing or yarding stress on ewe post-natal behaviour and the birth weight and post-natal behaviour of their lambs. Livestock Science 2006. 102, 121-9.

Abstract

This study was undertaken to determine whether a 24-hour fast plus yarding could duplicate the increase in lamb birth weight associated with mid-pregnancy shearing, thereby avoiding the potentially harmful effects of shearing ewes in a cold climate. Mixed-age Romney ewes bearing singleton (n=138) or twin lambs (n=115) were assigned to one of three treatment groups; control, yarded or shorn. Yarding and mid-pregnancy shearing took place on day 79 and 80 of pregnancy. Throughout pregnancy ewes were managed as a single group until 1 week prior to lambing at which time they were divided into 3 groups for lambing. Within twelve hours of birth lamb weight, body dimensions and behaviour were recorded. The maternal behaviour of the ewe was also determined during this period. The liveweight of twin-bearing ewes was greater than their singleton-bearing counterparts ($P<0.05$). Lambs born to ewes that were shorn during mid-pregnancy were heavier ($P<0.05$) at birth than lambs born to ewes in either the control or yarded treatment groups by 0.4 and 0.5 kg, respectively. Lambs born to ewes in the shorn treatment had larger girths (41.0 vs. 38.9 and 39.7 cm) and crown-rump-lengths (CRL) (56.9 vs. 54.9 and 55.6 cm) than lambs born to ewes in both the control and yarded treatments. Ewe treatment had little effect on ewe and lamb behaviour. Lambs born to shorn ewes were heavier ($P<0.05$) at weaning by 1.7 kg than lambs born to yarded ewes. The absence of a significant difference in the birth weight and body size between lambs born to yarded and control ewes and the significant difference between shorn and yarded treatments indicates that yarding stress is not the mechanism responsible for the birth weight increase from mid-pregnancy shearing.

Introduction

Under New Zealand conditions lamb mortality rates of 10-17% in singleton-born and 19-27% in twin-born lambs have been reported (Dalton et al., 1980; Hinch et al., 1985; Nicoll et al., 1999). It has been suggested that the optimum birth weight to achieve maximal lamb survival is between 3.5 and 5.5 kg (Dalton et al., 1980; Hinch et al., 1985). Therefore there is considerable interest in methods of increasing lamb birth weight of otherwise light-weight lambs as a means of increasing lamb survival.

The benefits of mid-pregnancy shearing include an increased number of ewes seeking shelter, a reduction in wool around the udder thus facilitating teat location and a reduction in number of ewes becoming recumbent (Kenyon et al., 2003) and increased lamb birth weights in winter-housed (Rutter et al., 1971; Adalsteinsson, 1972; Rutter et al., 1972; Kneale and Bastiman, 1977; Maund, 1980; Morgan and Broadbent, 1980) and pasture based production systems (Morris and McCutcheon, 1997; Morris et al., 2000; Smeaton et al., 2000; Kenyon et al., 2002a; Kenyon et al., 2002b, c; Revell et al., 2002). Mid-pregnancy shearing is associated with an increase in lamb survival which has been linked to the increase in lamb birth weight (Morris et al., 1999). It is also possible that mid-pregnancy shearing affects the dam’s behaviour at parturition, although this has not been previously investigated.

Mid-pregnancy shearing takes place during the winter months and can increase the ewe’s susceptibility to cold stress. If weather conditions are cold, wet and windy, mid-pregnancy shearing can result in ewe deaths (Dabiri et al., 1995b; Husain et al., 1997). The use of a ‘cover’ or ‘snow’ comb can reduce this risk by leaving a greater residual fleece depth. Elucidation of the mechanism for the increase in lamb birth weight produced by mid-pregnancy shearing may lead to the identification of methods which increase lamb birth weight and survival without risk to the ewe.

Shearing is both an acute (Hargreaves and Hutson, 1990b, a, c) and chronic stressor (Pierzchala et al., 1983). Acute stressors for sheep include; yarding (commonly involving dogs, people and vehicles), time spent off pasture (24-36 hours), isolation, noise of shearing machinery and handling by the shearer (Hargreaves and Hutson, 1990b, a, c). In addition chronic cold stress is produced by wool removal (Pierzchala et al., 1983).

To date the specific mechanism resulting in the increase in lamb birth weight from mid-pregnancy shearing has not been identified. The increase in lamb birth weight observed is not due to increased gestation lengths (Kenyon et al., 2002c), increased ewe feed intakes (Kenyon et al., 2002b; Revell et al., 2002), changes in the ewe's metabolism (Revell et al., 2002) or increased maternal thyroid hormone (T3 and T4) concentrations (Kenyon et al., 2005b). Other stressors such as transport and isolation increase fetal weight in late gestation. In sheep, repeated isolation during the last 5 weeks of pregnancy (day 112 to 145 of pregnancy) resulted in increased lamb birth weights (Roussel et al., 2004). In cattle, repeated transportation in early to mid-pregnancy (days 60, 80, 100, 120 and 140 of a 281 day pregnancy) resulted in increased fetal calf weights (Lay et al., 1997a). Therefore an increase in lamb birth weight may be achieved by producing a stress response in the ewe thus eliminating the need to remove the fleece, thus eliminating the risks of hypothermia to the ewe.

The objectives of this study were firstly to investigate whether yarding (24-36 hours off pasture and association with dogs and humans) produced an increase in lamb birth weight and secondly to determine if mid-pregnancy shearing affects ewe or lamb behaviour post-parturition.

Methods and materials

Animals and measurements

Oestrus was synchronised in 438 mixed age Romney ewes through the use of progesterone impregnated intravaginal devices (EziBreed CIDR, Pfizer, Auckland, New Zealand) for 13 days. Rams were introduced on the day of CIDR removal and individual breeding dates were recorded from daily observation of ewes for ram harness crayon marks for 4 days (01 Apr 2003 to 04 Apr 2003). Ewe pregnancy diagnosis was conducted using trans-abdominal ultrasound 62 days after the mid-point of the breeding period. Ewes diagnosed as bearing singleton (n=138) or twin (n=115) fetuses were randomly assigned to one of three treatment groups. The treatments included; control, 24 hour fast in covered yards and movement through yards driven by people and dogs (yarded) and mid-pregnancy shearing including a 24 hour fast (shorn). On day 79 of pregnancy (P79) all ewes were brought into the yards and drafted into treatment groups. Ewes in the control group were immediately returned to pasture. Ewes in the shorn treatment were shorn on P80 (20 June 03) using a cover comb (Sunbeam Corporation Ltd, New Zealand) leaving 5-7 mm of wool. All ewes had approximately 6 months of wool growth on day 80 of pregnancy. Ewe fleece weights were recorded to adjust ewe liveweight for the weight of the wool removed. Ewes in the yarded treatment were moved through yards by people and dogs for 30 minutes on two occasions (day 79 and 80 of pregnancy). Four people and two dogs (a loud Huntaway and an experienced heading dog) moved the ewes briskly through the yards. Ewes were moved through a drafting race (3 to 4 times during each session) and at the conclusion of the session ewes were difficult to move and unwilling to pass near the dogs. In New Zealand it is common practice to fast sheep for 24 hours prior to shearing, therefore yarded ewes were also housed off-pasture between the two treatment days.

During the remainder of pregnancy all ewes were managed as one group under commercial farming conditions. Ewes were weighed on days 76, 118 and 133 of pregnancy. On day 140 of pregnancy all ewes were moved to lambing paddocks with 15 and 12 ewes per hectare for singleton- and twin-bearing ewes, respectively. Within each pregnancy rank and treatment group ewes were randomly allocated to each lambing paddock. During the lambing period ewes were observed three times daily to identify newly born lambs and, if required, to provide assistance to ewes lambing.

Within 12 hours of birth, lambs were tagged and weighed. The sex and litter size of each lamb was recorded. Crown rump length (CRL), thoracic girth (circumference of the chest immediately posterior to forelimb), forelimb length (FL, distance from the shoulder joint to the tip of the hoof on the left leg) and hind limb length (HL, distance from the hip joint to the tip of the hoof on the left leg) were measured. The lamb's coat colour was ranked on a 4 point scale from 0 to 3 (0 = no staining, 1 = pale yellow, 2 = yellow and 3 = dark yellow - orange) as an indicator of birthing stress (Oliver et al., 2001).

Ewe maternal behaviour was observed during the tagging procedure and quantified using a maternal behaviour score (MBS) (O'Connor et al., 1985; Dwyer and Lawrence, 1998; Everett-Hincks, 2003). Observation of the behaviour of the ewe and lamb was made by one of 4 experienced shepherds. Immediately post-tagging the lamb was placed on its side while an observer stood at least 10 metres away from the lamb. After the lamb was released the behaviour of the lamb and the ewe was recorded for 5 minutes. The time taken for the lamb to stand, make contact with the ewe, suck and follow the ewe (if she moved more than 5 meters from the tagging site) was noted. During the observation period the number of both high and low pitch ewe bleats were recorded in addition to whether the lamb bleated. Lambs were weighed again at weaning 80 days after the mid-point of lambing. The presence of lamb on day 20 (docking) and day 80 (weaning) after the mid-point of the lambing period was used to determine survival.

This study was conducted at Massey University Keeble sheep and beef farm, 5km south of Palmerston North, New Zealand from March until October 2003. The study was conducted with the approval of Massey University Animal Ethics Committee (MUAEC 03/66).

Statistical analysis

Continuous data (ewe liveweight, gestation length, lamb birth weight, were subject to analysis of variance using the generalised linear model procedure from the statistical package 'Minitab' (Minitab Inc, 2003). The main effects of ewe treatment (control vs. yarded vs. shorn), pregnancy status (singleton vs. twin bearing) or litter size (singleton vs. twin), sex of lamb (female vs. male) and interactions between these parameters were included in the original models. All non-significant ($P>0.05$) interactions were removed and the models re-fitted.

Pregnancy-status and litter size were determined retrospectively as the number of lambs identified per ewe within 12 hours of birth. Litter size was not altered if a lamb was born dead or died after birth.

Ewe liveweight was used as a covariate in the models to partition variation in lamb girth and forelimb length. Ewe liveweight and gestation length were used as covariates in the models to partition variation in lamb crown rump length and hind limb length.

Count data (low and high pitched ewe bleats) were analysed using the Kruskal-Wallis procedure in Minitab (Minitab, 2003). The main effects were ewe treatment (control vs.

yarded vs. shorn), pregnancy status or litter size (singleton vs. twin) and lamb sex (female vs. male).

Categorical data (coat meconium score and maternal behaviour score) were analysed using the mixed model procedure in SAS (SAS, 2005). The effects of litter size and ewe treatment were examined. In addition, the proportion of lambs that had birth weights of less than 4 kg was compared using the PROC GENMOD procedure and included the fixed effect of ewe treatment.

Analysis of survival was conducted using the Kaplan-Meier survival analysis procedure in the statistical package SPSS (SPSS, 2003). The effects of litter size and ewe treatment were included in the model.

Results

Weather conditions at time of shearing

During the week after shearing the minimum and maximum air temperatures were 5°C and 14.7 °C. On 3 consecutive days (26-28 June 03) minimum temperatures dropped below 0°C. The average wind speeds during the week after shearing were 3.3 km/hr, the relative humidity was 84% and the average rainfall was 2.5 mm.

Ewe liveweight and gestation length

Singleton-bearing ewes were lighter ($P<0.05$) than twin-bearing ewes during pregnancy (Table 5). Ewe liveweight did not differ between ewe treatments at any time during pregnancy. Neither pregnancy status nor ewe treatment had any effect on the gestation length (Table 5). Shorn ewes tended to have a longer gestation than both the control and yarded ewes ($P=0.09$).

Table 5. Ewe live weights (kg) on days 79, 99 and 118 of pregnancy and gestation lengths of singleton- and twin-bearing ewes in the control, yarded and shorn treatments (least squares mean ± standard error)

		Ewe liveweight			Gestation
	(n)	P79	P99	P118	length
Pregnancy status					
Singleton	138	$51.9^{a} \pm 0.4$	$59.0^{a} \pm 0.5$	$62.2^{a} \pm 0.5$	147.0 ± 0.2
Twin	115	$56.0^{b} \pm 0.5$	$64.9^{b} \pm 0.5$	$67.8^{b} \pm 0.5$	146.6 ± 0.2
Ewe treatment					
Control	81	54.6 ± 0.6	61.5 ± 0.6	64.5 ± 0.7	146.6 ± 0.2
Yarded	82	53.5 ± 0.5	61.6 ± 0.6	64.7 ± 0.7	146.6 ± 0.2
Shorn	90	53.8 ± 0.5	62.7 ± 0.6	65.7 ± 0.7	147.2 ± 0.2

Means within columns with different superscripts are significantly different (P<0.05)

Lamb live weights at birth and weaning

At birth and on day 80 of lactation, singleton-born lambs were heavier (P<0.05) than their twin-born counterparts (Table 6). At birth, lambs born to shorn ewes were heavier (P<0.05) than lambs born to both control (by 0.5 kg) and yarded ewes (by 0.6 kg). The birth weight increase was greater for twin (0.5 kg; P<0.001) than singleton lambs (0.3 kg; P=0.09). Fewer lambs born to shorn ewes weighed less than 4 kg at birth than lambs born to control ewes (10.4 vs. 20.8 %; P<0.05). There was no difference in the birth weight of lambs born to ewes in the control and yarded treatments.

On day 80 of lactation lambs born to yarded ewes were lighter (P<0.05) than lambs born to shorn ewes and singleton-born lambs were heavier than twin-born lambs (Table 6).

Table 6. Live weight (kg) of singleton and twin lambs born to ewes in the control, yarded and shorn treatments at birth and weaning on day 80 of lactation (least squares mean ± standard error)

	Lamb liveweight (kg)			
	(n)	Birth	(n)	Weaning
Litter size				
Singleton	137	$5.7^{b} \pm 0.06$	120	$26.4^{b} \pm 0.3$
Twin	232	$4.5^{a} \pm 0.04$	204	$22.4^{a} \pm 0.3$
Ewe treatment				
Control	117	$5.0^{a} \pm 0.07$	100	$24.2^{ab} \pm 0.4$
Yarded	117	$4.9^{a} \pm 0.07$	104	$23.7^{a} \pm 0.4$
Shorn	135	$5.4^{b} \pm 0.07$	120	$25.4^{b} \pm 0.4$
Litter size x Ewe treatment				
Singleton x Control	43	$5.6^{a} \pm 0.13$		
Singleton x Yarded	49	$5.6^{a} \pm 0.12$		
Singleton x Shorn	45	$5.9^{a} \pm 0.13$		
Twin x Control	74	$4.3^{a} \pm 0.10$		
Twin x Yarded	68	$4.3^{a} \pm 0.10$		
Twin x Shorn	90	$4.8^{b} \pm 0.09$		

Means within columns with different superscripts are significantly different (P<0.05)

Lamb girth, crown rump length, forelimb and hind limb length

Litter size and ewe treatment had a significant (P<0.01) effect on lamb thoracic girth, crown rump length (CRL) and forelimb length (Table 7). Singleton-born lambs were larger (P>0.05) in all body dimensions than twin-born lambs. Lambs born to shorn ewes had larger (P<0.05) girths and CRL's than lambs born to ewes in both the control and yarded treatments. In addition lambs born to shorn ewes had larger (P<0.05) forelimb lengths compared to lambs born to yarded ewes (Table 7).

There was a interaction (P<0.05) between litter size and ewe treatment on lamb rear leg length (Table 7). Twin lambs born to shorn ewes had (P<0.05) longer hind limbs than lambs born to yarded ewes. Singleton lambs born to ewes in any of the treatments did not differ in hind limb length.

Table 7. Thoracic girth, crown-rump length (CRL), forelimb and hind limb length (cm) and coat meconium staining score of single and twin lambs born to ewes in the control, yarded and shorn treatments (least squares mean ± standard error)

	(n)	Girth (cm) [2]	CRL (cm) [2]	Forelimb (cm) [2]	Hind limb (cm) [1]	Meconium score [2]
Litter size						
Singleton	137	41.7^{b}±0.2	57.7^{b}±0.3	31.1^{b}±0.2	37.1^{b}±0.2	1.8^{b}±0.14
Twin	231	38.6^{a} ±0.2	53.9^{a}±0.3	29.7^{a}±0.4	35.5^{a}±0.2	1.6^{a}±0.13
Ewe treatment						
Control	116	38.9^{a}±0.2	54.9^{a}±0.4	30.4^{ab}±0.2	36.0^{a}±0.2	1.8^{b}±0.15
Yarded	117	39.7^{a}±0.2	55.6^{a}±0.4	29.9^{a}±0.2	35.9^{a}±0.2	1.9^{b}±0.14
Shorn	135	41.0^{b}±0.2	56.9^{b}±0.3	31.1^{b}±0.2	36.6^{a}±0.2	1.5^{a}±0.15
Litter size x Ewe treatment						
Singleton x Control	43				36.7^{cd}±0.3	
Singleton x Yarded	49				37.5^{d}±0.3	
Singleton x Shorn	45				37.1^{cd}±0.3	
Twin x Control	73				35.4^{ab}±0.2	
Twin x Yarded	68				34.3^{a}±0.2	
Twin x Shorn	90				36.1^{bc}±0.2	

Means within columns with different superscripts are significantly different ($P<0.05$)

[1] significant interactions of litter size and ewe treatment

[2] Non-significant interactions of litter size and ewe treatment

Lamb coat meconium score and survival

Lamb coat meconium scores were higher ($P<0.05$) in singleton than twin lambs indicating darker coat meconium staining (Table 7). Coat meconium scores were lower in lambs born to shorn ewes than in lambs born to control or yarded ewes ($P<0.05$). Lamb survival to weaning was not affected by ewe treatment or litter size (data not shown).

Ewe and lamb behaviour

Pregnancy rank had a significant ($P<0.05$) effect on the number of low and high pitched bleats and on the number of ewes that did not emit a high pitched bleat (Table 8). Singleton bearing ewes made fewer low pitched bleats and more high pitched bleats than twin-bearing ewes ($P<0.05$). More twin-bearing ewes did not emit a high pitched bleat during the observation period than singleton bearing ewes ($P<0.05$). Ewe treatment had little effect on the ewe’s behaviour at lambing.

Table 8. The maternal behaviour score (MBS), frequency of low and high pitched bleats and the number and percentage of singleton and twin-bearing ewes that failed to bleat in a low or high pitch in the control, yarded and shorn treatments (values presented as medians).

		MBS	Low bleats	Failed to low bleat		High bleats	Failed to high bleat	
	(n)		n	n	% [1]	n	n	% [1]
Pregnancy rank								
Singleton	133	2.9[a]	4.0[a]	22	14.5	6.0[b]	14[a]	9.2
Twin	114	3.2[b]	5.0[b]	9	7.5	3.0[a]	26[b]	21.7
Ewe treatment								
Control	77	3.1	5.0	14	16.7	5.0	12	14.3
Yarded	81	3.0	5.0	9	9.4	5.0	15	15.6
Shorn	89	3.0	4.0	8	8.7	5.0	13	14.1

Medians within columns with different superscripts are significantly different (P<0.05)

[1] The percentage of ewes that did not bleat during the 5 minute observation period.

Maternal behaviour scores were greater for twin- than singleton-bearing ewes (P<0.05). However scores did not differ between ewe treatments (Table 8).

The lamb's latency to make contact with and follow its dam was significantly different (P<0.05) between singletons and twins (Table 9). Singleton-born lambs required more time (P<0.05) to make contact with their dam but shorter time to follow their dam than twin born lambs. Twin lambs born to shorn ewes were more likely to suckle during the 5 minute observation period than their counterparts born to yarded ewes (P<0.05). Ewe treatment overall had no other effect on lamb behaviour during the observation period.

Table 9. The number and percentage of lambs that failed to stand, contact their dam, suck and follow their dam and the time taken to exhibit each of these behaviours during a 5-minute observation period for singleton and twin lambs born to control, yarded and shorn ewes (data are presented as medians and percentages)

	(n)	Time to stand	Failed to stand		Time to contact	Failed to contact		Time to suck	Failed to suck		Time to follow	Failed to follow		Failed to bleat	
		(sec)	n	%	(sec)	n	%	(sec)	n	%	(Sec)	n	%	n	%
Litter size															
Singleton	133	14.5	11	8.3	16.0^{b}	7	5.3	90.5	59	44.4	96.0^{a}	26	19.5	12	9.0
Twin	216	12.0	13	6.0	11.0^{a}	9	5.0	98.0	102	47.2	115.0^{b}	62	28.7	17	7.9
Ewe treatment															
Control	109	11.5	9	8.3	12.0	3	2.8	100.0	54^{b}	49.5	105.5	35	32.1	9	8.3
Yarded	112	15.0	9	8.0	15.0	5	4.5	90.0	60^{b}	53.6	106.0	25	22.3	7	6.3
Shorn	128	12.5	6	4.7	14.5	8	6.3	98.0	47^{a}	38.1	107.5	28	21.9	13	10.2
Litter size x Ewe treatment															
Singleton x Control	41								20^{ab}	48.8					
Singleton x Yarded	48								22^{ab}	45.8					
Singleton x Shorn	44								17^{ab}	38.6					
Twin x Control	68								34^{ab}	50.0					
Twin x Yarded	64								38^{b}	59.4					
Twin x Shorn	84								30^{a}	35.7					

Medians within columns with different superscripts are significantly different ($P<0.05$)

Discussion

The aims of this study were firstly to determine if fasting ewes for 24-hours plus a period of intensive yarding could duplicate the lamb birth weight increase associated with mid-pregnancy shearing. Secondly, to determine if mid-pregnancy shearing or yarding altered the behaviour of either the ewe or lamb around the time of birth.

Mid-pregnancy shearing increased lamb birth weights by 0.4 kg and 0.5 kg compared with lambs born to control or yarded ewes, respectively. It was apparent that this birthweight increase was greatest in twin born lambs. An increase in birth weight of only twin-born lambs has been reported by Morris and McCutcheon (1997), Smeaton et al. (2000) and Kenyon et al. (2002a). An increase in the birth weight of twin-born lambs is most likely to increase lamb survival as twin lambs are more likely to have birth weights under the optimum range of 3.5 to 5.5 kg (Dalton et al., 1980) than singleton-born lambs. The reduction in the proportion of lambs that weighed less than 4 kg at birth between the shorn and control treatments indicates that shearing has the potential to increase lamb survival rates.

The slight increase in gestation length in the shorn ewes was in agreement with the findings of Adalsteinsson (1972) and Revell et al. (2002). However, this increase does not account for the increase in lamb birth weight seen in the present study. In late pregnancy fetuses grow at a rate of 150-200 g/day (Rattray et al., 1974), therefore the 0.6 day increase would only account for a 90-120 gram increase in birth weight.

The lack of a birthweight effect of the 24-hour fast plus intensive yarding indicates that this treatment cannot replace mid-pregnancy shearing as a means of increasing lamb birth weights. Therefore the birthweight increase from mid-pregnancy shearing is not associated with handling stressors. Other mediating factors associated with shearing that warrant investigation include isolation, up-ending, handling by the shearer, noise, wool removal, cold stress post wool removal or a combination of these events.

Lambs born to ewes shorn during pregnancy were 1.7 kg heavier at weaning than lambs born to ewes that were yarded. However, there was no difference in weaning weights between lambs born to shorn ewes of lambs of control ewes. Across studies the effects of mid-pregnancy shearing on lamb liveweight at weaning have not been consistent. Mid-pregnancy shearing has been reported to have no effect on weaning weight (Dabiri et al., 1994; Dabiri et al., 1995a; Dabiri et al., 1996; Husain et al., 1997; Morris and McCutcheon, 1997; Morris et al., 2000; Kenyon et al., 2002b; Revell et al., 2002) or a small increase in the weaning weight of singletons (Smeaton et al., 2000), or twins (Morris et al., 1999) or both singletons and twins (Cloete et al., 1994).

Mid-pregnancy shearing and yarding had little effect on the behaviour of ewes and lambs during a 5-minute observation period at the completion of tagging within 12 hours of birth. Singleton-bearing ewes in the yarded and shorn treatments were more likely to emit a low-pitched bleat than control ewes. Ewe low pitched bleats are made exclusively to the lamb and are described as ‘care-giver’ bleats that aid in forming the ewe-lamb bond (Dwyer et al., 1998). Therefore an increase in low bleats may indicate a greater ability of ewes in the yarded and shorn treatments to bond with their lambs. However there were no differences in the frequency of low bleats of twin bearing ewes regardless of treatment groups.

The maternal behaviour score was used as a method to quantify maternal behaviour. Whilst the maternal behaviour scores were recorded it became apparent that the lamb’s behaviour greatly influenced the ewe’s behaviour and therefore MBS. Lambs that bleated and stood quickly tended to reunite more quickly with their dam than lambs that remained recumbent and did not bleat. In addition the maternal behaviour scoring system does not account for the ewe's response to disturbance or predators. Therefore the MBS should be used as a generalised indictor of maternal behaviour.

Twin lambs born to shorn ewes were more likely to suck after being reunited with their dam than twin lambs born to yarded ewes. However, in the twin lambs that did suck, there was no difference in latency to suck between ewe treatment groups. In the absence of other alterations in lamb behaviour it is suggested that mid-pregnancy shearing and yarding stress has little effect on lamb behaviour.

Coat meconium staining of amniotic fluid is an indicator of fetal hypoxia at the time of delivery in humans (Kimble et al., 1999), but coat meconium staining has not been well characterised in sheep. In this study coat meconium staining between litter sizes and ewe treatments varied significantly. Mid-pregnancy shearing resulted in lambs with lighter coat staining than both the control and yarded treatments. The relationship between fetal hypoxia and the degree of coat meconium staining in sheep has not been quantified and requires further investigation.

Conclusions

Mid-pregnancy shearing resulted in lambs that were heavier and larger at birth than lambs born to both control and yarded ewes. Yarding stress had no effect on lamb birth weight or size compared with lambs born to control ewes. The birthweight increase was still present at weaning between lambs born to shorn lambs compared to those born to yarded ewes. Therefore mid-pregnancy shearing produces long-term changes in lambs' growth both before and after birth.

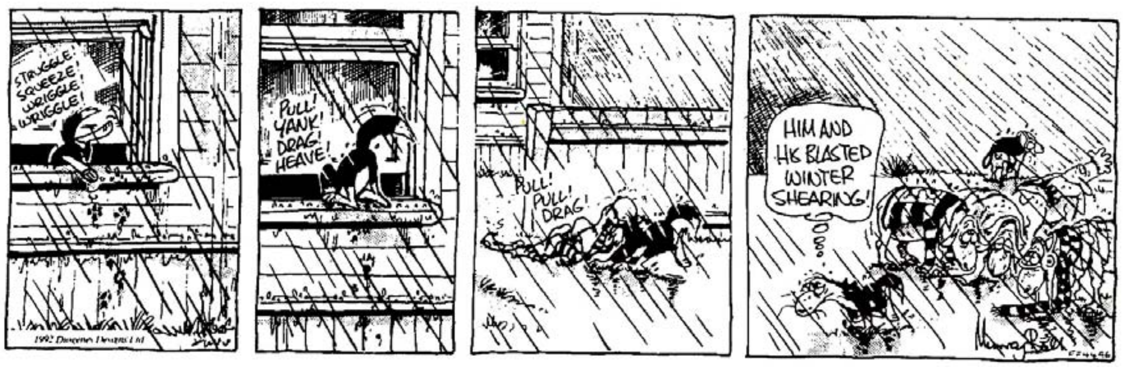
Further research

The results of this chapter indicate that yarding may not produce a stress response of sufficient intensity to alter lamb birth weights. Chapter 3a and b examines the effect of crutching and sham shearing which more closely reflect the events involved in shearing.

CHAPTER 3

A) The effect of mid-pregnancy shearing and litter size on lamb birth weight and postnatal plasma cortisol response.

B) The effect of mid-pregnancy stressors on twin-lamb live weight and body dimensions at birth.



Publications:

Corner R.A., Kenyon P.R., Stafford K.J., West D.M., Oliver M.H. The effect of mid-pregnancy shearing and litter size on lamb birth weight and postnatal plasma cortisol response. Small Ruminant Research 2007a. 73, 115-121

Corner R.A., Kenyon P.R., Stafford K.J., West D.M., Oliver M.H. The effect of mid-pregnancy stressors on twin-lamb live weight and body dimensions at birth. Livestock Science 2007b. 107, 126-31.

A) The effect of mid-pregnancy shearing and litter size on lamb birth weight and postnatal plasma cortisol response

Abstract

The study was conducted to determine the effect of mid-pregnancy shearing on lamb birth weight, body dimensions and postnatal plasma cortisol response to a stressor. Fetal exposure to excess maternal glucocorticoids at specific stages of pregnancy may influence postnatal function of the hypothalamic-pituitary-adrenal (HPA) axis. This in turn may have implications on the growth, development and survival of lambs. Romney ewes of various ages, bearing singletons (n=47) or twins (n=48) were shorn or not (control) on day 80 of pregnancy. Ewes were managed as a single flock throughout pregnancy under general commercial farming conditions. Within 12 h of birth the lamb's live weight and body dimensions were recorded. On day 39 of lactation the plasma cortisol response of the ram lambs to handling or castration was assessed. A random sample of ram lambs born to control (6 singleton-born and 9 twin-born ram lambs) or shorn (7 singleton-born and 11 twin-born ram lambs) ewes were allocated to either a handling or castration treatment. Singleton and twin lambs born to shorn ewes were 8% heavier (0.4 kg and 0.3 kg, respectively) at birth than their counterparts ($P<0.05$). Lambs born to ewes shorn in mid-pregnancy had longer ($P<0.05$) crown rump (53.1 vs. 51.7 cm) and forelimb (30.8 vs. 30.0 cm) measurements than the counterparts born to control ewes, respectively. However, when the lamb's live weight was considered in the statistical model these differences were not significant. The area under the plasma cortisol response curve of twin lambs to handling or castration was higher ($P<0.05$) than that of singletons (60.8 vs. 47.1 nmol/L.min). Mid-pregnancy shearing had no effect on the cortisol response of singleton or twin-born ram lambs to handling

or castration. Mid-pregnancy shearing resulted in increased lamb live weights and body dimensions in both singleton and twin lambs at birth - while plasma cortisol response of lambs to handling or castration were unaffected. The lamb live weight data suggest that mid pregnancy shearing had little effect on HPA axis development in singleton or twin offspring.

Introduction

Shearing ewes in mid-pregnancy has caused an increase in lamb birth weights in both winter-housed (Dyrmundsson and Lees, 1972; Austin and Young, 1977; Symonds et al., 1986; Vipond et al., 1987; Black and Chestnutt, 1990) and outdoor pasture-based sheep production systems (Morris and McCutcheon, 1997; Morris et al., 2000; Smeaton et al., 2000; Revell et al., 2002; Kenyon et al., 2004). This increase in lamb birth weight has been associated with an increase in lamb survival rate (Morris et al., 1999).

The shearing of sheep causes an elevation in plasma cortisol concentrations (Pierzchala et al., 1983; Hargreaves and Hutson, 1990a; Mears et al., 1999). During pregnancy, cortisol can be transferred from the maternal to the placental circulation and be carried to the fetal brain and other major organ systems (Zarrow et al., 1970; Weinstock, 1997). Transfer of blood cortisol from the mother to the fetus may account for all the circulating cortisol measured in both intact and adrenalectomised ovine fetuses between days 100 and 120 of gestation, however between days 122 and 135 maternal cortisol accounts for only 37% (Hennessy et al., 1982). Therefore an increase in maternal plasma cortisol concentrations resulting from mid-pregnancy shearing may temporarily increase fetal blood cortisol concentrations, which could impact on the development of the hypothalamic-pituitary-adrenal (HPA) axis.

In sheep, the artificial elevation of maternal glucocorticoids during mid pregnancy has a profound influence on fetal growth (Fowden et al., 1996; Jobe et al., 1998) and postnatal regulation of glucose tolerance (Nyirenda et al., 1998), blood pressure (Dodic et al., 1998) and HPA axis function (Sloboda et al., 2002). Prenatally stressed rat pups have shown an impaired stress-coping ability as adults (Fride et al., 1986). In sheep, the repeated isolation of ewes during late pregnancy (day 112 to 145 of pregnancy) resulted in increased basal plasma cortisol concentrations of their lambs at 25 days of age (Roussel et al., 2004) and repeated transport of cows during early and mid-pregnancy (day 60, 80, 100, 120 and 140 of a 281 day pregnancy) resulted in increased plasma cortisol responses of the calves to restraint at 10 and 150 days of age (Lay et al., 1997b). It is therefore possible that mid-pregnancy shearing may alter the lamb’s post-natal HPA function.

The objectives of this study were to confirm the effect of mid-pregnancy shearing on the birth weight of singleton- and twin-born lambs and to determine if mid-pregnancy shearing leads to an alteration in their lambs postnatal plasma cortisol response to a stressor.

Materials and methods

Oestrus was synchronised in 290 mixed-age Romney ewes through the use of progesterone impregnated intravaginal devices (EziBreed CIDR, Pfizer, Auckland, New Zealand) for 13 days. Romney rams were introduced to the ewes at a ratio of 1:15 on the day of CIDR removal. Individual breeding dates were recorded by daily observation of ram harness-crayon marks for 5 days after the introduction of the rams (30 Mar 04 to 02 Apr 04). Romney rams were removed after 5 days and replaced with Suffolk rams fitted with a different coloured crayon colour at a ram to ewe ratio of 1:125. Ewes marked by the Suffolk rams were excluded from the study. Ninety-six ewes diagnosed as twin bearing by trans-abdominal ultrasound scan 59 days after the mid-point of the breeding period (P59) were selected for the experiment. Ewes diagnosed as bearing singleton (n=47) or twin fetuses (n=48) were used in the experiment. On day 80 of pregnancy ewes were randomly assigned to either a control (n=44) or shorn (n=51) treatment group. The control ewes were returned to pasture and ewes in the shearing treatment were fasted for 24 h prior to shearing (16 June 04). The following day ewes were shorn with a cover comb (Sunbeam Corporation Ltd, New Zealand) that left a uniform residual fleece depth of 5-7 mm (Dabiri et al., 1995b). The weight of wool fleece removed was recorded and for all subsequent live weights the fleece weight was added to allow for a comparison of live weight between the shorn and control ewes. All ewes had approximately 6 months of wool growth. During the remainder of pregnancy all ewes were managed as one group under commercial farming conditions. On day 140 of gestation ewes were randomly allocated to one of 4 lambing paddocks with 15 and 12 ewes per hectare for singleton and twin bearing ewes, respectively. During pregnancy and lactation ewe live weights were recorded on day 79, 112, 140 of gestation (P79, 112, 140) and day 42 after the mid-point of the parturition period (L42).

During the lambing period ewes were observed three times daily by experienced shepherds to identify newly born lambs and, if required, to provide assistance to ewes lambing. Within 12 h of birth all lambs, alive or dead, were tagged and the sex and weight of the lamb was recorded. The lamb's crown rump length (CRL), thoracic girth, forelimb length and hindlimb length were measured. Lamb coat meconium staining was assessed as an indicator of parturition stress (Oliver et al., 2001). Coat meconium scores were ranked on a scale of 4, from 0 to 3 (0 = no staining, 1 = pale yellow, 2 = yellow and 3 = dark yellow – orange). During the postnatal period all ewes and lambs remained in the paddocks previously allocated before parturition. Lamb live weights were also recorded on days 42 and 94 after the mid-point of the parturition period.

On day 39, following the mid-point of the parturition period, plasma cortisol response of the ram lambs to handling or castration was assessed. Ram lambs born to the control (6 singletons and 9 twins) and shorn ewes (7 singletons and 11 twins) were randomly assigned to a handling or castration treatment. Lambs were assigned to treatments in such a way as to ensure that siblings were not allocated to the same treatment. Lambs in the handling treatment were restrained and the scrotum palpated. Lambs in the castration treatment were ring castrated by the application of a rubber ring (Allflex New Zealand Ltd, Palmerston North) to the scrotum, above the testes as described by Mellor and Stafford (2000). Blood samples (5 ml) were collected from lambs in both the castrated and handled treatment groups. Blood samples were obtained by jugular venipuncture using 20 G needles (BD Vacutainer Systems, Plymouth, UK) into 10 ml collection tubes containing sodium heparin (BD Vacutainer, Preanalytical solutions, Franklin Lakes, USA). The blood sampling commenced at 1300 h of each day and immediately prior to treatment (T=0) and 20, 40, 60, 90, 120, 150, 180, 210 and 240 minutes thereafter. At the completion of the blood sampling period all lambs were docked and any remaining entire ram lambs were castrated. Blood samples were immediately placed on ice and centrifuged at 3000 rpm for 10 minutes. Plasma was separated and frozen at -20°C and analysed by RIA kits for total plasma cortisol concentration using Clinical Assays™ GammaCoat™ ^{125}I-RIA (DiaSorin, Stillwater, MN, USA). The GammaCoat™ antiserum exhibits 100% cross reactivity with cortisol. The intra-assay coefficient of variation was 7.0% and the inter-assay variation was 9.2%.

This study was conducted with the approval of Massey University Animal Ethics Committee (MUAEC 04/55) at Massey University's Keeble sheep and beef farm, 5km south of Palmerston North, New Zealand from March to November 2004.

Statistics

Ewe live weight, gestation length, lamb live weight and lamb cortisol data were analysed using the repeated measures MIXED model (SAS, 2005). Lamb body dimensions were analysed using the MIXED model and the fixed effects included ewe pregnancy status or litter size and ewe treatment. The effect of litter size (singleton vs. twin) and ewe treatment (control vs. shorn) on lamb live weight (at birth, L42 and L94), body dimensions at birth and coat meconium staining were also analysed using this procedure. The effect of litter size, ewe treatment and time after treatment were analysed using the repeated measures MIXED model procedure (SAS, 2005).

Pregnancy status and litter size were determined retrospectively, based on the number of lambs identified per ewe within 12 h of birth. Pregnancy status or litter size were not altered if a lamb was born dead or subsequently died between L1 and L94. Lamb thoracic girth and hind limb length was not normally distributed (Anderson-Darling normality test, $P<0.05$). An X^2 transformation was used to normalise hindlimb length and a $X^{0.5}$ transformation was used for thoracic girth. Tables containing these variables (Table 12) set out the least squares means (± SE) and p-values of the transformed data. In addition means of the untransformed data (raw) are presented in parentheses. All analyses of the body dimension data were conducted both with and without birth weight included as a covariate in the model. The relationship of ewe live weight and gestation length on lamb birth weight was examined using a linear regression procedure (PROC REG) in SAS. The proportion of lambs with birth weights less than, within or below the optimal birth weight range (3.5-5.5 kg) was compared between ewe treatments using the PROC GENMOD procedure (SAS, 2005).

Lamb plasma cortisol concentrations and the integrated cortisol response were not normally distributed and a Log_e transformation was used to normalise the distribution. Table 13 presents the least squares means (± SE) and p-values of the transformed data and the mean of the raw data in parentheses. However, p-values were based on the analyses of transformed data. Plasma cortisol was analysed as the change from the baseline (T=0) concentration at each time point. Cortisol response curves were compared using the repeated measures mixed model procedure (SAS, 2005). For each lamb, the area under the curve between 0 and 180 minutes after treatment was calculated using the trapezoidal method.

Results

Weather conditions after shearing

The minimum temperature during the week after shearing was 6.3 °C and the maximum was 13.7°C. On two consecutive days minimum temperatures were below 0 °C (23rd and 24th June 04). The relative humidity during the week after shearing was 87.6% and the average wind speed was 3.6 km/h and average rainfall was 9.9 mm. The day after shearing was particularly wet with 30 mm of rain falling in 24 hours.

Ewe live weight and gestation length

Twin-bearing ewes were heavier (P<0.05) than singleton-bearing ewes throughout pregnancy, but not during lactation (Table 10). Shearing treatment had no effect on the ewe live weight between day 79 and 140 of pregnancy, however on day 42 of lactation control ewes were heavier (P<0.05) than shorn ewes. The gestation length of shorn ewes was on average 1.2 days longer than the control ewes (Table 10). No difference was recorded in the gestation length of singleton- and twin-bearing ewes. At birth, lambs born to shorn ewes were heavier (P<0.05) than lambs born to the control ewes, however, these differences did not persist to day 42 of lactation.

Table 10. Ewe live weight (kg) on days 79, 112 and 140 of pregnancy and days 42 and 94 of lactation and ewe gestation length (days) of singleton- or twin-bearing ewes in the control and shorn treatments (least squares mean ± standard error).

	Ewe live weight (kg)								Gestation
	n	P79	P112	P140	n	L42	n	L94	Length (days)
Pregnancy status									
Singleton	47	50^a±1	52^a±1	56^a±1	47	49±1	47	50±1	148.2±0.3
Twin	48	56^b±1	58^b±1	62^b±1	47	50±1	48	50±1	148.0±0.3
Ewe treatment									
Control	44	54±1	56±1	60±1	43	51^b±1	44	51±1	147.5^a±0.3
Shorn	51	53±1	54±1	58±1	51	48^a±1	51	50±1	148.7^b±0.3

Means within columns with different superscripts are significantly different (P<0.05)

Lamb live weight and body size

Singleton lambs were heavier (P<0.05) than twin lambs at birth, and days 42 and 94 of lactation (Table 11). The linear regression of ewe live weight and the lambs' gestational age had a significant effect on lamb birth weight (birth weight = -17.9 + 0.148 (gestation length) + 0.03 (ewe live weight), P<0.05). Therefore, an increase in gestational length of one day resulted in an increase of 148 g in birth weight. In addition, a 1 kg increase in ewe live weight at P140 contributed a 30 g increase in lamb birth weight. A higher percentage of lambs born to shorn ewes (29%) recorded birth weights of more than 5.5 kg compared to lambs born to control ewes (12%; P<0.05). However 11% of lambs born to control ewes and 9% born to shorn ewes weighed less than 3.5 kg.

Table 11. The live weight (kg) of singleton and twin lambs born to control and shorn ewes at birth and days 42 and 94 of lactation (least squares mean ± standard error)

	Lamb liveweight (kg)					
	n	Birth	n	L42	n	L94
Litter size						
Singleton	47	$5.7^{b} \pm 0.1$	40	$15.5^{b} \pm 0.4$	38	$21.9^{b} \pm 0.6$
Twin	97	$4.2^{a} \pm 0.1$	79	$12.8^{a} \pm 0.3$	77	$19.0^{a} \pm 0.4$
Ewe treatment						
Control	67	$4.8^{a} \pm 0.1$	58	14.1 ± 0.3	57	20.4 ± 0.5
Shorn	77	$5.1^{b} \pm 0.1$	61	14.2 ± 0.3	58	20.5 ± 0.5

Means within columns with different superscripts are significantly different (P<0.05)

Lambs born to mid-pregnancy shorn ewes recorded a longer (P<0.05) CRL and forelimb length than the control counterparts (Table 12). Singleton lambs were larger than twin lambs in all measures of body dimensions (P<0.05). When the lamb body dimensions were adjusted for birth weight the differences were no longer significant. No differences were observed in coat meconium scores between singletons and twins or between lambs born to control or shorn ewes.

Table 12. The crown-rump length, thoracic girth, forelimb, hind limb lengths (cm) and coat meconium score of singleton and twin lambs born to control or shorn ewes (least squares mean ± standard error)

	n	Lamb dimensions				Meconium
		CRL	Girth [1]	Forelimb	Hind limb [2]	score
Litter size						
Singleton	47	52.2±0.4	6.2±0.02 (37.9)	30.1±0.2	1293±24.0 (35.9)	2.9±0.3
Twin	98	51.7±0.3	6.1±0.02 (37.8)	30.2±0.2	1139±16.8 (33.7)	2.5±0.2
Ewe treatment						
Control	67	51.8±0.5	6.2±0.02 (38.1)	30.0±0.2	1196±19.7 (34.5)	2.8±0.3
Shorn	78	52.0±0.4	6.1±0.02 (37.6)	30.2±0.2	1199±13.2 (34.6)	2.6±0.2

[1] data $X^{0.5}$ transformed, untransformed means presented in parentheses

[2] data X^2 transformed, untransformed means presented in parentheses

Lamb plasma cortisol response to a stressor

Pre-treatment plasma cortisol concentrations were similar between litter sizes and ewe treatments (Table 13). Lamb live weight at birth and on day 42 of lactation were not related to pre-treatment plasma cortisol concentration. Castrated lambs had a 3-fold greater (P<0.05) peak cortisol concentration than handled lambs (Table 13). The change in plasma cortisol response above baseline levels was greater (P<0.05) for castrated than for handled lambs at 40, 60, 90, 120 and 150 minutes after treatment (Figure 5). Plasma cortisol concentrations in response to castration peaked 60 minutes after treatment, while handled lambs had a smaller peak at 20 minutes. Twin-born lambs tended (P=0.08) to have a greater plasma cortisol response than singletons (Figure 6). No differences were recorded in the cortisol response of lambs born to control or shorn ewes (data not shown).

Table 13. Pre-treatment and peak plasma cortisol concentrations (nmol/L) and area under the cortisol response curve (nmol/L.min) of singleton and twin lambs born to control and shorn ewes in response to handling or castration (least squares mean ± standard error)

		Cortisol concentration (nmol/L)		
	n	Pre-treatment cortisol [1]	Peak cortisol	Area under the curve[1] (nmol/L.min)
Litter size				
Single	13	3.5±0.2 (35.0)	56.1±11	4.03±0.1 (47.1)
Twin	20	3.3±0.1 (33.5)	80.7±8	4.21±0.1 (60.8)
Ewe treatment				
Control	15	3.5±0.1 (36.9)	72.8±10	4.14±0.1 (57.4)
Shorn	18	3.3±0.1(31.6)	64.0±9	4.09±0.1 (50.4)
Lamb treatment				
Handled	17	3.5±0.2 (40.8)	33.9[a]±9	3.81 [b]±0.1 (39.6)
Castrated	16	3.3±0.2 (20.5)	102.8[b]±10	4.42[a]±0.1 (68.2)
Litter size x lamb treatment				
Singleton x handled	8	3.52±0.2 (36.5)	21.1[a]±13.5	3.73[a]±0.1 (30.4)
Twin x handled	9	3.44±0.2 (43.2)	46.7[b]±12.8	3.89[b]±0.1 (48.9)
Singleton x castrated	5	3.52±0.2 (36.2)	91.3[a]±17.2	4.33[a]±0.2 (63.8)
Twin x castrated	11	3.08±0.2 (24.3)	114.3[b]±11.6	4.52[b]±0.1 (72.6)

[1] data were transformed using $\log_e$; non transformed means presented in parentheses

Means within columns with different superscripts are significantly different ($P<0.05$)

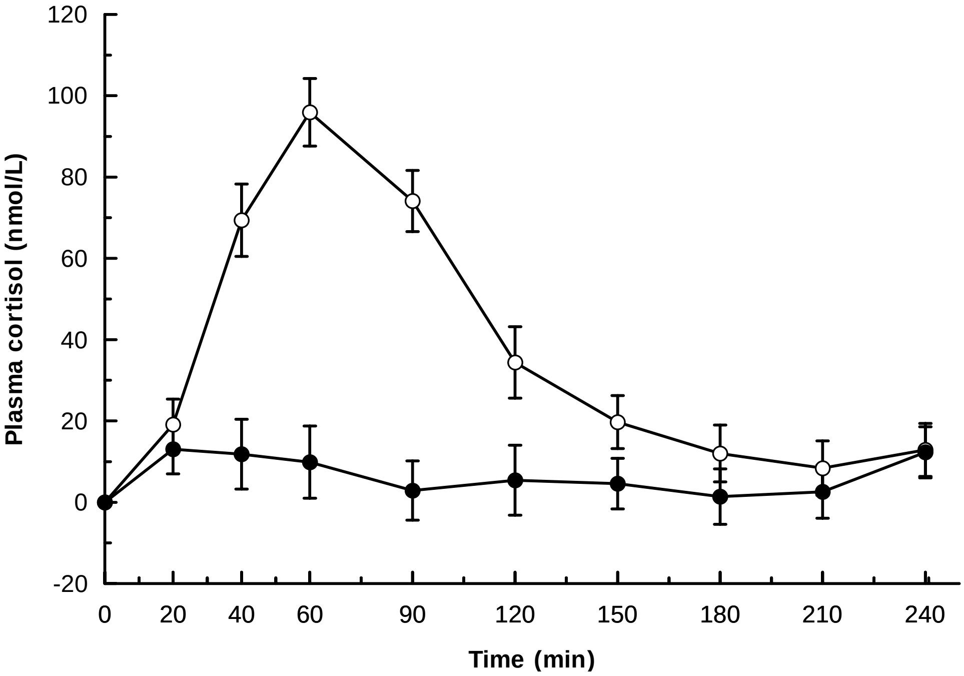


Figure 5. Plasma cortisol change from baseline concentrations over time lambs in response to handling (closed symbols) and castration (open symbols; least squares mean ± standard error).

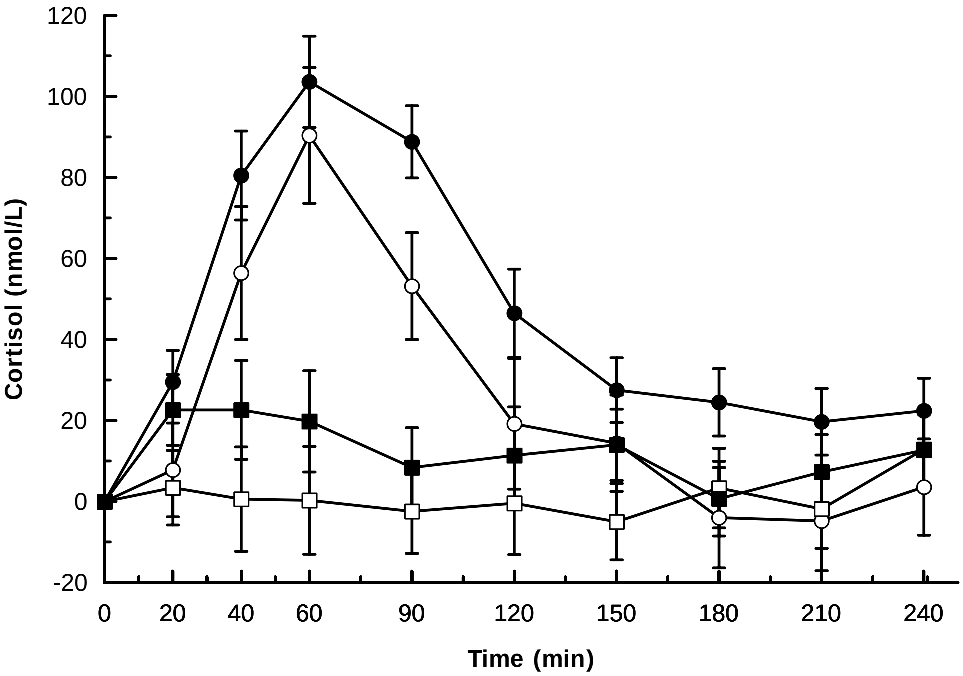


Figure 6. Plasma cortisol change from baseline concentrations over time of singleton (open symbols) and twin (closed symbols) lambs in response to castration (circle) or handling (square; least squares mean ± standard error)

Discussion

Mid-pregnancy shearing resulted in an 8% increase in the birth weight of both singleton and twin lambs. Increases in lamb birth weight have generally been reported in either singleton (Morris et al., 2000; Kenyon et al., 2002b; Revell et al., 2002) or twin-born lambs (Morris and McCutcheon, 1997; Smeaton et al., 2000; Kenyon et al., 2002a; Kenyon et al., 2004), but rarely in both (Kenyon et al., 2002c; Corner et al., 2006). The increase in lamb birth weight was due, in part, to an increase in gestation length of 1.2 days. Shearing ewes during pregnancy resulted in an increased gestation length of approximately 1 day (Adalsteinsson, 1972; Revell et al., 2002). During late gestation, fetal growth rates were 0.15 to 0.2 kg/day (Rattray et al., 1974). Thus the 1.2 day increase in gestation length would be unlikely to account for the entire increase in lamb birth weight observed from mid-pregnancy shearing. In addition, the inclusion of the lamb’s gestational age in the model of lamb birth weight did not eliminate the differences in birth weight of lambs born to control and shorn ewes.

Shorn ewes gave birth to lambs with a longer crown-rump and forelimb lengths compared to the control ewes. However, when the lamb’s birth weight was included in the statistical model these differences were no longer significant. Therefore the differences observed in the lambs’ body dimension were relative to birth weight.

A greater proportion of lambs born to shorn ewes weighed more than 5.5 kg at birth, compared to lambs born to the control ewes. It is generally accepted that the birth weight range for optimal lamb survival under New Zealand’s pastoral production systems is 3.5 to 5.5 kg (Hight and Jury, 1970; Dalton et al., 1980; Knight et al., 1988). Therefore an increase in lamb birth weight above 5.5 kg may result in an increase in the incidence of dystocia. However, there were insufficient lamb numbers to assess causes of mortality in this study. Mid-pregnancy shearing did not alter the proportion of lambs born weighing less than 3.5 kg. This suggests that mid-pregnancy shearing did not alter the fetal growth trajectory of these small lambs. Kenyon et al. (2006a) reported mid-pregnancy shearing to result in an increase in the survival rate of twin and triplet lambs due to a reduction in the proportion of lambs that weighed less than 3.5 kg at birth.

Mid-pregnancy shearing had no effect on the basal plasma cortisol concentration of ram lambs at 5-6 weeks of age. This finding is not in agreement with Roussel et al. (2004) who reported that at 25 days of age prenatally stressed lambs had a higher basal plasma cortisol concentration, compared with the control lambs.

In this study, twin-born lambs had a greater peak and area under the plasma cortisol curve in response to handling and castration than singleton-born lambs. This finding is in agreement with studies that examined cortisol and ACTH concentrations in fetal and post-pubertal female offspring. In late-pregnancy, singleton fetuses had higher concentrations of ACTH (between day 115 and 146 of gestation) and cortisol (between day 126 and 146) than twin fetuses (Edwards and McMillen, 2002). Bloomfield et al (2007) reported similar differences as in the present study, whereby female twin offspring at 10 months of age had greater ACTH, but not cortisol, response to a CRH+AVP challenge than singleton offspring. This finding however is contrary to that of Gardner et al. (2004) who concluded that basal adrenocortical function and responsiveness were blunted in twin relative to singleton fetuses. Additional studies are required to further examine the effect of litter size on the development of the HPA axis and consequences for offspring.

Plasma cortisol responses have been studied in either singletons (Peers et al., 2002; Sloboda et al., 2002; Orihuela et al., 2004) or twins (Molony et al., 2002) and in some studies the litter size of the lambs were not specified (Coppinger et al., 1991; Horton et al., 1991; Rhodes et al., 1994; Napolitano et al., 2003). The results of the present study indicate that when reporting the results of studies involving both singleton and twin offspring valuable information may be lost if the responses of each litter size are not reported separately.

Modifications of the postnatal physiology of twin lambs compared with singletons may reflect the maternal constraint of fetal growth due to space and nutrient supply (Gluckman and Hanson, 2004). Bloomfield et al. (2007) reported the postnatal HPA responsiveness to be related to birth weight. However, in this study lamb live weight at birth and L42 was not related to basal cortisol concentrations or area under the cortisol response curve of handled or castrated lambs. It would have been valuable to determine the change in plasma cortisol concentrations of ewes in response to shearing in an effort to quantify the stress response of the ewe. In the following chapter the cortisol response to shearing and other stressors will be measured.

Conclusion

Mid-pregnancy shearing produced a significant increase in lamb birth weight. Shorn ewes had a longer gestation length which alone did not account for the entire observed increase in lamb birth weight. The increase in lamb birth weight resulting from mid-pregnancy shearing did not appear to impact on the lambs' plasma cortisol response following castration. There was however a significant difference in plasma cortisol response to handling or castration between lamb litter sizes, which warrants further investigation.

Further research

The results of this chapter indicate that although mid-pregnancy shearing resulted in an increase in lamb birth weight and body size there did not appear to be alteration of the lamb's cortisol response to a stressor (handling or castration). In the following section of this chapter, the effect of a range of mid-pregnancy stressors on twin lamb birth weights and body size is examined. In addition, the relationship of ewe plasma cortisol response to treatment during pregnancy on lamb birth weight and size is also examined.

B) The effect of mid-pregnancy stressors on twin-lamb live weight and body dimensions at birth.

Abstract

This study was conducted to determine the effect of crutching, simulated shearing and conventional shearing in mid-pregnancy on maternal plasma cortisol levels and lamb live weight and body dimensions at birth. Twin-bearing Romney ewes (n=96) were subjected to one of four treatments (control, crutched, sham shorn and shorn) on day 80 of pregnancy. Ewes were maintained as a single flock throughout pregnancy under commercial farming conditions. Within 12 hours of birth the lambs were weighed and their body dimensions recorded. Lambs born to ewes shorn in mid-pregnancy were heavier ($P<0.05$) at birth than lambs born to control or sham-shorn ewes by 400 and 600 g (10 and 15%) respectively. The birth weight of lambs born to crutched and control ewes did not differ. Shearing and crutching produced a greater integrated cortisol response than sham-shearing ($P<0.05$). The failure of crutching to increase lamb birth weight suggests that the acute stress caused by shearing does not result in increased lamb birth weights.

Introduction

Many twin lambs born under New Zealand pastoral conditions weigh less than the optimum birth weight of 3.5 kg (Dalton et al., 1980). An increase in the birth weight of light-weight lambs has the potential to increase survival rates to weaning (Morris et al., 1999). Mid-pregnancy shearing has consistently increased lamb birth weights (Morris and McCutcheon, 1997; Morris et al., 2000; Smeaton et al., 2000; Kenyon et al., 2002a; b; c; Revell et al., 2002; Kenyon et al., 2004; Corner et al., 2006). However, in New

Zealand, mid-pregnancy shearing takes place in the winter when conditions can be cold, wet and windy and may result in hypothermia (Morris et al., 1962) with ewe mortality rates of 3-14% being recorded (Dabiri et al., 1995a). Therefore, identification of alternative techniques to increase lamb birth weight would alleviate these risks to the ewe.

The lamb birth weight increase caused by mid-pregnancy shearing was not due to increase in ewe feed intake (Kenyon et al., 2002b; Revell et al., 2002), longer gestation length (Kenyon et al., 2002c), elevated maternal thyroid hormone concentrations (Kenyon et al., 2005b) or events involved with being yarded (Chapter 2). Stressors during pregnancy caused by transport (cattle) and isolation (sheep) result in increased fetal (Lay et al., 1997a) and birth weights (Roussel et al., 2004), respectively. Therefore, it is possible that stress caused by the events associated with shearing may increase lamb birth weight.

Shearing involves mustering, time off pasture, handling, isolation, noise, tactile stimulation by the shearing machinery and fleece removal. These events combine to produce an acute stress response; increased plasma concentrations of cortisol, ß-endorphin, glucose and increased heart rate and haematocrit (Jephcott et al., 1987; Hargreaves and Hutson, 1990b, d, a; Mears et al., 1999). Shearing produced a larger cortisol response than crutching, isolation and handling (Hargreaves and Hutson, 1990a, d).

The objectives of this study were to determine the effects of mid-pregnancy shearing, simulated shearing (sham), and crutching on ewe plasma cortisol responses and lamb live weight and body dimensions at birth.

Materials and methods

Ewes

Oestrus was synchronised in 290 mixed-age Romney ewes through the use of progesterone impregnated intravaginal devices (EziBreed CIDR, Pfizer, Auckland, New Zealand) for 13 days. Romney rams were introduced to the ewes at a ratio of 1:15 on the day of CIDR removal. Individual breeding dates were recorded by daily observation of ram harness-crayon marks for 5 days after the introduction of the rams (30 Mar 04 to 02 Apr 04). Romney rams were removed after 5 days and replaced with Suffolk rams fitted with a different coloured crayon colour at a ram to ewe ratio of 1:125. Ewes marked by the Suffolk rams were excluded from the study. Ninety-nine ewes diagnosed as twin bearing by trans-abdominal ultrasound scan 59 days after the mid-point of the breeding period (P59) were selected for the experiment.

Ewes identified as bearing twin fetuses were randomly assigned to one of four treatments: unshorn (control), removal of wool from the belly and around the tail only (crutched), simulated shearing without wool removal (sham-shorn) and shearing (shorn). All ewe treatments took place on day 80 of pregnancy (17 June 04). Ewes in the crutched, sham-shorn and shorn treatments were held off pasture for 24 hours prior to treatment. During crutching the ewe was restrained in a similar manner to conventional shearing, but for a shorter period of time. Sham shearing involved all aspects of conventional shearing except removal of wool. Ewes were handled by the shearer for a similar duration to conventional shearing (approximately 2 minutes), the shearing machinery was turned on to generate the noise of shearing, but the hand piece was held above the fleece to ensure that no wool was removed. A cover comb which left a residual fleece depth of 5-7 mm (Sunbeam Corporation Ltd, New Zealand) was used for crutching and shearing (Dabiri et al., 1995b). All ewes had approximately six months of wool growth prior to shearing. The fleece of the shorn ewes was weighed and all subsequent ewe live weights were adjusted for fleece weight. The fleece removed by crutching was not weighed. During the remainder of pregnancy, all ewes were managed in one group under commercial farming conditions.

On days 80 and 83 of pregnancy, blood samples were collected by jugular venipuncture into 10 ml collection tubes containing sodium heparin (BD Vacutainer, Preanalytical Solutions, Franklin Lakes, USA) using 20 gauge 1 inch vacutainer needles (PrecisionGlide™, Becton Dickinson Vacutainers systems, Plymouth, UK). On P80, 10 ewes selected at random from each of the 4 treatments groups had blood samples collected immediately prior to treatment (t=0) and 10, 20, 30, 60 and 90 minutes after treatment. Blood samples were immediately placed on ice and then centrifuged at 3,000 rpm for 10 minutes. Plasma was separated and frozen at -20 °C. Plasma was analysed by radioimmunoassay for total cortisol concentration (Clinical Assays GammaCoat Cortisol ^{125}I RIA Kit, Diasorin Inc., Minnesota, USA). The GammaCoat™ antiserum exhibits 100% cross reactivity with cortisol. The published intra-assay coefficient of variation was 7.0% and the inter-assay variation was 9.2% (Anon., 2003a).

Ewe live weights were recorded on days 79, 112, 140 of pregnancy and day 42 of lactation. On day 130 of pregnancy all ewes were crutched in preparation for parturition. Ewes were randomly allocated to lambing paddocks on day 140 of pregnancy at a stocking rate of 12 ewes per hectare. During the lambing period ewes were observed three times daily to identify newly born lambs and, if required, to provide assistance to ewes lambing.

Lambs

Within 12 hours of birth, lambs were tagged, weighed and their body dimensions measured by an experienced shepherd. Lamb body dimension measures included crown rump length (CRL), thoracic girth (immediately posterior to the forelimb), forelimb length (shoulder to the tip of the hoof on the left leg) and hindlimb length (hip joint to the tip of the hoof on the left leg). The lamb's coat meconium staining was ranked on a 4-point scale from 0 to 3 (0 = white, 1 = pale yellow, 2 = yellow and 3 = dark yellow/orange) as an indicator of stress resulting from parturition (Oliver et al., 2001). Lamb live weights were recorded on days 42 and 94 of lactation.

This study was conducted with approval of the Massey University Animal Ethics committee (MUAEC 04/55).

Statistical analysis

All continuous data were subject to analysis of variance using the generalised linear model (GLM) procedure (SAS, 2005). Data that were not normally distributed were analysed using the Kruskal-Wallis test in the non-parametric procedure (NPAR1WAY) in SAS (2005). Categorical data (coat meconium score) were analysed using the GENMOD procedure in SAS (SAS, 2005). Data recorded over time were subject to repeated measures analyses using a MIXED procedure (SAS, 2005). Lamb survival data was treated as a binomial trait and logit transformed and analysed using the GENMOD procedure in SAS (SAS, 2005).

Lamb live weights at L42, CRL and forelimb length were not normally distributed (Anderson-Darling normality test, $P<0.05$). The distributions of CRL and lamb live weight at L42 were normalised using an X^2 transformation and forelimb length was normalised using a $\log_e$ transformation. Tables contain least squares means and p-values of the transformed data and the means of raw data in parentheses. Ewe gestation length was not normally distributed and transformations could not normalise the data; therefore, median gestation lengths are presented.

Analyses of lamb dimensions (CRL, girth, fore and hind limb lengths) were conducted with and without birth weight fitted as a covariate to determine whether differences in size were proportional to birth weight or if they represented differences in the shape of the lambs. In addition lamb live weights at birth, L42 and L94 were adjusted for the live weight of their dam at P140, L42 and L94, respectively.

Cortisol data were not normally distributed (Anderson-Darling normality test, $P<0.05$) and an $X^{0.5}$ transformation was used to normalise the distribution of the data. Figures contain means and standard errors of the raw data for simplicity, however, p-values were based on analyses of transformed data.

Results

Weather conditions after shearing

The mean minimum and maximum temperatures after shearing were 1.7 and 11.8 °C, respectively. The minimum temperature dropped below 0 °C on two days during this period (on the second and sixth day after shearing). The average rainfall in the seven days after shearing was 0.1 mm and the relative humidity was 84.4%.

Ewe live weight

Ewe treatment had no effect on ewe live weight during pregnancy and lactation (Table 14). Ewe live weights increased significantly ($P<0.05$) from day 79 to 112 and day 112 to 140 of pregnancy, however, after parturition (day 42 to 94 of lactation) ewe live weights did not change.

Table 14. Live weight (kg) of twin-bearing ewes in the control, crutched, sham-shorn and shorn treatments on days 79, 112, 140 of pregnancy and days 42 and 94 of lactation (least squares means ± standard error)

	n	Ewe live weight (kg)				
		P79	P112	P140	L42	L94
Ewe treatment						
Control	21	56.3±1.6	59.8±1.7	63.6±1.9	51.5±1.3	51.3±1.3
Crutched	26	55.3±1.5	60.1±1.5	65.0±1.7	50.3±1.1	51.5±1.2
Sham-shorn	22	56.3±1.6	58.3±1.7	63.3±1.9	52.6±1.3	52.0±1.3
Shorn [1]	27	55.4±1.4	58.9±1.5	62.8±1.7	50.7±1.1	50.9±1.2

[1] Ewe live weights from days 112, 140 of pregnancy and days 42 and 94 of lactation were adjusted for the weight of the fleece removed on day 80 of pregnancy

Ewe plasma cortisol responses

The plasma cortisol responses of ewes in the shorn and crutched treatments were similar (Figure 7). Control ewes had lower (P<0.05) plasma cortisol concentrations than all other treatments 10 minutes after treatment (Table 15 and Figure 7). Sham-shorn ewes had lower (P<0.05) cortisol concentrations than shorn ewes 20 and 30 minutes after treatment. Sham-shorn ewes had lower (P<0.05) cortisol concentrations 90 minutes after treatment than control and shorn ewes.

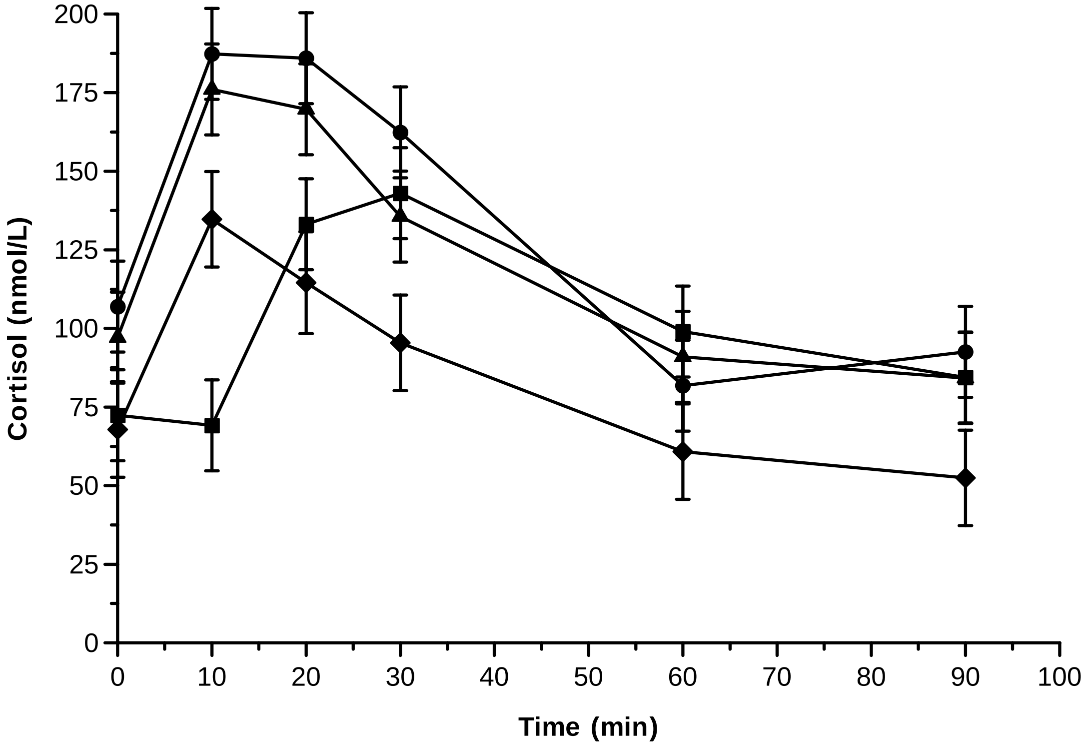


Figure 7. Plasma cortisol concentrations of twin-bearing ewes immediately before (0) and 10, 20, 30, 60, 90 minutes after treatment (control =square, crutched = triangle, sham-shorn =diamond, shorn = circle) on day 80 of pregnancy (least squares mean ± standard error)

Ten minutes after treatment ewes in the control treatment had a lower plasma cortisol concentration than all other treatments ($P<0.05$), however, crutched and shorn ewes had similar plasma cortisol concentrations. Ewes in the control treatment took longer ($P<0.05$) to reach peak cortisol concentration than all other treatments (Table 15). Sham-shorn ewes had a lower peak cortisol and integrated cortisol response than crutched or shorn ewes ($P<0.05$).

Table 15. Plasma cortisol concentration 10 minutes after treatment (nmol/L), time to reach peak level (min) and integrated cortisol response (nmol/L.min) of ewes in the control, crutched, sham-shorn and shorn treatments (least squares mean ± standard error)

	n	Cortisol conc. after 10 min (nmol/L) [1]	Time to reach peak concentration (min)	Area under response curve (nmol/L.min) [1]
Ewe treatment				
Control	10	4.21^{a}±0.2 (69.2)	30.2^{b} ± 2.7	4.62^{ab} ±0.1 (105.4)
Crutched	10	5.08^{c}±0.2 (176.0)	14.5^{a} ± 2.7	4.69^{b} ±0.1 (118.3)
Sham-shorn	10	4.84^{b}±0.2 (134.7)	12.8^{a} ± 2.7	4.34^{a} ±0.1 (79.8)
Shorn	10	5.19^{c}±0.2 (187.4)	16.4^{a} ± 2.7	4.78^{b} ±0.1 (126.2)

Means within columns with different superscripts are significantly different (P<0.05)

[1] data $X^{0.5}$ transformed, untransformed means presented in parentheses

Gestation length

The median gestation length of shorn ewes was longer (P<0.05) than either control or sham-shorn ewes by 1.5 to 2 days (Table 16). The gestation lengths were similar for ewes in the control, crutched and sham-shorn treatments.

Table 16. Median gestation length (days) of twin-bearing ewes in the control, crutched, sham-shorn and shorn treatments

	n	Gestation length (days)
Ewe treatment		
Control	21	147.0^{a}
Crutched	26	148.0^{ab}
Sham-shorn	22	147.5^{a}
Shorn	27	149.0^{b}

Medians within columns with different superscripts are significantly different (P<0.05)

Lamb live weight

Lambs born to shorn ewes were heavier (P<0.05) at birth than lambs born to control, crutched and sham-shorn ewes (Table 17). Ewe treatment had no effect on lamb live weight at either day 42 or 94 of lactation. The proportion of lambs that had a birth weight below, within or above the birthweight range for optimal for survival (3.5-5.5 kg) was uniform across ewe treatments.

Ewe live weight on day 140 of pregnancy and gestation length predicted lamb birth weight (P=0.01 and P=0.06, respectively). In the model, birth weight = -12.8 + 0.04 (ewe liveweight) + 0.1 (ewe gestation length); r2 = 0.18). Therefore for every 1 day increase in gestation length there was a corresponding 0.1 kg increase in lamb birth weight and for every 1 kg increase in ewe weight there was a 0.04 kg increase.

Table 17. Live weight (kg) of lambs at birth and on days 42 and 94 days of lactation (L42 and L94) and lamb survival (%) to day 94 of lactation (L94) of lambs born to ewes in the control, crutched, sham-shorn and shorn treatments (least squares mean ± standard error)

	Lamb live weight (kg)						Survival to
	n	Birth	n	L42 [1]	n	L94	L94 [2] (%)
Sex of lamb *							
Female	94	4.2^a±0.1	74	3.55±0.1 (12.7)	73	18.9±0.4	-1.3±0.3 (79)
Male	97	4.4^b±0.1	82	3.48±0.1 (12.2)	76	19.4±0.4	-1.4±0.3 (79)
Ewe treatment							
Control	44	4.2^a±0.1	37	3.54±0.1 (12.7)	37	18.8±0.6	-2.0±0.5 (88)
Crutched	52	4.3^a±0.1	43	3.53±0.1 (12.4)	41	19.4±0.6	-1.3±0.3 (79)
Sham-shorn	44	4.1^a±0.1	35	3.47±0.1 (12.4)	32	19.4±0.7	-1.0±0.3 (73)
Shorn	54	4.5^b±0.1	42	3.51±0.1 (12.2)	40	19.0±0.6	-1.0±0.3 (74)

[1] data was $X^{0.5}$ transformed; untransformed means are presented in parentheses

[2] data was logit transformed; back transformed percentages are presented in parentheses

Means within columns with different superscripts are significantly different (P<0.05)

* Sex of three lambs was not recorded

Lamb dimensions and coat meconium score

Lambs born to shorn ewes had longer (P<0.05) forelimb and hindlimb lengths than lambs born to control ewes, however, when adjusted for birth weight, these differences were no longer significant. An increase of 1 kg in birth weight resulted in an increase in forelimb length of 1.4 cm and hindlimb of 1.8 cm. Lamb coat meconium staining scores did not differ between lambs born to ewes in the control, crutched, sham shorn and shorn treatments (Table 18).

Table 18. Crown-rump (CRL), thoracic girth, forelimb and hind limb length (cm) and coat meconium score at birth of twin lambs born to control, crutched, sham-shorn and shorn ewes (least squares mean ± standard error).

		Lamb dimensions (cm)				Meconium
	n	CRL[1]	Girth	Forelimb[2]	Hind limb	Score
Ewe treatment						
Control	44	2548±36 (50.4)	36.7±0.3	3.37±0.01 (29.1)	33.6±0.3	1.6±0.1
Crutched	52	2563±31 (50.5)	36.7±0.2	3.39±0.01 (29.7)	34.0±0.2	1.6±0.1
Sham-shorn	44	2595±34 (50.8)	36.7±0.3	3.39±0.01 (29.9)	34.0±0.3	1.7±0.1
Shorn	54	2547±31 (50.4)	36.4±0.2	3.39±0.01 (29.8)	33.9±0.2	1.9±0.1

[1] Data was X^2 transformed; raw means are presented in parentheses

[2] Data were Log_e transformed; raw means are presented in parentheses

Discussion

Mid-pregnancy shearing caused an increase in lamb birth weight consistent with that observed in Chapter 2 and with previous reports (Morris et al., 2000; Kenyon et al., 2002b, c). The birth weight of lambs born to control, crutched and sham shorn ewes were similar.

Shearing caused an acute stress reaction in the ewe which was indicated by an increase in ewe plasma cortisol concentrations which returned to baseline levels 90 minutes after shearing. Mendoza et al. (2000) stated that an acute stress response concludes when plasma cortisol concentrations return to pre-treatment levels. It is uncertain however what effect previous events had on the basal plasma cortisol concentrations. It is likely that the presence of people in close proximity and drafting of ewes into pens caused an increase in plasma cortisol concentrations prior to the collection of the first blood sample. Therefore, it is likely that shearing produced an acute stress response for at least 90 minutes. It would have been prudent to measure plasma cortisol concentrations of ewes for 2 to 3 days following shearing to determine the duration of the cortisol increase. Astrup et al. (1988) reported that 3 days after shearing ewes had elevated corticosteroid concentrations compared to 8 days prior to shearing. Therefore the response measured in this study was only of the acute stress response to shearing rather than a chronic response.

Shearing and crutching produced a similar elevation in plasma cortisol. It was expected that the elevation in plasma cortisol would be greater and remain elevated for longer as a result of shearing. Shearing, but not crutching, resulted in an increase in lamb birth weight therefore it appears that the increase in birth weight was a result of the acute stress response to shearing. In order to exclude the stress response of the ewe to shearing as a cause of the increase in lamb birth weight the plasma cortisol response to shearing in the following 3 to 4 days is required.

The mechanism that produces the increase in lamb birth weight from mid-pregnancy shearing may be chronic stress. Prolonged activation of the HPA axis results from chronic stress or failure to adapt to a stressor and during pregnancy this may result in fetal disorders (Weinstock, 2005). Chronic cold stress resulting from a short fleece during winter was likely to have long term consequences on the ewe and the fetus. Thyroid hormone concentrations became elevated after mid-pregnancy shearing as a result of cold stress (Symonds et al., 1989), however, elevated thyroid hormones alone did not result in increased lamb birth weight (Kenyon et al., 2005b).

The plasma cortisol response of control ewes was unexpected. The study design was such that ewes in the shorn and crutched treatments remained inside the shearing shed during blood sampling. Sham-shorn and control ewes, however, were held in covered yards outside the shed to minimise the impact of noise from inside the shed on the control ewes. It appears from the plasma cortisol concentrations of the control ewes that these ewes were exposed to a stressor between 10 to 30 minutes after the start of the sampling period which resulted in elevated plasma cortisol concentrations. The cause for this response is unknown. Caution should be taken when examining the cortisol response to treatment that the response of control and sham-shorn ewes should not be compared to crutched and shorn ewes.

The gestation length of ewes that were shorn in mid-pregnancy was 1.5 days longer than for control ewes. The regression model developed in this study indicates that an increase in gestation length of 1 day would result in a 0.1 kg increase in lamb birth weight. (Rattray et al., 1974) reported that in late gestation, fetal growth rates were approximately 0.15-0.2 kg/day. Therefore, the increase in gestation length does not account for the entire increase in lamb birth weight observed in the present study. Increases in birth weight resulting from mid-pregnancy shearing have been observed without a corresponding increase in gestation length (Kenyon et al., 2002c; Corner et al., 2006). In studies where there was an increase in gestation length, the duration was insufficient to account for the entire difference in lamb birth weight (Kenyon et al., 2002b; Revell et al., 2002).

Conclusion

Mid-pregnancy shearing and crutching produced similar increases in maternal cortisol concentration; however, in contrast to shearing, crutching had no effect on lamb birth weight. Therefore, the acute stress response to shearing appears unlikely to be the mechanism by which mid-pregnancy shearing increases lamb birth weight.

Further research

Based on the result of the previous studies (Chapters 2, 3a and b) it was hypothesised that the chronic stress response to shearing may result in an increase in lamb birth weight. Therefore, repeated maternal stressors, including isolation, sham shearing and exposure to exogenous cortisol will be examined in chapter 4.

CHAPTER 4

The effect of stressors imposed on ewes during pregnancy on lamb live weight and body size at birth

Abstract

The effect of various stressors on ewes during mid- and late-pregnancy on lamb weight and body dimensions at birth was investigated. Romney ewes bearing twin fetuses (n=144) were allocated to one of six mid-pregnancy treatments: control, isolation on two or ten occasions, sham-shearing on ten occasions, intramuscular cortisol injections on ten occasions or shearing. Isolation, sham-shearing and cortisol treatments were conducted twice a week beginning on day 74 after the mid-point of the breeding period and shearing occurred on day 76. Ewe live weights and condition scores were recorded 48, 62 and 106 days after the mid-point of the breeding period. Ewe live weights during lactation were recorded 63 days after the mid-point of the parturition period. Plasma cortisol response of ewes in the cortisol, isolation x 2, isolation x 10, sham-shorn and shorn treatments was determined during their 1st treatment. In addition, the plasma cortisol response to the 5th and 9th treatments was determined for ewes in the isolation x 10, sham-shorn and cortisol treatments. Lamb live weight and body dimensions (crown rump length, girth, forelimb and hindlimb length) were recorded at birth and postnatal live weights were recorded 63 days after the mid-point of the parturition period (L63). During pregnancy, ewe treatment had no effect on ewe live weight. However, in late-pregnancy, shorn ewes had higher body condition scores than ewes in the sham-shorn and cortisol treatments ($P<0.05$). During lactation, shorn ewes were heavier than isolation x 2, isolation x 10 or sham-shorn ewes ($P<0.05$). Intramuscular injections of cortisol had a greater effect on ewe plasma cortisol concentrations than all other treatments ($P<0.05$). Shearing produced a greater plasma cortisol response during the hour after treatment than isolation x 10 and sham shearing, however, isolation x 2 had an intermediate cortisol response ($P<0.05$). Ewe plasma cortisol responses decreased during the five weeks of isolation and sham shearing but cortisol injections

produced a greater response during the 5th than the 1st and 9th treatments ($P<0.05$). Lambs born to shorn ewes were heavier and larger (CRL, forelimb and hindlimb lengths) than all other lambs ($P<0.05$). In addition, lambs born to cortisol ewes were lighter and smaller than lambs born to control ewes ($P<0.05$). These results indicate that the mechanism by which mid-pregnancy shearing increases lamb birth weight is unlikely to be chronic stress or greatly elevated maternal plasma cortisol concentrations.

Introduction

Mid-pregnancy shearing results in an increase in the birth weight of both singleton (Morris et al., 2000; Sherlock et al., 2003) and twin lambs (Morris and McCutcheon, 1997; Smeaton et al., 2000; Kenyon et al., 2004). Kenyon et al. (2006a) reported that mid-pregnancy shearing reduced the proportion of lambs that weighed less than 3.5 kg at birth and has been shown to increase lamb survival rates in New Zealand's pastoral production system (Morris et al., 1999; Kenyon et al., 2006a).

The component of shearing that produces an increase in lamb birth weight has not been identified. Several factors that have been eliminated as possible causes of the increase in lamb birth weight include: increased ewe feed intakes (Kenyon et al., 2002b; Revell et al., 2002), longer gestation lengths (Kenyon et al., 2002c) and elevated maternal thyroid hormone concentrations (Kenyon et al., 2005b). Roussel et al. (2004) reported that ewes socially isolated, for 1 h twice a week for 5 weeks beginning on day 112 of pregnancy, gave birth to lambs that were heavier than lambs born to control ewes. In addition, the repeated transport of cows on days 60, 80, 100, 120 and 140 of a 281 day pregnancy (Lay et al., 1997b) resulted in an increase in the birth weight of offspring. Repeated stressors produce an increase in maternal stress hormones such as cortisol (Niezgoda et al., 1987; Coppinger et al., 1991; Roussel et al., 2004). This increase in maternal cortisol increases the ewe's metabolism by stimulating gluconeogenesis and increasing glycogen in the liver which in turn produces an increase in circulating glucose concentrations (Cunningham, 2002). In addition, maternal cortisol may act directly on the development of the fetus, as cortisol can cross the placenta increasing fetal cortisol concentrations (Zarrow et al., 1970; Otten et al., 2004).

In the ewe, shearing produces both acute and chronic stress responses. The acute stress response is characterised by an increase in heart rate (Hargreaves and Hutson, 1990a), haematocrit (Hargreaves and Hutson, 1990d) and plasma concentrations of stress hormones such as cortisol (Pierzchala et al., 1983; Hargreaves and Hutson, 1990b) and β-endorphin (Jephcott et al., 1987; Mears et al., 1999). The chronic stress response is harder to quantify but may be a result of cold exposure due to fleece removal.

The aims of this experiment were twofold: to determine if repeated stressors produce an increase in lamb birth weight similar to mid-pregnancy shearing and to establish if elevated maternal plasma cortisol concentrations in between days 74 and 106 of pregnancy result in an increase in lamb birth weight.

Materials and Methods

Ewes

Oestrus in Romney ewes was synchronised using progesterone in controlled internal drug release devices (Eazi-Breed CIDR, Pfizer, Auckland, New Zealand) for 13 days before breeding. To increase the number of multiple pregnancies, ewes were given 400 IU serum gonadotrophin B (Folligon™, Intervet, Auckland, New Zealand) on the day of CIDR removal. Individual breeding dates were recorded by daily observations of ram crayon harness marks on ewes during the 5-day breeding period (14 Apr 05 to 18 Apr 05). Pregnancy diagnosis was conducted 49 days after the mid-point of the breeding period (day 49 of pregnancy) and ewes bearing twin fetuses (n=144) were selected for the trial. Twenty-four ewes were allocated to each of the six treatments on day 72 of pregnancy (25 June 05): control, sham shearing on ten occasions, isolation on two occasions (isolation x 2), isolation on ten occasions (isolation x 10), cortisol injections on ten occasions and mid-pregnancy shearing. All treatments were balanced for ewe live weight and all ewes had approximately 6 months of wool growth on day 75 of pregnancy. During pregnancy and lactation ewes were randomly allocated to two paddocks. On each treatment day all ewes were brought into the yards and drafted into their treatment groups.

Ewe treatments

Isolation x 2 ewes were isolated twice during one week (days 75 and 78 of pregnancy) and ewes in the isolation x 10 treatment were isolated 10 times over 5 weeks (days 75, 78, 82, 85, 89, 92, 96, 99, 103 and 106 of pregnancy). Ewes were subjected to isolation for an hour on each treatment day by placing ewes in individual pens constructed with solid walls and a wire mesh roof and gate. When in the isolation pen ewes were unable to see any other sheep. Twelve ewes were kept in a group pen while the remaining ewes were isolated for one hour. At the conclusion of the hour, ewes in the holding pen were moved into the isolation pens and ewes that had been isolated were held in the holding pen.

Ewes in the cortisol treatment were given hydrocortisone injected intramuscularly into the neck (Solu-Cortef, Pharmacia, Auckland, New Zealand) at a dose of 6 mg/kg (Jobe et al., 2003) twice a week for 5 weeks (on days 74, 77, 81, 84, 88, 91, 95, 98, 102 and 105 of pregnancy).

Ewes in the sham-shearing treatment had no fleece removed but were exposed to the events commonly associated with shearing; people, dogs and vehicles, noise of the shearing machinery, handling by the shearer and contact of the shearing machinery against the wool. This treatment deviated from shearing as ewes were not fasted prior to treatment. To ensure that each ewe experienced the same conditions, the duration of sham shearing was standardised to 2 minutes, which approximated the time taken to shear a ewe. Sham shearing began on day 74 of pregnancy and was conducted twice a week for 5 weeks (on days 74, 77, 81, 84, 88, 91, 95, 98, 102 and 105 of pregnancy).

Ewes in the shearing treatment were drafted from the flock on day 75 of pregnancy and fasted for 24 hours. On day 76 of pregnancy ewes were shorn with a cover comb (Sunbeam Corporation Ltd, New Zealand) which left a residual fleece depth of 5-7 mm. The weight of fleece removed was recorded for each ewe and subsequent live weights were adjusted for the weight of fleece removed.

Ewes in the control treatment were quietly walked to the yards, without the use of dogs, on days 74, 75, 76, 77, 78, 81, 82, 84, 85, 88, 89, 91, 92, 95, 96, 98, 99, 102, 103, 105 and 106 of pregnancy. Control ewes were the drafted from ewes in the other treatments and returned to pasture. Control ewes were off-pasture for no more than 30 minutes on each treatment day. To avoid restraining control ewes, and thus exposing ewes to a potential stressor, blood samples were not collected. In chapter 3b, control ewes showed a significant increase in plasma cortisol concentrations in response to blood collection (Figure 7).

Blood sampling

Blood samples were collected from ewes in each treatment group (except control ewes) on their first day of treatment (sham-shorn and cortisol on pregnancy day 74, isolation x

2 and isolation x 10 on day 75 and shearing on day 76). Blood sampling began at 9 am and sampling was completed by 1 pm. Blood samples (5 ml) were collected by jugular venipuncture using 20 gauge needles into 10 ml collection tubes containing sodium heparin (BD Vacutainer, Preanalytical solutions, Franklin Lakes, USA). Blood was collected immediately prior to treatment and 15 and 60 minutes after the start of treatment. Blood samples were collected from ewes in the sham-shorn, isolation x 10 and cortisol treatments during the 5th treatment (days 88 and 89 of pregnancy) and 9th treatment (days 102 and 103 of pregnancy), in accordance with the previous sampling procedure. The total plasma cortisol concentrations of the blood samples collected was determined using diagnostic kits (Clinical Assays GammaCoat 125I-RIA, DiaSorin, MN, USA). The GammaCoat™ antiserum exhibits 100% cross reactivity with cortisol. The published intra-assay coefficient of variation was 7.0% and the inter-assay variation was 9.2% (Anon., 2003a).

All ewes were weighed 12 days prior to breeding (day -12 of pregnancy) and on days 48, 62 of pregnancy and day 63 after the mid-point of the parturition period (day 63 of lactation). Ewe body condition scores were recorded, when ewes were weighted, during pregnancy but not lactation.

Lambs

During the lambing period ewes were observed three times daily by experienced shepherds to identify newly born lambs and, if required, to provide assistance to ewes lambing. Within 12 hours of birth all lambs were ear tagged, identified to their dam, weighed, body dimensions measured (crown rump length, girth, forelimb and hindlimb lengths) and coat meconium colour was ranked on a scale of 0 to 3 (Oliver et al., 2001). During the ear tagging procedure the maternal behaviour of the ewe was ranked on a scale from 1 (the ewe fled when shepherd approached her lambs and did not return) to 5 (ewe remained close to lamb and made contact with the lamb or the shepherd) (O'Connor et al., 1985; Everett-Hincks et al., 2005b). Lamb live weights were recorded on day 63 of lactation.

This study was conducted with approval of the Massey University Animal Ethic Committee (MUAEC 05/49)

Statistical analyses

This experiment involved a random design incorporating ewe treatment (control, cortisol, isolation x 2, isolation x 10, sham-shorn and shorn). Ewes were excluded from the analyses if they failed to give birth (n=5), did not give birth to twin lambs (n=3) or if a fetus died in utero (n=1). One ewe was euthanised during the treatment period due to poor health and body condition.

Ewe live weight, condition score and cortisol response to treatment was analysed using repeated measures analysis in the general linear model procedure (PROC GLM) in SAS (SAS, 2005) including the fixed effect of ewe treatment. The distribution of plasma cortisol concentrations was not normal, therefore a Log_e transformation was used to normalise the data. Tables contain the mean ± SEM of the transformed data and the mean of the untransformed data are presented in parentheses. The area under the cortisol response curve was calculated using the trapezoidal method.

Ewe condition scores, maternal behaviour scores (MBS) and lamb coat meconium staining scores were analysed using the PROC GENMOD procedure in SAS. Ewe condition score included the fixed effect of ewe treatment and the model of coat meconium score contained ewe treatment and sex of lamb.

Lamb live weight and body dimensions were analysed using the generalised linear model (PROC GLM) including the fixed effect of ewe treatment and sex of the lamb (male and female). Models of lamb body dimensions were run both with and without lamb birth weight included as a covariate in the model. The analysis of lamb girth contained only lambs born alive as lambs born dead did not breathe which then altered the girth measurement. Lamb girth, forelimb and hindlimb lengths were not normally distributed therefore the data were transformed (X^2 for girth and hindlimb and Log_e for forelimb). Tables containing these variables present transformed means and standard errors with the raw mean in parentheses.

Results

Ewe live weight and body condition scores

Ewe live weights increased during pregnancy (P-12 to 106) and then decreased between P106 and L63 ($P<0.05$; Table 19). Ewe treatment had no effect on ewe live weight during pregnancy, however, on L63 ewes in the shorn treatment were heavier ($P<0.05$) than ewes in the isolation x 2, isolation x 10 and sham-shorn treatments.

Table 19. Live weight (kg) on days 48, 62 and 106 of pregnancy and day 63 of lactation of ewes in the control, cortisol, isolation x 2, isolation x 10, sham shorn and shorn treatments (least squares mean ± standard error)

		Ewe live weight (kg)			
	n	P48	P62	P106	L63
Ewe treatment					
Control	24	65.7 ± 1.3	64.2 ± 1.3	70.8 ± 1.3	65.3^{ab} ± 1.6
Cortisol	23	65.3 ± 1.4	64.0 ± 1.4	68.8 ± 1.3	65.6^{ab} ± 1.6
Isolation x 2	21	65.0 ± 1.3	64.2 ± 1.4	71.2 ± 1.3	64.3^{a} ± 1.7
Isolation x 10	20	65.7 ± 1.4	63.7 ± 1.4	70.8 ± 1.3	63.6^{a} ± 1.7
Sham-shorn	23	65.2 ± 1.3	64.0 ± 1.4	69.1 ± 1.3	63.6^{a} ± 1.6
Shorn	23	65.0 ± 1.3	63.9 ± 1.4	71.6 ± 1.3	68.9^{b} ± 1.7

Means within columns with different superscripts are significantly different (P<0.05)

Ewe live weight change between days 48 and 62 of pregnancy were similar for ewes in all treatments (Table 20). Later in pregnancy (day 62-106), however, shorn ewes had a greater weight gain than ewes that were given cortisol or were sham-shorn. Ewes live weight decreased between day 106 of pregnancy and day 63 of lactation as a result of parturition. The weight loss of ewes after day 106 of pregnancy and day 63 of lactation was lower for shorn than isolated ewes (on either 2 or 10 occasions).

Table 20. Ewe liveweight change (kg/d) during pregnancy and lactation (from P48 to 62, P62 to 106 and P106 to L63) of ewes in the control, cortisol, isolation x 2, isolation x 10, sham shorn and shorn treatments (least squares mean ± standard error)

	n	Live weight change (kg/day)		
		P48 to 62	P62 to 106	P106 to L63
Ewe treatment				
Control	24	-0.14 ± 0.04	$0.15^{ab} \pm 0.02$	$-0.05^{ab} \pm 0.1$
Cortisol	23	-0.05 ± 0.04	$0.11^{a} \pm 0.02$	$-0.03^{a} \pm 0.1$
Isolation x 2	21	-0.06 ± 0.04	$0.16^{b} \pm 0.02$	$-0.08^{b} \pm 0.1$
Isolation x 10	20	-0.13 ± 0.04	$0.15^{ab} \pm 0.02$	$-0.07^{b} \pm 0.1$
Sham-shorn	23	-0.06 ± 0.04	$0.11^{a} \pm 0.02$	$-0.05^{ab} \pm 0.1$
Shorn	23	-0.08 ± 0.04	$0.17^{b} \pm 0.02$	$-0.02^{a} \pm 0.1$

Means within columns with different superscripts are significantly different (P<0.05)

Overall ewe body condition scores decreased (P<0.05) between successive recordings. Ewes were approximately 0.4 of a condition score lighter on day 106 of pregnancy than at day 48 (Table 21). In late-pregnancy (P106), shorn ewes had greater (P<0.05) body condition scores than ewes in the sham-shorn and cortisol treatments. When ewe live weight was included in the model as a covariate shorn ewes had higher (P<0.05) condition scores (3.1) than control, cortisol, isolation x 2, isolation x 10 and sham-shorn ewes (2.8, 2.8, 2.9, 2.8 and 2.6, respectively).

Table 21. Body condition scores on days 48, 62 and 106 of pregnancy of ewes in the control, cortisol, isolation x 2, isolation x 10, sham shorn and shorn treatments (least squares mean ± standard error)

	n	Body condition score		
		P48	P62	P106
Ewe treatment				
Control	24	3.0 ± 0.06	3.0 ± 0.08	3.0^{bc} ± 0.1
Cortisol	23	3.1 ± 0.07	3.0 ± 0.09	2.8^{ab} ± 0.1
Isolation x 2	21	3.1 ± 0.07	3.0 ± 0.09	3.0^{bc} ± 0.1
Isolation x 10	20	3.2 ± 0.07	3.0 ± 0.09	3.0^{bc} ± 0.1
Sham-shorn	23	3.0 ± 0.07	2.8 ± 0.09	2.7^{a} ± 0.1
Shorn	23	3.1 ± 0.07	2.9 ± 0.09	3.2^{c} ± 0.1

Means within columns with different superscripts are significantly different (P<0.05)

Ewe plasma cortisol response to treatment

Ewes given cortisol had approximately a 100-fold higher (P<0.05) area under the response curve than ewes in the isolation x 10 and sham-shorn treatments (Table 22). On the first day of treatment shorn ewes had a greater (P<0.05) area under the cortisol curve than ewes in the isolation x 10 and sham-shorn treatments (Figure 8). Ewes in the isolated x 2 treatment had an intermediate response to their first treatment.

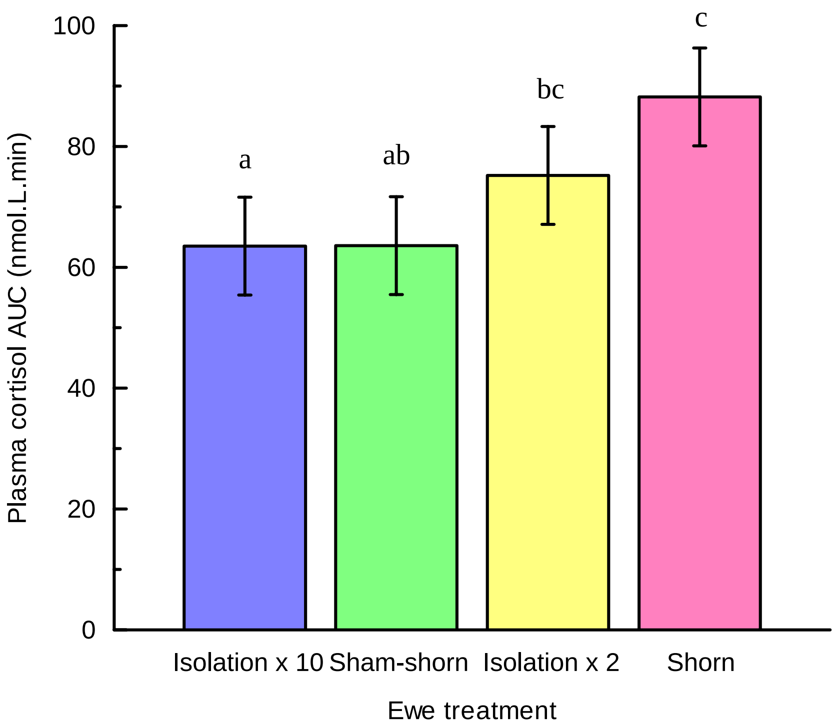


Figure 8. Area under the plasma cortisol response curve (nmol/L.min) of ewes in the isolation x 2, isolation x 10, sham-shorn and shorn treatments on the first day of treatment (least squares mean ± standard error). Means within columns with different superscripts are significantly different (P<0.05)

Repeated sham-shearing resulted in a decrease in area under the cortisol response curve between the first and ninth treatments, indicating that ewes habituated to the stressor (Table 22). The cortisol response of ewes to isolation was lower during the fifth treatment than the first, however, the response during the ninth treatment was greater than on the fifth treatment. Cortisol doses produced a greater (P<0.05) response after treatment in treatment 5 than treatments 1 and 9.

Table 22. Area under the cortisol response curve (AUC cortisol nmol/L.min) of ewes in the isolation x 2, isolation x 10 and sham shorn and shorn on the 1^{st}, 5^{th} and 9^{th} treatments (least squares mean ± standard error)

		AUC cortisol (nmol/L.min)[1]			P-value [2] Treatment	
	n	1st Treatment	5th Treatment	9th Treatment	1 vs. 5	5 vs. 9
Ewe treatment						
Cortisol	24	8.5^{d} ± 0.1 (5701)	8.9 ± 0.1 ^{b} (11240)	8.4 ± 0.1 ^{b} (5755)	*	**
Isolation x 2	24	4.2^{bc} ± 0.1 (75.2)	---	---	---	---
Isolation x 10	24	4.2^{a} ± 0.1 (63.5)	3.9 ± 0.1 ^{a} (62.0)	3.5 ± 0.1 ^{a} (37.8)	ns	**
Sham-shorn	24	4.1^{ab} ± 0.1 (63.6)	3.6 ± 0.1 ^{a} (38.1)	3.8 ± 0.1 ^{a} (53.0)	**	*
Shorn	24	4.4^{c} ± 0.1 (88.2)	---	---	---	---

Means within columns with different superscripts are significantly different (P<0.05)

[1] data was $\log_e$ transformed, untransformed means are presented in parentheses

[2] ns indicates no significant difference between treatments 1 and 5 or 5 and 9, + indicated P<0.1, * indicates P<0.05, and ** indicates P<0.01.

Ewe gestation length and maternal behaviour score

Ewe gestation lengths did not vary between treatments (Table 23). In addition there was no effect of ewe treatments on the ewes' maternal behaviour scores.

Table 23. Gestation length (days) and maternal behaviour scores (MBS) of ewes in the control, cortisol, isolation x 2, isolation x 10, sham shorn and shorn treatments (least squares mean ± standard error)

	n	Gestation length (days)	Maternal behaviour score
Ewe treatment			
Control	23	145.7	3.0±0.2
Cortisol	23	145.7	3.1±0.2
Isolation x 2	21	145.4	2.7±0.2
Isolation x 10	20	145.5	3.1±0.3
Sham-shorn	22	145.6	2.7±0.2
Shorn	22	146.4	3.2±0.2

Means within columns with different superscripts are significantly different (P<0.05)

Lamb birth weight and body dimensions

At birth, ram lambs were heavier than ewe lambs by 0.3 kg (P<0.05; Table 23). In addition, ram lambs had larger girths (by 0.6 cm) and longer forelimb and hindlimb lengths than ewe lambs (by 0.8 and 0.7 cm, respectively).

Lambs born to shorn ewes were heavier than lambs born to ewes in all other treatments (P<0.05). In addition, lambs born to ewes in the cortisol treatment were lighter than lambs born to ewes in all treatments but sham-shorn. All lambs born to shorn ewes weighed more than 4 kg at birth compared to 92, 67, 88, 88, 85% of lambs born to control, cortisol, isolation x 2, isolation x 10 and sham-shorn treatments, respectively.

Lambs born to control and shorn ewes had longer (P<0.05) CRL than all other lambs (Table 23). The girth of lambs born to cortisol ewes was shorter (P<0.05) than all other lambs, except lambs born to sham shorn ewes. Forelimb length and hindlimb length showed a similar pattern with lambs born to control and shorn ewes having longer limbs than lambs born to ewes in the cortisol, isolation x 2 and sham-shorn treatments.

When lamb birth weight was included in the model as a covariate for CRL, forelimb and hindlimb lengths significant differences remained between ewe treatments. Lambs born to shorn ewes had smaller CRL relative to their birth weight (51.9 cm; $P<0.05$) than control and cortisol lambs (53.5 and 53.3 cm, respectively). In addition, control lambs had longer forelimb lengths (29.7 cm; $P<0.05$) than lambs born to ewes in the cortisol, isolation x 2, isolation x 10 and sham shorn treatments (29.1, 28.9, 29.0 and 29.1 cm, respectively). Lambs born to control ewes also had longer hind limbs (36.2; $P<0.05$) than lambs born to ewes given cortisol or isolated twice (35.4 and 35.3 cm, respectively). Lamb coat meconium staining was lighter ($P<0.05$) for ram than ewe lambs and for lambs born to shorn ewes than lambs born to ewes in all other treatments (Table 23).

Ram lambs were heavier than ewe lambs 63 days after the mid-point of the lambing period (Table 23). In addition lambs born to ewes in the cortisol treatment were lighter at L63 than lambs born to ewes in the isolation x 2 and isolation x 10 treatments ($P<0.05$).

Table 24. Live weight (kg) at birth, CRL, girth, forelimb and hindlimb lengths (cm) and coat meconium staining of ewe and ram lambs born to ewes in the control, cortisol, isolation x 2, isolation x 10, sham shorn and shorn treatments (least squares means± standard error)

		Birth	Body dimensions				Coat		Live weight
	n	Weight	CRL	Girth [1,2]	Forelimb [3]	Hindlimb [2]	Meconium	n	(L63)
Sex of lamb									
Ewe	144	4.6^{a}±0.06	52.9±0.2	1325^{a}±13 (36.3)	3.35^{a}±0.1(28.8)	1245^{a}±14 (35.2)	2.0^{b}±0.1	117	18.6^{a}±0.3
Ram	122	4.9^{b}±0.06	52.5±0.2	1369^{b}±15(36.9)	3.38^{b}±0.1(29.6)	1298^{b}±15(35.9)	1.8^{a}±0.1	104	20.6^{b}±0.4
Ewe treatment									
Control	48	4.8^{b}±0.1	53.6^{b}±0.3	1365^{bc}±24(36.9)	3.39^{b}c±0.1 (29.7)	1321^{b}c±24(36.3)	2.2^{b}±0.2	36	19.4^{ab}±0.6
Cortisol	46	4.4^{a}±0.1	52.3^{a}±0.3	1270^{a}±24(35.5)	3.35^{a}±0.1(28.8)	1213^{a}±24(34.7)	2.1^{b}±0.2	39	19.1^{a}±0.6
Isolation x 2	42	4.7^{b}±0.1	52.3^{a}±0.3	1358^{bc}±25(36.8)	3.36^{a}±0.1(29.1)	1236^{a}±25(35.1)	1.8^{b}±0.2	38	20.3^{b}±0.6
Isolation x 10	40	4.8^{b}±0.1	52.8^{a}±0.3	1357^{bc}±25(36.8)	3.37^{ab}±0.1(29.0)	1266^{ab}±25(35.5)	2.0^{b}±0.2	33	20.4^{b}±0.6
Sham-shorn	46	4.6^{ab}±0.1	52.0^{a}±0.3	1330^{ab}±24(36.4)	3.36^{a}±0.1(28.9)	1243^{a}±24(35.2)	1.9^{b}±0.2	40	19.0^{ab}±0.6
Shorn	46	5.9^{c}±0.1	53.7^{b}±0.3	1403^{c}±24(37.4)	3.40^{c}±0.1(29.9)	1348^{c}±24(36.7)	1.4^{a}±0.2	35	19.8^{ab}±0.6

Means within columns with different superscripts are significantly different ($P<0.05$)

[1] Lambs born dead were excluded from the analysis

[2] Data X^2 transformed, untransformed means are presented in parentheses

[3] Log_e transformed, untransformed means are presented in parentheses

Discussion

Lambs born to shorn ewes were larger and heavier than lambs born to unshorn control ewes. This finding adds to the growing body of evidence which shows that mid-pregnancy shearing of ewes increases lamb birth weight (Morris et al., 1999; Smeaton et al., 2000; Revell et al., 2002; Sherlock et al., 2003; Kenyon et al., 2004). In chapters 2, 3a and 3b increases in lamb size (CRL, forelimb and hindlimb length) in response to mid-pregnancy shearing were identified, however in those studies the inclusion of birth weight in the statistical model of body dimensions rendered the differences non-significant. In the present study, however, lambs born to shorn ewes had shorter CRL relative to body weight, but had similar girth and limb lengths, than lambs born to ewes in the control treatment.

The component of shearing that may increase lamb birth weight is unknown. To date, increased ewe feed intake, gestation length and thyroid hormones have been eliminated as possibilities (Kenyon et al., 2003). Although ewe feed intake has been eliminated as a cause of the increase in lamb birth weight, the shorn ewes in this study gained more weight in mid-pregnancy (between days 62 and 106 of pregnancy) than ewes that were given cortisol or sham shorn and lost less weight between day 106 of pregnancy and day 63 of lactation than ewes that were isolated. It should be noted, that differences were not observed between shorn and control ewes therefore the increase in live weight does not explain the increase in lamb birth weight between these two treatments.

Studies that examined the effect of repeated isolation and transport during pregnancy suggested that chronic stressors may influence fetal growth and lamb birth weight (Lay et al., 1997b; Roussel et al., 2004). Therefore, it was hypothesised that chronic stress may be the component of mid-pregnancy shearing that increased lamb birth weight. In this study, however, repeated isolation and sham shearing had no effect on lamb birth weight.

A component of shearing that may alter lamb birth weight is chronic cold exposure. In response to shearing pregnant ewes can have reduced rectal temperatures for up to 20 days. Cold stress resulting from shearing, produces a series of hormonal and metabolic adaptations in the ewe (Clarke et al., 1997a). In response to shearing, pregnant ewes have elevated plasma concentrations of the thyroid hormones thyroxine (T4) and triiodothyronine (T3; (Symonds et al., 1988a; Morris et al., 2000; Sherlock et al., 2003). Elevated T4 and T3 concentrations are associated with an increase the ewe's basal metabolic rate (Tortora and Grabowski, 1996) and the stimulation of heat production (Symonds et al., 1989). In a study that examined the role of T3 and T4 in the birth weight response to mid-pregnancy shearing, Kenyon et al. (2005b) concluded that these hormones were not the sole endocrine mechanism for the increase in lamb birth weight.

Heat production in the shorn pregnant ewe has been reported to be fuelled by an increase in fat catabolism (Symonds et al., 1986). In that study, however, shorn and unshorn ewes had similar plasma concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (β-OHB). The proposal that an increase in fat oxidation of shorn ewes to meet the energy requirements for elevated heat production is difficult to reconcile against the reported changes in ewe live weight and body condition after mid-pregnancy shearing. Studies that examined ewe body condition scores, including the present study, reported no difference between shorn and unshorn ewes (Dabiri et al., 1996; Clarke et al., 1997a; Kenyon et al., 2002b; Revell et al., 2002).

Glucose is the most likely candidate for producing the increase in lamb birth weight in response to mid-pregnancy shearing. The infusion of exogenous glucose into late-gestation fetuses resulted in an increase in their weight compared to saline infused lambs (Stevens et al., 1990). Glucose diffusion through the placenta is dependent on the maternal and fetal concentration gradient, therefore, fetal glucose concentrations can be altered by maternal glucose concentrations (Takata and Hirano, 1997). This hypothesis is supported by the findings of Hay et al. (1983b) who reported uterine glucose uptake had a linear relationship with maternal glucose production. Shearing in mid-pregnancy has inconsistent effects on maternal plasma glucose concentrations. The majority of studies reported that shorn ewes had greater glucose concentrations than unshorn ewes (Symonds et al., 1988a; Clarke et al., 1997a; Morris et al., 2000). However, several studies reported similar concentrations of shorn and unshorn ewes (Symonds et al., 1988b; Sherlock et al., 2003). Indeed, Revell et al. (2000) found that shorn twin-bearing ewes had lower basal glucose concentrations than unshorn ewes even though they produced heavier lambs.

Based on the work of Roussel et al. (2004), it was hypothesised that isolation would result in an increase in lamb birth weight, however, that was not the case. Roussel et al. (2004) reported that lambs born to ewes that were isolated, for 1 h twice a week for 5 weeks in mid-pregnancy, were 0.5 kg heavier than lamb born to control ewes. Roussel et al. (2004) reported that ewes had decreased plasma cortisol concentrations in response to successive exposures to isolation thus indicating habituation to the stressor. This is consistent with the present study in which a reduction in plasma cortisol response to isolation was observed between the 5th and 9th treatments. The study by Roussel et al. (2004) was fundamentally different from the present study in that ewes were selected based on their cortisol response to isolation prior to the start of the study (low and high reactivity). In the present study ewes were selected at random from a larger flock. In addition, ewes in the current study were of mixed-age whereas Roussel et al. (2004) had only primiparous ewes. These studies also differed in the breed of ewe used. The present study was of Romney ewes whereas Roussel et al. (2004) used a Merino and Border Leicester cross. The Merino is a highly gregarious breed that flock together when disturbed (Lynch et al., 1992a). In preparation for parturition, hill breed ewes (Blackface and Romney) are more likely to seek isolation than Merinos (Dwyer and Lawrence, 2005). Therefore, isolation may be a greater stressor for primiparous Merino x Border Leicester than the mixed-age Romney ewes. The previous handling of the ewes and the familiarity of the ewes with the environment that isolation took place in the study by Roussel et al. (2004) is unknown. The ewes in the current study, however, are often yarded and handled in the same shed as isolation took place. The response to isolation is likely to be greater for ewes that are handled infrequently and that are in an unfamiliar environment than ewes that are handled often and are in familiar surroundings (Price and Thos, 1980; Dwyer and Bornett, 2004).

In this study cortisol was given to ewes as a positive control to determine if elevated maternal cortisol concentrations altered lamb birth weight. Ewes given cortisol gave birth to lambs that were lighter and smaller than control, isolated and shorn ewes. This finding is contrary to a study by Jobe et al. (2003) that found a maternal injection of hydrocortisone on day 124 of pregnancy, at the same dose as in the present study, did not alter fetal weight on day P131. In that study, the maternal cortisol response to the cortisol injection was similar to concentrations reported after mid-pregnancy shearing (Chapter 3). In the present study, however, the maternal cortisol response to the exogenous cortisol injection was much higher. Therefore, this very large increase in plasma cortisol does not provide evidence for determining the effect of an elevation in plasma cortisol resulting from shearing.

Conclusion

In this study, exposing ewes to isolation, sham-shearing and elevated maternal cortisol concentrations did not produce an increase in lamb birth weight. Therefore it appears that either these alternative stressors do not adequately simulate the stress response to shearing or that the stress associated with mid-pregnancy shearing is not the mechanism by which mid-pregnancy shearing increases lamb birth weight. Further research should examine the role of cold stress resulting from mid-pregnancy on the birth weight response. In the scope of this thesis cold stress was not examined but an examination of cold stress could provide valuable information on the mechanisms of pregnancy-shearing that increase lamb birth weight.

Further research

The focus for the remainder of the thesis is to examine of the effects of ewe nutrition during mid- and late-pregnancy on lamb birth weight, size and behaviour. In addition, the longer term effects on the behaviour of ewe progeny will also be examined.

CHAPTER 5

The effect of nutrition from mid- to late-pregnancy on the performance of twin- and triplet-bearing ewes and the behaviour of ewes and lambs 24 h after birth.

Related publications:

Submitted to the Australian Journal of Experimental Agriculture.

Corner R.A., Kenyon P.R., Stafford K.J., West D.M., Lopez-Villalobos N., Morris S.T., Oliver M.H. The effect of nutrition from mid to late pregnancy on the performance of twin- and triplet-bearing ewes and their lambs.

Abstract

This experiment was conducted to evaluate the effect of offering ewes two different feeding levels during mid- and late-pregnancy, on ewe and lamb behaviour 12-24 h after birth. Romney ewes, bearing twin (n=80) or triplet fetuses (n=56), were allocated to a feeding treatment (pasture sward height of 2 or 4 cm) during mid-pregnancy (between 70 and 107 days after the mid-point of the breeding period, P70-107). In late-pregnancy (days 108 to 147 of pregnancy) half of the ewes were reallocated to a different feeding treatment, which produced 4 treatments: 2-2, 2-4, 4-2 and 4-4 cm sward heights. Two days after birth all ewes and lambs were offered pasture with an average sward height of 4 cm where they remained until weaning. Ewe live weights were recorded on days 65, 92, 107 and 130 of pregnancy and lamb live weights were recorded 12-24 h after birth. During mid-pregnancy (days 70 to 107 of pregnancy), ewes provided with 4 cm swards had liveweight gains of 262-290 g/day compared with 12-31 g/day for ewes provided with 2 cm swards. In late-pregnancy (days 108 to 147 of pregnancy), ewe liveweight gains were influenced by the ewes' previous nutritional treatment. Ewes in the 2-4 treatment had higher daily gains (538 g/day) than 4-4 ewes (343 g/day). In addition, 4-2 treatment ewes gained 90 g/day compared with 247 g/day for 2-2 ewes. Throughout pregnancy triplet-bearing ewes were heavier ($P<0.05$) than twin-bearing ewes, but during lactation ewe weights were similar. On day 130 of pregnancy, ewes provided with 2 cm swards (4-2 and 2-2) had greater ($P<0.05$) β-OHB and lower glucose ($P<0.05$) plasma concentrations than ewes provided with 4 cm swards (2-4 and 4-4). On P139, however, ewes provided with 4-4 swards had higher NEFA and β-OHB than ewes in all other treatments. Lambs born to ewes in the 4-4 treatment had the greatest birth weights ($P<0.05$), whereas lambs born to 2-2 treatment ewes had the lowest. The birth weight of lambs born to 2-4 treatment ewes was similar to that of lambs born to

ewes provided with 4-2 and 4-4 cm swards. At weaning ewe weights were similar between ewe nutritional treatments and ewes bearing twin or triplet fetuses. The ewe's maternal behaviour score was determined whilst her lambs were handled, and after the lambs were released the behaviour of the ewe and lambs was observed for 5 minutes. Ewe treatment and litter size had no effect on ewe maternal behaviour score. However, as MBS increased (ewes stayed closer to lambs during tagging), ewes bleated in a high-pitched less but were quicker to make contact with their lamb. During the observation period, ewes in the 2-4 cm sward height treatment bleated more in a low pitch ($P<0.05$) than ewes in the 2-2 treatment (17 vs. 8 bleats). A greater percentage of lambs born to 4-4 ewes (95%) bleated than lambs born to 2-2 ewes (84%; $P<0.05$). However, lambs born to ewes in the 2-2 treatment bleated earlier than lambs in all other treatments ($P<0.05$). Lambs born to 4-4 ewes were less likely ($P<0.05$) to move towards their dam in order to make contact than lambs born to 2-2 or 4-2 ewes (3.1 vs. 16.9 and 16.7, respectively). These findings suggest that when pasture availability is inadequate to provide an average pasture sward height of 4 cm throughout mid- and late-pregnancy, restricted intake in mid pregnancy may be compensated by providing additional pasture in late pregnancy. Ewe nutrition in this study had little effect on maternal behaviour, but, lambs born to ewes offered low pasture heights displayed behaviour that demonstrated greater 'need'.

Introduction

During the last decade lambing percentages in New Zealand have increased from 100 to 129% (M&WNZ - Economic Service, 2006a) with an increase in twin and triplet litters (Amer et al., 1999). The birth weights of triplet-born lambs are 19 to 24% lower than twin lambs (Hinch et al., 1985; Morris and Kenyon, 2004; Everett-Hincks et al., 2005a) and 36 to 40% lower than singleton lambs (Scales et al., 1986; Hinch et al., 1996). In addition, ewes bearing twin or triplet fetuses require more energy to support fetal growth than ewes bearing a single fetus (Geenty and Rattray, 1987).

Feeding requirements of single- and twin-bearing ewes have received a great deal of attention (Geenty and Rattray, 1987; Morris et al., 1993; Morris et al., 1994), however, only one study to date has examined the feeding requirements of triplet-bearing ewes on New Zealand's pastures (Morris and Kenyon, 2004). Morris and Kenyon (2004) recommended that twin- and triplet-bearing ewes be provided with pasture with an average sward height of 4 cm (equivalent to 1200 kg DM / ha) throughout mid- and late-pregnancy. Published nutrient requirements of sheep state that maintenance requirements of a 50 kg ewe bearing twin or triplet fetuses are 1.47 and 1.41 kg DM/day, respectively. Morris and Kenyon (2004) reported that feed intakes of twin and triplet ewes of 50-53 kg ewes offered pasture with a 2 cm sward height had daily dry-matter intakes of 1.2 kg between day 73 and 76 of pregnancy and 1.0 kg between day 120 to 123 of pregnancy. Therefore, 2 cm swards provide ewes with approximately 82-88% in mid-pregnancy and 68-71% of their late-pregnancy maintenance requirements. Ewes offered 4 cm sward heights had intakes of 2.2 kgDM/day between days 73-76 and 1.9 kgDM/day between day 120 and 123of pregnancy (Morris and Kenyon, 2004), this equates to 150-156% and 130-135% of their maintenance requirements in each period, respectively.

In some regions of New Zealand winter pasture growth rates are insufficient to allow for these relatively high pasture masses during the last two thirds of pregnancy (Matthews et al., 2000). A potential consequence of ewe undernutrition is that fetal growth may be retarded resulting in low birth weights (Morris and Kenyon, 2004) which are associated with increased mortality rates due to starvation and exposure (Alexander, 1984).

The establishment of an exclusive ewe lamb bond is essential for lamb survival (Nowak, 1996), and ensures that a ewe will only allow her own lambs to suck (Poindron et al., 2007). An attempt to suck by an alien lamb can result in a violent rejection by the ewe (Lynch et al., 1992b). Ewes learn to recognise their young within 30-120 minutes of contact (Poindron and Le Neindre, 1980; Lynch et al., 1992b), however, the ewe's attraction towards a young lamb will fade quickly if she is separated from the lamb (Poindron et al., 1984; Lynch et al., 1992b). The incidence of ewe and lamb separation increases as the time a ewe and her lambs remain at the birth site decreases (Lindsay et al., 1990). However, Murphy et al. (1994) suggested that improved lamb survival resulting from the increase in time spent at the birth site was due to the ewe remaining with all of her lambs rather than the ewe and lambs remaining at the birth site. Ewes that underfed during pregnancy may show an increased tendency to move away from the birth site thereby increasing the risk of separation.

Ewes undernourished during the last 6 weeks of pregnancy exhibit fewer appropriate maternal behaviours and inadequate maternal care compared to well-fed ewes (Putu et al., 1988; Nowak, 1996). The effects of nutrition on maternal behaviour score (MBS) vary between extensive and intensive production systems (Dwyer et al., 2003; Everett-Hincks et al., 2005a). Housed ewes that were under-fed were more likely to exhibit low MBSs than well-fed ewes (Dwyer et al., 2003). However, under pastoral conditions MBSs were similar for ewes offered 2, 4, 6 and 8 cm pastures in mid- and late-pregnancy (Everett-Hincks et al., 2005a).

The aim of this study was to compare the effects of feeding pasture with a maximum sward height of 2 or a minimum of 4 cm in mid and late pregnancy on the performance and behaviour of twin- and triplet-bearing ewes and their lambs.

Methods and materials

Experimental design and animals

Mixed-age Romney ewes (80 bearing twin and 56 triplet fetuses) that were mated during a 17-day period (14 April – 1 May 2004) to Romney rams were randomly allocated to replicated mid- and late-pregnancy feeding levels. From day 70 to 107 after the mid-point of the breeding period (P70-107) ewes were offered pastures with a sward height of either less than 2 cm (~700 kg DM/ha) or greater than 4 cm (~1300 kg DM/ha). Ewe feeding levels were balanced for pregnancy rank and live weight. In late-pregnancy (days 108 to 147 of pregnancy) half of the ewes were reallocated to a different feeding level which produced 4 feeding treatments: 2-2, 2-4, 4-2 and 4-4 cm swards.

Pasture management

The sward height of established ryegrass/white clover pasture was recorded weekly using a sward stick (Jenquip, New Zealand, 100 readings per paddock) and herbage dry matter was estimated fortnightly using a rising plate meter (Ashgrove Pastoral Products, New Zealand, 50 readings per paddock). Sward height was estimated using the equation: sward height cm = (50-average reading)/2. Dry matter was estimated using the equation: (average reading x 1.49) + 250. Sward height and dry matter estimates were based on the entire sward, ground to tip. Sward heights were maintained by rotating ewes between 9 paddocks, four with a maximum target sward height of 2 cm and five with a minimum of 4 cm. It was initially intended that ewes would be offered pastures with a sward height of 2 or 6 cm, however, 6 cm pastures could not be maintained therefore a target of 4 cm was selected prior to the start of ewe treatments commencing.

To ensure that pasture heights were maintained at an average height of 2 cm, ewes were moved off pastures if the swards dropped below 1.5 cm to another paddock with a sward height of 2 cm. In addition, if the swards of another designated 2 cm paddock rose above 2 cm ewes were moved onto those paddocks to prevent the sward heights increasing above the level required. Minimum sward heights of 4 cm were maintained by moving ewes off paddocks when the sward dropped below 4 cm to paddocks with a sward height minimum of 5 cm. This rotation resulted in ewes in the 2 cm treatment being provided with a range of sward heights from 1 to 2 cm and ewes in the 4 cm treatment 3 to 6 cm. Two days after parturition ewes and their lambs were moved to paddocks that had a minimum sward height of 4 cm and remained there until day 90 of lactation (L90).

Ewe measurements

Ewe live weights were recorded within 1 hour of removal from pasture on days 65, 92, 107 and 130 of pregnancy and days 42 and 90 of lactation. Blood samples were collected from all ewes on days 130 and 139 of pregnancy by jugular venipuncture using 20 gauge needles into 10 ml collection tubes containing sodium heparin (BD Vacutainer, Preanalytical solutions, Franklin Lakes, USA). Blood samples were immediately placed on ice and once chilled, samples were centrifuged at 3000 rpm for 10 minutes. The separated plasma was collected and frozen at -20 °C for further analysis. Plasma concentrations of glucose, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (β-OHB) were determined using diagnostic kits. Glucose concentrations were determined using the hexokinase enzymatic method (Roche Diagnostics NZ Ltd, Auckland, New Zealand), NEFA using the acyl-CoA synthase/acyl-CoA oxidase enzymatic method (Wako Pure Chemical Industries Ltd, Osaka, Japan) and β-OHB using the β-hydroxybutyrate dehydrogenase enzymatic method (Sigma Aldrich (Cat A310), Auckland, New Zealand). The published intra-assay coefficient of variation for each method was 1% for glucose, 4% for β-OHB and 5% for NEFA.

During the lambing period ewes were observed three times daily by experienced shepherds to identify newly born lambs and, if required, to provide assistance to ewes lambing. Twelve to twenty-four hours after parturition the ewe's maternal behaviour was quantified using the MBS (O'Connor et al., 1985; Everett-Hincks et al., 2005b). In addition, the bleating behaviour of the ewe was assessed for 5 minutes after her lambs were released. The frequency of ewe high and low-pitched bleats was recorded.

Lamb measurements

Within 12 to 24 hours of birth, lambs were identified to their dam, ear tagged and their sex and birth weight was recorded. At the completion of the ear-tagging procedure all lambs in the litter were placed on their side and released. The frequency of lamb bleats was recorded and the latency for the lamb to stand, contact the ewe, successfully suck and follow its dam if she moved more than 5 meters from the tagging site ewe was also recorded (Everett-Hincks et al., 2005a).

This study was conducted with the approval of the Massey University Animal Ethics Committee (MUAEC 04/25).

Statistical analysis

This experiment involved a crossover design incorporating litter size (twin vs. triplet) and ewe feeding level (2-2 vs. 2-4 vs. 4-2 vs. 4-4). Ewes were excluded from the study if the ewe died (n=4) or was diagnosed as twin-bearing but gave birth to only one lamb (n=4) or a lamb was born dead (n=2). Therefore, data from 126 ewes were included in the statistical analyses. The behaviour of 119 of the ewes and their lambs was observed.

Ewe pregnancy status was determined retrospectively based on number of lambs born to each ewe regardless of whether the lambs were born dead or alive. Ewe live weight and blood metabolite concentrations were analysed using the MIXED procedure for repeated measures in SAS (2005). The model included the fixed effects of treatment, day of pregnancy, age of the ewe and pregnancy status and their interactions, and the random effect of animal within each treatment (Littell et al., 1998). Non-significant interactions ($P>0.05$) were removed and the model was re-run. Least square means and their standard errors (S.E.) were obtained for each time point of measurement. Ewe liveweight gain was analysed using the MIXED procedure in SAS. The model included the fixed effects of ewe treatment, pregnancy status and ewe age. Ewe weight at the beginning of the period (mid- or late-pregnancy) was included in the model as a covariate. Lamb live weight was analysed using the MIXED procedure in SAS. The models included the fixed effects of litter size, sex of the lamb, ewe treatment and their interactions.

Lamb litter size was determined retrospectively based on the number of lambs born, regardless of whether they were born alive or dead. The number of high and low-pitched ewe bleats and the time for the ewe to leave the tagging site were analysed using the generalised linear model (GLM) procedure in SAS. The model included the fixed effects of litter size and ewe treatment and their interactions. Interactions were included in the model and any non-significant interactions were removed and the model was re-run. Ewe weight was included in the model as a covariate but was removed if non-significant.

The frequency of lamb bleats and the time to bleat, stand, contact, suck, follow was also analysed using the GLM procedure in SAS. The fixed effects included in the model were litter size and ewe treatment. Lamb birth weight was included in the model as a covariate but was removed if not significant.

The distribution of the frequency of ewes bleats (both high- and low-pitched), lamb bleats and the time for the lamb to bleat, stand, contact and suck were not normal, therefore, a $X^{0.5}$ transformation was used to normalise the data. Tables containing these variables present least squared means and standard errors of the transformed data plus the means of the untransformed data in parentheses.

The presence or absence of behaviours of the ewe (bleating in a low or high pitch, ewe moving toward lamb to make contact and leaving tagging site) and lamb (bleat, stand, contact, suck, follow and move towards ewe to make contact) were analysed using a binomial distribution and logit transformation in the GENMOD procedure in SAS. Tables containing these variables present least squares means and standard errors of the transformed data and the back-transformed means in parentheses. Ewe maternal behaviour score was also analysed using the GENMOD procedure in SAS. The model included fixed effects of ewe treatment and litter size.

Results

Pasture

Target sward heights were successfully achieved during both mid- and late-pregnancy (Table 25).

Table 25. Sward heights and herbage masses during between days 70 and 107 and between days 108 and 147 of pregnancy offered to ewes in the 2 and 4 cm sward height treatment groups (least squares mean ± standard error)

	Mid pregnancy (P70-107)		Late pregnancy (P108-147)	
	Sward height (cm)	Herbage mass (kgDM/Ha)	Sward height (cm)	Herbage mass (kgDM/Ha)
Ewe sward height treatment				
2 cm	1.6 ± 0.1	736 ± 19	1.4 ± 0.1	686 ± 6
4 cm	4.4 ± 1.6	1340 ± 38	4.2 ± 0.1	1331 ± 12

Ewe live weights and ewe average daily gain

Triplet-bearing ewes were heavier (P<0.05) than twin-bearing ewes throughout pregnancy however after parturition these live weight differences disappeared (Table 26). The live weight gain of ewes between days 65 and 130 of pregnancy were influenced by both ewe pregnancy rank and treatment group. Twin- and triplet-bearing ewes had similar live weight gains during pregnancy and lactation (data not shown).

Table 26. Live weight of ewes offered 2 or 4 cm sward heights between days 65 and 107 and between days 108 and 147 of pregnancy (least squares mean ± standard error)

	n	Ewe live weight (kg) P65	P92	P107	n	P130	n	L42	n	L90
Pregnancy status										
Twins	80	53.1^{a}±1.0	57.9^{a}±1.0	58.4^{a}±1.0	77	66.6^{a}±1.0	74	60.6±1.0	74	59.0±1.0
Triplets	56	58.7^{b}±1.1	62.7^{b}±1.1	65.0^{b}±1.1	54	72.3^{b}±1.1	48	62.2±1.1	51	58.9±1.1
Ewe sward height treatment										
4-4	34	54.9±1.2	62.4^{a}±1.2	67.0^{a}±1.2	31	75.1^{c}±1.2	28	62.7^{ab}±1.2	29	59.7±1.2
4-2	35	57.0±1.2	64.1^{a}±1.2	68.0^{a}±1.2	35	70.2^{b}±1.2	32	62.9^{b}±1.2	33	59.5±1.2
2-4	34	55.8±1.4	57.1^{b}±1.4	56.9^{b}±1.4	32	69.9^{b}±1.4	30	60.4^{ab}±1.4	30	58.7±1.4
2-2	33	56.0±1.4	57.5^{b}±1.4	56.6^{b}±1.4	33	62.6^{a}±1.4	32	59.6^{a}±1.4	33	58.0±1.4

Means within columns with different superscripts are significantly different (P<0.05)

From day 65 to 107 of pregnancy, the average daily weight gain of ewes provided with 2 cm swards was lower than ewes on 4 cm (P<0.05; Table 27). After feeding levels were changed on day 108 of pregnancy, ewes that remained on 4 cm swards (4-4) maintained their rate of weight gain, whilst ewes changed to the 2 cm treatment (4-2) had the lowest gains (P<0.05). Ewes initially provided with 2 cm swards and then 4 cm (2-4) had the greatest weight gains during the same period (P<0.05). At day 130 of pregnancy ewes in the 2-2 treatment were lighter (P<0.05) than ewes in all other treatments and ewes in the 4-4 treatment were heavier (P<0.05) than ewes in all other treatments.

Table 27.Ewe liveweight gain (g/day) of twin- and triplet-bearing ewes in the 4-4, 4-2, 2-4 and 2-2 treatments between days 65 and 107 and day 108 and 130 of pregnancy (least squares mean ± standard error)

		Ewe liveweight gain (g/day)		
	n	P65-P107	n	P108-P130
Ewe sward height treatment				
4-4	34	$280^{b} \pm 12$	31	$338^{c} \pm 26$
4-2	35	$258^{b} \pm 12$	35	$90^{a} \pm 24$
2-4	34	$19^{a} \pm 12$	32	$542^{d} \pm 26$
2-2	33	$9^{a} \pm 12$	33	$247^{b} \pm 25$

Means within columns with different superscripts are significantly different (P<0.05)

Ewe metabolite concentrations

Twin-bearing ewes had lower concentrations of β-OHB and NEFA than triplet-bearing ewes on day 130 and 139 of pregnancy (P<0.05) however there were no differences in the plasma glucose concentrations (P>0.05; Table 28). On day 130 of pregnancy, ewes provided with 4 cm swards (4-4 and 2-4) had lower β-OHB (P<0.05) and higher glucose concentrations (P<0.05) than ewes on 2 cm swards (4-2 and 2-2). In addition, ewes in the 2-4 sward height treatment had lower NEFA concentrations than ewes in all other treatments (P<0.05). On day 139 of pregnancy, ewes in the 4-4 sward height treatment had higher β-OHB concentrations than ewes in all other treatments. Also on day 139, concentrations of NEFA were different (P<0.05) between all treatments such that ewes offered 4-4 swards had the highest concentrations followed by ewes offered 2-4, 4-2 and 2-2 swards with the lowest.

From day 130 to 139 of pregnancy, ewes in the 4-4 sward height treatment had increased (P<0.05) plasma concentrations of β-OHB and NEFA and decreased concentrations of glucose (P<0.05; Table 284). During the same period ewes in the 2-4 treatment had increased plasma β-OHB concentrations (P<0.05) and also tended to have higher NEFA levels ($P<0.1$). Ewes in the 4-2 and 2-2 sward height treatments showed a reduction in both β-OHB and NEFA concentrations ($P<0.05$) however there was no difference in plasma glucose concentrations.

Table 28. Plasma concentrations (mmol/L) of β-hydroxybutyrate, non-esterified fatty acids and glucose of twin- and triplet-bearing ewes in the 4-4, 4-2, 2-4 and 2-2 sward height treatments on days 130 and 139 of pregnancy (least squares mean ± standard error)

			Plasma concentrations (mmol/L)								
	n		β-hydroxybutyrate[2]		P-value[1]	Non-esterified fatty acids		P-value[1]	Glucose		P-value[1]
	P130	P139	P130	P139	P130 vs. 139	P130	P139	P130 vs. 139	P130	P139	P130 vs. 139
Pregnancy status											
Twins	68	65	0.77^a±0.03 (0.62)	0.78^a±0.02 (0.65)	ns	0.84^a±0.06 (0.74)	0.80^a±0.05 (0.70)	ns	2.07±0.03 (4.3)	2.02±0.03 (4.1)	ns
Triplets	50	45	0.88^b±0.03 (0.80)	0.85^b±0.02 (0.72)	ns	0.99^b±0.02 (1.03)	0.87^b±0.03 (0.81)	ns	2.13±0.04 (4.1)	2.07±0.03 (4.4)	ns
Ewe sward height treatment											
4-4	28	24	0.78^a±0.03 (0.63)	0.91^b±0.02 (0.85)	***	0.91^b±0.05 (0.86)	1.04^d±0.04 (1.11)	*	2.18^b±0.04 (4.8)	2.03±0.04 (4.2)	**
4-2	31	32	0.90^b±0.03 (0.83)	0.75^a±0.02 (0.59)	***	0.99^b±0.05 (0.99)	0.77^b±0.04 (0.61)	***	1.99^a±0.04 (4.0)	2.05±0.04 (4.2)	ns
2-4	28	25	0.73^a±0.04 (0.55)	0.80^a±0.03 (0.67)	*	0.77^a±0.05 (0.64)	0.92^c±0.05 (0.87)	+	2.20^b±0.04 (4.9)	2.03±0.04 (4.2)	**
2-2	31	29	0.90^b±0.03 (0.84)	0.80^a±0.02 (0.64)	**	0.99^b±0.05 (1.05)	0.63^a±0.04 (0.43)	***	2.02^a±0.04 (4.1)	2.08±0.03 (4.4)	ns

Means within columns with different superscripts are significantly different (P<0.05)

[1] Comparison of sample day within litter size and ewe treatment, ns P>0.05, + P<0.1, * P<0.05, ** P<0.01, ***P<0.001

Lamb live weight and postnatal growth

Twin-born lambs were heavier (P<0.05) than triplets at birth, L42 and L90 (Table 29). In addition, lambs born to ewes offered 4-4 swards were heavier (P<0.05) than lambs born to ewes offered 2-2 swards by 0.5, 1.7 and 2.5 kg at birth, day 42 and 90 of lactation, respectively. Lambs born to ewes in the 4-2 sward height treatment were lighter at birth than their counterparts born to ewes offered 4-4 swards (P<0.05). In addition, there was a tendency (P=0.06) for lambs born to ewes offered 2-4 swards to be heavier than ewes offered 2-2 swards at birth. There was no difference in birth weight between lambs born to ewes offered 2-4 and 4-4 swards, however, on day 42 of lactation lambs born to ewes offered 2-4 swards were lighter than lambs born to ewes provided with 4-4 cm swards (P<0.05).

The growth rate of twin lambs was greater than triplet lambs between birth and day 42 of lactation and between day 43 and day 90 of lactation (Table 29). The growth rate of lambs between birth and 42 of lactation was greater for lambs born to ewes offered 4-4 swards than 2-4 and 2-2 swards (P<0.05) with lambs born to ewes offered 4-2 swards having intermediary growth rates. Growth rates between days 42 and 90 of lactation, however, were greater (P<0.05) for lambs born to ewes offered 2-4 than 2-2 swards. A higher proportion of triplet-born lambs died prior to weaning than twin-born lambs (P<0.05), however, mortality rates of lambs born to ewes in each of the sward height treatments did not differ.

Table 29.Lamb live weight at birth and days 42 and 90 of lactation of twin and triplet lambs born to ewes in the 4-4, 4-2, 2-4 and 2-2 cm sward height treatments (least squares mean ± standard error).

	Lamb live weight (kg)						Average daily gain (kg/day)	
	n	Birth	n	L42	n	L90	L0-42	L43-90
Litter size								
Twin	164	$4.5^{b}\pm0.1$	139	$17.0^{b}\pm0.2$	139	$25.2^{b}\pm0.3$	$0.25^{b}\pm0.01$	$0.19^{b}\pm0.01$
Triplet	132	$3.8^{a}\pm0.1$	96	$14.4^{a}\pm0.3$	93	$21.7^{a}\pm0.4$	$0.22^{a}\pm0.01$	$0.16^{a}\pm0.01$
Ewe sward height treatment								
4-4	71	$4.4^{b}\pm0.1$	54	$16.6^{c}\pm0.4$	54	$24.7^{b}\pm0.5$	$0.25^{b}\pm0.01$	$0.18^{ab}\pm0.01$
4-2	77	$4.0^{a}\pm0.1$	58	$15.9^{bc}\pm0.3$	60	$23.4^{ab}\pm0.5$	$0.24^{ab}\pm0.01$	$0.17^{ab}\pm0.01$
2-4	67	$4.2^{ab}\pm0.1$	58	$15.4^{ab}\pm0.3$	57	$23.5^{ab}\pm0.5$	$0.23^{a}\pm0.01$	$0.18^{b}\pm0.01$
2-2	81	$3.9^{a}\pm0.1$	60	$14.9^{a}\pm0.3$	61	$22.2^{a}\pm0.5$	$0.22^{a}\pm0.01$	$0.17^{a}\pm0.01$

Means within columns with different superscripts are significantly different (P<0.05)

Ewe and lamb behaviour

More twin-bearing ewes bleated in a high-pitch than triplet-bearing ewes (P<0.05). Ewes that bleated in a high pitch had a similar number of bleats between litter sizes and ewe treatments (Table 30). Fewer ewes in the 4-2 sward height treatment bleated in a low pitch than ewes in the 4-4 treatment. Of ewes that bleated in a low pitch, ewe treatment and litter size had no effect on the number of bleats. Ewe MBS did not vary between ewe sward treatments.

Table 30. Maternal behaviour score (MBS), the percentage of ewes that bleated in a high or low pitch and the number of high and low pitched bleats of twin- and triplet-bearing ewes in the 2-2, 2-4, 4-2 and 4-4 cm sward treatments (least squares mean ± standard error)

			Ewe high-pitched bleats		Ewe low-pitched bleats	
	n	MBS	%Bleated [2]	No. bleats [1]	Bleated (%) [2]	No. bleats [1]
Litter size						
Twin	77	2.5 ± 0.1	98.7^{b}	2.1 ± 0.1 (10.8)	82.9	2.2 ± 0.1 (12.1)
Triplet	43	2.6 ± 0.2	88.4^{a}	1.9 ± 0.1 (8.2)	88.4	2.1 ± 0.2 (12.3)
Ewe sward treatment						
2-2	31	2.7 ± 0.2	96.8	2.0 ± 0.2 (10.1)	80.7^{ab}	1.9^{a} ± 0.2 (7.8)
2-4	30	2.4 ± 0.2	96.6	2.1 ± 0.2 (9.5)	89.7^{ab}	2.5^{b} ± 0.2 (16.7)
4-2	32	2.8 ± 0.2	93.8	2.0 ± 0.2 (10.6)	75.0^{a}	2.0^{b} ± 0.2 (10.7)
4-4	27	2.4 ± 0.2	92.6	1.8 ± 0.3 (7.8)	96.3^{b}	2.3^{b} ± 0.2 (13.5)

Means within columns with different superscripts are significantly different ($P<0.05$)

1 Data were logit transformed, means of back transformed data are presented in parentheses

2 Data were $X^{0.5}$ transformed, untransformed means are presented in parentheses

Fewer lambs born to ewes in the 2-2 sward treatment bleated during the 5 minute observation period than lambs born to ewes in the 4-4 treatment ($P<0.05$; Table 31). However, of lambs that bleated, those born to ewes offered 2-2 swards bleated earlier than lambs born to ewes in all other sward height treatments ($P<0.05$). The number of bleats emitted by lambs that bleated was similar between litter sizes and ewe treatments.

Table 31. The percentage of lambs that bleated, time taken to bleat and the number of bleats during the 5-minute observation period of twin and triplet lambs born to ewes in the 2-2, 2-4, 4-2 and 4-4 cm sward height treatments (least squares mean ± standard error)

		Lamb bleats		
	n	Lambs that bleated (%) [1]	Time to bleat (sec) [2]	Bleats (n) [2]
Litter size				
Twin	149	89.9 (-2.2)	1.8±0.1 (17.2)	2.4±0.1 (14.5)
Triplet	118	89.0 (-2.1)	2.0±0.1 (23.7)	2.1±0.1 (12.8)
Ewe sward height treatment				
2-2	69	84.1^{a} (-1.7)	1.4^{a}±0.2 (10.7)	2.6±0.1 (16.9)
2-4	63	88.9^{ab} (-2.1)	2.1^{b}±0.2 (27.1)	2.3±0.1 (14.5)
4-2	71	90.1^{ab} (-2.2)	2.0^{b}±0.2 (23.3)	2.1±0.1 (12.4)
4-4	64	95.3^{b} (-3.0)	2.0^{b}±0.2 (20.8)	2.1±0.1 (10.8)

Means within columns with different superscripts are significantly different ($P<0.05$)

[1] Data were logit transformed, means of transformed data are presented in parentheses

[2] Data were $X^{0.5}$ transformed, untransformed means are presented in parentheses

To make contact with their dam, fewer lambs born to ewes in the 4-4 sward treatment ($P<0.05$) moved toward their dam than lambs born to ewes offered 2-2 or 4-2 swards (Table 32), however, a similar proportion of ewes moved towards their lamb. Triplet lambs and their dam were quicker ($P<0.05$) to make contact than twin lambs but did not differ between sward treatments.

Table 32. The percentage of twin and triplet lambs born to ewes in the 2-2, 2-4, 4-2 and 4-4 cm sward height treatments that made contact with their dam by the ewe moving to the lamb or the lamb moving to the ewe and lambs, the time for the ewe and lamb to make contact and the percentage that successfully made contact with their dam

	n	Contact [1] (%)	Ewe to lamb [1,2] (%)	Lamb to ewe [1,2] (%)	Time to contact [3] (sec)
Litter size					
Twin	144	91.9	88.9	12.5	3.3^{b}±0.1 (46.0)
Triplet	109	88.1	82.6	10.0	2.9^{a}±0.1 (33.6)
Ewe sward treatment					
2-2	64	88.4	81.5	16.9^{b}	3.1±0.2 (41.6)
2-4	66	87.3	84.5	8.6^{ab}	3.2±0.2 (36.5)
4-2	58	94.4	89.4	16.7^{b}	3.0±0.1 (39.8)
4-4	65	91.6	89.1	3.1^{a}	3.1±0.2 (41.4)

[1] P-values are based on analysis of logit transformed data

[2] A ewe and lamb that approached each other were classed as both “ewe to lamb” and “lamb to ewe”

[3] Data were Log_e transformed, untransformed means are presented in parentheses

The percentage of lambs that stood or successfully sucked was similar between litter sizes and ewe sward treatments (Table 33). In addition, the time for lambs to stand and successfully suck was similar between ewe sward treatments and litter sizes.

Table 33. The percentage of twin and triplet lambs to that stood and successfully sucked and time required to exhibit each behaviour during the 5-minute observation period for twin and triplet lambs born to ewes in the 2-2, 2-4, 4-2 and 4-4cm sward height treatments (least squares mean ± standard error)

	n	Lamb Stand		Lamb Suck	
		% [1]	Time (sec) [2]	% [1]	Time (sec) [2]
Litter size					
Twin	149	87.3	2.8±0.1 (37.1)	31.5	4.8±0.1 (145.4)
Triplet	118	93.2	2.6±0.1 (30.1)	35.6	4.8±0.1 (144.5)
Ewe sward treatment					
2-2	69	92.8	2.9±0.2 (42.0)	35.6	4.9±0.1 (155.8)
2-4	63	85.7	2.9±0.2 (35.1)	30.2	4.8±0.2 (147.4)
4-2	71	88.7	2.5±0.2 (23.8)	35.2	4.7±0.1 (122.6)
4-4	64	92.2	2.7±0.2 (33.4)	32.8	4.9±0.1 (154.0)

Means within columns with different superscripts are significantly different (P<0.05)

[1] P-values of comparisons within columns are based on analysis of logit transformed data

[2] Data were $X^{0.5}$ transformed, untransformed means are presented in parentheses

The time taken for the ewe and lamb to make contact was negatively correlated with ewe MBS and live weight and positively correlated with the time for the ewe to leave the tagging site (P<0.05; Table 34). Time to make contact was also correlated with lamb behaviours including number of bleats, and the time taken to bleat, stand and suck (P<0.05). The time required for the lamb to stand was negatively correlated with number of lamb bleats and positively correlated with time to bleat, contact, suck and follow and with time taken for ewe to leave the tagging site.

Table 34. Correlations of ewe and lamb post-tagging behaviours with correlation coefficients and p-values presented above the diagonal and number of animals included below.

	1. Ewe wt	2. MBS	3. High bleat	4. Low bleat	5. Lamb bleat	6. Time to bleat	7. Time to stand	8. Time to contact	9. Time to suck	10. Time to leave	11. Time to follow
1. Ewe weight (P130)	---	0.17**	-0.19**		-0.28***			-0.20**		-0.21*	
2. Ewe MBS	265	---	-0.29***					-0.36***			
3. Ewe high bleats	266	265	---		0.16**			0.58***	0.20*		-0.13*
4. Ewe low bleats	267	265	266	---						0.29**	
5. Lamb bleats	263	261	262	263	---	-0.14*	-0.12+	0.15*			
6. Lamb time to bleat	239	237	238	239	235	---	0.56***	0.19**	0.24*	0.25*	0.16*
7. Lamb time to stand	240	238	239	240	236	222	---	0.40***	0.21*	0.31**	0.16*
8. Lamb time to contact	238	236	237	238	234	219	222	---	0.32**	0.20*	
9. Lamb time to suck	89	89	89	89	86	84	89	88	---		0.27*
10. Ewe time to leave	108	107	108	108	106	98	106	104	28	---	
11. Lamb time to follow	267	265	266	267	263	239	240	238	89	108	---

Blank cells indicate non-significant correlations (P>0.1), + P<0.1, * P,0.05, ** P<0.01 and *** P<0.001

The proportion of ewes that moved further than 5 meters from the tagging site during the observation period and the time taken to move from tagging site was similar between litter sizes and ewe treatments (Table 35). The proportion of lambs that followed their dam after she moved away from tagging site was greater for lambs born to 2-4 ewes than lambs born to ewes in all other treatments (P<0.05). However, of the lambs that followed their dam, the time to follow was similar between litter sizes and ewe treatments.

Table 35. Percentage of ewes move more than 5 meters from the tagging site during the 5-minute observation period and the time taken move away and the percentage of lambs that followed their dam and the time taken to follow for twin and triplet lambs born to ewes in the 2-2, 2-4 4-2 and 4-4 cm sward height treatments

		Ewe moved >5m		Lambs followed ewe	
	n	Dams that moved [1] (%)	Time to leave [2] (sec)	Lambs that followed [1] (%)	Time to follow [2] (sec)
Litter size					
Twin	149	60.5	4.7±0.2 (128.9)	77.1	0.4±0.2 (3.3)
Triplet	118	48.8	4.5±0.2 (109.5)	66.1	0.5±0.2 (12.2)
Ewe sward treatment					
2-2	69	51.6	4.5±0.1 (113.1)	69.7^{a}	0.7±0.2 (9.4)
2-4	63	48.3	4.8±0.1 (127.2)	93.1^{b}	0.3±0.2 (13.2)
4-2	71	68.8	4.7±0.1 (139.2)	69.8^{a}	0.4±0.2 (4.5)
4-4	64	55.6	4.4±0.1 (97.4)	61.8^{a}	0.2±0.2 (4.0)

[1] P-values were based on analysis of logit transformed data

[2] Data were $X^{0.5}$ transformed, untransformed means are presented in parentheses

Discussion

This experiment evaluated the effect of providing twin- and triplet-bearing ewes pastures with a sward height of less than 2 cm (~700 kg DM/ha) or greater than 4 cm

(~1300 kg DM/ha) in mid and late pregnancy on ewe and lamb performance and behaviour. Paddock target sward heights were achieved in both mid and late pregnancy.

Ewe and lamb performance

Ewe sward treatments altered the rate of ewe live weight gain during both feeding periods (day 70 to 107 and day 108 to 147 of pregnancy). In addition, live weight gains in late-pregnancy were altered by sward height treatment in mid-pregnancy.

The weight of the gravid uterus (the uterus including fetuses, placenta and amniotic fluids) at day 140 of pregnancy for twin-bearing ewes has been reported to range between 12 to 17 kg and for triplet-bearing ewes 21-22 kg (McCoard et al., 1997; McCoard et al., 2000; Grazu-Bilska et al., 2006; Kenyon et al., 2007). In this study, ewe live weight change between days 65 to 130 of pregnancy can be calculated, although, these values may be an underestimation of the ewe's live weight change over the entire pregnancy period. However, these data suggest that twin-bearing ewes in the 4-4, 4-2 or 2-4 treatments may have achieved a live weight gain additional to that of the gravid uterus during pregnancy. The weight change of ewes in the 2-2 treatment was lower than the predicted gravid uterus weight suggesting these ewes may have lost live weight. Triplet-bearing ewes provided with 4-4 cm sward heights also may have achieved live weight gain additional to the weight of the gravid uterus but those in the 4-2, 2-4 and 2-2 cm treatments may have lost live weight during pregnancy.

The pregnant ewe is not always able to meet the glucose demands of the growing fetus and therefore must utilise energy reserves during times of undernutrition (Morgante, 2004). Mobilisation of fat reserves is indicated by elevated plasma concentrations of NEFA and β-OHB (Robinson et al., 2002). β-hydroxybutyrate concentrations of less than 0.8 mmol/L indicate an adequate level of feeding (Russel, 1984), however concentrations greater than 1.0 mmol/L are deemed high and indicate fat mobilisation (Morgante, 2004). On day 130 of pregnancy, ewe plasma concentrations of β-OHB indicate that ewes provided with 2 cm swards (4-2 and 2-2) were mobilising body reserves to a greater degree than ewes provided with 4 cm swards in the same period (4-4, 2-4). On day 139 of pregnancy, however, ewes in the 4-4 treatment had higher β-OHB and NEFA concentrations than all other ewes which may suggest that only ewes provided with 4-4 had body reserves that could be mobilised in the later stages of pregnancy. There is a need to examine the fat mobilisation of ewes under these conditions.

Lambs born to ewes provided with 2 cm swards between days 70 and 107 of pregnancy and 4 cm between days 108 and 147 of pregnancy (2-4) had similar birth weights to lambs born to ewes provided with 4 cm between days 70 and 147 of pregnancy (4-4). In addition, lambs born to ewes provided with 2 cm swards between days 70 and 147 of pregnancy (2-2) had similar birth weights to lambs born to ewes in the 4-2 treatment. This suggests that for twin and triplet-born lambs, nutrition between days 108 and 147 of pregnancy was more important for lamb birth weight than between days 70 and 107 of pregnancy. This finding is in agreement with Brink (1990) who reported that ewes fed 0.5, 1.0 and 1.5 times their recommended energy requirements from breeding to day 109 of pregnancy and then fed *ad libitum* during last 40 days of pregnancy gave birth to lambs with similar live weights. In addition, single- and twin-bearing ewes on a low or high level of nutrition in mid pregnancy (P50-P100) and then offered supplements in late pregnancy gave birth to lambs with similar birth weights (Kleemann et al., 1993). It is important to note, however, that these studies do not distinguish between litter sizes when reporting birth weight effects of the sward height treatments.

Lambs that were born to 4-4 ewes were heavier at weaning (L90) and had greater average daily weight gains to L42 than lambs born to 2-2 ewes. These findings differ from those of Morris and Kenyon (2004) who reported that on day 37 of lactation, but not day 87, lambs born to ewes provided with 4 cm pastures in mid and late pregnancy were heavier than lambs born to ewes on 2 cm. This increase in growth rates may be due to greater milk production. Undernutrition during late pregnancy can restrict mammary growth thus resulting in a delay in the ewe reaching full milk production and a lower total milk production over lactation (Wallace, 1948; Peart, 1967). Kenyon et al. (2005c) concluded that twin and triplet ewes should be provided with pastures with a sward height of at least 4 cm during mid and late-pregnancy to ensure that lamb colostrum was not adversely affected.

Curll et al. (1975) reported that lambs born to ewes that gained weight in mid pregnancy had greater postnatal daily weight gains than lambs born to ewes that lost weight during the same period. In this study, ewes whose sward height treatment was altered between mid- and late-pregnancy (2-4 and 4-2) had the greatest and lowest live weight gains in late pregnancy, respectively. However, the growth rate of their lambs from birth to L42 was intermediate between lambs born to ewes in the 4-4 and 2-2 sward treatment. Therefore it appears that the ewe’s rate of weight gain or loss during mid- and late-pregnancy did not affect their lambs’ postnatal growth.

At weaning, ewe live weights were similar across all ewe treatment groups indicating there was no carryover effect of nutrition during pregnancy when ewes were provided with pasture with a minimum sward height of 4 cm during lactation. This finding contrasts with those of Morris and Kenyon (2004) who reported that ewe live weight differences due to offering ewes 2 cm swards between day 64 of pregnancy and parturition compared with ewes offered either 4, 6 or 8 cm in the same period remained in lactation when ewes were provided with sward heights of 4 or 8 cm in lactation.

Ewe and lamb behaviour

Ewe bleats, particularly low-pitched bleats, aid in the establishment of the ewe lamb bond and therefore are important for lamb survival (Nowak, 1996; Dwyer and Lawrence, 2005). The maternal low-pitched bleat is considered to be a ‘care-giver’ bleat made exclusively to the lamb whereas a high-pitched bleat is a ‘protest’ or distress bleat (Dwyer et al., 1998). In this study, ewes offered 4 cm swards in both mid and late pregnancy (4-4) were more likely to bleat in a low pitch than ewes in the 4-2 treatment. In contrast, Everett-Hincks et al. (2005a) reported that twin-bearing ewes offered 2 cm swards from day 64 of pregnancy until first day after parturition had a higher rate of low-pitched bleats than twin-bearing ewes offered 6 cm swards. The similarity in the number of high pitched bleats between ewe treatments and litter sizes observed in this study is in agreement with previous studies of Dwyer et al. (2003) and Everett-Hincks et al. (2005a).

Lambs born to ewes with high MBS have greater survival rates to weaning than lambs born to ewes with low scores (O'Connor et al., 1985; Everett-Hincks et al., 2005b). In the current study, MBSs were similar between nutritional treatments and litter sizes. This finding was in agreement with O’Connor (1996), however, the majority of other studies, including Chapter 2 of this thesis, reported increases in MBS as litter size increased (O'Connor et al., 1985; Dwyer et al., 2003; Everett-Hincks et al., 2005b). Everett-Hincks et al. (2005b) reported that there was no effect of ewe nutrition from day 64 of pregnancy to parturition on MBS. However, Dwyer et al. (2003) reported that ewes offered high intakes (100% of maintenance requirements) were more likely to receive high scores and less likely to receive low scores than low intake ewes (65%).

This difference in findings may be due to differences in birth weight of lambs observed. In the study of Dwyer et al. (2003) the average birth weight of twin lambs born to low- and high-intake ewes was 3.0 and 3.3 kg, respectively whereas in the current study twin lambs born to ewes offered 2-2 or 4-4 swards were 3.9 and 4.4 kg, respectively. In addition, the environment in which ewe and lamb behaviour was observed also differed: in the current study behaviour of the ewes and lambs was observed in the paddock whereas Dwyer et al. (2003) observed ewes indoors. The environmental factors may have influenced ewe and lamb behaviour.

The behaviour of the lamb is believed to be the major limiting factor to lamb survival (O'Connor and Lawrence, 1992). Lambs that stand soon after birth, suck soon after standing and maintain close contact with their dam are more likely to develop an exclusive bond with their dam (Alexander, 1988). Lamb bleating contributes to the establishment and maintenance of the ewe-lamb bond (Lindsay, 1996; Nowak, 1996). Vocal behaviour of the neonate has been suggested to be an expression of need (Weary and Fraser, 1995) and is inversely related to maternal care (Dwyer and Lawrence, 1998). In this experiment fewer lambs born to ewes in the 2-2 treatment bleated during the observation period. Lambs born to 2-2 ewes that bleated, however, did so earlier than all other lambs and with a greater frequency than lambs born to in the 4-2 and 4-4 ewes. This bleating behaviour indicates that lambs born to 2-2 ewes had greater ‘drive’ to be with their dam than lambs born to ewes in all other treatments.

The time taken for the lamb to bleat was positively correlated with the time taken for the lamb to make contact with its dam, stand, successfully suck and follow its dam if she moved away from the tagging area. This indicates that a short latency for the lamb to bleat is also an indication of greater ‘drive’ to be with their dam.

An increase in lamb birth weight is associated with an increase in lamb vigour (Moore et al., 1986) and a more rapid progression in the expression of neonatal behaviours after birth (Dwyer et al., 2003). The frequency of lamb bleats during the observation period was negatively correlated with lamb birth weight which is in agreement with Dwyer et al. (1998) who reported that low birth weight was associated with an increased bleat rate in the Blackface breed, but not Suffolk breed. In this study, the frequency of lamb bleats during the 5-minute observation period was also positively correlated with the total number of ewe high-pitched or ‘protest’ bleats and the latency for the ewe and lamb to make contact and was negatively correlated with the latency for the lamb to bleat. Therefore this finding adds to the hypothesis that lambs born to ewes that were well-fed during pregnancy had a lesser ‘need’ of their dam.

Behavioural progress, immediately after birth, of heavy birth weight lambs is faster than light lambs (Dwyer et al., 2003). Behavioural progress can be used as an indictor of neonatal vigour, however, in this study lamb birth weight was not correlated with the time taken to stand, contact or suck. This may be due to a non-linear relationship between birth weight and the time to exhibit these behaviours. For example very light or heavy lambs may be less active at birth than lambs with intermediate birth weights. The time taken for the ewe and lamb to make contact however was positively correlated with the time taken for the lamb to bleat, stand and successfully suck. Therefore time to contact may provide an indication of the ‘need’ a lamb has for their dam.

Triplet lambs made contact with their dam faster than twin lambs. Everett-Hincks et al. (2005a) also reported that triplets were quicker to make contact with their dam after tagging than twin lambs. This difference may be due to a stimulatory effect of litter mates on both lamb behaviour and ewe response to her lamb. This finding, however, is contrary to that of Dwyer et al., (2003) who found that triplet lambs were slower to stand, suck and play than twins. These findings may differ due to the age of the lamb. In the study by Dwyer et al. (2003) lambs were observed immediately after birth, whereas, in the present study lambs were 12-24 h old. It may be that Dwyer et al. (2003) observed lamb behavioural progression whereas the present study observed the lamb's desire to reunite with its dam.

Conclusion

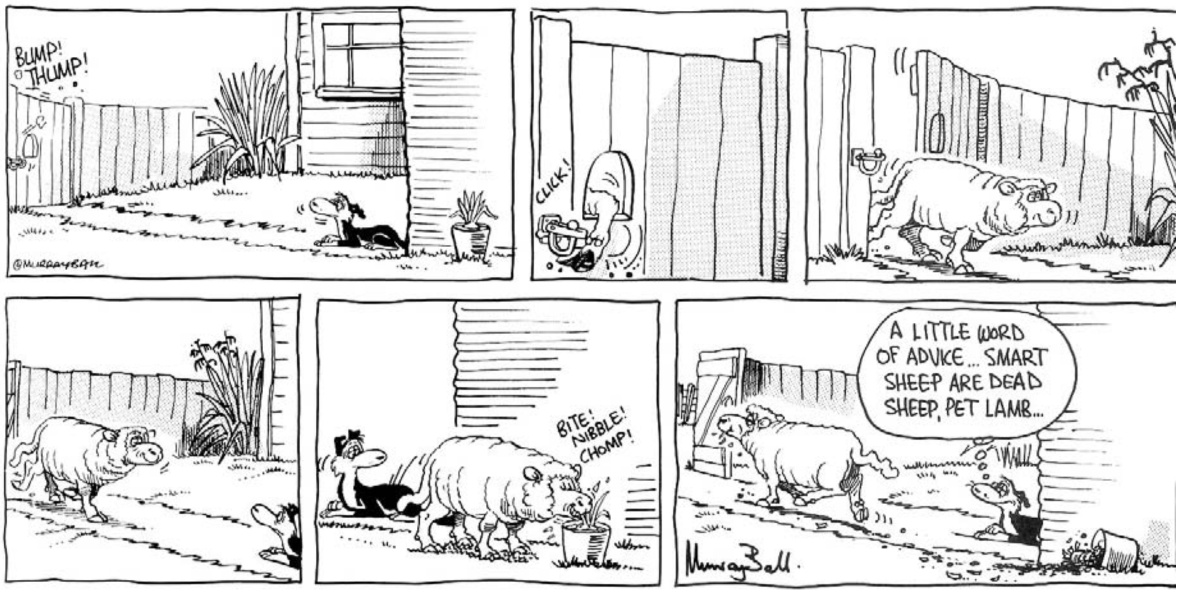
The results from this study reinforce previous findings which indicate that a minimum sward height of 4 cm throughout mid and late pregnancy improves lamb birth and weaning weight compared to lambs born to ewes provided with 2 cm. However, under conditions where pasture growth and availability is inadequate to maintain a minimum sward height of 4 cm in mid pregnancy, a sward height treatment with a minimum sward height of 4 cm in late pregnancy (i.e. 2-4) can still result in adequate lamb performance. The nutritional treatments had no effect on the ewe's maternal behaviour score. However, lambs born to ewes offered 4 cm pastures in between days 70 and 147 of pregnancy appear to have a reduced 'need' for their dam than lambs born to ewes offered 2 cm pastures between either days 70 and 107 or days 108 and 147 of pregnancy.

Further research

The effect of ewe nutrition on the lamb has received a great deal of attention however the longer-term effects on the progeny has received less interest. Chapter 6 examines the effects of maternal nutrition on the behaviour of ewe offspring at 1 and 2 years of age.

CHAPTER 6

Does the ewe nutrition during pregnancy affect the behaviour of ewe offspring at 1 and 2 years of age?



Related publications:

Corner R.A., Kenyon P.R., Stafford K.J., West D.M., Morris S.T., Blair H.T. Does ewe nutrition during pregnancy affect the behaviour of ewe lambs at 1 and 2 years of age? Proceedings of the New Zealand Society of Animal Production, Lincoln University, New Zealand, New Zealand Society of Animal Production 2005. 65, 29-32.

Abstract

The effects of the nutrition of the dam during pregnancy on the behaviour of her lambs in the days after birth has been examined but the longer term effects on lamb behaviour and mothering ability are not known. The behaviour of twin and triplet ewes born to dams offered herbage with an average sward height of 2 or 6 cm from day 64 to 132 of pregnancy was recorded at 1 year of age in an arena test and at 2 years of age after lambing. A greater proportion ($P<0.05$) of the progeny born to dam's offered 6 cm sward heights bleated in a high pitch during a 5 minute arena test. However there were no differences in minimum, median and maximum distance of the ewe from the observer or on the frequency of low and high-pitched bleats. In addition there was no difference in the behaviour of twin- and triplet-born ewes in the arena test. At 2 years of age twin-born ewes had a significantly greater maternal behaviour score than their triplet-born counterparts. Ewe nutrition during pregnancy did not affect the maternal behaviour score of their ewe lambs after lambing at 2 years of age. These results suggest that feeding levels during pregnancy are less important than the litter size in terms of the long-term effects on behaviour.

Introduction

The effects of ewe nutrition during pregnancy on the behaviour of the lamb in the 2-3 days after birth have been examined (Thomson and Thomson, 1949; Moore et al., 1986; Dwyer et al., 2003) but the longer term effects of nutrition during pregnancy on the maternal behaviour of the female progeny as adult are not well known.

Dwyer et al. (2003) reported that lamb birth weight, not maternal nutrition influenced lamb behaviour while Moore and Power (1986) concluded that maternal undernutrition reduced lamb vigour. In chapter 5, ewes offered 4 cm swards between day 70 of pregnancy and parturition gave birth to lambs that appeared to have a reduced ‘drive’ to remain with their dam than lambs born to ewes offered 2cm swards during the same period. To date the only study to examine undernutrition in adult offspring is Erhard et al. (2004) who reported that at 18 months of age the offspring of ewes undernourished for the first 95 days of pregnancy were more active when restrained and approached a novel stimulus more slowly than progeny of ewes fed maintenance requirements. It is hypothesised that maternal nutrition during pregnancy may result in offspring with reduced exploratory behaviours and altered maternal behaviour.

The effect of nutrition during pregnancy on the maternal behaviour of offspring is also of interest in terms of long-term ewe reproductive success. It is not known if the behavioural differences seen just after birth are still present in the mature ewe during the post-parturition period. If the maternal behaviour of female offspring is affected by their dams’ nutrition during pregnancy this could be used to improve mothering ability and success in rearing lambs to weaning.

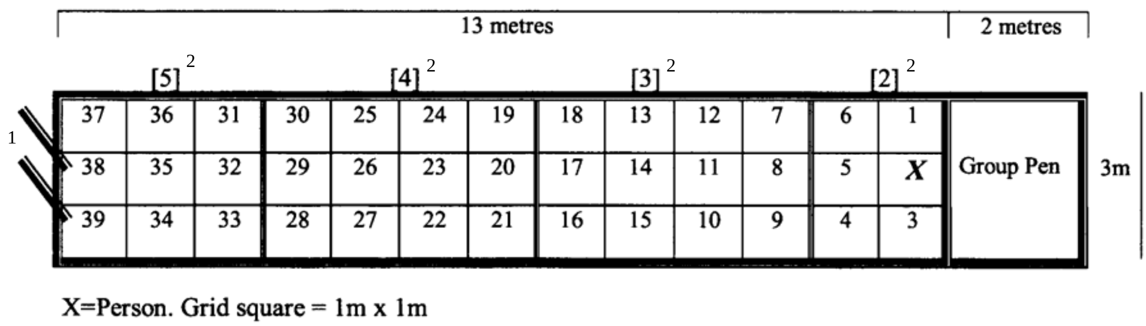
The behaviour of sheep can be measured in many ways. The arena test provides information on an animal’s response to a conflict situation (Kilgour and Szantar-Coddington, 1995, 1997) and provides information on the nervousness and emotivity of the ewe. This may predict future mothering ability (Kilgour, 1998). The maternal behaviour score (MBS) measures the interaction of the ewe and lamb after birth (O'Connor et al., 1985). High maternal behaviour scores are related to increased lamb survival and weaning weight (O'Connor et al., 1985; Parker and Nicol, 1993) and behaviour in the arena test has been linked to subsequent maternal behaviour (Everett-Hincks, 2003).

In 2000, ewes scanned as bearing twin and triplet fetuses were offered a sward at a height of 2 or 6 cm from day 64 to 132 of pregnancy. At birth lambs born to ewes offered the 2 cm sward were significantly lighter than those offered 6 cm sward (Morris and Kenyon, 2004). Behavioural observations were made of the lambs 6 to 12 hours after birth. Triplet lambs were less likely to bleat, make contact with the ewe and stand after being separated from their dam than twin lambs (Everett-Hincks, 2003). Therefore the effect of this alteration in the behaviour of the neonate has the potential to result in altered behaviour later in life.

The female offspring of ewes that were offered pastures with a sward height of 2 or 6 cm (Morris and Kenyon, 2004) were retained and the behaviour of these ewe progeny was assessed in an arena test at 1 year of age and after parturition at 2 years of age.

Methods and materials

At 1 year of age, 28 twin and 28 triplet Romney ewes born to Romney dams offered a sward height of 2 cm (n=33) or 6 cm (n=23) (Morris and Kenyon, 2004) were observed in an arena test (on 17th and 18th Sep 2003) as described by Everett-Hincks (2003). Prior to this test the ewes in this study were maintained as a mob with other sheep from the same farm (i.e. not prenatally stressed). The arena was a 13 x 3m pen marked into a 1 x 1m grid (Figure 9). Test sheep were held in a pen out of sight of the test area. A group of 10 sheep (test sheep not tested on that day) were held in a pen separated from the test arena by a wire gate which allowed visual contact with the test sheep (Figure 9). An observer who recorded the behaviour of the test sheep stood directly in front of the group sheep (Figure 9). The observer was the same for all sheep tested. A single test sheep entered the arena at the end opposite to the group sheep and its position was recorded every 15 seconds for 5 minutes. The movement of the sheep was determined from recordings of the position of the test sheep. Position was defined as the grid square in which the left front foot rested. Vocalisation, urination, defecation and sniffs of group sheep were also recorded during the 5 minute period. Ewe vocalisations were classified as low and high pitched bleats. Three measures of ewe position in the arena were calculated: minimum (the distance closest to observer), median (midpoint between the minimum and maximum distance from observer) and maximum (the distance furthest from observer) distance of the test sheep from the observer during the 5 minute observation period. The estimated total distance that the test sheep travelled in the test period was also calculated. In addition the arena was divided into 4 zones (Figure 9) and the proportion of time the test sheep spent in each zone was determined.



[1] Entrance to the arena
[2] Arena zone

Figure 9. Diagram of the arena showing dimensions of the arena, grid layout and zones (Everett-Hincks, 2003).

Between 6 and 12 hours after parturition the maternal behaviour score (MBS) of the 2 year old primiparous twin-born (n=22) and triplet-born (n=26) ewes born to dams offered herbage of 2 cm (n=25) or 6 cm (n=21) sward heights was recorded. Lambs were separated from their dam for tagging purposes and during this period a maternal behaviour score similar to that described by O’Connor et al. (1985) was recorded on a

5-point scale (1, ewe flees as shepherd approaches her lambs and 5, ewe make contact with the shepherd or lambs while lambs are being handled).

This study was conducted with the approval of the Massey University Animal Ethics Committee (MUAEC 03/100).

Statistical analysis

The records of 4 ewes in the arena test were excluded because of incomplete data due to test sheep jumping out of the arena before completion of the test. All data from the arena test were not normally distributed and methods tried failed to normalise the data. Therefore non-parametric methods such as Kruskal-Wallis and Chi squared tests were used (Minitab, 2003). The effect of the dam sward height (2 cm vs. 6 cm) and litter size (twin vs. triplet) on the minimum, median and maximum distance from the observer, total distance travelled, proportion of time spent in each zone, the frequency of sniffs of the group sheep and bleats (low or high pitched) was tested using the Kruskal-Wallis test. In addition the presence and absence of behaviours such as bleats (low, high and total), urination, defecation and sniffing group sheep was analysed using a Chi^2 analysis.

The MBS was normally distributed and therefore the effect of dam sward height and litter size was analysed using a general linear model (GLM). The number of ewes in each MBS category was analysed using Chi^2 test. Correlations between MBS and arena data were analysed using the Pearson product moment correlation coefficient.

Results

In the arena test the maximum distance from the observer (X) ranged from 4 to 13 metres and the minimum distance of 1 to 7 metres with the median distance being 1 to 11 metres. There were no significant effects of dam nutrition or litter size on either the distance of the test sheep from the observer (minimum, median and maximum) or the estimated total distance travelled during the recording period (Table 36).

Ewes spent the greatest proportion of time in zone 3 (median = 0.5) and least in zone 2 (median = 0). No significant difference in the proportion of time spent in each zone was found between dam nutrition or litter sizes (Table 36).

Table 36. The minimum, median and maximum distance from the observer in the arena test and the median proportion of time spent in each zone, for twin- or triplet-born lambs born to dams offered 2 or 6 cm pasture sward heights in mid- and late-pregnancy (medians compared using Kruskal-Wallis test of medians).

	Litter size		Dam sward treatment	
	Twin	Triplet	2 cm[1]	6 cm[2]
(n)	28	28	33	23
Minimum distance from X (m)	2.0	2.0	2.0	2.0
Median distance from X (m)	4.0	5.0	5.0	4.0
Maximum distance from X (m)	12.0	11.0	12.0	11.0
Total distance travelled (m)	26.5	29.0	26.0	30.0
Proportion of time in zone 2	0.0	0.0	0.0	0.0
Proportion of time in zone 3	0.5	0.5	0.5	0.45
Proportion of time in zone 4	0.08	0.15	0.1	0.1
Proportion of time in zone 5	0.05	0.1	0.05	0.1

[1] Ewes offered a 2 cm sward height in mid-late pregnancy

[2] Ewes offered a 6 cm sward height in mid-late pregnancy

Of the test sheep, 43.6% urinated, 10.9% defecated, 38.2% sniffed group sheep and 85.5% bleated during the 5 minute observation period. Of the sheep that bleated 31.9% emitted only high pitched bleats, 8.5% only low pitched and 59.6% emitted both high and low pitched bleats. A greater proportion of sheep born to ewes offered 6 cm high swards bleated with a high pitch compared with those born to ewes offered 2 cm($P<0.05$). No other differences between dam nutrition or litter size were identified in sheep arena behaviours (Table 37).

Table 37. The number (and percentage) of twin- or triplet-born ewe progeny born to dams offered 2 or 6 cm sward heights between days 64 and 132 of pregnancy that urinated, defecated, bleated in a high or low pitch or sniffed group sheep in the arena test and the frequency of each of these behaviours

	Litter size		Dam sward treatment	
	Twin	Triplet	2 cm	6 cm
(n)	28	28	33	23
Urination [1]	10 (37.0)	14 (50.0)	14 (43.8)	10 (43.5)
Defecation [1]	4 (14.8)	2 (7.1)	4 (12.5)	2 (8.7)
High bleats [1]	22 (81.5)	21 (75.0)	22[a] (68.8)	21[b] (91.3)
Number of high bleats [2]	2.0	6.0	3.5	5.0
Low bleats [1]	17 (63.0)	15 (53.6)	19 (59.4)	13 (56.5)
Number of low bleats [2]	1.0	1.0	1.0	1.0
Bleated [1]	25 (92.6)	22 (78.6)	26 (81.3)	21 (91.3)
Total number of bleats [2]	4.0	7.5	6.0	6.0
Sniffed group sheep [1]	11 (40.7)	10 (35.7)	14 (43.8)	7 (30.4)
Number of sniffs [2]	0	0	0	0

Frequencies or medians within treatments with different superscripts are significantly different (P<0.05).

[1] Chi^2 test showing number (percentage)

[2] Kruskal-Wallis test showing median

Maternal behaviour scores of 2-year-old primiparous ewes ranged from 1 to 4 with no ewes classified as 5. Most ewes were classified as a 2 (34.8%) or 3 (34.8%) with fewer classified as 1 (19.6%) and 4 (10.9%). The mean MBS for twin-born ewes was significantly (P<0.05) greater than for triplet-born ewes. However, no significant differences were seen in the frequency of ewes within each of the maternal behaviour scores between dam nutrition and litter sizes (Table 38). MBS was significantly correlated with urination (r = -0.36, P=0.017) but with no other variables measured in the arena test.

Table 38. The number (and percentage) of ewes in each maternal behaviour category at 6-12 hours after parturition for twin- and triplet-born ewe progeny born to dams offered 2 or 6 cm sward height from mid-pregnancy (least squares mean ± standard error)

		Maternal behaviour score					
Litter size	(n)	1	2	3	4	5	Mean±S.E.
Twin	22	2 (9.9)	7 (31.8)	10 (45.5)	3 (13.6)	0 (0)	$2.6^{b} \pm 0.20$
Triplet	26	7 (29.2)	9 (37.5)	6 (25.0)	2 (8.3)	0 (0)	$2.1^{a} \pm 0.20$
Dam sward treatment							
2 cm	25	4 (16.0)	9 (36.0)	10 (40.0)	2 (8.0)	0 (0)	2.4 ± 0.19
6 cm	21	5 (23.8)	7 (33.3)	6 (28.6)	3 (14.3)	0 (0)	2.3 ± 0.21

Means within treatments with different superscripts are significantly different (P<0.05)

Discussion

This study was conducted to investigate the long-term effects of maternal nutrition on ewe progeny behaviour at 1 and 2 years of age using an arena test and maternal behaviour score. Behaviour of ewes in the arena test has previously been correlated with maternal behaviour (Everett-Hincks, 2003). The behaviour of the ewe progeny in the arena test and after parturition can be used as an indicator of mothering ability and success in rearing lambs to weaning.

In this study the proportion of ewes that bleated with a high pitch during the arena test was greater for those ewes whose dam was offered a herbage height of 6 cm compared with those offered 2 cm. The high pitched bleat is considered to be a protest bleat (Dwyer et al., 1998). Therefore progeny born to well-fed ewes appear to be less at ease in the arena test at 1 year of age. This is not in agreement with the findings of Erhard et al. (2004) who concluded that undernutrition during pregnancy increased emotional reactivity of the progeny at 18 months of age. Erhard et al. (2004) examined the effect of underfeeding for the first 95 days of pregnancy and the provision of adequate feed for the remainder of pregnancy. They found that although lamb birth weights were unaffected by ewe nutrition the behaviour of the offspring was altered and concluded that there may be abnormal development of the HPA axis. Lesage et al. (2006) observed that an early insult was likely to have its most severe impact during the period of rapid brain ‘growth’ therefore undernutrition during early pregnancy may have a greater impact on long-term behaviour of ewe lambs than in mid- to late-pregnancy.

As primiparous 2 year-old ewes the mean maternal behaviour score was significantly greater in twin than triplet-born ewes although no differences were observed between twin and triplet-born ewes during the arena test. MBS results may be a result not only of the offspring but also of their dam for example the litter size of the dam may influence the maternal behaviour of offspring. In this study the litter size of the dam was not known and therefore could not be incorporated in the study design. O’Connor et al. (1985) found that with each unit increase in maternal behaviour score there was a 6% increase in lamb survival. This suggests that the survival of lambs born to twin-born ewes may be greater than of lambs born to triplet-born ewes.

Conclusion

Ewe nutrition between day 64 and 132 of pregnancy resulted in very minor differences in the behaviour of ewe offspring in the arena test and had no effect on MBS at their first lambing. However, twin-born ewes had a greater average MBS than triplet-born ewes. Therefore the effects of feeding levels during pregnancy seem to be less important than litter size on the long-term effects on ewe behaviour.

Further research

In addition to the examination of behaviour of the female offspring the metabolism and reproductive success of these ewes was also examined in Chapter 7a) and b).

CHAPTER 7

A) Does ewe nutrition during pregnancy affect the metabolism of twin-born lambs?

B) The effect of maternal nutrition during gestation on the reproductive success of female progeny as 2-tooths.

Related publications:

Corner R.A., Kenyon P.R., Stafford K.J., West D.M., Oliver M.H., Morris S.T., Blair H.T. Does nutrition during pregnancy affect the metabolism of twin-born lambs? Proceedings of the New Zealand Society of Animal Production, Lincoln University, New Zealand, New Zealand Society of Animal Production 2005. 65, 163-167.

Corner R.A., Kenyon P.R., Stafford K.J., West D.M., Morris S.T. The effect of maternal nutrition during gestation on the reproductive success of female progeny as 2-tooths. Proceedings of the New Zealand Society of Animal Production 2006. 66, 434-439.

A) Does ewe nutrition during pregnancy affect the metabolism of twin-born lambs?

Abstract

An insulin tolerance test was conducted on 8-month old twin-born ewe lambs whose dams were offered sward heights of either 2 or 6 cm between day 64 and 132 of pregnancy in order to identify any alterations in the metabolism of the lamb. Changes in metabolism may have long term effects on growth and productivity. An insulin challenge (0.2 U/kg) was administered to female lambs at 8 months of age and their plasma insulin, glucose and cortisol responses were monitored. The plasma cortisol concentration of ewe lambs born to ewes offered 2 cm sward height was significantly ($P<0.05$) higher than ewe lambs born to ewes offered 6 cm sward height at 50 minutes after the insulin challenge. No differences were found in the insulin or glucose responses to the insulin challenge between dam sward height groups (2 vs. 6 cm). Birth weight had no effect on the insulin, glucose or cortisol response to the insulin challenge. These results show that both dam nutrition and lamb birth weight had little to no effect on the metabolic responses measured. These results suggest that lambs born to ewes offered a herbage sward height of only 2 cm or lambs with low birth weights may not show altered metabolism.

Introduction

Currently in New Zealand there is a trend towards greater lambing percentages through an increase in the number of multiple births (Geenty, 1997). It is possible that twin and triplet lambs may be more susceptible to nutritional restriction during gestation than singletons (Geenty, 1997).

Maternal undernutrition and impaired fetal growth have been linked with postnatal glucose intolerance, insulin resistance, hyperinsulinemia and increased blood pressure in humans and sheep (Oliver et al., 2002; Kind et al., 2003). To date the effect of ewe nutrition during pregnancy on the metabolism of twin-born lambs has not been examined in a pasture based farming system. It was hypothesised that maternal undernutrition between day 64 and 132 of pregnancy would result in altered insulin sensitivity of offspring.

The insulin tolerance test (ITT) has been used to examine differences in glucose homeostasis of singleton (Oliver et al., 2002) and twin lambs (Clarke et al., 2000). At 30 months of age, singleton lambs born to ewes underfed for 20 days in late pregnancy displayed higher plasma insulin concentrations than lambs born to ewes underfed for 10 days during the same period (Oliver et al., 2002). Clarke et al. (2000) reported that low birth weight twin lambs (4.1 ± 0.3 kg) had a lower area under the insulin tolerance curve in response to a glucose challenge than high birthweight lambs (5.1 ± 0.1 kg) indicating enhanced insulin tolerance at 1 year of age. Oliver et al. (2002) concluded that birth weight is more important than dam nutrition during pregnancy on postnatal glucose tolerance. In that study differences observed at 5 months of age were not present at 30 months. These studies suggest that both dam nutrition during pregnancy and birth size can have long term effects on the metabolism of the offspring.

This study was designed to examine the effects of maternal nutrition and lamb birth weight on ewe offspring glucose and cortisol response to an ITT. The ewe offspring involved in this study were born to twin- and triplet-bearing ewes offered an average sward height of either 2 or 6 cm between day 64 and 132 of pregnancy (Morris and Kenyon, 2004). In that study lambs to ewes offered 2 cm sward heights were 0.3 kg lighter at birth than those born to ewes offered 6 cm. In addition, the change in dam live weight was 7.3 kg vs. 18.7 kg from day 64 to day 132 of pregnancy for ewes offered either 2 or 6 cm sward heights respectively. These results suggest that these multiple bearing ewes offered sward heights of 2 cm were undernourished during the mid- to late-pregnancy period in comparison to those offered 6 cm.

Methods and materials

This experiment was conducted at Massey University's Keeble farm, 5km south of Palmerston North, New Zealand. An ITT was conducted to determine the effect of herbage sward height on some aspects of metabolism of twin-born ewe lambs during puberty (8 months of age). In addition the effect of birth weight irrespective of dam nutrition was examined. Lambs weighing less than 4 kg at birth were classified as "light" or those greater than 4 kg as "heavy".

The day before the ITT each ewe lamb was weighed (range 48 to 57.5 kg) and an indwelling catheter was inserted into the jugular vein (Oliver et al., 2002). The ewe lambs were fasted overnight and at 0900 h were given an intravenous injection of 8 U insulin (≈0.2 U/kg Humulin R; Eli Lilly, Indianapolis, IN, USA). Heparinised blood samples were collected at 0, 10, 20, 30, 40, 50, 60 and 120 minutes after i.v. insulin injection. Blood samples were placed on ice and once chilled centrifuged for 10 minutes at 4000 rpm. The resulting plasma was harvested and frozen at -20 °C for analysis.

Samples were analysed for insulin, glucose and cortisol. Plasma glucose concentration was analysed using standard enzymatic micro-methods modified for 96-well micro-plates (Kunst et al., 1984; Ashour et al., 1987). Plasma insulin concentration was measured by radioimmunoassay (RIA) (Oliver et al., 1993). Endogenous and exogenous insulin could not be distinguished therefore total insulin concentrations are reported.

Plasma cortisol concentration was measured by RIA using diagnostic kits (Clinical Assays GammaCoat Cortisol 125I RIA Kit, DiaSorin Inc., Minnesota, USA).

Statistical analysis

Plasma concentrations of insulin, glucose and cortisol were not normally distributed. The natural $\log_e$ of insulin and glucose and the square root of cortisol were calculated. The effect of dam's sward height (2 cm vs. 6 cm) and the birth weight group (light vs. heavy) were tested using the analysis of variance for repeated measures (SPSS, 2003). Individual time points were tested using generalised linear model (GLM, (Minitab, 2003). The relationship of the birth weight and current weight of the ewe lambs to their plasma insulin, glucose and insulin response to the insulin challenge was examined using linear regression procedure (PROC REG) in SAS (SAS, 2005).

Results

The insulin challenge produced an increase in plasma insulin concentrations at 10 minutes which then retuned to baseline levels by 20 minutes (Table 39). The insulin challenge failed to produce a significant difference in the plasma insulin concentrations between the progeny of ewes offered either 2 or 6 cm sward heights over the sampling period or between light and heavy birthweight lambs. The weight of the ewe lambs at birth and at the time of the challenge had no effect on the insulin response.

Table 39. The effect of dam sward surface height and progeny birthweight group (low or high) on plasma insulin concentrations (ug/ml) at 0, 10, 20, 30, 40, 50, 60 and 120 minutes after insulin challenge (least squares mean ± standard error).

	n	Time from insulin challenge (min)[1] 0	10	20	30	40	50	60	120
Dam sward height treatment									
2 cm	14	-2.4±0.2 (0.10)	3.6±0.4 (169.8)	1.8±0.1 (6.5)	0.8±0.2 (2.5)	-0.04±0.1 (1.0)	-0.7±0.2 (0.6)	-1.1±0.1 (0.4)	-2.2±0.1 (0.13)
6 cm	11	-2.4±0.2 (0.11)	3.3±0.4 (49.8)	1.8±0.2 (7.5)	1.0±0.2 (3.8)	-0.04±0.1 (1.0)	-0.5±0.2 (1.5)	-1.3±0.1 (0.3)	-2.2±0.2 (0.13)
Birthweight group									
Light	12	-2.6±0.2 (0.13)	3.5±0.4 (79.2)	1.8±0.2 (7.3)	0.9±0.2 (3.5)	-0.09±0.1 (1.0)	-0.7±0.2 (1.4)	-1.2±0.1 (0.3)	-2.1±0.2 (0.12)
Heavy	13	-2.3±0.2 (0.09)	3.4±0.4 (140.3)	1.8±0.1 (6.7)	0.9±0.2 (2.8)	0.01±0.1 (1.0)	-0.6±0.2 (0.7)	-1.2±0.1 (0.3)	-2.2±0.1 (0.14)

[1] Data were Log_e transformed, untransformed means presented in parentheses

Glucose concentrations decreased steadily after the insulin challenge for 50 minutes and began to rise from 60 to 120 minutes (Table 40). There were no significant differences in the plasma glucose concentrations at any time after the insulin challenge between the progeny of ewes offered either 2 or 6 cm sward heights or between light and heavy birthweight ewe lambs. The weight of the ewe lambs at birth and at the time of the challenge had no effect on the glucose response.

Table 40. The effect of dam sward surface height and progeny birthweight group (low or high) on the plasma glucose concentrations (mM) at 0, 10, 20, 30, 40, 50, 60 and 120 minutes after insulin challenge(least squares mean ± standard error).

		Time after insulin challenge [1] (min)							
	n	0	10	20	30	40	50	60	120
Dam sward height treatment									
2 cm	14	1.34±0.05 (3.8)	1.16±0.05 (3.2)	0.88±0.06 (2.4)	0.62±0.06 (1.9)	0.55±0.06 (1.8)	0.59±0.05 (1.8)	0.65±0.05 (1.9)	1.12±0.05 (3.1)
6 cm	11	1.39±0.05 (4.1)	1.24±0.06 (3.6)	0.97±0.07 (2.7)	0.77±0.07 (2.3)	0.68±0.06 (2.0)	0.67±0.06 (2.0)	0.73±0.06 (2.1)	1.06±0.06 (2.9)
Birthweight group									
Light	12	1.33±0.05 (3.8)	1.19±0.06 (3.3)	0.91±0.07 (2.5)	0.69±0.07 (2.0)	0.58±0.06 (1.8)	0.59±0.06 (1.8)	0.71±0.05 (2.0)	1.06±0.06 (2.9)
Heavy	13	1.39±0.05 (4.1)	1.21±0.05 (2.7)	0.94±0.06 (2.7)	0.71±0.07 (2.1)	0.65±0.06 (2.0)	0.67±0.06 (2.0)	0.66±0.05 (2.0)	1.12±0.05 (3.1)

[1] Data were Log_e transformed, non transformed means are presented in parentheses

After the insulin challenge, the plasma cortisol concentration decreased for the first 20 minutes and then increased until 60 minutes post challenge. It then returned to baseline levels at 120 minutes (Table 41). There was a significant interaction ($P<0.05$) of dam sward height group x lamb birth weight group the baseline cortisol concentration. Light birthweight lambs born to ewes offered 2 cm sward heights had a higher cortisol concentration than heavy ewe lambs. However this relationship was not observed in lambs born to ewes offered 6 cm sward heights. Lambs born to ewes fed 2 cm sward heights had a significantly ($P<0.05$) higher cortisol concentration at 50 minutes after the insulin challenge than the lambs born to ewes fed 6 cm sward heights. The cortisol response to the insulin challenge was influenced by the weight of the ewe lamb at the time of the test (cortisol = 258.8 – 2.2 (weight), $r^2 = 0.02$, $P<0.05$).

Table 41. The effect of dam sward height treatment and progeny birthweight group (low or high) on plasma cortisol concentrations (nmol/L) at 0, 10, 20, 30, 40, 50, 60 and 120 minutes after insulin challenge (least squares mean ± standard error).

		Time after insulin challenge [1] (min)							
	n	0	10	20	30	40	50	60	120
Dam sward height treatment									
2	14	11.9±0.4 (147.8)	10.5±0.7 (117.3)	8.7±0.5 (80.5)	10.1±0.5 (104.2)	14.3±0.4 (205.8)	15.9b±0. 4 (252.4)	16.4±0.5 (268.4)	10.9±0.7 (123.6)
6	11	11.7±0.5 (143.8)	10.1±0.8 (106.4)	8.2±0.6 (76.5)	9.9±0. 6 (101.6)	13.4±0.4 (181.1)	14.6a±0. 4 (214.4)	15.3±0.5 (239.2)	10.2±0.8 (110.5)
Birthweight group									
Light	12	12.3±0.5 (160.7)	10.8±0.8 (121.8)	8. 5±0.6 (80.8)	9.5±0.5 (93.7)	13.5±0.4 (183.0)	15.2±0.4 (232.8)	15.9±0.5 (257.4)	10.8±0.8 (123.4)
Heavy	13	11.3±0.4 (130.9)	9.8±0.7 (102.0)	8.5±0.5 (76.3)	10.5±0.5 (112.0)	14.2±0.4 (203.9)	15.3±0.4 (234.0)	15.8±0.5 (250.3)	10.3±0.7 (110.7)
Dam sward height treatment x Birthweight group									
2 x Light	8	13.2^{b}±0.6 (177.4)							
2 x Heavy	6	10.6^{a}±0.6 (113.3)							
6 x Light	4	11.3^{ab}±0.8 (129.3)							
6 x Heavy	7	12.0^{ab}±0.6 (145.7)							

Means within treatments with different superscripts are significantly different (P<0.05)

[1] Data were $X^{0.5}$ transformed, non transformed data are given in parentheses

Discussion

This study was conducted to investigate the effects of dam nutrition between day 64 and 132 of pregnancy on the metabolic response of twin-born ewe lambs to an ITT. Maternal undernutrition during pregnancy has been linked to impaired fetal growth and postnatal metabolism (Oliver et al., 2002). The ITT provides information on the ewe lambs sensitivity to insulin in terms of the degree and rate of glucose depletion and stimulation of the hypothalamic-pituitary-adrenal axis. Therefore an ITT will provide some information on the potential long-term effects of maternal undernutrition on lamb productivity.

In general, dam nutritional treatment had no effect on the metabolic response of the ewe lambs at 8 months of age. However, the plasma cortisol concentration of ewe lambs born to dams offered 2 cm sward heights were greater at 50 minutes after the insulin challenge than lambs born to ewes offered 6 cm. This suggests that dam nutrition in mid- to late-pregnancy has little effect on cortisol production in the resulting progeny.

Birth weight (light vs. heavy) had no effect on the insulin, glucose and cortisol response to an ITT. This finding is contrary to Clarke et al. (2000) who found a significant difference in insulin tolerance between light (4.1 ± 0.3 kg) and heavy lambs (5.1 ± 0.1 kg) at one and 6 months of age. However, Clarke et al. (2000) compared the birth weights within sets of twins whose birth weights differed by more than 25%. In this study, the birth weights of unrelated twin lambs classified as light (3.3 ± 0.1 kg) and heavy (4.8 ± 0.1 kg) differed by 69%. The mean weights of both the light and heavy groups were lower than those reported by Clarke et al. (2000). In addition, Clarke et al. (2000) did not have a ewe nutritional treatment thereby examining the effect of in utero nutrition on postnatal metabolism.

There was no difference in plasma insulin concentration at any time after insulin challenge between dam nutrition or birthweight groups. In contrast, Oliver et al. (2002) found that at 5 months of age, plasma insulin concentrations were higher in lambs born to dams severely underfed for 20 days in late pregnancy. They also found that the area under the glucose and insulin response curves was related to the lamb's weight at the time of challenge. However Oliver et al. (2002) employed severe underfeeding (underfed = 0.3 MJ/day vs. *ad libitum* = 15 MJ/day) for a short period while in this trial a longer period (70 days) of moderate underfeeding was utilised.

Conclusion

These data suggest that maternal undernutrition in mid- to late-pregnancy and birth weight of twin-born ewes may only have a minor affect on their offspring's subsequent long-term metabolism. In biological terms these differences are small and are probably of no consequence.

B) The effect of maternal nutrition during gestation on the reproductive success of female progeny as 2-tooths.

Abstract

The inter-generational effect of litter size and nutrition during pregnancy was assessed by examining the reproductive success of female progeny at their first breeding as 2-tooths (1.5 years of age). Fifty-six 2-tooth ewes born as a twin or triplet and whose dam was maintained on 2 or 6 cm sward height between day 64 and 132 of pregnancy were synchronised and bred to Romney rams. Pregnancy diagnosis was conducted using ultrasound on day 71 from the start of the breeding period (P71). All ewes were maintained as one group under commercial farming conditions throughout pregnancy. Within 12 hours of birth lambs were ear tagged, weighed and body dimensions (CRL, girth, forelimb and rear limb lengths) were recorded. Lamb live weights were also recorded at day 48 of life (L48). Fifty-one of the 56 ewes that were mated, lambed. Twin-born ewes had longer ($P<0.05$) gestation lengths (by 1.3 days) than triplet-born ewes. Singleton lambs born to triplet-born ewes were ($P<0.05$) lighter (4.6 kg) at birth than singleton lambs born to twin-born ewes (5.1 kg). Singleton lambs whose grand-dams were maintained on 2 cm pastures during pregnancy and whose dam was born as a twin had significantly ($P<0.05$) longer rear limbs than their counterparts whose grand dam was maintained on 6 cm and whose dam was born a triplet but they did not differ in birth weight. Dam litter size and the nutritional treatment of their grand-dam had no significant effect on twin-lamb liveweight and body dimensions at birth. These results suggest that selecting triplet ewe lambs for breeding could have detrimental effects on flock productivity. In addition, a previous study involving the same animals reported triplet born ewes displayed poorer maternal behaviour. To the authors knowledge this is

the first finding of this kind and could have implications for ewe selection on New Zealand farms.

Introduction

The consequences of maternal undernutrition on the first generation of progeny has received a great deal of attention (Thomson and Thomson, 1949; Everitt, 1964; Mellor and Murray, 1981; Holst et al., 1986; Moore et al., 1986; Scales et al., 1986; Gunn et al., 1995; Heasman et al., 1998; Morris and Kenyon, 2004). However, relatively little is known about the effects of maternal nutrition during pregnancy and litter size on the following generations. Undernutrition during pregnancy generally results in lighter lambs (Mellor and Murray, 1982; Holst et al., 1992; Morris and Kenyon, 2004) and can also alter the body dimensions of the lamb by increasing the crown-rump length (CRL) (Heasman et al., 1999) and reducing girth size (Holst et al., 1992; Morris and Kenyon, 2004) or both (Mellor and Murray, 1982). Reduced weight at birth can reduce lamb survival (Dalton et al., 1980; Scales et al., 1986).

Little is known about the effects of maternal nutrition on the body size and birth weight of the second generation in sheep. In rats a low protein diet before breeding and during pregnancy resulted in reduced birth weight, litter size and increased pup mortality (McLeod et al., 1972). In addition the female progeny displayed reduced fertility and produced litters with reduced growth rates. Gunn et al. (1995) found that the female progeny of ewes on a high level of nutrition for the last 100 days of pregnancy or first 100 days of lactation displayed greater embryo and fetal losses at 3 and 4 years of age. In chapter 6, triplet born ewes had poorer maternal behaviour than twin-born ewes. In rats a low protein diet prior to and during pregnancy resulted in pups with retarded development and intelligence which persisted in the next generation (Cowley and

Griesel, 1966). These combined results suggest that there is potential for pregnancy nutrition and ewe litter size to have long-term effects on flock performance.

The present study was designed to determine if lambs born to twin or triplet born 2-tooth ewes whose dams were maintained on 2 or 6 cm sward heights differed in live weight and body size at birth.

Materials and Methods

Fifty-six primiparous twin- and triplet-born ewes born to dam's maintained on either 2 or 6 cm sward heights from day 64 of pregnancy to parturition (Morris and Kenyon, 2004) were bred as 2-tooths. Ewes were oestrus synchronised and bred to Romney rams. Breeding dates were determined from daily ram crayon marks (10 Apr 04 to 12 Apr 04). Pregnancy diagnosis was conducted using ultrasound on day 71 from the start of the breeding period (P71). All ewes were maintained as one group under commercial farming conditions throughout pregnancy. Ewe live weights were recorded twelve days prior to breeding (P-12) and on days 107 and 143 of pregnancy.

During the lambing period ewes were observed three times daily by an experienced shepherd to identify newly born lambs and provide assistance to ewes lambing if required. Within 12 hours of birth lambs were ear tagged and their dam was identified. The lambs' live weight and body dimensions (CRL, girth, forelimb and rear limb lengths) were recorded. Forelimb length was the distance from the shoulder joint to the tip of the hoof on the left leg and rear limb length was the distance from the hip joint to the tip of the hoof on the left leg.

Statistical analyses

Data were analysed using the Proc Mixed procedure in SAS version 8.02 (SAS, 2005) with the fixed effects of ewe litter size (twin vs. triplet), grand-dam sward height treatments (2 cm vs. 6 cm) and lamb litter size (singleton vs. twin) and the linear covariate effects of gestation length and lambing date deviation from the start of lambing. The relationship between gestation length and lamb birth weight was determined using the Proc GLM procedure in SAS adjusting for the fixed effect of litter size. The models of lamb body dimensions were run with and without the inclusion of birth weight as a covariate. Tables present least squares means ± standard error.

Results

The liveweight of ewes mated in this study did not differ between their litter size or dam's nutritional treatment at breeding and throughout pregnancy (Table 42). Of the 56 ewes that were mated, 50 ewes lambed producing 76 lambs. Lambing percentages were similar between ewe litter sizes (twin = 144% and triplet=159%) and dam nutrition treatments (2 cm=154% and 6 cm=150%).

Table 42. Ewe liveweight (kg) prior to breeding and throughout pregnancy of twin- and triplet-bearing ewes born to dams offered pasture with an average sward height of 2 cm or 6 cm between day 64 and 132 of pregnancy (least squares mean ± standard error)

		Ewe live weights (kg)		
	n	P-14	P107	P143
Ewe litter size				
Twin	28	52.2±1.1	56.7±1.1	59.1±1.2
Triplet	31	50.9±0.9	55.1±1.0	57.2±1.1
Maternal dam sward height treatment				
2 cm	35	51.2±0.9	55.1±0.9	57.7±1.0
6 cm	24	51.9±1.1	56.6±1.1	58.7±1.3
Ewe pregnancy status				
Singleton	24	50.8±1.1	54.1±1.1	56.7±1.3
Twin	29	52.4±1.0	57.5±1.0	58.7±1.2
Ewe litter size x maternal dam sward height treatment				
Twin x 2 cm	17	50.6^{a}±1.3	54.7^{a}±1.3	
Twin x 6 cm	11	54.8^{b}±1.6	58.9^{b}±1.6	
Triplet x 2 cm	18	51.4^{ab}±1.3	55.7^{ab}±1.5	
Triplet x 6 cm	13	49.8^{a}±1.5	53.8^{a}±1.6	

Means within columns with different superscripts are significantly different (P<0.05)

Gestation lengths were significantly longer for twin-born (146.4 days) than triplet-born (145.2 days) 2-tooth ewes (Table 43). There was no variation in the gestation length of ewes bearing singleton or twin fetuses or between grand-dam sward height treatments.

Table 43. The gestation length of single- and twin-bearing ewes that were born as part of a twin or triplet litter and whose dam was offered pasture with an average sward height of 2 or 6 cm in mid- and late-pregnancy (least squares mean ± standard error).

	n	Gestation length (days)
Lamb litter size		
Singleton	25	145.7±0.5
Twin	26	145.8±0.4
Ewe litter size		
Twin	25	146.4[b]±0.4
Triplet	26	145.2[a]±0.4
Maternal grand-dam's sward height treatment		
2 cm	28	145.9±0.4
6 cm	23	145.6±0.5

Means within columns with different superscripts are significantly different (P<0.05)

Singleton lambs born to triplet-born ewes were lighter (by 0.5 kg; P<0.05) at birth than their counterparts born to twin-born ewes (Table 44). Lamb birth weight and ewe gestation length were related such that for every 1 day increase in gestation length (GL) greater than 142 days there was an average increase in birth weight of 0.148 kg ($y = 3.3 + 0.148$ GL).

Singleton lambs were significantly heavier and larger in all measures of body dimension than their twin-counterparts (Table 44). Hind limb length had a significant (P=0.05) interaction of lamb litter size, ewe litter size and maternal grand-dam sward height treatment. Therefore, singleton lambs born to twin- and triplet-born ewes whose maternal grand-dam was maintained on 6 cm pastures during pregnancy had longer (P<0.05) rear limbs than singletons born to triplet-born ewes whose maternal grand-dams were maintained on 2 cm sward heights. When birth weight was included in the model of body dimensions differences in hindlimb length were no longer significant

(P>0.05). Relative to birth weight the forelimb length of singletons was smaller than twins. In addition, lambs of ewes that were born a twin had longer forelimbs relative to their birth weight than lambs of ewes born a triplet. Ewe litter size and the grand-dam sward height treatment had no significant effect on the live weight and body dimensions of twin lambs.

Table 44. The birth weight (kg), crown rump length (CRL), girth, forelimb (FL) and hind limb (HL) lengths (cm) of singleton and twin lambs born to ewes that were born a twin or triplet and whose maternal grand-dam was offered pastures with an average sward height of 2 or 6 cm in mid- and late-pregnancy (least squares mean ± standard error).

		Birth	Lamb dimensions (cm)			
	n	Weight (kg)	CRL	Girth	FL	HL
Lamb litter size (LBR)						
Single	25	4.7^{b}±0.1	52.3^{b}±0.8	36.3^{b}±0.6	28.5^{b}±0.4	34.7^{b}±0.5
Twin	27	3.6^{a}±0.1	48.0^{a}±0.5	33.1^{a}±0.39	27.5^{a}±0.3	32.4^{a}±0.3
Ewe litter size (EBR)						
Twin	25	4.3±0.1	50.6±0.6	34.5±0.5	28.7±0.4	34.1±0.4
Triplet	26	4.1±0.1	49.7±0.7	34.9±0.5	27.3±0.4	33.0±0.4
Maternal grand-dam sward height treatment (Sward height treatment)						
2 cm	28	4.1±0.1	49.7±0.6	34.6±0.4	27.9±0.4	32.9±0.4
6 cm	23	4.3±0.1	50.5±0.7	34.9±0.5	28.1±0.4	34.2±0.4
Lamb litter size x Ewe litter size						
Singleton x Twin	14	5.1^{c}±0.2				
Singleton x Triplet	10	4.6^{b}±0.2				
Twin x Twin	11	3.6^{a}±0.2				
Twin x Triplet	16	3.6^{a}±0.1				
LBR x EBR x Sward height treatment						
Singleton x Twin x 2 cm	6					34.9^{bc}±0.9
Singleton x Twin x 6 cm	8					35.7^{b}±1.0
Singleton x Triplet x 2 cm	5					32.3^{ac}±1.1
Singleton x Triplet x 6 cm	5					35.9^{b}±1.1
Twin x Twin x 2 cm	6					32.2^{a}±0.7
Twin x Twin x 6 cm	5					33.5^{abc}±0.8
Twin x Triplet x 2 cm	9					32.3^{a}±0.6
Twin x Triplet x 6 cm	7					31.7^{a}±0.7

Means within columns with different superscripts are significantly different ($P<0.05$)

Discussion

The ewes' litter size at 2 years of age had a significant effect on the gestation length and birth weight of singleton lambs. Triplet born ewes had significantly shorter gestation lengths and gave birth to lighter singleton lambs that their twin-born counterparts. The relationship of gestation length to litter size was such that for every 1 day increase in gestation length there was in increase of 0.148 kg in lamb birth weight which is similar to the late gestation fetal growth rates described by Rattray et al. (1974). The increase in gestation length of twin-born ewes in comparison to triplet-born ewes would therefore account for less than half of the increase in the birth weight of singleton lambs observed in this study.

Lamb survival is greatest in lambs that weigh 3.5 - 5.5 kg (Dalton et al., 1980) therefore, triplet born ewes are at a greater risk of giving birth to lambs that weigh less than 3.5 kg. Small lambs have higher mortality resulting from starvation-exposure due to increased heat loss resulting from a greater surface area to body weight ratio (McDonald, 1962) and low energy reserves with which to maintain homeothermy (Robinson, 1981).

The behaviour of these ewes was also influenced by their litter size (Chapter 6) such that triplet-born ewes had significantly poorer maternal behaviour than twin-born ewes. Poor maternal behaviour within the first day after parturition is a major cause of lamb mortality (Alexander, 1984). Therefore poor maternal behaviour of triplet born ewes may result in low lamb survival rates.

There was no significant effect of the maternal grand-dam's sward height treatment on their daughters lambing percentage and their lambs birth weight and body dimensions. The number of the ewe offspring available in this study was limited and therefore the power in this study may have been insufficient to detect differences between grand-dam sward height treatments. In addition, the effect of ewe and lamb litter size further reduced the likelihood of observing differences, if present.

The rear limb length of singleton lambs was the only variable significantly affected by grand dam nutrition. These findings are in agreement with a study on wild house mice (Mus musculus) which found no difference in the birth or weaning weight of offspring born to daughters of underfed females compared to daughters of control females (Meikle and Westberg, 2001).

The underlying mechanism for the differences observed between twin- and triplet-born ewes is unclear. The effect of the weight of the ewe has been eliminated as twin- and triplet-born ewes has similar live weights prior to breeding and during pregnancy. This finding is in agreement with Gunn et al. (1995) who also reported no difference in the live weight of offspring at the time of breeding. The underlying mechanism by which nutrition can alter reproduction of offspring may influence cellular processes within the ovary, neuroendocrine regulation of reproduction and neural stimulation of the reproductive axis {Martin, 2004 #548). At present there is little information regarding the effect of maternal nutrition or fetal number on these systems therefore additional work is required to determine the factors at work in this study.

Conclusions

These results suggest that selecting triplet ewe lambs as replacements could have detrimental effects on their ability to successfully rear a lamb as a 2-tooth. To the author's knowledge this is the first finding of this kind and could have implications for selection on New Zealand farms.

Further research

The previous studies provided an opportunity to examine the effects of maternal stressors in mid-pregnancy on the HPA axis of lambs. Chapter 8 contains 4 studies that examined the effects of ewe treatment on the plasma cortisol response of ram lambs to handling or castration.

CHAPTER 8

The effect of maternal stressors in mid- and late-pregnancy on basal and stimulated cortisol concentrations of lambs between 2 and 7 weeks of age

Abstract

A series of studies were conducted to examine the effects of mid-pregnancy shearing and ewe nutrition in mid- and late-pregnancy on lamb birth weight. These studies provided an opportunity to examine the effect of maternal stressors on the plasma cortisol response of lambs to a painful stressor. Exposure of ewes to stressors and artificial elevation of maternal glucocorticoid concentrations can result in altered development of the fetal HPA axis. The objective of these studies was to determine if exposing ewes to a stressor in mid-pregnancy or altering ewe nutrition in mid- and late-pregnancy affects the plasma cortisol response of their male offspring to handling or castration. Four studies were conducted with either singleton and twin (studies 1 and 2) or twin and triplet lambs (studies 3 and 4). In study 1, lambs were born to ewes that were yarded (n=40), shorn (n=40) or remained unshorn (n=40) on day 76 of pregnancy. The lambs in study 2 were born to ewes that were shorn (n=18) or not shorn (n=15) on day 79 of pregnancy. In study 3 the lambs were born to ewes offered pastures with a sward height of 2 or 6 cm (n=11 and 24, respectively) from day 64 of pregnancy until parturition. In study 4, lambs were born to ewes that were offered pastures with a sward height of 2 or 4 cm (n=27 and 26, respectively) from day 70 of pregnancy until parturition. The average age of lambs in the study 1, 2, 3 and 4 was 14, 40, 37 and 50 days, respectively. In all 4 studies, lambs were randomly allocated to a handling or castration treatment. Lambs in the handling treatment had their scrotum palpated and were restrained for blood sampling. Castration involved the application of a rubber ring to the scrotum. The plasma cortisol concentration for each lamb was determined from blood samples collected immediately prior to treatment (T=0) and 20, 40, 60, 90, 120, 150, 180, 210 and 240 minutes after treatment. The plasma cortisol concentrations of castrated lambs were greater than that of handled lambs in all studies ($P<0.05$). Ewe

treatment had no effect on the basal plasma cortisol concentrations of lambs in all 4 studies. In study 1, the basal cortisol concentrations of singleton lambs was lower than twins (P<0.05). In studies 2, 3 and 4 there was no difference in the plasma cortisol response to castration or handling between ewe treatments and lamb litter sizes. In study 1, handled lambs born to shorn ewes had lower maximum plasma cortisol concentrations than lambs born to control and yarded ewes (P<0.05). In addition, in response to castration, there was an interaction of ewe treatment and lamb litter size. Singleton lambs born to shorn ewes had a greater cortisol response than singleton lambs born to control ewes, but in twin lambs the opposite was found. These findings suggest that the stressors in mid-pregnancy had little effect on the function of the HPA axis of the lamb. However, lamb litter size appears to have an effect on postnatal basal and stimulated plasma cortisol concentrations.

Introduction

Sheep are subjected to a myriad of stressors during their lifetime. Pregnant ewes in extensive production systems may experience undernutrition (Mellor, 1983) and may be shorn (Chapter 2, 3 and 4). The effect of ewe nutrition and shearing in mid-pregnancy on lamb birth weight and growth has been documented. However, little is known of the effects of these stressors on the HPA axis of lambs.

In the pregnant ewe, an increase in plasma cortisol concentrations can result in elevated fetal cortisol concentrations (Zarrow et al., 1970; Barbazanges et al., 1996). Jensen et al. (2002) reported that maternal infusion of cortisol in late-pregnancy produced an increase in fetal cortisol concentrations. It has been hypothesised that the elevation of fetal cortisol concentrations alters the development of the fetal HPA axis (Challis et al., 2000a; Symonds et al., 2001). Stressors during pregnancy in sheep, including undernutrition (Hawkins et al., 2000; Bloomfield et al., 2003a) and social isolation (Roussel et al., 2004) have been reported to alter the activity of HPA axis of offspring, however, these effects have been inconsistent (Table 45). In early pregnancy (14 days before mating to day 30 of pregnancy) undernutrition (0.85 or 0.5 of maintenance requirements) resulted in elevated cortisol and ACTH concentrations in lambs at approximately 2 months of age. In older lambs, however, no differences were observed. Undernutrition for 10, but not 20 days from day 105 of pregnancy also resulted in an elevated ACTH concentrations in 30 month old offspring (Bloomfield et al., 2003a). In contrast, the injection of cortisol or dexamethasone in between day 22 and 29 of pregnancy had no effect on offspring but in late-pregnancy basal ACTH concentrations were elevated in offspring between the ages of 1 and 3 years. It was hypothesised that the exposure of ewes to stressors in mid- and late-pregnancy would result in elevated basal cortisol concentrations and an elevated cortisol response of male offspring to a stressor.

The activity of the HPA axis of lambs has been examined in a number of ways. The most common method is a hormonal challenge (ACTH, or CRH plus AVP). An alternative approach to examine the function of the HPA axis of lambs is to use painful stimuli. Castration produces a highly repeatable increase in plasma cortisol concentrations above basal levels for 2-3 hrs (Mellor and Stafford, 2000).

The aim of these studies was to determine the effect of mid-pregnancy shearing and ewe nutrition in mid-and late-pregnancy and lamb litter size on the basal cortisol concentrations and cortisol response to castration of offspring between 2 and 7 weeks of age.

Methods and materials

A series of 4 experiments were conducted to examine the effect of mid-pregnancy shearing and nutrition in mid- and late-pregnancy on the cortisol response of lambs to a stressor. The plasma cortisol response of lambs to handling or castration was examined between 2 and 7 weeks of age.

Study 1 – Mid-pregnancy stressors

The plasma cortisol response of singleton (n=60) and twin (n=60) ram lambs born to ewes in the yarded, shorn or unshorn treatments described in chapter 2 was examined on days 13 to 17 after the mid-point of the parturition period. On days 75 and 76 of pregnancy, ewes were moved through undercover yards for 30 minutes on each day thereby exposing ewes to people, dogs and loud noises. On day 76 of pregnancy all remaining ewes were either shorn or remained as unshorn controls. Lambs were randomly assigned to the castration or handling treatments and had serial blood samples collected from the jugular vein in order to determine their plasma cortisol response to treatment.

Study 2 – Mid-pregnancy shearing

The plasma cortisol response of singleton (n=19) and twin (n=38) ram lambs was examined on day 39 after the mid-point of the parturition period. Lambs were born to ewes that were shorn on day 79 of pregnancy or that remained unshorn as controls (Chapter 3). Lambs were randomly allocated to the handling or castration treatment and serial blood samples were collected from the jugular vein to determine their plasma cortisol responses.

Table 45. Summary of the effects of ewe treatment (maternal undernutrition, glucocorticoid doses and isolation) during pregnancy on postnatal HPA axis function of offspring

Maternal treatment [1]	Day of pregnancy [2]	Litter size [3]	Offspring age [4]	HPA axis test [45]	Result [6]	Hormone reported [7]	Reference
Undernutrition (0.85M + 1 M)	-14 to 70	1 + 2	85 days	CRH+AVP	UN > C	Cortisol + ACTH	(Hawkins et al., 2000)
Undernutrition (0.5 M + 1 M)	0-30	2	2 mth	CRH	UN > C	ACTH and cortisol AUC	(Chadio et al., 2007)
			5.5 mth	CRH	No effect	ACTH and cortisol AUC	
			10 mth	CRH	No effect	ACTH and cortisol AUC	
Undernutrition (0.5 M + 1 M)	1-30	1+2	4 m	Basal conc.	No effect	Basal cortisol	(Gardner et al., 2006)
			1 yr	CRH+AVP and ACTH	UN > C	(females only), basal cortisol and stimulated cortisol and ACTH	
Undernutrition (0.3 + 13 MJ/day)	105-115	1	30 mth	CRH+AVP and Insulin	UN > C	ACTH response	(Bloomfield et al., 2003a)
					UN > C	ACTH response	
Undernutrition (0.3 + 13 MJ/day)	105-125	1	30 mth	CRH+AVP and Insulin	No effect	ACTH and cortisol	(Bloomfield et al., 2003a)
					No effect	ACTH and cortisol	

Maternal treatment [1]	Day of pregnancy [2]	Litter size [3]	Offspring age [4]	HPA axis test [5]	Result [6]	Hormone reported [7]	Reference
Cortisol (0.28 mg/kg/day)	27-29	1+2	5 yr	Haemorrhage stress	No effect	ACTH and cortisol	(Dodic et al., 2002)
Dexamethasone (0.28 mg/kg/day)	22-29	1 (Male)	4, 10, 19 mth	ACTH	No effect	Cortisol	
Betamethasone (0.5 mg/kg)	104	?	6 mth	CRH+AVP	No effect	ACTH and cortisol	(Sloboda et al., 2002)
			1 yr	CRH+AVP	β > C	Basal and stimulated cortisol	
					No effect	ACTH	
Betamethasone (0.5 mg/kg)	104+111+118+124	1	2 yr	CRH+AVP	β > C	Basal ACTH	(Sloboda et al., 2007)
			3 yr	CRH+AVP	β > C	Basal ACTH	
					β < C	Basal and stimulated cortisol	
Stressor (Isolation + Control)	112-147	1 + 2 + 3	25 d	Basal concentrations	I > C	Basal cortisol	(Roussel et al., 2004)
			8 mth		No effect	Basal cortisol	

[1] Ewe treatments during pregnancy, M=nutrient requirements for the maintenance of ewes during pregnancy

[2] The days of pregnancy that ewes were treated (negative values indicate treatment began prior to mating)

[3] The litter size of the offspring assessed, plus sign indicates that results were polled for litter sizes

[4] The postnatal age of the offspring when the HPA axis test was conducted

[5] The method of assessing the activity of the HPA axis (CRH and AVP challenge, ACTH challenge, basal concentrations only, or haemorrhage test)

[6] The differences observed between lambs born to ewes in each treatment group (UN=Lambs born to undernourished ewes, I= Lambs born to isolated ewes, β=Lambs born to ewes given betamethasone and C=lambs born to control ewes)

[7] The variables that were identified as being significantly different between treatment groups

[8] The variables that were measured

Study 3 –Nutrition in mid- to late-pregnancy

The plasma cortisol response of twin (n=16) and triplet (n=19) ram lambs was examined on day 37 after the mid-point of the parturition period. Ram lambs born to ewes that were offered pastures with an average sward height of 2 or 6 cm from day 64 of pregnancy until parturition (Chapters 6 and 7) were randomly assigned to the handling or castration treatment. Serial blood samples were collected from the jugular vein to determine plasma cortisol concentrations.

Study 4 –Nutrition in mid- to late-pregnancy

The plasma cortisol response of twin (n=26) and triplet (n=27) ram lambs was examined on day 46 after the mid-point of the parturition period. Ram lambs born to ewes that were offered pastures with an average sward height of 2 or 4 cm from P70 to parturition (Chapter 5) were randomly allocated to a handling or castration treatment. Serial blood samples were collected from the jugular vein to determine plasma cortisol concentrations.

Lamb treatment and blood sampling procedures

The handling treatment involved the physical restraint of lambs and palpation of the scrotum. The castration treatment involved the application of a rubber ring (Allflex New Zealand Ltd, Palmerston North) to the scrotum (Mellor and Stafford, 2000). Handling was used as the control. Blood samples were collected by jugular venipuncture into 10 ml tubes containing sodium heparin (BD Vacutainer, Preanalytical solutions, Franklin Lakes, USA). Blood samples were taken immediately prior to treatment (T=0) and 20, 40, 60, 90, 120, 150, 180, 210 and 240 minutes after. Blood samples were immediately placed on ice and centrifuged at 3000 rpm for 10 minutes. Plasma was separated and frozen at -20°C for analysis. Plasma was analysed for total cortisol concentration using a radioimmunoassay kit (Clinical Assays™ GammaCoat™ ^{125}I-RIA, DiaSorin, Stillwater, MN, USA). The GammaCoat™ antiserum exhibits 100% cross reactivity with ovine cortisol and the published intra-assay coefficient of variation was 7.0% and the inter-assay variation was 9.2% (Anon., 2003a).

Statistics

For all the experiments described above plasma cortisol concentrations were not normally distributed. To normalise the distribution a Log_e transformation was applied to the data. Basal plasma cortisol concentrations (t=0) were assessed using the Proc GLM procedure (SAS, 2005) which included the fixed effects of litter size and ewe treatment. The change in plasma cortisol concentrations from basal levels was calculated for all subsequent time points. Cortisol response curves were compared using the repeated measures analysis in the PROC MIXED procedure (SAS, 2005). The models included the fixed effects of litter size and ewe treatment. The area under the cortisol response curve was calculated and peak cortisol concentrations were determined for each animal and compared using PROC GLM in SAS (SAS, 2005). Models were run both without and without covariates (Study 1 included lamb birth weight; Study 2 included lamb birth and docking weights; Study 4 included birth and docking weights). The figures illustrating these results present the means ± standard error of the untransformed data for ease of understanding however p-values were based on the analyses of transformed data.

Results

Basal plasma cortisol concentrations

In study 1, basal plasma cortisol concentrations were greater for twin lambs born to shorn ewes than their singleton counterparts (P<0.05). In addition, twin lambs born to yarded ewes tended (P<0.10) to have greater basal plasma cortisol concentrations than singleton lambs (Table 46). Ewe treatment, however, had no effect on basal cortisol concentrations in all 4 studies. There was also no effect of lamb litter size on basal plasma cortisol concentrations in studies 2, 3 and 4. There was no effect of lamb birth weight or docking weight on the lamb's basal plasma concentrations.

Table 46. Basal plasma cortisol concentrations of singleton, twin and triplet lambs born ewes in each study (least squares mean ± standard error).

Ewe	Basal cortisol (nmol/L) [1]						Litter size
treatment	n	Singleton	n	Twin	n	Triplet	P-value
Study 1							
Control	20	3.3 ± 0.1 (31.7)	20	3.4 ± 0.1 (25.7)			ns
Yarded	20	3.2 ± 0.1 (29.6)	20	3.5 ± 0.2 (38.0)			+
Shorn	20	3.0 ± 0.1 (24.4)	20	3.7 ± 0.1 (42.5)			***
Study 2							
Control	6	3.6 ± 0.2 (38.8)	7	3.3 ± 0.2 (36.0)			ns
Shorn	9	3.5 ± 0.2 (34.0)	11	3.2 ± 0.2 (29.9)			ns
Study 3							
2 cm			5	3.7 ± 0.2 (44.1)	6	3.9 ± 0.1 (60.2)	ns
6 cm			11	3.9 ± 0.2 (52.9)	13	4.2 ± 0.1 (74.0)	ns
Study 4							
2 cm			12	2.8 ± 0.2 (21.5)	15	2.4 ± 0.1 (13.5)	ns
4 cm			14	2.9 ± 0.2 (23.6)	12	2.8 ± 0.1 (19.2)	ns

[1] Data were $\log_e$ transformed, untransformed means are given in parentheses

Means within columns with different superscripts are significantly different (P<0.05)

ns indicates P>0.1, + P<0.1, * P<0.05, ** P<0.01, *** P<0.001

Plasma cortisol response to handling and castration

Study 1

The plasma cortisol response of lambs to castration was greater ($P<0.05$) than handling at 20, 40, 60, 90, and 120 minutes after treatment (Figure 10). The plasma cortisol concentration of lambs in response to castration dropped below that of handled lambs 210 and 240 minutes after castration.

There was a significant 4-way interaction of lamb treatment, ewe treatment, litter size and time. At 90, 120, 180 and 240 minutes after castration, singleton lambs born to ewes in the shorn treatment had greater plasma cortisol concentrations than singleton lambs born to control ewes. In addition, 210 minutes after castration singleton lambs born to shorn ewes also had greater plasma cortisol concentrations than singleton lambs born to yarded ewes. Twin lambs born to shorn ewes had lower plasma cortisol concentrations in response to castration than twin lambs born to control ewes 150 minutes after treatment. In response to handling, twin lambs born to yarded ewes had a greater plasma cortisol concentration than twin lambs born to control ewes 20 minutes after treatment. Twin lambs born to shorn ewes had lower plasma cortisol concentrations 120 minutes after handling than twin lambs born to control ewes. There was no effect of lamb birth weight on the plasma cortisol response of lambs in this study.

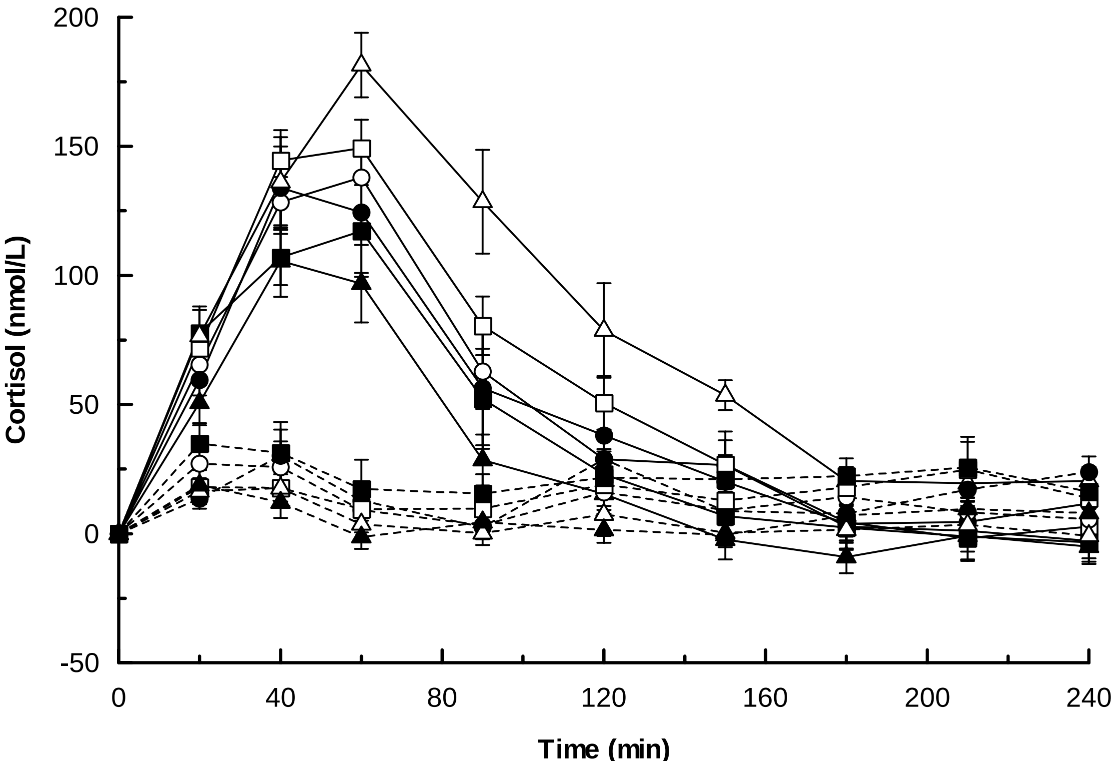


Figure 10. Plasma cortisol change from basal concentration over time in response to handling (dashed lines) or castration (solid lines) of singleton (open symbols) and twin lambs (closed symbols) born to control (circles), yarded (squares) and shorn ewes (triangles)

Study 2

The plasma cortisol response of lambs to castration was greater than handling 40, 60 and 90 minutes after treatment and tended (P=0.05) to be greater at 120 minutes (Figure 11). In response to castration, twin lambs had higher plasma cortisol concentrations than singleton lambs 90 and 180 minutes after treatment (P<0.05). There was no effect of lamb birth or docking weight on the plasma cortisol responses of lambs in this study.

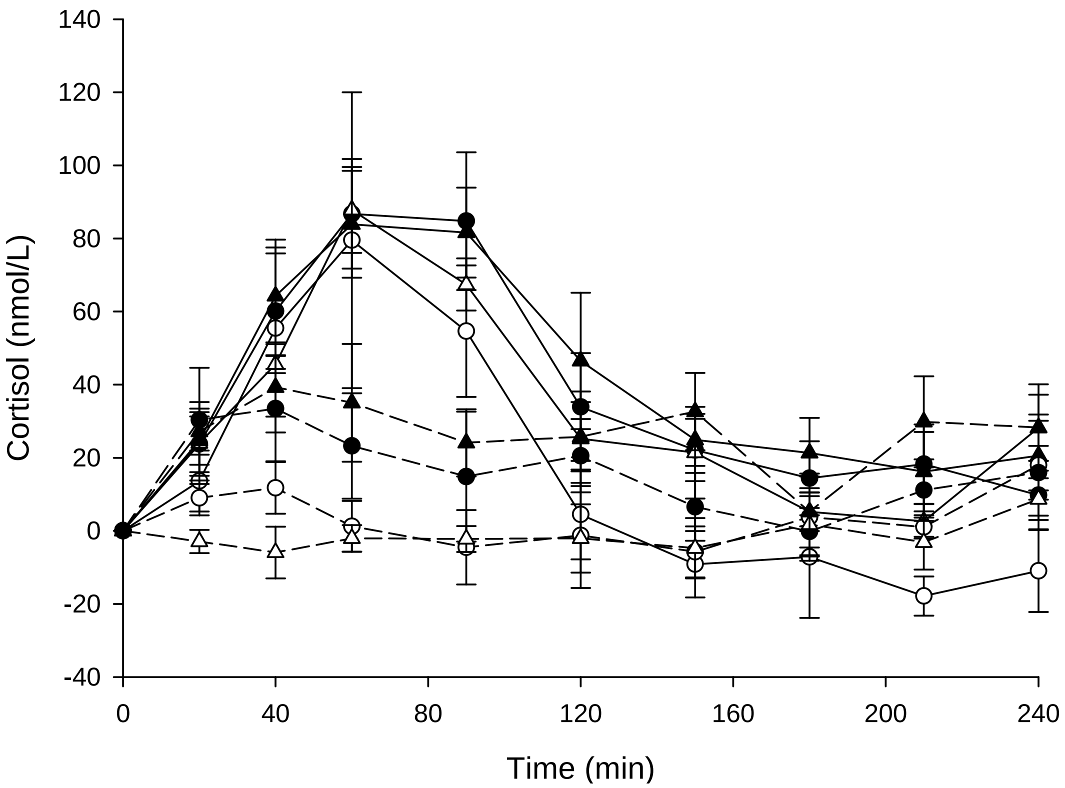


Figure 11. Plasma cortisol change from basal concentration over time in response to handling (dashed lines) or castration (solid lines) of singleton (open symbols) and twin lambs (closed symbols) born to control (circles) and shorn ewes (triangles)

Study 3

The plasma cortisol response of lambs to castration was greater (P<0.05) than handling between 20 and 120 minutes after treatment (Figure 12). Twenty minutes after castration, twin lambs born to ewes offered 2 cm pastures during mid- and late-pregnancy had greater cortisol concentrations than twin lambs born to ewes offered 6 cm. At all other time points there was no effect of ewe treatment or lamb litter size on the lambs' cortisol response to handling or castration.

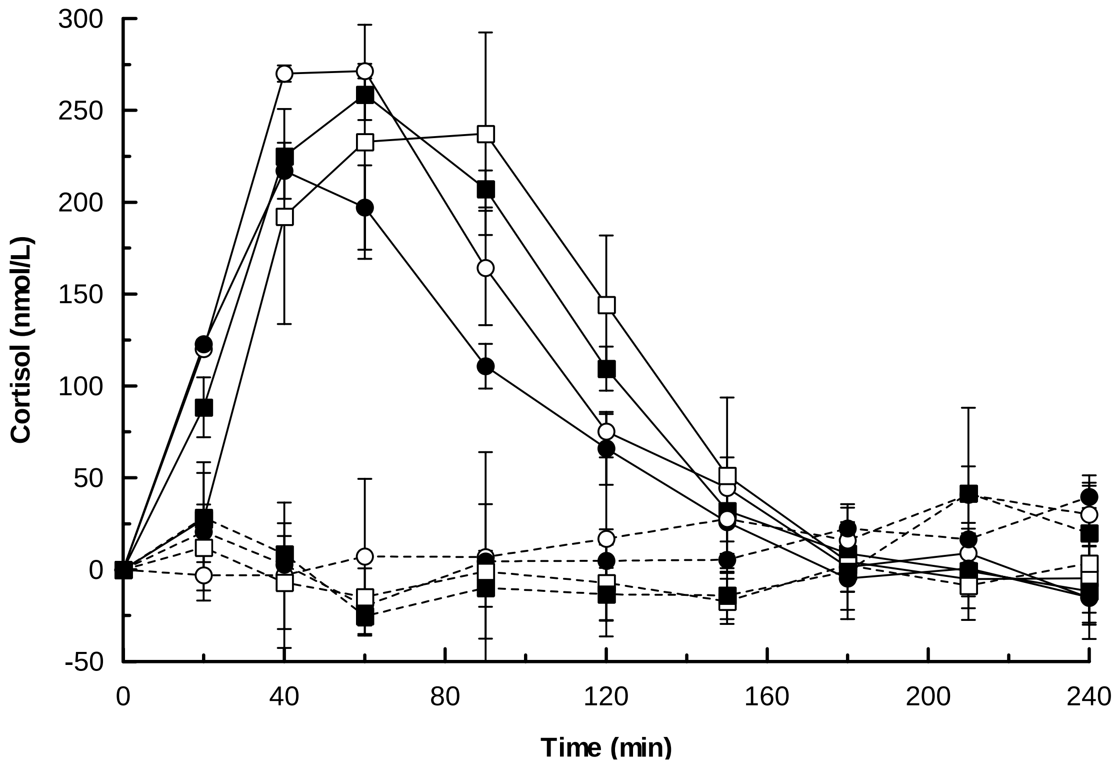


Figure 12. Plasma cortisol response to handling (dashed lines) or castration (solid lines) of twin (open symbols) and triplet (closed symbols) born to ewes offered 2 (circles) or 6 cm pastures (squares) in mid- and late-pregnancy

Study 4

Lambs that were castrated had greater plasma cortisol concentrations than handled lambs between 20 and 150 minutes after treatment (Figure 13). The plasma cortisol response of twin and triplet lambs to handling was similar at all time points except 210 minutes after treatment (P<0.05). The plasma cortisol response to castration did not differ between litter size or ewe nutrition treatments. There was no effect of lamb birth or docking weight on the plasma cortisol responses of lambs in this study.

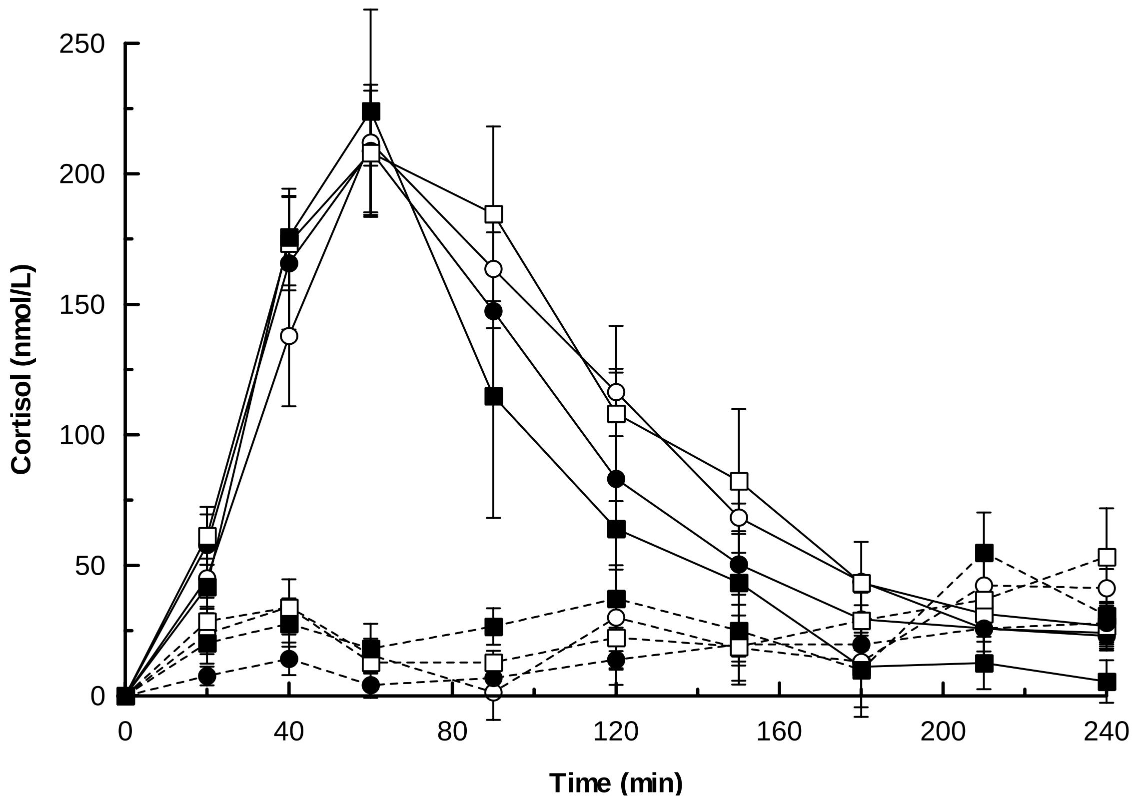


Figure 13. Plasma cortisol response to handling (dashed lines) or castration (solid lines) of twin (open symbols) and triplet (closed symbols) born to ewes offered 2 (circles) or 4 cm pastures (squares) in mid- and late-pregnancy

Maximum plasma cortisol concentrations and area under the cortisol response curve

Lambs that were castrated had a greater maximum plasma cortisol concentration than handled lambs in all studies. A similar pattern was seen in the area under the cortisol response curve, however in study 2, the area under response curve in response to handling and castration was not different for lambs born to control ewes (P>0.05).

Study 1

Maximum plasma cortisol and area under the response curve had a significant interaction of litter size and ewe treatment (P<0.05; Table 47). Twin lambs born to shorn ewes (37.0 nmol/L/min) had a lower area under their cortisol response curve than singleton lambs born to control, yarded and shorn ewes (60.6 vs. 71.5 vs. 94.0 nmol/L/min, respectively). The maximum plasma cortisol concentration for singleton lambs born to shorn ewes was greater than singleton lambs born to control ewes. In addition, the cortisol response of singleton lambs born to shorn ewes was greater than twin lambs born to shorn ewes (P<0.05). In addition, twin lambs born to shorn ewes had a lower maximum cortisol concentration to castration than twin lambs born to control, yarded and shorn ewes (P<0.05).

Study 2

There was no effect of ewe treatment or lamb litter size on the area under the curve or maximum cortisol response to handling or castration (Table 48).

Study 3

There was no effect of litter size or ewe treatment on maximum cortisol concentrations or area under the cortisol response curve (Table 49).

Study 4

The maximum plasma cortisol concentration and area under the cortisol response curve was similar between litter sizes and ewe nutritional treatments in response to castration and handling (Table 50). The maximum cortisol concentration in response to castration tended to be lower for triplet lambs born to ewes offered 4 cm pastures than their counterparts born to ewes on 2 cm ($P<0.1$).

Table 47. Maximum plasma cortisol concentration (nmol/L) and area under the cortisol response curve (AUC; nmol/L.min) of castrated and handled single and twin lambs born to control, yarded and shorn ewes (least squares mean ± standard error)

		Maximum (nmol/L)[1]		AUC (nmol/L.min)[2]	
	n	Handled	Castrated	Handled	Castrated
Litter size					
Singleton	60	3.43 ± 0.1 (36.8)	5.06^{b} ± 0.1 (162.3)	2.68 ± 0.1 (10.8)	4.23^{b} ± 0.1 (75.4)
Twin	60	3.51 ± 0.1 (44.0)	4.79^{a} ± 0.1 (129.1)	2.40 ± 0.1 (13.4)	3.72^{a} ± 0.1 (48.6)
Ewe treatment					
Control	40	3.71^{b} ± 0.1 (47.1)	4.93 ± 0.1 (144.7)	2.73 ± 0.1 (13.5)	3.98 ± 0.1 (59.4)
Yarded	40	3.71^{b} ± 0.1 (46.2)	4.93 ± 0.1 (143.0)	2.63 ± 0.1 (17.5)	3.97 ± 0.1 (61.1)
Shorn	40	3.00^{a} ± 0.1 (28.0)	4.91 ± 0.1 (149.3)	2.28 ± 0.1 (5.3)	3.97 ± 0.1 (65.5)
Litter size x Ewe treatment					
Singleton x Control	20	3.67^{b} ± 0.1 (45.7)	4.93^{b} ± 0.1 (142.9)	2.81^{b} ± 0.1 (13.1)	4.00^{bc} ± 0.1 (60.6)
Singleton x Yarded	20	3.66^{b} ± 0.1 (40.3)	5.03^{bc} ± 0.1 (155.2)	2.57^{ab} ± 0.1 (13.7)	4.22^{bc} ± 0.1 (71.5)
Singleton x Shorn	20	2.95^{a} ± 0.1 (24.6)	5.21^{c} ± 0.1 (188.7)	2.68^{ab} ± 0.1 (5.8)	4.49^{c} ± 0.1 (94.0)
Twin x Control	20	3.75^{b} ± 0.1 (48.5)	4.93^{b} ± 0.1 (146.6)	2.65^{ab} ± 0.1 (14.0)	3.97^{abc} ±0.1 (58.2)
Twin x Yarded	20	3.75^{b} ± 0.1 (52.1)	4.84^{ab} ± 0.1 (130.8)	2.69^{ab} ± 0.1 (21.3)	3.73^{ab} ± 0.1 (50.7)
Twin x Shorn	20	3.03^{a} ± 0.1 (31.5)	4.61^{a} ± 0.1 (109.8)	1.87^{a} ± 0.1 (4.8)	3.45^{a} ± 0.1 (37.0)

[1] The maximum cortisol concentrations recorded for each lamb

[2] Area under the cortisol response curve

[3] Data were $\log_e$ transformed, untransformed means are given in parentheses

Means within columns with different superscripts are significantly different (P<0.05)

Table 48. Maximum plasma cortisol concentration (nmol/L) and area under the cortisol response curve (AUC; nmol/L.min) of castrated and handled singleton and twin lambs born to control and shorn ewes (least squares mean ± standard error)

	n	Maximum (nmol/L)[1]		AUC (nmol/L.min)[2]	
		Handled	Castrated	Handled	Castrated
Litter size					
Singleton	13	2.80 ± 0.4 (21.1)	4.41 ± 0.5 (95.3)	3.63 ± 0.2 (-0.6)	4.29 ± 0.3 (34.1)
Twin	20	3.42 ± 0.3 (48.4)	4.69 ± 0.3 (114.1)	3.69 ± 0.2 (13.6)	4.52 ± 0.2 (55.7)
Ewe treatment					
Control	15	3.38 ± 0.4 (37.5)	4.61 ± 0.4 (115.4)	3.78 ± 0.2 (7.7)	4.39 ± 0.3 (44.6)
Shorn	18	2.82 ± 0.3 (32.0)	4.49 ± 0.4 (94.0)	3.53 ± 0.2 (5.2)	4.42 ± 0.2 (45.1)

[1] The maximum cortisol concentrations recorded for each lamb

[2] Area under the cortisol response curve

[3] Data were $\log_e$ transformed, untransformed means are given in parentheses

Means within columns with different superscripts are significantly different (P<0.05)

Table 49. Maximum plasma cortisol concentration (nmol/L) and area under the cortisol response curve (AUC; nmol/L.min) of castrated and handled twin and triplet lambs born to ewes offered 2 or 6cm pasture in mid- and late-pregnancy (least squares mean ± standard error)

		Maximum (nmol/L)[1]		AUC (nmol/L.min)[2]	
	n	Handled	Castrated	Handled	Castrated
Litter size					
Twin	16	3.06 ± 0.4 (30.7)	5.56 ± 0.4 (274.3)	3.91 ± 0.2 (2.3)	5.13 ± 0.3 (128.8)
Triplet	19	3.49 ± 0.4 (63.1)	5.45 ± 0.4 (244.4)	3.69 ± 0.2 (-0.8)	5.04 ± 0.2 (114.0)
Ewe Nutrition					
2 cm	11	3.68 ± 0.4 (45.6)	5.45 ± 0.5 (245.0)	4.00 ± 0.3 (7.2)	5.04 ± 0.3 (113.5)
6 cm	24	2.87 ± 0.3 (48.2)	5.55 ± 0.3 (273.7)	3.60 ± 0.3 (-5.6)	5.12 ± 0.2 (129.4)

[1] The maximum cortisol concentrations recorded for each lamb

[2] Area under the cortisol response curve

[3] Data were $\log_e$ transformed, untransformed means are given in parentheses

Means within columns with different superscripts are significantly different ($P<0.05$)

Table 50. Maximum plasma cortisol concentration (nmol/L) and area under the cortisol response curve (AUC; nmol/L.min) of castrated and handled twin and triplet lambs born to ewes offered 2 or 6 cm pasture in mid- and late-pregnancy (least squares mean ± standard error)

		Maximum (nmol/L) [1]		AUC (nmol/L.min) [2]	
	n	Handled	Castrated	Handled	Castrated
Litter size					
Twin	26	4.02 ± 0.1 (61.9)	5.33 ± 0.1 (219.5)	3.50 ± 0.1 (19.0)	4.81 ± 0.1 (128.8)
Triplet	27	3.81 ± 0.1 (58.1)	5.36 ± 0.1 (220.8)	3.48 ± 0.1 (17.4)	4.72 ± 0.1 (98.2)
Ewe Nutrition					
2 cm	27	4.06 ± 0.1 (65.2)	5.40 ± 0.1 (223.7)	3.63 ± 0.1 (21.8)	4.79 ± 0.1 (107.2)
4 cm	26	3.78± 0.1 (48.2)	5.30 ± 0.1 (216.6)	3.35 ± 0.1 (14.7)	4.74 ± 0.1 (107.3)

[1] The maximum cortisol concentrations recorded for each lamb

[2] Area under the cortisol response curve

[3] Data were $\log_e$ transformed, untransformed means are given in parentheses

Means within columns with different superscripts are significantly different ($P<0.05$)

Discussion

The aim of these studies was to examine the effect of mid-pregnancy shearing and ewe nutrition in mid- and late-pregnancy on the plasma cortisol response of lambs to handling and castration between 2 and 7 weeks of age.

In all 4 studies castration produced a greater plasma cortisol response than handling. Therefore, the use of castration was an effective method to stimulate an increase in the lamb's plasma cortisol concentrations. In one of the four studies, ewe treatment during pregnancy and lamb litter size was found to affect the lamb postnatal cortisol concentrations (Study 1). The remaining 3 studies there were no differences in basal or stimulated plasma cortisol concentrations between ewe treatments and lamb litter sizes. This experiment could have been strengthened by measuring the plasma concentrations of ACTH in addition to cortisol. The combination of these two hormones would have provided more information of the function of the HPA axis.

In study 1, lamb litter size had a greater effect than ewe treatment on the plasma cortisol response of lambs to handling and castration. Twin lambs born to shorn and yarded, but not control ewes, had greater basal plasma cortisol concentrations than their singleton counterparts. In addition, there was a significant interaction of ewe treatment and lamb litter size on the plasma cortisol response to castration. Singleton lambs born to shorn ewes had a greater cortisol response to castration than singleton lambs born to control ewes, however, the opposite was observed in twin lambs. The differential effect of ewe stressors during mid- and late-pregnancy on singleton, twin and triplet lambs is an area of study that has received little attention. Previously, studies containing more than one litter size did not report the responses of each litter size separately (for example (Dodic et al., 2002; Moritz et al., 2002; Roussel et al., 2004). To date one study has set out to examine the effects of litter size (singleton and twin) on postnatal HPA axis function. Bloomfield et al. (2007) reported that post-pubertal twin-born ewes had greater ACTH, but not cortisol, response to a CRH + AVP challenge at 10 months of age. They concluded that the reduced growth of twin fetuses compared to singletons during gestation altered postnatal HPA function and that this was determined by factors specific to the fetus rather than maternal factors. In the present studies, however, lamb birth weight had no effect on the lamb's plasma cortisol response. Therefore, the cause of the difference in lamb response to treatment remains unclear.

In response to handling at 2 weeks of age, lambs born to ewes that were shorn in mid-pregnancy had a lower plasma cortisol response than lambs born to control or yarded ewes. This finding is contrary to the majority of studies of ruminants which report that prenatally stressed offspring had elevated HPA axis activity compared to offspring born to control dams (Lay et al., 1997b; Hawkins et al., 2000; Sloboda et al., 2002; Bloomfield et al., 2003a; Roussel et al., 2004; Chadio et al., 2007). The cause of this difference is unclear, however the observed difference in the response of singleton and twin lambs observed may have influenced this response.

The effect of prenatal stress can be altered by the postnatal age of the lamb. Fowden et al. (2005) noted that newborn and juvenile animals have blunted adrenocortical responses, but hypothesised that after puberty glucocorticoid responses to stress were likely to be enhanced. In the present study, lambs born to ewes shorn in mid-pregnancy had a lower maximum cortisol response to handling than control lambs at 2 weeks of age (study 1), but no differences were observed at 2 months (study 2). Chadio et al. (2007) reported that lambs born to ewes undernourished in early pregnancy had greater ACTH and cortisol responses to a CRH challenge than lambs born to well-fed ewes at 2 months of age, but not at 5.5 or 10 months. They concluded that puberty-related changes in HPA axis responsiveness may be responsible. In the current study all lambs were all pre-pubertal at the time the stressor was imposed so that explanation does not fit in this case.

Maternal undernutrition in mid- and late-pregnancy had no effect on the basal or stimulated cortisol concentrations of the lamb. In these studies, ewes were offered pastures to provide approximately 80 or 140% of the late-pregnancy maintenance requirements for 50 kg ewes bearing twin or triplet fetuses (NRC, 2007). Offspring of ewes severely underfed for 10, but not 20 days, in late-pregnancy showed increased ACTH responses to a corticotrophin releasing hormone plus arginine vasopressin (CRH+AVP) and an insulin challenge (Bloomfield et al., 2003a). Therefore, the intensity and duration of the undernutrition imposed in this study may not have been great enough to alter the development of the fetal HPA axis.

Stressors in mid-pregnancy may have little effect on the development of the lamb. In mid-pregnancy the phase of rapid placental growth is complete (P90; (Ehrhardt and Bell, 1995) but the rapid growth and development of the brain has not yet commenced (P120; (Challis et al., 2000b). The majority of studies that have examined the effects of prenatal stressors on lamb postnatal HPA axis function exposed ewes to stressors in late-gestation (day 100 to 145 of pregnancy; (Bloomfield et al., 2003a; Borwick et al., 2003; Roussel et al., 2004; Sloboda et al., 2007). In early pregnancy (P27 to 29) treatment of ewes with dexamethasone had no effect on adult HPA function of offspring (Dodic et al., 2002) whereas a single injection of betamethasone on day 104 of pregnancy enhanced HPA axis function in lambs at 1 year of age (Sloboda et al., 2002). Therefore the development of the fetal HPA may be insensitive to the effects caused by shearing the ewe in mid-pregnancy.

Conclusion

It appears that lamb litter size had a greater effect than prenatal stressors on the basal and stimulated cortisol concentrations of lamb at 2 weeks of age. However, differences in lamb cortisol concentrations were not observed in older lambs (between 4 - 7 weeks of age). Therefore, no definitive conclusions can be made about the effects of mid-pregnancy shearing and nutrition on the postnatal HPA function of lambs.

GENERAL DISCUSSION

Lamb mortality costs New Zealand farmers an estimated ~$NZ264 million per annum in lost potential earnings from the sale of lambs for slaughter (Statistics New Zealand, 2004; M&WNZ - Economic Service, 2006b). In New Zealand, lambing percentages have increased from 100 to 128% between 1990 and 2005 (M&WNZ - Economic Service, 2006a). Amer et al. (1999) has shown that this increase in lambing percentage has been driven by an increase in twin and triplet lambs. Twin and triplet lambs have lower survival rates to weaning than singleton lambs which is primarily due to their lower birth weights (Geenty, 1997; Kenyon et al., 2007; Stafford et al., 2007).

The behaviour of both the ewe and lamb plays an in important role in lamb survival. The expression of appropriate ewe and lamb behaviours leads to the establishment of an exclusive ewe and lamb bond (Poindron et al., 1984; Dwyer et al., 2003). The failure to develop this bond will result in the ewe rejecting the lamb and in most cases, death of the lamb due to starvation and exposure (Nowak and Poindron, 2006). The behaviour of both the ewe and lamb can be influenced by undernutrition during pregnancy (Dwyer et al., 2003) however the effect of mid-pregnancy shearing has not been examined.

The lambs' postnatal hypothalamic-pituitary-adrenal (HPA) axis function can be altered by exposing the ewe to experimentally imposed stressors in pregnancy, such as severe undernutrition, isolation or injections of glucocorticoids (Sloboda et al., 2002; Bloomfield et al., 2003a; Roussel et al., 2004). The effects of maternal nutrition on lamb HPA responses has been examined in both early and late pregnancy however the effects in mid-pregnancy have received little attention. In addition, the effect of shearing on the activity of the HPA axis of the lamb has not been examined.

The experiments in this thesis examined the effect of shearing and differing nutrition on offspring at birth, weaning and at 1 and 2 years of age. The results of this work identified that shearing ewes at approximately day 80 of pregnancy resulted in an increase in lamb birth weight, however, other stressors had no effect. In terms of ewe nutrition, it was found that underfeeding ewes between days 108 and 147 of pregnancy had a greater effect on lamb birth weight than between days 70 and 107 of pregnancy. Lamb live weight at weaning was altered by mid-pregnancy shearing in one of three studies however undernutrition from day 70 to 147 of pregnancy resulted in decreased live weight gain between birth and day 42 of lactation but not between day 43 and 90 of lactation.

In the studies that examined mid-pregnancy shearing the behaviour of the ewe and lamb was influenced more by litter size than by ewe treatment in pregnancy. Ewe nutrition during pregnancy however had a greater effect on ewe and lamb behaviour than litter size. The behaviour of the female offspring at 8 months of age was not affected by litter size however offspring of ewes offered 6 cm swards from day 64 to 132 of pregnancy bleated more in a high pitch than offspring of ewes offered 2 cm. At 2 years of age female offspring born a twin had a greater maternal behaviour score than their triplet-born counterparts.

Mid-pregnancy shearing

New Zealand farmers currently use mid-pregnancy shearing as a management tool to increase lamb birth weights and survival (Kenyon et al., 2003). However, this increase in lamb birth weight may be achieved at the expense of the dam. In poor weather shearing results in cold stress and places the ewe at risk of hypothermia that can result in death. Therefore, the management of newly shorn ewes is critical to reduce the impact on the ewe.

Shearing has been generally regarded as a stressor however the removal of fleece produces a greater increase in plasma cortisol concentrations than transport, isolation and up-ending alone (Pierzchala et al., 1983; Hargreaves and Hutson, 1990b). The plasma cortisol response of sheep increases as each additional stressor occurs. Up-ending plus shearing noise and wool removal produced a greater plasma cortisol peak than up-ending and shearing noise which produced a greater plasma cortisol peak than up-ending alone (Hargreaves and Hutson, 1990a).

It was hypothesised that stressors associated with the shearing process produced the observed increase in lamb birth weight (Roussel et al., 2004). If this was the case, lamb birth weights could be increased by exposing ewes to an alternative stressor, eliminating the need to remove the fleece from the ewe and thus considerably reduce the risk of ewe mortality.

In a series of 3 experiments, the effect of exposing ewes to a series of stressors was assessed in order to test this hypothesis. In each of the 3 studies the effect of stressor on the birth weight and size of lambs, ewe and lamb behaviour and the plasma cortisol response of ram lambs to castration and handling was assessed. The magnitude of the stressors and the stress response of the ewe varied greatly between studies. This variation in intensity of the stressor provided a structured program to assess the effects on the lamb.

The ewe treatments imposed in each study are listed below:

- Chapter 2, control (unshorn), yarding and shearing in mid-pregnancy
- Chapter 3a and 3b, control (unshorn), crutching and sham-shearing and shearing in mid-pregnancy
- Chapter 4, control (unshorn), shearing in mid-pregnancy, repeated isolation, repeated sham-shearing and repeated cortisol injection in mid- and late-pregnancy

In the Chapter 3, an unbalanced trial design was used due to the unexpectedly low number of ewes bearing a single fetus. Singleton-bearing ewes were allocated to the control and shorn treatments while twin-bearing ewes were allocated to control, crutch, sham-shorn and shorn treatments.

In all of these studies, the birth weight of lambs born to ewes shorn in mid-pregnancy was greater than lambs born to control ewes. However, all other stressors examined failed to produce the increase in lamb birth weight compared to their shorn counterparts. Therefore, it was concluded that the cause of the increase in lamb birth weight was not the stress response associated with shearing. It is possible however that a series of stressors (for example mustering, fasting and wool removal) may be required to reach a threshold which then results in an increase in lamb birth weight. In addition, the role of cold stress resulting from mid-pregnancy shearing was not examined and warrants investigation. Cold stress results in metabolic, hormonal and behavioural adaptations in the ewe (Symonds et al., 1988a; Gregory, 1995; Clarke et al., 1997a; Morris et al., 2000).

The mid-pregnancy stressors examined, with the exception of shearing, did not alter lamb postnatal growth. Shearing, however, had an inconsistent effect on postnatal growth. In chapter 2, lambs born to shorn ewes were heavier on day 80 of lactation than lambs born to yarded, but not control ewes. In chapters 3 and 4, however, lamb postnatal weights were unaffected by ewe treatment. These findings suggest that while mid-pregnancy shearing increased lamb birth weights it did not always alter postnatal growth.

Mid-pregnancy stressors, including shearing, had little effect on ewe or lamb behaviour after birth (Chapters 2 and 4). However, in Chapter 2, twin lambs born to ewes exposed to intensive yarding were less likely to successfully suck in a 5-minute observation period following tagging than twin lambs born to shorn ewes. Lamb litter size had a greater influence on ewe behaviour than ewe treatment in Chapter 2. Twin-bearing ewes had higher maternal behaviour scores and exhibited bleating behaviours associated with enhanced ewe lamb bonding compared to singleton-bearing ewes. The behaviour of the lamb was less affected by litter size, however, twin lambs made contact with their dam sooner after they were released than singleton lambs but were slower to follow their dam when she moved more than 5 m away from the tagging site.

The plasma cortisol response of ram lambs to castration and handling appeared to be altered to a greater degree by litter size than ewe stressors in mid-pregnancy. In chapter 8, study 1 showed that singleton lambs had lower basal plasma cortisol concentrations and a smaller plasma cortisol response to handling than twin lambs. After castration, singleton lambs born to shorn ewes had a greater cortisol response than their twin counterparts. In study 2 of chapter 8, there were no differences between ewe treatments or lamb litter sizes on the plasma cortisol response of ram lambs to handling and castration. In these studies, plasma adrenocorticotropic hormone (ACTH) response of lambs was not examined and may have provided additional information on the function of the HPA axis (Chapter 8). In addition, alternative HPA axis tests may have been employed such as an ACTH or CRH + AVP challenge. These tests provide information on the activity of HPA axis for example an ACTH challenge provides information on adrenocortical function whereas the CRH + AVP challenge relates to the function of the adenohypophysis (Figure 1).

The coat meconium staining of lambs was recorded within 12 hours of birth. This measure was previously used by Oliver et al. (2001) who described the measure as an indictor of fetal stress before birth. However, Westgate et al. (2002) concluded that meconium is normally passed into the amniotic fluid but cleared rapidly so that amniotic fluid is normally not obviously stained. This observation is also supported by Cajal et al. (2003) who reported that all 240 human fetuses studied between weeks 15 and 41 of gestation were observed to have one or more defecations. They also reported that the frequency of defecations was highest between week 28 and 34 of gestation. Therefore the variation in meconium score observed in this thesis may be related to the interval between defecation and birth rather than as an indicator of stress.

Ewe nutrition in mid- and late-pregnancy

The feeding recommendations for twin- and triplet-bearing ewes suggest that ewes should be offered pastures with an average sward height of 4 cm in mid- and late-pregnancy (Morris and Kenyon, 2004; Kenyon et al., 2006a). In some regions of New Zealand, however, pasture growth during the winter months is insufficient to meet these pasture covers (Matthews et al., 2000).

The focus of Chapter 5 was to determine during which period of pregnancy, mid or late, ewe nutrition has the greatest effect on lamb birth weight, ewe and lamb behaviour and ram lamb cortisol response to handling and castration. Ewes were offered pasture with an average sward height of less than 2 cm or greater than 4 cm. Lamb birth weight was unaffected by offering ewes 2 cm swards in mid-pregnancy, if ewes were offered 4 cm swards in late-pregnancy. However, lamb postnatal weight gain was altered by the nutrition the ewe received (Chapter 5). Lambs born to ewes offered pastures with a sward height of less than 2 cm in mid- and late-pregnancy had lower average daily gains between birth and docking than lambs born to ewes offered 4 cm swards in both mid- and late-pregnancy. However, between docking and weaning the average daily gain of lambs in these two groups were similar.

Ewe maternal behaviour was not altered by their plane of nutrition during pregnancy. However, lambs born to ewes offered swards of less 2 cm in mid- and late-pregnancy, showed a greater 'drive' to maintain contact with their dam than lambs born to ewes on the higher plane of nutrition (Chapter 5). The lambs 'drive' to maintain contact with their dam was based on the correlation of 'time to contact' with other lamb behaviours such as number of bleats and the time taken to bleat and suck. It appears from this combination of behaviours that the lambs have a greater focus on remaining with their dam.

In Chapter 8, the plasma cortisol response of twin and triplet ram lambs to handling or castration was examined at approximately 1 month of age. There was no effect of either ewe nutritional treatment or lamb litter size on the lambs' cortisol response.

The metabolism of the twin-born female progeny of ewes offered pastures with a sward height of 2 or 6 cm in mid- and late-pregnancy was investigated at 8 months of age (Chapter 7a). Plasma concentrations of insulin, glucose and cortisol in response to an insulin tolerance test were examined. It was concluded that underfeeding ewes during pregnancy (2 cm swards) did not alter the metabolic response of the female progeny to insulin at 8 months of age.

At 1 year of age, the behaviour of 56 twin- and triplet-born ewe lamb born to ewes offered 2 or 6 cm pastures in mid- and late-pregnancy was observed in an Arena test (Chapter 6). Lambs born to ewes offered 2 cm swards appeared to be less at ease in the arena test than progeny of ewes offered 6 cm swards. However, the locomotive behaviour of the lambs of ewes offered 2 and 6 cm swards was similar.

The reproductive success and maternal behaviour of these ewes was also examined at their first lambing at 2 years of age. Ewe nutrition during pregnancy had no effect on the maternal behaviour and gestation length of their female progeny or on the birth weight of their live lambs. However, triplet-born ewes showed poorer maternal behaviour, shorter gestation lengths and gave birth to singleton lambs that were 0.5 kg lighter than twin-born ewes. Therefore, the use of triplet-born ewes as replacement stock could have detrimental effects on flock productivity. Additional work is required to determine the repeatability and long-term implications of this finding.

Implications of these findings

The underlying mechanism by which mid-pregnancy shearing effects birth weight was not identified in this thesis. Therefore, at present there is no alternative management practice to mid-pregnancy shearing as a method of increasing lamb birth weight. Shearing during pregnancy consistently increased lamb birth weight, but the effect on lamb postnatal growth was variable. The stressors examined, including shearing, had only minor effects on the behaviour of the ewe and lamb 12 to 24 h after birth. This series of studies supports the use of mid-pregnancy shearing as a management strategy to increase lamb birth weight since few adverse effects on ewe and lamb behaviour or postnatal growth of the lamb were identified. However, care should be taken to reduce the environmental impact on newly shorn sheep.

The effect on lamb birth weight of ewe nutrition was greater in late-pregnancy than mid-pregnancy. Mild undernutrition in mid- and late-pregnancy had no effect on ewe maternal behaviour but did result in lambs that had a greater ‘drive’ to maintain contact with their dam. Ewe nutrition during mid- and late-pregnancy had a minor effect on the behaviour or metabolism of ewe progeny in the first year of life. However, litter size had a greater effect than ewe nutrition during mid- and late-pregnancy on female offspring. Triplet-born ewes had poorer maternal behaviour and gave birth to lighter singleton lambs than twin-born ewes. Therefore, it is recommended that ewes should be offered 4 cm pastures in both mid- and late-pregnancy, however, if this is not possible ewes can be offered lower swards in mid-pregnancy provided they are offered 4 cm in late-pregnancy. In addition, farmers should not use triplet-born ewes as replacement stock.

Potential limitations of the study design

In order to bring the two halves of this thesis together a combination of shearing and ewe nutrition in mid- and late-pregnancy could have been examined. However, in 2002, Kenyon et al. (2002c) conducted an experiment to examine the relationship of ewe nutrition and mid-pregnancy shearing. They concluded that ewe nutrition had no effect on the increase in lamb birth weight observed from mid-pregnancy shearing. They did not, however, examine the effect of offering sub-maintenance rations in mid- and late-pregnancy. It is unclear at present whether the effects of mid-pregnancy shearing on lamb birth weights are modified by or occur regardless of ewe nutrition.

Where to from here?

The component of shearing that produces the increase in lamb birth weight remains elusive. It was concluded from the experiments within this thesis that those stressors associated with shearing that were examined are not the cause of the increase in lamb birth weight. The exposure of ewes to cold stress in mid- and late-pregnancy requires examination. The effect of cold stress on lamb birth weight could be examined by placing fully fleeced and newly shorn ewes in both ‘warm’ and ‘cold’ temperature controlled environments during mid- and late-pregnancy. If the mechanism of the increase in lamb birth weight was cold stress then it would be expected that the birth weight of lambs born to shorn ewes kept in a cold environment would be greater than shorn ewes kept in warm conditions. Indeed, an optimal degree of cold exposure to increase lamb birth weight could be identified to minimise risks to the ewe.

Intensive investigation of the metabolic profile of the ewe and the developing fetus(es) during the first 24 h after shearing and at weekly intervals until parturition would provide information on the changes in ewe metabolism that may lead to the increase in lamb birth weight. The effect of shearing on plasma glucose, insulin, non-esterfied fatty acids and β-hydroxybutyrate concentrations of both the ewe and fetus would provide additional information on the ewe's metabolism and utilisation of fat reserves after shearing. It has been hypothesised that an increase in maternal glucose concentrations produces the increase in lamb birth weight (Symonds et al., 1988b; Sherlock et al., 2003), however, the effects of shearing on ewe glucose concentrations are variable (Morris et al., 2000; Revell et al., 2000). To determine the effects of mid-pregnancy shearing on the fetus placental function needs to be examined systematically in order to determine how fetal growth is increased. In addition, effects of maternal nutrient partitioning and fetal metabolism also require examination.

Examination of supplementary feeding of concentrates to ewes in mid- and late-pregnancy could extend the work conducted on the effect of ewe nutrition on the ewe and her lambs. Providing ewes with concentrates could be particularly important for triplet ewes whose intake can be restricted in late-pregnancy by the size of the conceptus (Kenyon and Webby, 2007). The birth weight of twin and triplet lambs may be enhanced to a greater degree by the provision of supplementary feed than pasture alone. In addition, identifying an optimal time and duration during pregnancy that providing ewes with concentrate feeds resulted in an increase lamb birth weight could reduce the costs of this practice thereby increasing the number of farmers that can afford to offer ewes concentrates. In addition, it would be valuable to know how the ewe partitions the supplementary feed either into fat reserves or to the fetus.

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