Chapter 1: General Introduction

Sheep production in Australia is characterised by large flock sizes, across extensive areas where animals graze outdoors year round. Lambing also occurs outdoors and in many cases under adverse environmental conditions leading to significant lamb losses. Under these extensive conditions lamb losses from birth to marking are often greater than 20% (Hinch 2008) and in economic terms these losses are estimated to cost the sheep industry at least $A56 million/year (Sackett *et al.* 2006). Low lamb survival rates not only represent a loss in lamb production but also an opportunity cost in terms of a reduction in wool production or quality, reduced surplus sheep for sale, a reduction in selective breeding capabilities and a reduction in options available to adjust flock structure (Alexander 1984). Improving lamb survival continues to be an important production issue for the Australian sheep industry despite many years of research focusing on this area.

In addition to the economic cost of low lamb survival rates, lamb losses also represent an animal welfare concern for both the lamb and the ewe. Some of the welfare challenges for the lamb soon after birth include hunger, hypothermia, pain and injury from the birth process and distress from maternal separation (Dwyer 2008b). Welfare challenges for the ewe may include pain and injury from the birth process and the impact of the loss of her lamb, although the welfare implications of this have never been studied (Dwyer 2008b). As the welfare of farmed animals becomes more important to consumers, improvement in lamb survival rates will be of paramount importance in the continued viability of the sheep industry.

The majority of lamb losses occur in the first 24 hours after birth with Hall *et al.* (1995) reporting that 67% of lambs that die do so within the first day of birth. Dystocia or starvation-mismothering are implicated in the majority of losses at this time and losses are often higher in multiple litters compared to single born lambs (Hinch *et al.* 1985) as outlined in Table 1.1. Table 1.1 also highlights the lack of improvement in lamb survival rates despite it being the focus of much research over many years. Much of the research to date has focused on management strategies aimed at improving ewe nutrition (Alexander *et al.* 1962; Banchero *et al.* 2004b; Fogarty *et al.* 1992; Geenty 1997; Scales *et al.* 1986; Vincent *et al.* 1985) or modifying the environment to make it more favourable at lambing time (Alexander 1961c; Lynch and Alexander 1980; Mottershead *et al.* 1982; Watson *et al.* 1968). Therefore there appears to be some merit in a continued focus on improving lamb survival; however, more focus may need to be placed on the early neonatal period and the role of the lamb in its own survival.

Table 1.1: Lamb loss rates from various historical studies categorised by litter size.

Author	**Period**	**Single lamb losses (%)**	**Twin lamb losses (%)**
Lax *et al.* (1965)	To weaning	15 – 29	
Egan *et al.* (1972)	To marking	9.3 – 12	
Knight *et al.* (1975)	To marking	9.4 – 13.3	
Cumming *et al.* (1978)	To marking	23 – 29	
Atkins (1980)	To weaning	13.4 – 19.3	19.6 – 35
Caple *et al.* (1982)	Not stated	17.2 – 27.8	37.5 – 56.3
Beetson (1984)	Not stated	8 – 10	29 – 38
Jordan and LeFeuvre (1989)	To marking	9 – 20	
Kleeman *et al.* (1991)	To weaning	12-13	38 – 39
Kelly (1992)	To marking	6 – 19.9	19.1 – 63.2
Hall *et al.* (1995)	To 3 days	19	
Holst *et al.* (2002)	To 3 days	11.1	20.8
Kleeman and Walker (2005)	To marking	16.6	43.8
Fowler (2007)	To weaning	16.5	31.5

There are several factors that determine whether a lamb will survive in the immediate post-natal period. These include breed (Holst 2002; Wiener *et al.* 1973; Woolliams *et al.* 1983), ewe nutrition (Hinch *et al.* 1985), litter size (Purser and Young 1964), birth weight, climatic conditions (e.g. cold exposure), ewe age and parity, ease of birth, lamb behaviour and maternal behaviour. Factors affecting survival have been widely reviewed (Alexander 1984; Dennis 1972; Dwyer and Lawrence 2005; Haughey 1981; Hinch 2008; Nowak 1996; Nowak and Poindron 2006) so will not be repeated here, but the following review will examine in some detail the relative contributions to lamb survival made by both the ewe and the lamb particularly focusing on the early neonatal period.

Chapter 2: Literature review

2.1 Lamb survival

Lamb survival is a key contributor to high reproductive rates. Nowak *et al.* (2009) have attempted to outline the relative contributions made to survival by the ewe and lamb, particularly in relation to their behaviours (Figure 2.1). Figure 2.1 highlights the large variety of ways that the ewe and the lamb contribute to survival variation and also the interrelationships. The interactions between mother and young makes it difficult to fully differentiate and quantify the contribution of each to survival probability and this will be examined in greater detail in this review.

The Australian sheep industry is dominated by the Merino breed and initially it was thought that high mortality rates in Merino lambs were due to the "poor" mothering ability of the ewes (Nowak 1996). However, it is understood that both the ewe and the lamb have important parts to play (Dwyer and Lawrence 1999; O'Connor and Lawrence 1992) although the relative contributions may vary with breed (Lindsay *et al.* 1990; Nowak *et al.* 1989; Stevens *et al.* 1984). Several authors report that the behaviour of the lamb is at least as important as the behaviour of the mother (Dwyer 2003; Dwyer and Lawrence 1999).

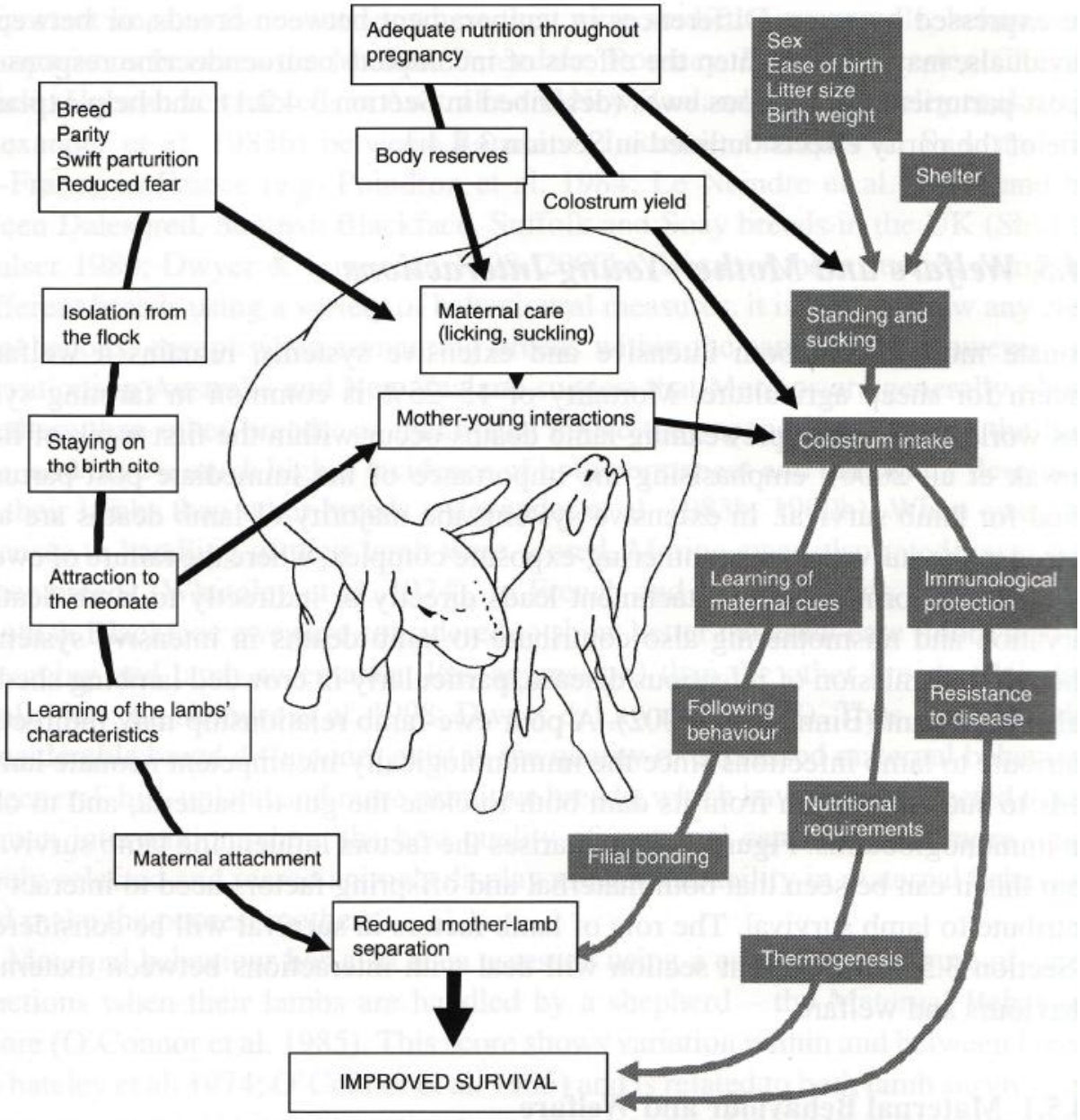

Figure 2.1: Relationships between ewe and lamb contributions to lamb survival (from Nowak *et al*. 2009). White boxes indicate maternal factors and grey boxes indicate lamb factors.

2.2 Ewe contribution to lamb survival

2.2.1 Energy supply

In the prenatal and early postnatal period, the lamb relies solely on the ewe for provision of their energy needs. Survival is compromised when the ewe is inadequately nourished during pregnancy which can also translate into problems associated with reduced availability of colostrum for the lamb. Nutrition during pregnancy is probably the primary factor that influences the energy passed to the lamb both before and after parturition (Banchero *et al.* 2004a; Budge *et al.* 2004; Langlands and Sutherland 1968; Prior and Christenson 1976). This aspect of ewe management has been studied extensively in the context of lamb survival (Alexander 1984; Alexander *et al.* 1956; Corner *et al.* 2008; Fogarty *et al.* 1992; Holst *et al.* 1986; Kerslake *et al.* 2009; Khalaf *et al.* 1979) and in short, adequate ewe nutrition before, during and after pregnancy is known to be important to the survival of the lamb (Everett-Hincks *et al.* 2005a; Gardner *et al.* 2007; Holst *et al.* 1986; Scales *et al.* 1986).

2.2.1.1 Birth weight

The effect of pregnancy nutrition on energy supply in newborn lambs is mainly reflected in differences in birth weight. The impact of birth weight on lamb survival has been well documented (Alexander *et al.* 1959; Davies 1964; Egan *et al.* 1977; Hall *et al.* 1995; Hinch *et al.* 1985; Mullaney 1969). It is generally accepted that birth weight is the most important factor affecting lamb survival as it accounts for much of the survival variation between years, seasons, ewe age, type of birth (Hinch *et al.* 1985), strain, sex and chill value on the day of birth (Hall *et al.* 1995). Very light lambs and very heavy lambs have the highest mortality rates (Alexander *et al.* 1959). A birth weight of 5.0 kg is considered to be optimal for survival (Fogarty *et al.* 1992), as shown in Figure 2.2, however, this may vary depending on

breed and age of the ewe (Alexander 1984). The mean birth weight of a population is usually below the optimum weight for survival (Mullaney 1969) which implies there needs to be increases in lamb birth weights to improve survival. Mid- to late- pregnancy nutrition is thought to be particularly important for optimising birth weight as this is when 90% of foetal growth occurs. In contrast to the general dogma, Thomson *et al.* (2004) reported high survival rates across a much wider birth weight range (3 – 9 kg) and in different sheep breeds. They attributed the improved survival to better management practices including improved ewe nutrition, heavier ewes and selection for easy care lambing (Thomson *et al.* 2004).

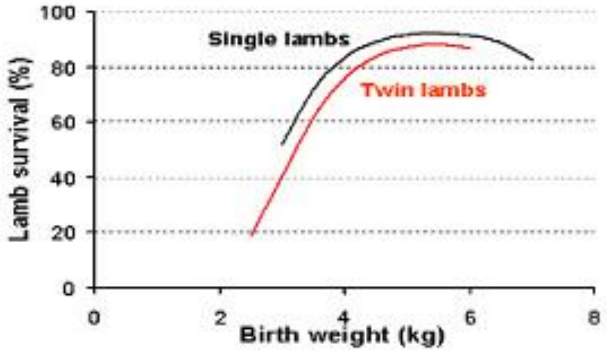

Figure 2.2: Effects of birth weight on lamb survival for single and twin Merino lambs (Hatcher 2007).

The reduction in survival rates at high birth weights is often attributed to the effects of dystocia (Fogarty *et al.* 1992) which is usually caused by foeto-pelvic disproportion arising from an oversized foetus and/or a small maternal pelvic canal (Alexander 1984). In one study, dystocia accounted for 53% of lamb deaths, particularly in heavy lambs (Hall *et al.* 1995).

Mortalities in light birth weight lambs are often attributed to the starvation-exposure-mismothering complex (Fogarty *et al.* 1992), with lighter lambs known to have lower amounts of energy reserves and a reduced thermogenic capacity (Alexander 1984). Hall *et al.* (1995) reported that lambs dying from starvation-exposure-mismothering were an average 0.5 kg lighter at birth than those that survived. Type of birth may also impact on birth weight. As litter size increases, birth weight declines and associated survival levels also decline (Mullaney 1969). In a study by Hinch *et al*. (1985), survival declined markedly for triplets and quadruplets when compared to singles and twins in highly fecund Booroola Merinos and the differences were largely associated with birth weight differences.

2.2.1.2 Placental insufficiency

Placental insufficiency is another way in which lambs may have reduced energy supplies during gestation and at birth. The placenta provides all the energy, respiratory and waste cycling needs of the foetus so factors reducing the efficiency of circulation may result in lambs that are low birth weight, hypoxic, metabolically immature, have reduced energy supplies and impaired cognition. Placental insufficiency may result from inadequate maternal nutrition during pregnancy and increasing litter size (Reynolds *et al.* 2005). Severe maternal undernutrition may result in reduced placental size (Mellor and Murray 1981) and therefore reduced availability of nutrients for the lamb. Likewise, increasing litter size may result in a decrease in availability of nutrients per foetus with a smaller amount of placental tissue available per foetus (Alexander 1978a; Bleker *et al.* 1979; Dwyer *et al.* 2005; Mellor 1983; Mellor and Murray 1981).

The effect of low birth weight on survival has been discussed, however, low birth weights for gestational age may provide an indication that the lamb is metabolically immature

(Greenwood *et al.* 2002; Stafford *et al.* 2007). Metabolic immaturity refers to lambs that are not sufficiently developed to cope efficiently with the extra-uterine environment (Greenwood *et al.* 2002; Mellor 1988). They may appear to be normal, however, vital organs such as the kidneys, lungs and heart may not be sufficiently developed (Greenwood and Bell 2003). Immature lambs often have increased urea levels in the blood stream due to a continued reliance on protein metabolism to supply energy needs instead of carbohydrate metabolism (Thompson *et al.* 2006).

Chronic hypoxia (oxygen deprivation) may develop in lambs suffering placental insufficiency (Mellor 1988) due to inefficient circulation of respiratory gases through the placenta. This often results in lamb deaths due to a compromised ability to thermoregulate due to hypoxia and will be discussed in more detail in a later section.

2.2.1.3 Colostrum availability

Lambs require colostrum soon after birth to not only meet their energy needs but also to obtain immunoglobulins vital for protection against infection. Colostrum production begins just prior to or soon after parturition (McCance and Alexander 1959) and the volume and quality produced may depend on ewe nutrition and litter size (Banchero *et al.* 2004a; Banchero *et al.* 2004b; Mellor and Murray 1986). It has been estimated that lambs require at least 180 – 210 ml/kg birth weight of colostrum in the first day of life to meet their energy demands particularly in cold, outdoor conditions (Mellor and Murray 1986). The volume actually obtained may be compromised when ewes are undernourished during pregnancy resulting in low quality, low volume colostrum that is highly viscous and difficult for the lamb to suckle (McCance and Alexander 1959). High energy diets particularly in late pregnancy have been shown to have a positive influence on colostrum production. Banchero

et al. (2004a; 2004b) reported that feeding cracked corn provided high amounts of glucose to the ewe which was conducive to higher milk production. These authors also reported that the improved diet resulted in earlier lactation, so higher volumes of low viscosity colostrum were available to the lamb at the first suckling attempt. Other authors have also reported that a high plane of nutrition during pregnancy results in low viscosity milk that is easily suckled (Hall *et al.* 1992b; McCance and Alexander 1959; Shubber *et al.* 1979).

Twin- and multiple-bearing ewes may produce higher volumes of colostrum than single-bearing ewes however the volume per lamb may be reduced (Alexander and Lloyd Davies 1959; Hall *et al.* 1992a; Shubber *et al.* 1979). This means that twin lambs are competing with each other to meet their energy needs and in the development of their bond with the ewe. Shubber *et al.* (1979) found that triplet lambs ingested 40% less colostrum in the first 24 hours than single lambs suggesting multiple lambs may not be able to consume as much colostrum as single lambs even if it was available. The onset of lactation may sometimes be delayed in twin bearing ewes (Hall *et al.* 1990; McNeill *et al.* 1998) meaning that milk is not necessarily available when the lamb first successfully attaches to the teat. Hall *et al.* (1990) reported that triplet-bearing crossbred ewes fed to maintain weight had no colostrum in the udder at 90 minutes after parturition whereas single ewes had 218 g at the same time point. This is perhaps not surprising considering triplet bearing ewes had lost more body condition then single bearing ewes (344g compared to 221g) so would have less energy reserves to draw on for colostrum production (Hall *et al.* 1990).

The consequences of a reduced volume and quality of colostrum include insufficient energy leading to starvation, compromised immunity and potentially an inferior quality ewe-lamb

bond. Holst *et al.*(1996) showed that lambs suckling ewes with low volumes of highly viscous colostrum had increased frequency and duration of suckling bouts and this was particularly noticeable in twin lambs. It is thought that receipt of colostrum provides positive feedback to the lamb thereby improving the establishment of the ewe-lamb bond (Nowak *et al.* 1997). Alexander and Williams (1966a) have also demonstrated the importance of positive feedback where lambs that were prevented from suckling at the udder discontinued their teat seeking behaviour compared to lambs that were allowed to suckle.

2.2.2 Birth environment

The ewe also has a role in providing a benign environment for the newborn lamb. This may be through a short, uncomplicated parturition (see Alexander 1984; Haughey 1980a for reviews) but also behaviourally through selection of an appropriate birth site to limit stressors such as inclement weather effects and interference from other ewes.

2.2.2.1 Birth process

Parturition is by nature a stressful process and necessarily so to stimulate proper function in the neonate that must change rapidly from a fluid breathing foetus to an air breathing neonate along with associated changes in thermoregulation and energy supply. However, this stress can be exacerbated by dystocia due to foeto-pelvic disproportion or malpresentation, or prolonged due to slow birth which may result in lambs with central nervous system damage and hypoxia that in turn, affects the ability of the lamb to function normally (Mellor 1988). Dystocia can also affect the ability of the ewe to adequately groom and suckle the lamb (Dwyer *et al.* 2001; Shelley 1970).

Parturition is under tight physiological control and relies on hormonal signals from both the foetus and the ewe. Parturition is initiated by a cortisol surge in the neonate that is necessary to ensure all maturation of vital tissues such as lungs, kidneys and the gut has occurred (Mellor 1988), although other hormones may also be involved. Disruptions to this hormonal control from either the ewe or the lamb may result in a prolonged birth which has consequences for the subsequent survival of the lamb. This may be from birth injury (Haughey 1980b) or disruptions to the physiological control of maternal behaviour due to an extended birth and recovery time (Poindron *et al.* 1984) leading to increased desertions of lambs (Alexander 1960; Alexander *et al.* 1959).

Dystocia occurs in 4 – 36% (Mellor 1988) of parturient ewes for a number of reasons including large foetal size, malpresentation and nutritional status of the ewe. It is thought to account directly for 30% of total lamb losses (Duff *et al.* 1982) but may be implicated in up to 65% of lamb losses (Haughey 1980a). Dystocia may also be influenced by other factors such as parity, genetics and litter size (Kerslake *et al.* 2005). Lambs may die as a direct result of dystocia while others may die hours or days later due to injury or trauma caused during parturition which affects their physical ability to behave appropriately in the neonatal period (Dwyer 2003).

Central nervous system (CNS) damage is one of the main indicators used to identify death associated with dystocia or difficult/prolonged birth. In some instances lambs may be diagnosed as dying from starvation-mismothering-exposure but this is a consequence of the

trauma of a difficult birth. However, some CNS damage is often evident in lambs born through a normal delivery (Haughey 1980a) although it is typically less severe than in lambs that die following dystocia. Lambs with more severe birth trauma to the CNS tend to die nearer to birth than lambs that die from other causes associated with birth trauma (Haughey 1982). Presumably, lambs that do not die during or soon after parturition (<6 h) from birth trauma probably die as a result of starvation-exposure due to impaired teat seeking ability caused by CNS damage and therefore a lack of energy and an associated inability to thermoregulate.

Acute hypoxia may also occur with dystocia. Elevated blood lactate levels provide an indication of those lambs suffering hypoxia (Barlow *et al.* 1987). Acute hypoxia, associated with the birth process, is usually transient, lasting only about 30 minutes (Eales and Small 1985), but during this time the ability of the lamb to thermoregulate is impaired (Alexander and Williams 1970). This may result in lamb deaths particularly in cold, wet and windy weather.

Difficult and prolonged births can also have implications on the development of critical neonatal behaviours (Haughey 1980a) and subsequent development of the ewe-lamb bond (Dwyer 2003; Shelley 1970). Dwyer (2003) found that manual delivery (required due to malpresentation or failure to progress through parturition after two hours) of Suffolk and Scottish Blackface lambs resulted in delayed progression of all behaviours from shaking the head through to suckling. Lambs that were assisted during birth took an extra 10 minutes to suckle compared to lambs born without assistance (Dwyer 2003). Similarly, Haughey (1980a) also showed that lambs that had an artificially delayed vaginal birth were less likely

to suckle successfully than those lambs delivered normally (28% vs. 1%). Birth difficulty can also influence maternal behaviour (Shelley 1970) and therefore subsequent ewe-lamb bonding. Dwyer *et al.* (2001) found that ewes that had been assisted (full manual delivery due to prolonged or difficult birth) at lambing took significantly longer to begin grooming their lambs than ewes that did not require assistance (348 s vs. 54 s, respectively).

2.2.2.2 Critical peri- and post-parturient behaviours in the ewe

A way of providing a benign environment for the lamb is through the selection of a birth site and self-isolation from the flock, although these behaviours are not necessarily exhibited in all breeds (Alexander *et al.* 1990a). Birth site selection may be important to lamb survival in terms of protection from environmental influences such as wet, windy weather and from predation. The ability of ewes to select appropriate birth sites appears to be breed or management dependent with wild breeds, such as Soay and Rocky Mountain Bighorn, being more likely to seek protection from the elements (Geist 1971; Shillito and Hoyland 1971) than domesticated breeds such as Corriedale, Merino or Scottish Blackface (Alexander *et al.* 1990a; Lynch *et al.* 1992; Stevens *et al.* 1981). Geist (1971) reported that Bighorn ewes may seek shelter and isolation up to two weeks before parturition while Stevens *et al.* (1981) reported that it is unclear if Merino ewes seek isolation or get left behind as the mob moves away.

An example of a management dependent influence on shelter seeking is that if Merino ewes are shorn within a few weeks of lambing they are more likely to seek shelter at lambing time (Alexander and Lynch 1976; Alexander *et al.* 1979; Lynch and Alexander 1977; Mottershead *et al.* 1982) than when in full wool. Even in these studies, selection of a birth site was not

always clear and may have been related to where other ewes had recently given birth as peri-parturient ewes are attracted to fresh foetal fluids (Lynch *et al.* 1992). Isolation from the flock also becomes important following parturition as it allows the ewe and the lamb to bond without interference from other peri-parturient ewes that may steal a newborn lamb and subsequently abandon it following the birth of their own lamb (Nowak and Poindron 2006).

The length of time the ewe spends alone with her lamb/s at the birth site will also facilitate the development of the ewe-lamb bond and is particularly important for twins (Nowak 1996). Alexander *et al.* (1983) found that the proportion of ewes permanently separated from a twin decreased (from nearly 80% down to 5%) as the time spent on the birth site increased (from 0 to >4 hours). It has been suggested that the longer time spent on the birth site allowed the ewe to recognise the number of offspring and bond with the whole litter (Alexander *et al.* 1983) although the role of the lamb in this bonding process was not considered in this study. Six hours spent on the birth site is considered ideal for bond formation and subsequent lamb survival (Nowak 1996), however, some breeds, in particular the Merino, may move from the birth site much earlier with Alexander *et al.* (1983) reporting an average of two hours. In the latter study, drought conditions prevailed so ewes were on very poor pastures and were being fed supplementary grain twice a day which may have impacted on their post-parturient behaviour. However, there appears to be no clear data to confirm nutritional impacts on time at the birth site.

2.2.3 Stimulus for the lamb

2.2.3.1 Maternal behaviour and formation of the ewe-lamb bond

Following birth, quick progression by the ewe from expulsion to standing and grooming the lamb is considered normal (Lynch *et al.* 1992) but this can be delayed in primiparous ewes or ewes that experienced difficult/prolonged births (Arnold and Morgan 1975a). The ewe licks the lamb vigorously, due to a strong attraction to foetal fluids, which dries the lamb and results in maternal recognition of the offspring (Nowak 1996). While performing this grooming behaviour, the ewe typically emits a deep, soft bleat or rumbling (Dwyer *et al.* 1998) which may facilitate recognition of the ewe by the lamb (Nowak 1996; Sebe *et al.* 2007).

It is thought that ewe behaviour influences the activity of the lamb and vice versa. It is likely that grooming behaviour will have more influence on time to suckle rather than time to stand as ewe behaviour can influence the ability of the lamb to access the udder. For example, ewes may move away from the lamb or circle which will affect their ability to suckle. Wassmuth *et al.* (2001) suggested that more active cleaning and licking behaviour by the ewe will stimulate the lamb to stand earlier and therefore present an earlier opportunity to suckle. However, other authors have found a difference in the time taken for Mule and Blackface lambs to stand and suckle despite there being no observed breed differences in maternal behaviour suggesting that ewe and lamb behaviour did not affect each other (O'Connor and Lawrence 1992). Several authors report that the behaviour of the lamb is at least as important as the behaviour of the mother (Dwyer 2003; Dwyer and Lawrence 1999; Nowak and Lindsay 1992) and this is possibly best demonstrated when ewes display reduced maternal

behaviour when they have a dead lamb that provides no reciprocal stimulus for the ewe to respond to (Sawalha *et al.* 2007).

Once the lamb is standing, ewe behaviour remains important in the initiation of suckling. Ideally, the ewe will stand still and allow the lamb to locate the udder and suckle. However, some ewes, especially primiparous ewes, may display aberrant behaviours such as butting, circling or abandoning the lamb (Cloete and Scholtz 1998; Everett-Hincks *et al.* 2005b; Grandinson 2005; Vince and Billing 1986). Cloete and Scholtz (1998) found that a higher proportion of maiden ewes backed, circled and butted their lambs than mature ewes. These authors also observed differences between ewes selected for a high or low rearing ability where the former were less likely to back, circle or butt their lambs.

Formation of a robust ewe-lamb bond is essential to the survival of the lamb as the ewe is the sole source of nutrition for the lamb in the first few weeks of life. Bond formation assists in mutual recognition and acceptance thereby allowing the lamb to suckle. The stimulus for bonding begins as soon as the ewe begins licking birth fluids (Vince and Billing 1986) and continues after the lamb is expelled and is facilitated by grooming. Ideally this grooming phase will continue for a few hours, however, interruptions are common. These interruptions may be from other peri-parturient ewes, litter mates, human interference or due to the ewe moving away to graze or rejoin the flock (Alexander *et al.* 1983; Dwyer 2008a).

Nutrition and body condition during pregnancy may also impact on ewe behaviour following birth. Dwyer *et al.* (2003) found that under-nourished, low body condition score Scottish

Blackface ewes spent less time grooming and more time eating in the first 30 minutes after birth compared to adequately nourished ewes. Similarly, Everett-Hincks *et al.* (2005a) reported that ewes lambing on a higher pasture allowance were more likely to remain with their lambs after tagging than ewes at lower pasture availability. Other authors (Putu 1990; Putu *et al.* 1988b; Thomson and Thomson 1949) have found similar patterns with adequately fed ewes being more attentive and spending more time at the birth site than undernourished ewes. This improved behaviour may well be particularly important for twin lamb survival (Putu *et al.* 1988b).

2.2.4 Conclusion

The role of the ewe in the subsequent survival of her lamb is well documented. It is clear that management strategies, such as the provision of adequate nutrition throughout pregnancy, have a critical influence on lamb survival. Likewise, the display of appropriate maternal behaviour such as grooming and spending time on the birth site to allow for the formation of a strong ewe-lamb bond is also paramount. There appears to be few areas of limited knowledge in the contribution of the ewe to lamb survival (Ferguson *et al.* 2007) so the next section will focus on the role of the lamb and how important they are in contributing (both behaviourally and physiologically) to their own survival.

2.3 Lamb contribution to survival

2.3.1 Access energy by suckling soon after birth and regularly

Prior to suckling, lambs rely on stored energy in fat and muscle for their energy needs. The energy available from these sources is limited so it is essential that the lamb suckles soon

after birth to supplement their energy supply. Lambs with a high probability of survival tend to have higher pre-suckling levels of plasma glucose, fructose and NEFAs (Barlow *et al.* 1987; Mellor and Pearson 1977; Stafford *et al.* 2007), metabolites associated with energy supply.

Following birth, the lamb performs innate behaviours essential to life including shaking the head and giving several gasps to settle into regular respiration before progressing to standing and suckling. Progression to standing usually occurs within 30 minutes of birth and most lambs begin to suckle within 1–2 hours of birth (Nowak *et al.* 2008), although significant breed differences in these times have been reported (Cloete and Scholtz 1998; Slee and Springbett 1986). Slee and Springbett (1986) found that Scottish Blackface and wild breeds (Soay and Boreray) stood and reached the udder in an average of 17 and 30 minutes, respectively, while other domesticated breeds such as Finnish Landrace took an average of 54 minutes to stand and 94 minutes to suckle.

Once the initial behaviours have been performed, the lamb must be ready to follow and maintain contact with the ewe as she moves away from the birth site soon after birth. This is particularly evident in Merino ewes who exhibit a high degree of gregariousness (Alexander *et al.* 1983). The ability of the lamb to maintain contact may in part depend on the availability of adequate lamb energy supplies but also on the cognitive ability of the lamb to register and respond to the ewe's behavioural cues. Oppong-Anane (1991) found that Merino lambs often had the physiological capability to follow the ewe, as measured by maximal oxygen consumption, but there was considerable variability in the actual following and recognition behaviour displayed by lambs, although the reason for this was unclear.

2.3.2 Adapt to a new environment

The newborn lamb has to be able to maintain contact with its mother, often under challenging conditions. Its behavioural responsiveness needs to be maintained while it is also adapting to weather and other stressors. The adaptation process may be impacted by the trauma associated with a difficult birth (and associated hypoxia or physical injury/bruising), immaturity at birth (associated hypoxia) and exposure to extreme weather conditions. Figure 2.3 outlines the interactions between stressors and how this may impact on lamb survival. These stressors may have a physiological and/or behavioural impact (impaired mobility, recognition or cognition) on the ability of the lamb to survive. Cold stress in particular is a major factor known to influence lamb behaviours in the neonatal period (Alexander and Williams 1966b) however, effects of cold on lamb behaviour have not been well documented. Under cold, wet and windy conditions, teat seeking activity is inhibited and if cold exposure continues and the lamb's rectal temperature falls below 38°C, teat seeking activity continues to be depressed even if environmental conditions improve (Alexander and Williams 1966b). Other authors have reported that lambs kept under cold conditions (-1°C) ingest less milk than those in warm conditions (20°C) although this study did not look specifically at lamb behaviours (Thompson 1983). In such stressful situations, it is important for the lamb to maintain contact with the ewe to ensure food supply and physical protection for survival. The lamb must be able to respond and adapt their behaviour in a way which responds to the cues being given by the ewe.

2.3.2.1 Thermoregulation

The thermoregulatory capacity of the lamb is essential for its subsequent survival in a stressful new environment. Losses due to cold are estimated to account for approximately 30% of total lamb losses in New Zealand (McCutcheon *et al.* 1981). In the milder conditions experienced during lambing in Australia, losses are more likely to be around 10% (Alexander *et al.* 1980), although under extreme weather conditions, losses due to hypothermia have been reported to be up to 52% (Arnold and Morgan 1975b). Hypothermia is likely to be a primary cause for those lambs that are diagnosed of dying from starvation/mismothering, therefore, hypothermia may account for a much higher percentage of lamb losses. Following birth, the lamb must adapt rapidly to a cold environment by increasing heat production through metabolism of energy substrates which include brown adipose tissue initially, followed by the energy obtained through colostrum ingestion. If the lamb is unable to meet this thermoregulatory demand, then starvation due to depletion of body reserves or death from exposure due to hypothermia may ensue.

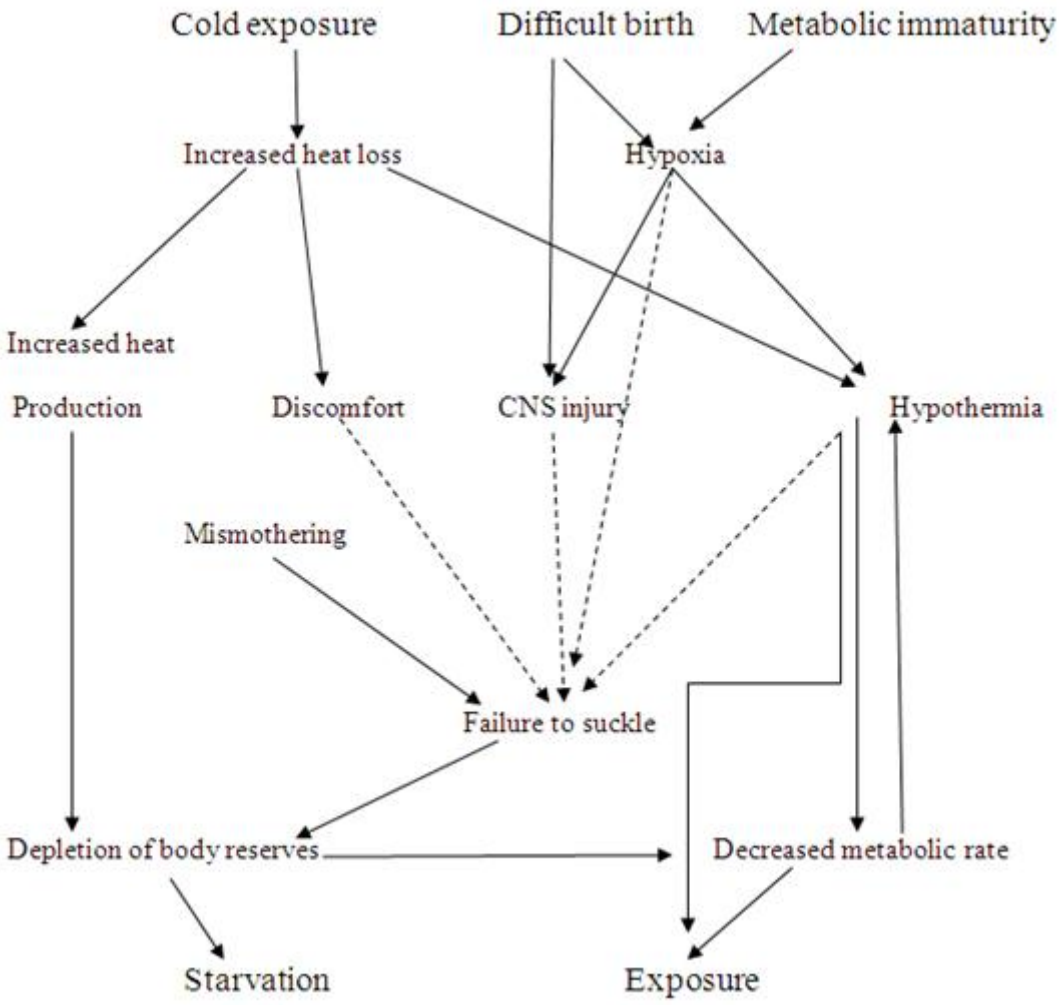

Figure 2.3: Stressors faced by the neonatal lamb and interactions with factors affecting lamb survival (adapted from McCutcheon *et al.* 1981). Dashed lines indicate behavioural factors while solid lines indicate physiological factors.

Lambs increase heat production through an increase in their metabolic rate to summit metabolism. Summit metabolism refers to the maximum metabolic response to cold (Alexander 1979) after which body temperature begins to decline under continued cold exposure without access to further energy substrates (either body reserves or colostrum). Rapid attainment of summit metabolism is necessary for the lamb to maintain body

temperature under cold conditions, however, there is significant variation in summit metabolism due to factors such as breed (Slee 1981; Slee *et al.* 1991; Slee and Stott 1986), pregnancy nutrition (Alexander 1962d) and current physiological state (e.g. whether the lamb is hypoxic or has suckled or not). The ability of the lamb to maintain summit metabolism may provide an indicator of their resistance to hypothermia.

Slee and Springbett (1986) reported that lambs that found the udder quickly tended to have greater resistance to hypothermia across 10 breeds with Merino and Finn lambs being the slowest. Lambs of these breeds were also more likely to be susceptible to hypothermia as measured by rectal temperatures one hour after cold exposure.

2.3.2.2 Recover from hypoxia

Lambs may be under stress in the early neonatal period due to acute or chronic hypoxia. Acute hypoxia is associated with a prolonged birth and can be seen by high levels of plasma lactate soon after birth (Barlow *et al.* 1987). Acute hypoxia is usually transient and lactate levels are generally back to normal levels within an hour after birth (Comline and Silver 1972). Chronic hypoxia on the other hand is caused by placental insufficiency during pregnancy and can be distinguished from acute hypoxia by high packed cell volume and low plasma fructose and glucose levels (Barlow *et al.* 1987; Mellor and Pearson 1977) along with high levels of lactate soon after birth. The effects of chronic hypoxia do not disappear after birth and can have implications for the subsequent survival of the lamb. Hypoxia, both chronic and acute, has been implicated in 35 – 59% of lambs that die soon after birth (Barlow *et al.* 1987). However, this report is from high litter size flocks (90% of lambs from twin and

triplet litters) and percentage losses may be lower in Australian Merino flocks where twinning is usually less than 30% (Kelly 1992; Kilgour 1992; Kleeman and Walker 2005).

It has been shown that pre-partum hypoxia has a detrimental effect on the ability of the lamb to reach and maintain summit metabolism (Eales and Small 1980) and it is therefore essential that the lamb recovers from hypoxia in order to be able to thermoregulate adequately. Likewise, the cognitive capacity of the lamb may also be affected by hypoxia, particularly chronic hypoxia, which may have consequences for the subsequent behaviour of the lamb and development of recognition of the ewe. Cock *et al*. (2001) have shown that lambs suffering intra-uterine growth retardation perform poorly in behaviour tests (maze and obstacle course) designed to test their cognitive ability up to five weeks of age compared to normal lambs. It is also well established in the human literature that infants suffering hypoxia in the neonatal period may have impaired cognitive ability that may be sustained over time (Volpe 2008).

2.3.3 Lamb behavioural responses to the ewe

The critical early lamb behaviours of standing and suckling need to occur quickly to ensure the lamb has access to an energy supply other than their endogenous reserves. Progression through these behaviours must occur regardless of the stimulation provided by the ewe but as discussed in an earlier section, may progress more rapidly with appropriate grooming from the ewe. The time to perform these behaviours has often been used to assess the likelihood of lamb survival along with other subjective behavioural criteria (Cloete 1993; Dwyer *et al.* 2005; Owens *et al.* 1985; Slee and Springbett 1986).

As discussed in a previous section, it is essential for the lamb to perform critical early behaviours as soon as possible to establish contact and a food supply. However, after these behaviours occur and the ewe begins to move away from the birth site to feed or rejoin the flock, the lamb must be ready to follow and maintain contact with the ewe. This may be more important for particular breeds such as the Merino which are more gregarious and have high twin lamb losses (Stevens *et al.* 1982; Stevens *et al.* 1984).

It has been established that the ewe ceases the grooming behaviour necessary for lambs to stand and suckle within a few hours of birth (Poindron *et al.* 1984). This suggests that from the time grooming ceases the lamb's capacity to respond to and follow the ewe becomes more important in maintaining the contact necessary to continue the ewe-lamb relationship. However, there are no studies that have clearly tested whether this is true nor identified the age of the lamb when these changes might impact on survival.

2.3.4 Lamb vigour

Lamb vigour has often been used as an indicator of the probability of survival (Alexander *et al.* 1959; Alexander *et al.* 1990b; Wassmuth *et al.* 2001). However, a consistent and well defined explanation of lamb vigour is often lacking (O'Connor and Lawrence 1992; Owens *et al.* 1985). Published definitions of lamb vigour include; general alertness, activity or 'strength' of the lamb (Alexander *et al.* 1990b; Nash *et al.* 1996; Owens *et al.* 1985); time taken to perform certain behaviours, generally standing and suckling (Dwyer *et al.* 2005; Owens *et al.* 1985); growth rate to weaning (Wassmuth *et al.* 2001); or in some cases, it is not defined at all but still used as a causal factor for lamb survival (Alexander *et al.* 1959). When lamb vigour has been assessed, these definitions have been used alone or in combination (Alexander *et al.* 1959; Owens *et al.* 1985; Pfister *et al.* 2006b).

To date, most of the methods for measuring lamb vigour have focused on behavioural (time it takes the lamb to stand and suckle) and/or physiological measures such as rectal temperature within 1 – 3 hours of birth. One aspect of vigour assessment that warrants further consideration is the timing of the assessment. Ideally, it should coincide with the time when vigour is of greatest importance to survival in a particular production environment. This may be at birth in the case of intensive lambing systems (Dwyer 2008a) or alternatively, it may be when the ewe is moving away from the birth site in extensive systems (Murphy *et al.* 1994a; Putu *et al.* 1988a) and/or when the lamb is under a physiological stress such as that experienced due to cold, a difficult birth, placental insufficiency or starvation.

2.3.5 Measures of lamb vigour

2.3.5.1 Behavioural

Subjective assessments have often been used to describe lamb vigour (Alexander *et al.* 1959; Alexander *et al.* 1990b; Owens *et al.* 1985; Pfister *et al.* 2006b) and the scales used vary between studies. Alexander *et al.* (1959) described lambs that died as being of poor vigour while lambs that survived were vigorous but no scale or description of what vigour meant was provided. In this study, timed behaviours, including time to stand and suckle, were also measured but these were not used in the assessment of lamb vigour (Alexander *et al.* 1959). Similarly, Owens *et al*. (1985) used a classification of poor, fair or good to describe lamb vigour but decided that this was inadequate so incorporated timed behaviours (time to attempt to stand, time to stand, time to attempt to suckle and time to suckle successfully) in their assessment. None of the data for the subjective assessments was presented in their study, so a comparison between subjective and timed behaviours was not possible.

Various numerical scoring systems have been developed to measure lamb vigour (Holst 1987) with some requiring multiple observations while others are scored on one observation. Holst (1987) used a scale of 1 – 5 to describe lamb vigour using the definitions in Table 2.1. This scoring system was more useful than the arbitrary use of terms poor, fair or good to describe lamb vigour (Alexander *et al.* 1990b) as they are defined with some measurable behavioural parameters.

Table 2.1: Definition of lamb vigour score (Holst 1987).

Score	Description
1	Doesn't stand for at least 40 min; little or no teat-seeking drive
2	Attempts to stand after 30 min; low teat-seeking drive and tendency to follow ewe
3	Shakes head within 30 sec; attempts to stand within 15 min; seeking teat within 10 min of standing; follows ewe but distracted by other moving objects
4	Attempts to stand within 10 min of birth; seeking teat within 5 min of standing; strong tendency to follow ewe
5	Attempts to stand within 5 min of birth; follows ewe closely

Simple additive measures have also been used where lambs were observed and scored a number of times in the first 24 to 40 hours of life. Measures were then added to give a total vigour score. Theriez and Villette (1985) observed lambs six times, from birth to 40 hours after birth, and assigned a score of 0 (lamb lying down) or 1 (lamb attempting to stand or standing) at each observation. These scores were added to give a total score of between 0 and 6, with 0 being least vigorous. Lambs with low scores were considered to be "high-risk" in terms of survival (Theriez and Villette 1985).

Some UK groups (North of England Mule Sheep Association, Suffolk Sheep Society) are advocating the use of vigour scoring and recording in genetic improvement programs to improve lamb survival (Macfarlane *et al.* 2010; Matheson *et al.* 2010). This scoring system uses a scale of 1 – 4 with 1 being highly vigorous and the assessment is made at 10 minutes of age (Matheson *et al.* 2010). This score is combined with a similar measure of lambing ease and mothering ability to assist the producer when deciding whether to breed from these lambs in the future.

Subjective lamb vigour scores are currently being applied in a large scale progeny testing program in Australia (Brien *et al.* 2009). The score is based on a 1 – 5 scale with a score of 1 indicating a very vigorous lamb. The following table (Table 2.2) outlines the definition of each score which is measured when the lamb has been restrained and then within 30 seconds of release. Lambs are scored at various ages from birth to 24 hours of age and an age is estimated at the time of assessment.

Table 2.2: Lamb vigour score used in the information nucleus flock by the Sheep CRC (Brien *et al.* 2009).

Score	Description
1	Constant struggle – bleat in response to ewe – on release reaches ewe quickly and follows
2	Regular struggle while held – moves to the ewe on release – bleating common
3	Some struggle – walking in direction of ewe bleats but no contact – may bleat
4	Some struggle – attempts to walk but aimless – no apparent response to ewe bleats
5	Little movement when held – lies on release

Subjective lamb vigour scores are useful for comparing animals within a study, however, accurate comparisons between studies are usually difficult due to the wide range of

methodologies used and the subjective nature of the assessment. It is important to adequately describe the scale being used for vigour scores otherwise the measures used are arbitrary and interpretation of the data is therefore difficult. Some authors have combined physiological measures with behavioural scores to achieve more robust assessments of lamb vigour (Pfister *et al.* 2006b). Pfister *et al.* (2006b) measured five factors using a revised version of the APGAR scoring system used for human infants (Volpe 2008). Heart rate, respiration, activity, appearance and sucking ability were all measured on a scale of 0 – 2 and then added together to produce a final score. Heart and respiration rates were measured objectively using monitors and the 0 – 2 scores were then applied while activity and appearance were based on the struggling attempts of the lamb under restraint and the lamb's attentiveness (Pfister *et al.* 2006b). Sucking ability was scored as 2 if the lamb suckled without assistance, 1 if it required assistance to attach but then suckled and 0 if it did not suckle. When added together, lambs scoring 0 – 3 were categorised as "probable death", 4 – 6 "survive with intervention" and 7 – 10 "strong healthy lambs with no intervention" (Pfister *et al.* 2006b). The utility of this vigour scoring system in the context of lamb survival requires further investigation.

Lamb vigour has also been described using measures of time taken for lambs to progress through a series of behaviours (Cloete 1993; Dwyer *et al.* 2005; Owens *et al.* 1985; Slee and Springbett 1986). These behaviours commonly include time taken to stand and suckle. Some authors use further classifications such as time to: attempt to stand, stand for a certain time period, first seek teats, reach the udder and apparently suckle (Cloete 1993; Dwyer 2003; Owens *et al.* 1985). Some also use the amount of time the lamb spends close to the mother and playing as measures of vigour (Dwyer 2003). The behaviours measured and their definitions for a number of studies (Dwyer 2003; Dwyer *et al.* 1996) are outlined below

(Table 2.3). Similar measures and definitions are used in other studies where timed lamb behaviours are measured.

Table 2.3: Definitions of lamb behaviours (Dwyer 2003).

Behaviour	Definition
Shakes head	Lamb raises and shakes head
To knees	Lamb on chest, pushes up on knees, supporting part of body off the ground
Attempts to stand	Lamb on knees, supports part of its weight on at least one foot
Stands	Lamb supports itself on all four feet for at least 5 s
To udder	Lamb in parallel inverse position with head nudging ewe in udder region
Suck attempt	Lamb in parallel inverse position, head beneath ewe in udder region, prevented from sucking by ewe movement or leaves udder region within 5 s
Successful suck	Lamb has teat in its mouth, in correct position, appears to be sucking for at least 5 s
Playing	Lamb running, jumping or frolicking, in a coordinated manner, with no apparent purpose

Timed behaviours have some limitations as a measure of lamb vigour due to the fact that the behaviour of the ewe may influence the expression of these behaviours (Dwyer 2003). The visual, auditory, oral and tactile stimuli provided by the ewe, can influence the time taken for the lamb to stand and seek the udder (Lynch *et al.* 1992). If the ewe does not start grooming the lamb or does not emit a low rumbling sound then the lamb may remain quiet and still for a longer period of time (Lynch *et al.* 1992). Notwithstanding this, timed behaviours still provide an objective means for assessing vigour.

From a practical perspective, timing the progression of behaviours in neonatal lambs is a long and laborious process and often requires the presence of more than one person. It is most useful in experimental situations where, generally, only a small number of animals are being

observed and there may be the option for video recording. However, on farm, it would be too time consuming and the application of a subjective assessment as discussed above would be more practicable and have wider appeal. Ideally, an objective, easy to measure parameter would have the most appeal when measuring lamb vigour particularly for genetic improvement of the trait. To date, no such measure has been identified.

2.3.5.2 Behaviour tests

Lamb behavioural tests have been used for various applications including to test how the lamb discriminates its mother from other ewes (Alexander and Shillito-Walser 1978; Nowak 1990; Nowak and Lindsay 1992; Nowak *et al.* 1989; Sebe *et al.* 2007); the following ability of the lamb (Oppong-Anane 1991; Shillito Walser *et al.* 1985); the cognitive ability of the lamb (Pfister *et al.* 2006b) and to test impairments to the lamb due to birth difficulty or other stressors (Haughey 1980a; Nowak *et al.* 1987; Pfister *et al.* 2006a). Although not considered specifically in these studies, all these tests are providing an assessment of lamb vigour and it would be expected that more vigorous lambs would be able to discriminate between their mother and an alien ewe and reach her more quickly than a less vigorous lamb. A vigorous lamb should also be able to progress through a maze or some other test arena more successfully, particularly if viability is associated with cognitive function as appears to be the case in the human neonate (Volpe 2008).

Behavioural tests could also be used to test the ability of the lamb to cope with a stressor such as cold. Lambs exposed to cold challenges tend to have attenuated responses to maternal signals (Alexander and Williams 1966b) and therefore, it would seem that a test should be completed in a stressful environment where contrasts are likely to be highlighted. In general,

it would be expected that the more vigorous lambs would be able to perform such tests more effectively than a less vigorous lamb as they are likely to have suckled, had a less stressful birth and therefore, not have impaired cognitive function.

The age of the lamb is also an important consideration when using behavioural tests. Various authors (Bickell *et al.* 2009; Lindsay *et al.* 1990; Nowak *et al.* 1987; Nowak *et al.* 1989) have reported that lambs younger than 12 hours of age are not able to perform discriminatory tests and consistent performance in these tests is not developed until the lamb is at least 24 hours of age (Nowak *et al.* 1989). The type of test used may also influence the performance of the lamb. The development of a test of the responsiveness of a lamb may be more useful than its ability to discriminate or its cognitive ability under benign conditions.

Aside from the age of the lamb, the timing of the test according to other events, such as when the ewe is moving away from the birth site, also needs to be considered. Movement away from the birth site is a critical time for the lamb as it needs to respond to the visual and auditory cues from the ewe to maintain contact with her as she moves. The lamb needs to be able to do this in a selective way to reduce the likelihood of mismothering and therefore ensure a continued energy supply.

2.3.5.3 Physiological measures of vigour

Blood hormones and metabolites

Blood metabolites can provide a means of assessing lamb vigour by providing information on the viability of the lamb particularly in relation to pre and parturient stress. Barlow *et al.*

(1987) outlined a number of blood parameters that are useful in determining if a lamb is compromised due to placental insufficiency, hypoxia or metabolic immaturity as outlined in Table 2.4. However, the time at which these measures are taken and the preceding conditions that the lamb has been exposed to can alter metabolite levels. It may be more important to sample blood a number of times in order to gain a more accurate profile of the specific metabolite and how this in turn relates to the expression of vigour.

Table 2.4: Blood metabolites associated with placental insufficiency, hypoxia and metabolic immaturity (adapted from Barlow *et al.* 1987).

Category	Diagnostic variable	Results in
Placental insufficiency	High PCV	Chronic hypoxia
	High lactate	Foetal undernutrition and
	Low fructose	growth retardation
	Low glucose	Inhibited heat production
	Low T4	
	High cortisol	
	Low birth weight	
	Low rectal temperature	
	Age at death <12h	
Acute intra-partum hypoxia	PCV not high	Acute foetal hypoxia
	High lactate	Inhibited heat production
	Low T4	
	Low rectal temperature	
	Age at death <12h	
Metabolic immaturity	Low T4	Inhibited heat production
	Low insulin	
	Low fructose	
	Low rectal temperature	

Blood concentrations of thyroid hormones, both triiodothyronine (T3) and thyroxine (T4), in neonates have been shown to relate to their thermogenic capacity (Mellor and Pearson 1977; Schermer *et al.* 1996). Lambs with higher levels of thyroid hormones are more likely to survive (Barlow *et al.* 1987) due to an improved thermoregulatory capacity. This is particularly important as a vast number of lamb deaths are related to hypothermia. Low levels of plasma thyroid hormones are associated with an increased reliance on shivering

thermogenesis for heat production (Clarke *et al.* 1997b) which is considered to be an inefficient means of heat production. Stafford *et al*. (2007) measured plasma T4 levels in neonatal twin and triplet lambs and found that triplet lambs have lower levels and were less likely to survive.

Blood measures such as glucose, fructose (Stafford *et al.* 2007) and non esterified fatty acids also provide indications of energy reserves in the neonate. These measures are particularly important prior to suckling as levels, particularly glucose, increase once the lamb has suckled (Daniels *et al.* 1974). Lambs with higher levels of these energy indicators at birth are considered to be at an advantage compared to lambs with low levels (Barlow *et al.* 1987). Fructose levels may also provide an indicator of metabolic maturity as triplet lambs have been shown to have lower levels than twin lambs (Stafford *et al.* 2007) and are usually smaller for gestational age indicating immaturity. Protein levels as measured by amino acids or urea also provide an indication of the degree of maturity of the lamb (Thompson *et al.* 2006). Protein metabolism for energy production is associated with foetal development whereas postnatally, carbohydrate metabolism predominates (Greenwood *et al.* 2002). Lambs with high protein levels would therefore be considered to be less mature as they are still relying on foetal metabolic mechanisms.

Blood lactate levels in the new born provide an indication of the degree of hypoxia suffered by the lamb during gestation and/or birth. Lactate levels are only useful if taken very soon after birth as lactate is quickly metabolised (Comline and Silver 1972). High levels indicate that the lamb has been excessively stressed during the birth process often associated with a difficult or a prolonged birth. High lactate levels have been associated with lambs that are

more likely to die and also with increased litter size (Barlow *et al.* 1987). High lactate levels along with high packed cell volume may indicate lambs that are suffering chronic hypoxia due to placental insufficiency, however, low PCV and high lactate would indicate lambs undergoing acute hypoxia (Barlow *et al.* 1987).

Rectal temperature

Rectal temperature at various ages after birth has been used as a measure of the viability of the newborn lamb (Barlow *et al.* 1987; Dwyer and Morgan 2006; Miller *et al.* 2010; Stafford *et al.* 2007). Lambs with rectal temperatures <37°C are generally less likely to survive (Barlow *et al.* 1987). Some authors have attempted to relate rectal temperatures to timed behaviours and various vigour scores of lambs (Dwyer and Morgan 2006; Miller *et al.* 2010; Slee and Springbett 1986). Miller *et al.* (2010) found no association between rectal temperature and time to stand and suckle, however, Dwyer and Morgan (2006) reported that lambs that are slow to stand and suckle have lower rectal temperatures within 1 hour of birth and at 24 hours of age than those that stood and suckled quickly and this effect was still evident at 72 hours of age. Similarly, Slee and Springbett (1986) reported that lambs with low rectal temperatures were more likely to fail to reach the udder. The discrepancy between the findings of Miller *et al.* (2010) and the other authors may be due to breed differences as their study used Merino lambs only. However, Slee and Springbett (1986) found this association across ten breeds of lambs including Merinos.

2.3.5.4 Response to cold

The ability of the lamb to respond to a cold challenge is another potential measure of lamb vigour. Lamb vigour is thought to be associated with the lamb's thermoregulatory capacity

with more vigorous lambs having a better ability to thermoregulate (Wassmuth *et al.* 2001). Slee and Springbett (1986) found that lambs that were able to find the udder quickly tended to have greater resistance to hypothermia across ten breeds.

Lambs respond to cold via two mechanisms, namely shivering and non-shivering thermogenesis. Non-shivering thermogenesis accounts for approximately 40% of heat generation (Alexander and Williams 1968) and is predominately associated with brown adipose tissue (BAT) metabolism. BAT is located in the peri-renal, abdominal, inguinal and pre-scapular regions of the lamb (Alexander and Bell 1975). BAT is highly vascularised with many mitochondria that contain uncoupling proteins. Cold exposure leads to stimulation of the sympathetic nervous system resulting in the release of noradrenaline. Noradrenaline binds to β-adrenoreceptors in the plasma membrane of adipocytes stimulating adenylcyclase to form cAMP. cAMP activates a hormone sensitive lipase, via protein kinases, to mobilise fatty acids from the stored triacylglycerols within the adipose cell. These fatty acids are then transported to the mitochondria where they are oxidised. Fatty acid oxidation is uncoupled from the synthesis of ATP by the proton conductance pathway so that the potential energy associated with the proton gradient is dissipated as heat rather than being used for the generation of ATP (Himms-Hagen 1985). This process is regulated by uncoupling proteins (UCP) (Cannon and Nedergaard 2004) and the activity of brown adipose tissue can be measured by the level of GDP binding to uncoupling protein (Symonds and Lomax 1992).

The presence and volume of UCP within BAT can be used to determine the capacity of BAT for thermogenesis (Trayhurn *et al.* 1993). Uncoupling protein levels increase when the animal is exposed to cold (Clarke and Symonds 1998) thereby improving the ability of the

animal to metabolise BAT to produce heat and maintain thermoneutrality. However, if the animal is unable to suckle and is exposed to cold, then atrophy of BAT may occur (Alexander 1962a) leading to a reduction in thermoregulatory capacity.

Thyroid hormones are also important in BAT catabolism with T3 levels regulating fatty acid oxidation through its action in controlling the transcription of UCP (Cannon and Nedergaard 2004). Noradrenaline also stimulates the activity of an enzyme, 5' monodeiodinase, which catalyses the conversion of T4 to T3 (Trayhurn 1993). This can also lead to higher levels of circulating T3 following exposure to cold (Himms-Hagen 1985).

Methods for inducing and measuring cold stress

Many workers have studied the effects of cold stress in neonatal lambs. Cold stress challenges have been achieved using cooling water baths, climate controlled chambers or environmental conditions. Alternatively cold stress has been simulated via pharmacological treatments. The cold response measures include; changes in rectal temperature, metabolic rate as measured by oxygen consumption and blood metabolites.

Inducing cold stress using cooling water baths was a technique developed by various workers including Eales and Small (1980), Slee *et al.* (1980) and Samson and Slee (1981), and requires the lamb to be immersed and restrained in a water bath that generally starts at a thermoneutral temperature of around 38.5°C (Eales and Small 1980; Stott and Slee 1985). The temperature of the water is gradually reduced by replacing the warm water with cool water at a steady rate (Slee *et al.* 1990). Rectal temperatures are logged regularly and the

lamb removed from the water bath when the rectal temperature reaches a pre-determined critical temperature between 35 and 37°C (Slee *et al.* 1990). Slee *et al.* (1990) compared variations in the length of time allowed for acclimatisation, the rate of water cooling and the critical temperature for the use of this technique and found that fast cooling (thermoneutral to 15°C in 50 minutes vs. 110 minutes) with a self-rewarming procedure was satisfactory for inducing and measuring non-shivering thermogenesis in neonatal lambs. Rectal temperatures and oxygen consumption, which can be used to calculate metabolic rate, are used to assess the physiological response of the lamb to this cold challenge. The acclimatisation period allows a basal metabolic rate and rectal temperature to be determined which is then pushed to summit metabolism (maximum metabolic rate). Figure 2.4 shows typical response curves for rectal temperature and metabolic rate. The application of this technique is limited in that it is very time consuming, requires specialised equipment and expertise and requires the lamb to be moved away from the ewe. Consequently, it is only useful under intense experimental conditions rather than for broader observational studies.

Exogenous administration of adrenaline, noradrenaline (Alexander 1969; Mills *et al.* 1967), isoprenaline (Slee *et al.* 1987a) and thyroid hormones (Alexander 1970) are pharmacological methods that have been used to induce non-shivering thermogenesis by simulating cold exposure. These pharmacological treatments work by stimulating BAT metabolism without the need for actual cold exposure to stimulate the parasympathetic nervous system. Noradrenaline has been found to produce the most consistent results (Alexander 1970; Slee *et al.* 1987a) when administered via infusion or subcutaneous injection. Slee *et al.* (1987a) compared noradrenaline and isoprenaline via subcutaneous or intravenous injection and found that subcutaneous noradrenaline injection at a dose of 150 µg/kg birth weight was the simplest and most effective treatment for large scale experiments. Using this method, a

consistent rectal temperature response was obtained in lambs of unspecified ages from six different sires (Slee *et al.* 1987a).

Various authors (Alexander and Williams 1966b; Gudex 2001; Slee *et al.* 1980) have taken advantage of prevailing environmental conditions to study the effects of cold on neonatal responsiveness. The disadvantage of this approach is that it is difficult to repeat conditions or to have the lambs in the conditions at a set critical age. Climate controlled chambers, designed to mimic environmental conditions, can overcome this shortcoming and have been used extensively in the early work on neonatal thermogenesis by Alexander *et al.* (1958b; 1961a; b; 1962b; 1970; 1966b) and also in more recent work by Kerslake *et al.* (2009). Temperature, wind and rain conditions are able to be simulated in these chambers, using refrigeration, fans and sprinklers. Ambient temperatures as low as -15°C have been achieved using such chambers (Alexander 1962c). Lambs are generally placed in these chambers alone, however, in larger chambers both the ewe and lamb can be accommodated which overcomes the additional stress of separation from the dam during testing.

Exposure to cold produces a characteristic change in rectal temperature and metabolic rate, as measured by oxygen consumption (Figure 2.4), where rectal temperatures initially increase to a peak then gradually decline to a basal level and eventually drop off to critical levels whereas metabolic rate starts low and gradually increases to summit metabolism. The time over which this happens varies depending on the severity of the cold, other prevailing weather conditions (eg. wind and rain); on the age of the lamb and on the individual lamb itself. Lennon *et al.* (pers. comm.) and Kenyon *et al.* (pers. comm.) have identified large individual variation in rectal temperature and metabolic rate in response to cold as induced by

cooling water baths and temperature control chambers. Much of the early cold tolerance work also presents data on individual responses rather than averages due to this high individual variation (Alexander 1961d; 1962c; Slee *et al.* 1990).

The blood concentrations of glucose, lactate and free fatty acids all increase in lambs exposed to cold (Alexander *et al.* 1968; Alexander and Mills 1968; Mills *et al.* 1967) as shown in Figure 2.5. Free T4, total T4 and total T3 concentrations also increase during cold exposure (Wrutniak and Cabello 1989) with increases observed within an hour of exposure (Cabello 1983) despite increases in thyroid hormones also being associated with long term cold exposure. Ideally, blood samples should be collected before, during and/or after cold exposure to reliably assess the lamb's response profile.

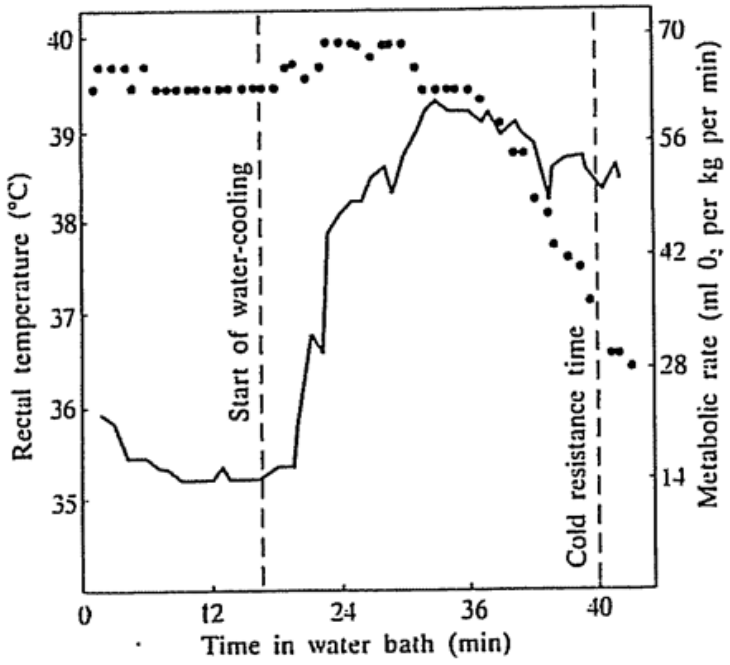

Figure 2.4: Typical change in rectal temperature (.....) and metabolic rate (___) of a lamb in a cooling water bath (Slee *et al.* 1990).

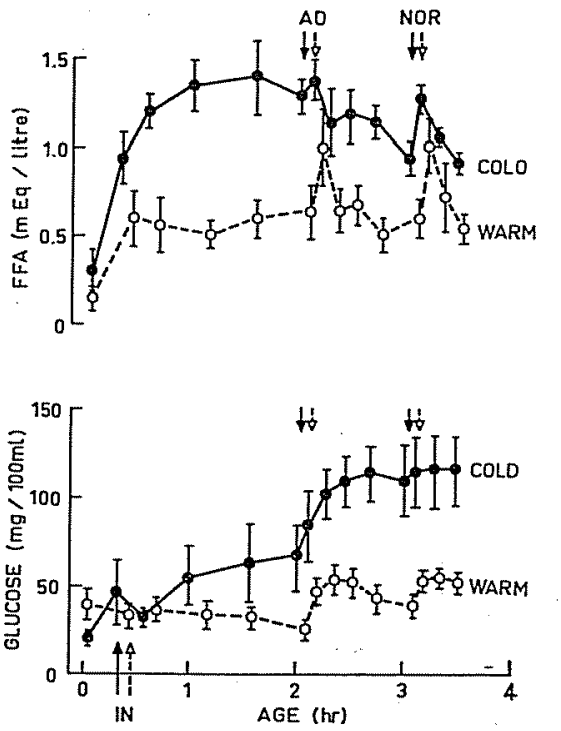

Figure 2.5: Effect of cold on blood plasma levels of free fatty acids and glucose with warm lambs kept at 27°C and cold lambs at 4°C (Alexander and Mills 1968).

Most of the methods used to induce cold stress are suitable for small numbers of animals under intensive experimental conditions, however, lambs are often required to be away from the ewe thereby confounding the stress due to cold with isolation stress. Many of the methods used have also been applied to lambs of a wide age range within studies and these lambs may vary in their response to cold stress due to age. There is a need for a method of inducing cold stress while the lamb is still in the presence of the ewe and at a specific age in a uniform way so that variation in lamb responses to cold can be studied. Measures of cold responses have generally focused on physiological parameters as outlined above and very little work has been done on the impact of cold stress on behavioural responses of neonatal lambs. Some authors have considered the effect of cold on the suckling behaviour of the lamb and found that it suppresses suckling (Alexander and Williams 1966b; Thompson

1983), however, no studies on the ability of the lamb to respond to the ewe while under stress appear to have been reported.

2.3.6 Conclusions

Although it is clear that both the ewe and the lamb have a role in contributing to lamb survival, the relative contribution of the lamb to its own survival is less clearly defined and understood compared to the role of the ewe. The ability of the lamb to discriminate its own mother from an alien ewe has been studied extensively, however, in these tests it appears that the lamb cannot discriminate its mother before 12 hours of age. The ewe generally moves away from the birth site much earlier than this age and the ewe and lamb must maintain contact during this time. However, to date it has not been shown that the lamb can actually recognise its mother until much later. Lamb vigour in the very early neonatal period, up until the lamb suckles, has been studied extensively. However, there appears to be very little information on how the lamb contributes to its survival from the time it first suckles until 12 hours of age particularly in Merino lambs and how the physiological responses of the lamb contribute to subsequent behavioural responses. Therefore, this thesis aims to explore further ways of measuring lamb vigour particularly at a time when the ewe is beginning to focus on things other than the lamb (feeding, rejoining the flock) and when the lamb may be undergoing a physiological stress such as cold.

2.4 General aims

The general focus of this thesis is on the methods of assessing neonatal lamb vigour and how this could be improved. It extends the concept of lamb vigour from being important to the lamb as measured by how quickly it stands and suckles to the responsiveness of the lamb to

the ewe under stressful situations. This was done to assess how important the lamb is in contributing to its own survival particularly at times when the ewe would be moving to rejoin or maintain contact with the flock.

The specific aim of this thesis was to develop objective methods of assessing neonatal lamb vigour both soon after birth and later in the neonatal period (up to 12 hours of age). This was done through a series of experiments examining:

1. The effect of late pregnancy nutrition on time to perform early lamb behaviours and other lamb vigour measures (Chapter 4).
2. Differences between purebred and crossbred Merino lambs in time to perform early lamb behaviours and behavioural responses during cold stress (Chapter 5).
3. Progeny from sires selected for differences in lamb loss and subsequent effects on lamb behaviour (Chapter 6).
4. Novel methods for inducing cold stress to assess the behavioural responses of lambs while under a physiological stress (Chapter 3).

Chapter 3: Novel methods for inducing and measuring cold stress in neonatal lambs

This chapter outlines a number of experimental techniques developed to induce thermoregulatory responses in neonatal lambs and to measure the subsequent impact on physiology and behaviour. The aims were to develop a method whereby lambs could be exposed to cold in a uniform way without the need to isolate them from the ewe and at a standard age and to subsequently measure the impact of the cold challenge on lamb physiology and behaviour in the first 12 hours of life.

Section 3.1 outlines a method that assesses the timing of cold exposure within the first 12 hours of life on the physiological responses (rectal temperature and blood measures) of the lamb. It uses a standard method of a noradrenaline challenge that has been used for many years by many authors. This pilot study allowed the timing of the noradrenaline challenge for the experiment outlined in Chapter 4 to be decided.

Section 3.2 outlines a novel way of assessing changes in body temperature following a noradrenaline challenge. Infra red thermal imagery was used in this method and from this pilot study it was decided to use thermal imaging to assess responses to cold in the experiment reported in Chapter 5.

Section 3.3 outlines the use of an ice vest to induce cold stress in neonatal lambs. The vest is worn by the lamb in the presence of the ewe for the desired amount of time to induce cold stress. In this method the response to the ice vest was compared to the response to a noradrenaline challenge. This method was used in the experiment reported in Chapter 5.

Section 3.4 outlines a behavioural test that was developed to test the ability of the lamb to respond to ewe vocalisations. This method was tested on Scottish Blackface and Suffolk lambs at the Scottish Agricultural College. It was subsequently used to assess responses following a cold challenge or under thermoneutral conditions in the experiments reported in Chapters 5 and 6.

3.1 Timing of noradrenaline challenge in the first 12 hours of neonatal life does not affect the thermoregulatory response of neonatal lambs

3.1.1 Introduction

Metabolism of brown adipose tissue (BAT) during cold exposure is essential for effective thermoregulation in the neonatal lamb (Alexander and Williams 1968). On exposure to cold, endogenous noradrenaline is released binding to receptors on BAT cells thereby inducing non-shivering thermogenesis. Exogenous administration of noradrenaline has been used to mimic cold exposure to induce a thermoregulatory response in lambs (Alexander 1969; Cooper *et al.* 1976; Simpson and Slee 1988; Slee and Simpson 1991; Slee *et al.* 1987a; Symonds *et al.* 2000; Thompson and Jenkinson 1968). Various methods of administration and dose rates of exogenous noradrenaline have been used, however, a subcutaneous injection at a rate of 150 µg/kg has been found to be the most efficient and effective means (Slee *et al.* 1987a). The subsequent metabolic responses following an injection have been measured using oxygen consumption and changes in rectal temperature (Simpson and Slee 1988; Slee *et al.* 1987a; Slee *et al.* 1987b).

Many of the previous studies using exogenous noradrenaline administration have used lambs of a wide age range (0.5 hours to 30 days) (Alexander *et al.* 1970; Slee and Simpson 1991; Thompson and Jenkinson 1968) or the age of the lambs has not been specified (Slee *et al.* 1987a). Alexander and Williams (1968) considered the age of lambs but looked at lambs younger than one day old and then at various ages up until two months of age. Their results showed that lambs less than one day old (12 hours) produced a temperature change response to noradrenaline infusion while older lambs did not which corroborated the results of Thompson and Jenkinson (1968). Similarly, Alexander *et al.* (1970) found that the increase in oxygen consumption due to noradrenaline infusion declined rapidly with increasing age. It is known that in lambs, BAT is replaced by white adipose tissue in the first few days of life (Alexander 1978b; Gemmell *et al.* 1972) and therefore it was hypothesised that the timing of exposure to exogenous noradrenaline in the first day of life may affect thermoregulatory responses in neonatal lambs and that this change would be reflected in changes in plasma glucose, free triiodothyronine (T3) and free thyroxine (T4) levels.

3.1.2 Materials and Methods

All procedures in this experiment were conducted with the approval of the University of New England Animal Ethics Committee, AEC no. 08-096.

3.1.2.1 Animals and management

Forty pregnant multiparous Merino ewes were randomly selected from a group of ewes that had been oestrus synchronised for artificial insemination but were joined to Merino rams on their second oestrus following synchronisation. These ewes were maintained on pasture throughout pregnancy and were given supplementary triticale and corn grain every second

day from day 120 at 100 g/ewe/feed. The ewes were brought into the animal house 145 days after joining and held individually in pens 1.2 m x 1 m. Ewes were under constant monitoring throughout their time in the animal house and remained there until their lambs were 24 hours of age. Ewes were fed approximately 700 g of a triticale and corn grain mix along with 300 g of lucerne chaff daily and *ad libitum* pasture hay.

3.1.2.2 Treatments

Three treatments and two controls were used in this experiment:

Treatment A: Noradrenaline injection at 3 and 12 hours of age (n=11).

Treatment B: Noradrenaline injection at 6 and 12 hours of age (n=10).

Treatment C: Noradrenaline injection at 12 hours of age only (n=10).

Control 1: Sham injection (no noradrenaline) at 3 hours of age (n=3).

Control 2: Sham injection (no noradrenaline) at 6 hours of age (n=3).

Lambs were allocated to treatment group sequentially as they were born to ensure each treatment was applied over time. Controls were included at the end of the experiment and were given a sham injection. Both single and twin lambs were used with twin siblings allocated to different treatments.

3.1.2.3 Measures and protocols

The precise time of birth was recorded for each lamb with birth defined as complete expulsion of the lamb. Before 3 hours of age, the lamb was weighed and an iButton (DS1921H with a range of 15°C – 46°C and resolution of 0.125°C) temperature logger was inserted into the rectum of each lamb and secured *in situ* by fastening it to the tail. Rectal temperatures were logged every minute until approximately 14 hours of age allowing measurement of rectal temperature changes throughout the experiment without the need to rehandle the lamb.

Lambs were given a 150 µg/kg birth weight subcutaneous injection of noradrenaline (Levophed®) in the mid dorsal region according to treatment. Control lambs were given a sham injection. Immediately prior to and half an hour after injection, all lambs had a 5 ml blood sample taken via jugular venipuncture in lithium heparin vacutainers. Blood samples were immediately centrifuged at 3000 rpm for 10 minutes, the plasma separated and refrigerated. All plasma was frozen within 12 hours of collection.

Plasma was assayed for glucose, free T3 and free T4 levels. Glucose was tested using the hexokinase-glucose-6-phosphate dehydrogenase method on the Dimension® clinical chemistry system (DADE Behring). Free T3 and T4 were determined using a Siemens competitive analog immunoassay on the IMMULITE® system. These are direct or single test assays rather than equilibrium dialysis methods. Inter assay coefficients of variation were <0.53%, <7.1% and <1.6% for glucose, free T3 and free T4 respectively.

3.1.2.4 Statistical analysis

Data were analysed using PROC GLM and PROC MIXED in SAS. Treatment was the only fixed effect and no covariates were included in the final model. All data were tested at $P<0.05$ for significance. iButton data was sorted before analysis so the following information was available for each lamb: basal rectal temperature, peak rectal temperature, time to peak, time at peak, length of response (time of injection to time temperature reaches two standard deviations above basal), slope and area under the response curve.

The response to the first noradrenaline challenge in each treatment was compared to determine if the timing of the challenge was important. The first challenge in Treatment A and B were also compared to the 12 hour treatment in each of those groups to determine if the second response to noradrenaline differs due to prior challenge. The 12 hour challenge for Treatments A, B and C were also compared to confirm this.

3.1.3 Results

Birth weight did not differ significantly across treatment groups (Table 3.1). Rectal temperature parameters are shown in Table 3.1. When comparing the first noradrenaline challenge across treatments a number of variables differed significantly. The two control groups had a significantly shorter length of response compared to treatments A, B and C. Time to reach peak and area under the response curve also differed significantly in the same manner. Peak rectal temperature differed between treatment groups as shown in Table 3.1. Peak rectal temperature was significantly ($P<0.05$) lower in the control groups compared to treatment A, however it did not differ from treatment C. The 6 hr control had the lowest peak rectal temperature which was significantly ($P<0.05$) lower than both Treatments A and B.

Peak rectal temperature did not differ significantly (P<0.05) between the three noradrenaline challenged groups.

At the 12 hour noradrenaline challenge, the time at peak was the only variable to differ significantly (P<0.05, Table 3.2) between treatments and did not differ between Treatment A and B but was significantly longer for Treatment C. Time to reach peak tended (P=0.09) to be shorter for Treatment A compared to Treatment B but did not differ from C.

When comparing the first noradrenaline challenge to the second challenge in Treatments A and B there were no significant (P<0.05) differences between treatments or between challenge times (1 or 2) in any of the variables (Table 3.3). Peak rectal temperature tended to differ between challenge times (P=0.09) and time to peak also approached significance (P=0.06) between treatments.

3.1.3.1 Blood measures

Figure 3.1a shows the change in plasma glucose levels from the first noradrenaline challenge across all treatments. There was no significant difference in glucose levels due to treatment and the control groups did not differ with sampling time. Plasma glucose levels in all treatments increased significantly following noradrenaline challenge. For free T3 and T4 at the first noradrenaline challenge, there was no significant difference with sampling time but there were significant treatment effects. Figure 3.1b shows that the two control groups did not differ from each other but the 3hr control group had significantly higher free T3 levels than treatments A, B or C. The 6 hr control group also tended to be higher but not

significantly (P=0.06). The two control groups had significantly higher free T4 levels before and after the noradrenaline challenge compared to treatments A, B and C (Figure 3.1c).

No significant differences were observed in plasma free T3 and free T4 levels before and after the 12 hour noradrenaline challenge (Table 3.4) although free T3 levels tended to decrease (P=0.07). However, plasma glucose concentration increased significantly (P<0.05) following noradrenaline challenge.

Within treatments A and B, the change in blood plasma measures following noradrenaline challenge differed significantly across the first and second challenge for glucose but not free T3 or T4 (Table 3.5). The rise in glucose level following noradrenaline challenge was greater following the first challenge than the second for both treatments.

Table 3.1: Least square means and standard errors for iButton parameters for first noradrenaline challenge (A, B, C and controls).

	Treatment				
	3hr control	**6hr control**	**A (3hr)**	**B (6hr)**	**C**
	n=3	*n=3*	*n=7*	*n=8*	*n=3*
Birth weight (kg)	4.83±0.35	5.2±0.35	4.43±0.18	4.43±0.19	4.2±0.19
Basal rectal temperature (°C)	38.79±0.18	38.65±0.18	38.68±0.12	38.5±0.11	38.59±0.18
Response cut off temperature (°C)	39.04±0.18	38.9±0.18	38.98±0.12	38.73±0.11	39.01±0.18
Length of response (mins)	11.67±12.39^{a}	6±12.39^{a}	68.71±8.11^{b}	70.75±7.59^{b}	57.67±12.39^{b}
Peak rectal temperature (°C)	39.21±0.31^{ab}	38.88±0.31^{a}	40.04±0.21^{c}	39.91±0.19^{bc}	39.67±0.31^{ac}
Time to reach peak (mins)	4.33±5.90^{a}	4.00±5.90^{a}	18.86±3.86^{b}	26.75±3.61^{b}	21.67±5.90^{b}
Time spent at peak (mins)	4.67±3.86	4.67±3.86	6.86±2.53	10.25±2.36	12.67±3.86
Area under the response curve (°Cmin)	456.09±519.40^{a}	252.73±636.10^{a}	2721.65±320.40^{b}	2782.60±340.03^{b}	2619.59±519.41^{b}
Slope of response curve (°C/min)	0.07±0.02	0.07±0.03	0.07±0.01	0.05±0.01	0.05±0.02

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 3.2: Least square means and standard errors for iButton parameters for the 12 hour noradrenaline challenge (A12hr, B12hr and C).

	Treatment		
	A (12hr)	**B (12hr)**	**C**
	n=8	*n=8*	*n=3*
Basal rectal temperature (°C)	38.75±0.12	38.58±0.12	38.59±0.19
Response cut off temperature (°C)	39.07±0.12	38.73±0.12	39.01±0.19
Length of response (mins)	45.63±11.62	78.5±11.62	57.67±18.97
Peak rectal temperature (°C)	39.71±0.24	39.69±0.24	39.67±0.39
Time to reach peak (mins)	19.75±2.88	29.13±2.88	21.67±4.71
Time spent at peak (mins)	3.38±1.36^{a}	6.63±1.36^{a}	12.67±2.22^{b}
Area under the response curve (°Cmin)	2070.27±484.45	3087.06±484.45	2619.59±791.10
Slope of response curve (°C/min)	0.04±0.01	0.04±0.01	0.05±0.01

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 3.3: Least square means and standard errors for differences within treatments between challenge time (A and B).

	Treatment			
	A3hr	**A12hr**	**B6hr**	**B12hr**
	n=7	*n=8*	*n=8*	*n=8*
Length of response (mins)	68.06±10.78	45.66±10.13	70.75±10.17	78.5±10.17
Peak rectal temperature (°C)	40.02±0.21	39.75±0.20	39.91±0.21	39.69±0.21
Time to reach peak (mins)	18.87±3.70	20.38±3.48	26.75±3.50	29.13±3.50
Time at peak (mins)	6.85±2.32	3.37±2.17	10.25±2.17	6.63±2.17
Area under the response curve (°Cmin)	2661.36±435.04	2074.04±409.77	2782.6±412.58	3087.06±412.58
Slope of response curve to peak (°C/min)	0.07±0.01	0.05±0.01	0.05±0.01	0.04±0.01

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 3.4: Least square means and standard errors for blood parameters at the 12 hour noradrenaline challenge for treatments A, B and C.

	A12hr		**B12hr**		**C**	
	Before	**After**	**Before**	**After**	**Before**	**After**
	n=11	*n=11*	*n=10*	*n=10*	*n=10*	*n=9*
Glucose (mmol/L)	8.98±0.93^{a}	9.69±0.93^{b}	9.54±0.98^{a}	10.68±0.98^{b}	9.12±0.98^{a}	10.12±0.98^{b}
Free T3 (pg/mL)	6.55±0.42	6.41±0.42	6.41±0.44	6.18±0.44	7.14±0.44	6.88±0.44
Free T4 (ng/dL)	1.84±0.13	1.83±0.13	1.57±0.13	1.55±0.13	1.80±0.13	1.73±0.13

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 3.5: Change in plasma free T3 and free T4 levels following noradrenaline challenge within treatment.

	A3hr	**A12hr**	**B6hr**	**B12hr**
	n=9	*n=11*	*n=10*	*n=10*
Glucose (mmol/L)	1.38±0.36^{a}	0.71±0.33^{b}	1.96±0.34^{a}	1.14±0.34^{b}
Free T3 (pg/mL)	0.075 ± 0.21	-0.15 ± 0.20	0.20 ± 0.23	-0.23 ± 0.21
Free T4 (ng/dL)	-0.05 ± 0.057	-0.018 ± 0.054	-0.099 ± 0.06	-0.20 ± 0.057

Different superscripts within rows indicate means that differ significantly (P<0.05)

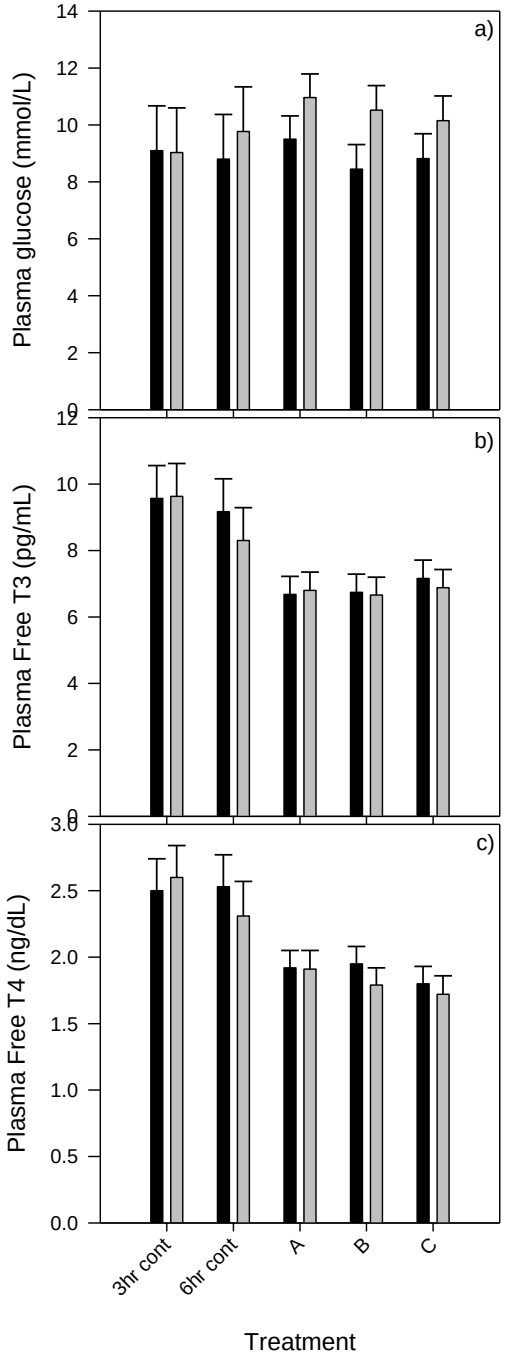

Figure 3.1: Plasma glucose (a), free T3 (b) and free T4 (c) concentrations immediately before (black bars) and 30 mins after (grey bars) the first noradrenaline challenge or sham injection for control lambs. Error bars represent standard errors.

3.1.4 Discussion

These results show that within the age range of 3 – 12 hours, lamb responses to a noradrenaline challenge are similar. This confirms Alexander's (1969) findings that all lambs under a day old responded to adrenaline or noradrenaline injection even though age within this period was not defined in his study. This also supports Eales and Small (1985) who reported that lambs up to five hours of age did not differ in their ability to respond to a cooling water bath as measured by respiratory quotient. It was expected that lamb responses to noradrenaline would be more age sensitive with younger lambs (i.e. 3 hours of age) having a greater response to injection than the older lambs in this study. This was not the case and the results also confirm the findings of Slee and Simpson (1991) where a wide age range (3 – 29 hours) of lambs was used.

Previous exposure to noradrenaline also appears to have little effect on the ability of the lamb to mount a response to a second noradrenaline challenge within the first day of life. This was unexpected as it was thought that the lambs would have used some of their BAT reserves to mount the first response and would therefore have limited stores available for the second response. This may be reflected in the trend (P=0.06) for lambs to have a shorter time taken to reach peak rectal temperature after the second challenge. Other authors have found similar results in that multiple exposures to adrenaline or noradrenaline have continued to produce a change in metabolic response as measured by oxygen consumption (Alexander and Mills 1968). Perhaps this level of noradrenaline challenge is not equivalent to a severe cold stress in which BAT reserves would be depleted and the lamb would not be able to mount an adequate subsequent thermogenic response. A comparison between physiological responses to an actual cold stress and a noradrenaline challenge needs to be performed to test this.

Plasma glucose concentrations increased following noradrenaline injection which was expected (Alexander *et al.* 1968; Alexander and Mills 1968). However, the lack of an effect on free T3 and T4 levels was not expected. It was thought that thyroid hormone levels would increase following noradrenaline challenge as lambs exposed to cold have increased plasma T3 and T4 levels (Wrutniak and Cabello 1989). However, other authors have reported that delivery temperature can influence the thyroid response and therefore, the levels of circulating thyroid hormones following various challenges (Symonds *et al.* 2000). In the present study, the lambs had suckled prior to noradrenaline challenge and this may have influenced thyroid hormone response. It has been reported that free fatty acids can influence thyroid hormone levels and upon cold exposure can cause an initial decrease followed by an increase in thyroid hormone concentrations (Wrutniak and Cabello 1989). This may mean that the lambs in this study did have a change in thyroid hormone levels but blood samples were taken when that change was less evident. Other authors have measured total thyroid hormone levels rather than free thyroid hormones, however the pattern of change would not be expected to differ for this reason (Wrutniak and Cabello 1987a; b).

Control animals were measured at the end of the experimental period which may affect the validity of their results as they were not randomly treated throughout the experimental lambing period as animals in other treatments were. However, the lack of treatment differences suggests this was not an issue. Free T3 and T4 levels were different in control animals compared to treated animals prior to the noradrenaline challenge, however this was not seen for glucose.

It was concluded that the timing of noradrenaline injection was not critical with respect to the thermoregulatory response in neonatal lambs less than 12 hours of age. However, changes in blood parameters are not as consistent as rectal temperature changes. Plasma glucose concentrations increase following noradrenaline injection but free T3 and T4 levels do not appear to change and time of sampling may be important particularly for lambs that have suckled milk. In light of these results, a noradrenaline challenge could be used at any age, from 3 – 12 hours, to elicit a thermogenic response in neonatal lambs.

3.2 Changes in lamb body temperature can be detected using infrared thermal imaging technology

3.2.1 Introduction

Thermal imaging technology has been used for many biological applications such as determining energy expenditure in pre-term infants (Adams *et al.* 2000), quantifying non-shivering thermogenesis in field voles (Jackson *et al.* 2001), assessing huddling behaviour in infant rats (Sokoloff and Blumberg 2001), measuring wing temperature in bats (Lancaster *et al.* 1997) and assessing bruising in apples (Varith *et al.* 2003) with varying degrees of success. Thermal imaging technology relies on radiated heat that can be measured in the infrared region of the light spectrum (Clark *et al.* 2003) and is very useful for measuring relative differences in temperature across an object. However, measures of absolute temperature may be more difficult as more information regarding atmospheric temperature, relative humidity and emissivity of the object are required.

Thermal imaging has been used to detect changes in surface temperature in humans, both adults and infants (Adams *et al.* 2000; Shuran and Nelson 1991) and other animals at various ages (Jackson *et al.* 2001; Lancaster *et al.* 1997; McCafferty *et al.* 1998; Sokoloff and Blumberg 2001) but its use has not been reported in neonatal lambs. Several authors (Adams *et al.* 2000; McCafferty *et al.* 1998; Shuran and Nelson 1991) have compared thermal imaging to quantify heat production/loss with other methods such as indirect calorimetry in humans (Adams *et al.* 2000; Shuran and Nelson 1991) or standard equations for the particular species under investigation (McCafferty *et al.* 1998). Collectively, they found thermal imaging technology to be a useful way of measuring heat production. Of these studies, only two specifically quantified heat production from brown adipose tissue (BAT), in neonatal rats during huddling (Sokoloff and Blumberg 2001) and field voles following a noradrenaline injection (Jackson *et al.* 2001). Measuring heat production from BAT provides a means of assessing the thermoregulatory capacity of the animal which for the neonatal lamb is essential for survival prior to suckling. These authors found that thermal imagery was useful in measuring relative surface temperature changes but it was more difficult to attribute this to BAT activity and to quantify this activity.

The major BAT depots in the neonatal lamb are in the pericardial and perirenal regions (Everett-Hincks and Duncan 2008). However, subcutaneous fat in the shoulder and hind limb regions is also functionally BAT (Trayhurn *et al.* 1993). Following cold exposure or exogenous noradrenaline stimulation, lambs mobilise BAT to thermoregulate and it would be expected that this extra heat production could be observed through surface temperature changes of the lamb as well as metabolic measures (rectal temperature, oxygen consumption). Jackson *et al.* (2001) showed that in field voles, the interscapular region where BAT was found could easily be distinguished using infra red thermal technology. However, infra red

temperature measurements were not found to be highly correlated with BAT activity as measured by oxygen consumption following a noradrenaline challenge. A study by Adams *et al.* (2000) in human infants found that infra red thermal technology could be used to determine energy expenditure in a comparable way to indirect calorimetry but this study was not specifically concerned with heat production from BAT.

In the current study it was hypothesised that thermal imaging could be used in lambs to identify the areas of heat production associated with BAT metabolism following a noradrenaline challenge and that surface temperatures would be correlated with rectal temperatures.

3.2.2 Materials and Methods

This experiment was conducted, as part of another experiment reported in Chapter 4 (pp 94), with the approval of the CSIRO FD McMaster Laboratory, Chiswick, Animal Ethics Committee, AEC No.08/11.

Single (n=11) and twin (n=8) Merino lambs were used in this experiment. At 6 hours of age, each lamb was subjected to a noradrenaline challenge at a dose of 150 µg/kg birth weight injected subcutaneously into the mid dorsal region. Immediately prior to and half an hour after the challenge, a dorsal thermal image was taken. Rectal temperatures were measured during the noradrenaline challenge using a temperature logger (iButton®) inserted into the rectum at 5.5 hours of age and removed at 8 hours of age.

Thermal images were taken using an infrared thermal camera (ThermaCAM^{TM}, FLIR systems) mounted on a frame with a fixed focal length (135 cm) between it and the lamb. Lambs were placed ventrally in a cradle with their legs restrained through holes in the base of the cradle to maintain a fixed position relative to the camera. Ambient temperature and relative humidity were recorded when an image was taken.

Images were analysed using ThermaCAM Researcher Professional software (FLIR systems). Images were adjusted for temperature, humidity and distance from camera assuming an emissivity of 0.96 (Porter and Gates 1969). Following adjustment, the maximum temperature for each image and the relative location (neck, shoulder, mid section, rump) of the maximum surface temperature, assessed visually, was recorded. A dorsal temperature profile of the lamb from shoulder to rump just to the right of the midline was plotted and the area under the curve calculated (Plate 3.1). The difference in area under the curve before and after the noradrenaline challenge was then determined for analysis.

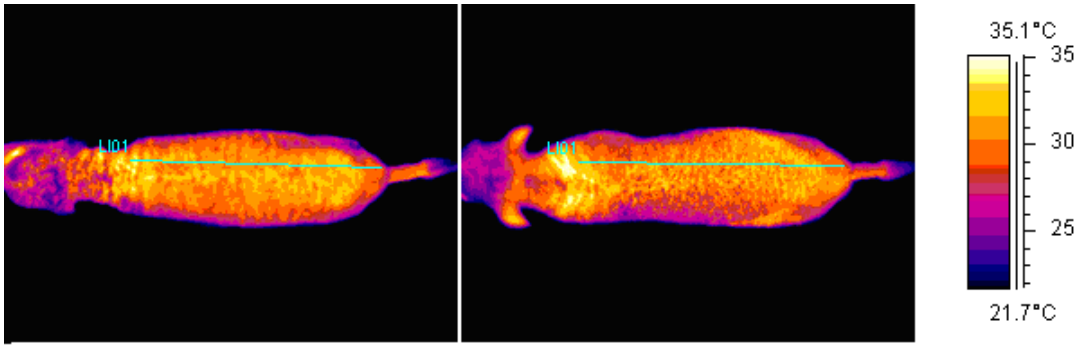

Plate 3.1: Lamb before noradrenaline injection on the left and following noradrenaline injection on the right. The lines show where the profile from shoulder to rump was taken.

All data were checked for normality prior to analysis. Analysis was carried out using PROC GLM and PROC MIXED in SAS using litter size as a fixed effect and nutrition treatment as a covariate (see statistical analysis section in Chapter 4, pp. 99, for more detail). PROC CORR was used to determine Pearson's correlation coefficients between maximum surface temperature and rectal temperature.

3.2.3 Results

There was a significant ($P<0.05$) increase in the maximum surface temperature following noradrenaline injection for both single and twin lambs (Figure 3.2a). In 71% of cases, the highest temperature was observed over the shoulder region. For 23% and 6% of the lambs, the highest temperature was found over the neck and mid-dorsal regions, respectively. Maximum surface temperature and the difference in area under the temperature curve did not differ significantly due to litter size although singles tended to have higher temperatures than twins ($P=0.09$).

There was a positive correlation (Pearson's correlation coefficient=0.54, $P<0.001$) between maximum surface temperature and rectal temperature before and after noradrenaline challenge. Rectal temperature increased following the noradrenaline challenge and differed ($P<0.05$) between singles and twins (Figure 3.2b) with lower rectal temperatures and a smaller response observed in twin lambs.

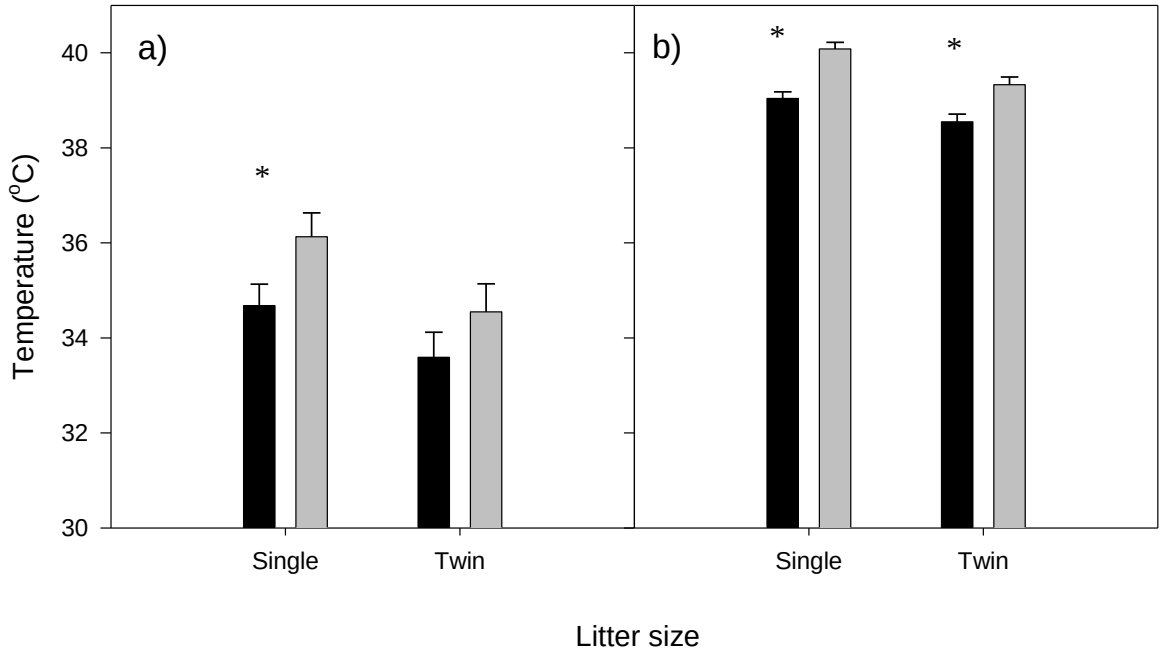

Figure 3.2: Increase in maximum surface temperature (a) and rectal temperature (b) for single and twin lambs following noradrenaline challenge (black bars are immediately before noradrenaline challenge and grey bars are 30 mins after the challenge). Error bars represent standard errors and asterisks indicate means that differ significantly ($P<0.05$).

3.2.4 Discussion

From this study, it appears that infra red thermal technology could be used to show the relative areas of heat production in neonatal lambs. However, using this technology to quantify heat production due to BAT metabolism remains more challenging (Jackson *et al.* 2001). Although there was an increase in the maximum surface temperature of the lamb and the area under the curve following noradrenaline challenge, we did not attempt to quantify the activity of BAT. Adams *et al.* (2000) were able to quantify energy expenditure in human infants but did not partition this into heat produced by BAT.

In the majority of cases, the maximum temperature was identified in the shoulder region of the lamb. It was expected that this may have occurred in the lumbar region as the underlying perirenal fat depot is one of the major sources of BAT. However, it is possible that as the subcutaneous fat depot in the shoulder is closer to the surface, it may be more easily identified using this technique than a deeper depot. Noradrenaline injections were given in the shoulder region therefore, this could have been a localised effect associated with injection. The pericardial region is also a major BAT depot however, it was expected that this depot would be too deep in the body to be readily seen through infra red imaging. In neonatal lambs, pericardial fat is used first (Macfarlan 1965 cited in Everett-Hincks and Duncan 2008) so the high temperature in the shoulder region may be due to this fat depot not just subcutaneous catabolism of BAT.

The moderate correlation between rectal temperature and maximum surface temperature suggests that infra red thermal imaging technology may be useful in determining a metabolic response. There was a trend suggesting single lambs had higher maximum surface temperatures compared with twin lambs; however, the fact that there was a significant difference in rectal temperatures due to litter size may suggest that rectal temperatures are a more sensitive measure of metabolic response compared to surface temperature measurements. The data set used was very small which may account for the lack of a significant difference in surface temperature.

This experiment has shown that infra red thermal technology was useful in identifying areas of heat production in the neonatal lamb. However, further work on a larger data set is needed to determine if this can be quantified as a measure of BAT metabolism. For future use of this method it is recommended that a number of changes to improve the accuracy of this technique should be implemented. These include greater restraint of the lambs to achieve more consistently shaped images across lambs and imaging times to allow images to be overlayed and differences to be assessed more easily. As such, this method of assessing changes in lamb body temperatures during a cold challenge was used again in the experiment reported in Chapter 5.

3.3 An ice vest can be used to elicit a cold response in neonatal lambs

3.3.1 Introduction

Cold responses in neonatal lambs have been the focus of much research work. Various methods to induce cold responses have been used including cooling water baths (Slee *et al.* 1990), temperature controlled chambers (Alexander 1962c; Kerslake *et al.* 2009), natural weather conditions and a range of pharmacological treatments such as noradrenaline and isoprenaline (Slee *et al.* 1987a). A number of methods have been applied to measure the responses including changes in rectal temperature, open circuit calorimetry, quantifying changes in blood metabolites and behavioural changes. Most of these methods require the lamb to be away from the ewe during testing resulting in a possible confounding of stress induced by separation with the stress induced by exposure to cold. To reduce issues with disrupting the ewe-lamb bond, these methods may not be used until the lamb has at least suckled (Slee *et al.* 1987a; Slee and Stott 1986) or is older, often up to a number of days old (Kerslake *et al.* 2009). During this time, it would be expected that the activity and volume of

brown adipose tissue would be reduced as it changes to white adipose tissue (Gemmell *et al.* 1972). Therefore, the responses measured may not be an accurate reflection of the ability of the lamb to respond to cold in the critical early neonatal period (i.e. <3 h post-partum).

The lamb needs to be able to respond to cold in this period not only to survive but also to be able to respond to the ewe in such a way as to maintain contact as she moves away from the birth site. Movement away from the birth site in Merinos has usually occurred before the lamb is 6 hours of age (Murphy *et al.* 1994b) and consequently it is important to know how lambs younger than 6 hours respond to cold exposure.

The pharmacological methods that have been used previously allow the lamb to remain with the ewe and can be administered at an early age without disrupting the ewe-lamb bond. Although these methods stimulate brown adipose tissue metabolism they may not provide an accurate reflection of the effect of a real cold exposure on non-shivering thermogenesis.

A method to test a neonatal lamb's (<12 hours of age) cold response while still in the presence of the ewe and using actual cold rather than a simulated exposure is required. It was hypothesised that fitting a neonatal lamb with an ice vest may be such a method. It was also hypothesised that this cold challenge would produce a different rectal temperature response to a noradrenaline challenge.

3.3.2 Materials and Methods

All procedures were carried out with the approval of the University of New England Animal Ethics Committee, AEC no. 09/116.

3.3.2.1 Animals

Forty pregnant multiparous Merino ewes, at approximately 143 days gestation and joined to Merino sires, were sourced from the Sheep CRC information nucleus flock (INF) (van der Werf *et al.* 2010) in Armidale and housed in individual pens (approximately 1.2 m x 1.2 m) until 1 day post-partum. Ewes were fed 1.4 kgDM/ewe/day of a mixed ration of corn, cottonseed meal pellets (43% crude protein) and lucerne based animal house pellets (9.04 MJME/kg, 16.2% crude protein) along with *ad libitum* lucerne hay while in the animal house. Straw bedding was provided for the ewes. Ewes were under constant video monitoring so an accurate time of birth could be determined. Minor assistance during parturition was provided for one ewe only.

Following birth, ewes and lambs were not disturbed until the lamb was at least 3 hours of age. Between 3 and 6 hours of age, each lamb had a temperature logger inserted rectally (Star-Oddi DST centi®) and underwent a behavioural test (see below). An ice vest (similar to a small dog coat but with an ice pack inside, see Plate 3.2) was then placed on the lamb for either 0, 10, 20 or 30 minutes and the lamb was put back in the pen with the ewe. Lambs in the 0 minute treatment group had the vest on for 20 minutes however there was no ice pack inserted. Ten lambs were used for each treatment. After the appropriate time, the ice vest was removed and a second behavioural test performed. The temperature logger remained in the lamb for at least an hour after vest removal. At this time the lamb had further measurements taken for the INF data collection (see Brien *et al.* 2009) including birth weight, crown rump length, birth coat score etc. Ewes and lambs were left undisturbed until the lamb was 12 hours of age. At 12 hours, a temperature logger was again inserted rectally and a behavioural test performed. A noradrenaline injection (150 µg/kg birth weight) was administered and the lamb placed back with the ewe. Half an hour following injection, a

final behavioural test was performed. The temperature logger was removed one hour after the final behavioural test. Ewes and lambs remained in the animal house until the lamb was at least 24 hours of age when they were released into a small paddock surrounding the animal house for further monitoring according to the INF protocol (Brien *et al.* 2009).


a)

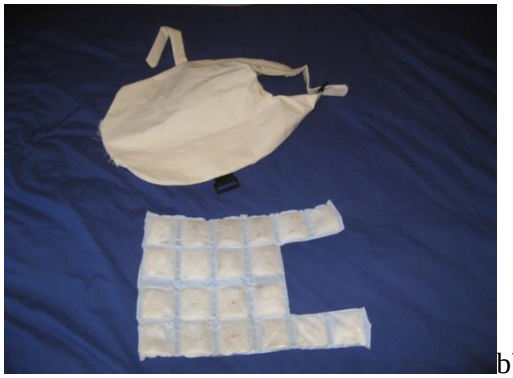
b)

Plate 3.2: Lamb wearing ice vest (a) and ice vest components (b).

3.3.2.2 Behavioural test

The behavioural test arena, as shown in Figure 3.3, consisted of a short race (4 m) with a small release pen midway along the race. The arena was made of sheep panels covered in hessian so the lamb could not be distracted by movement outside the race. Lambs were placed standing up in the release pen and an audio recording, of a recently lambed ewe (not related to the lamb) bleating was played from one end of the race. The end of the race that the audio cue was played from was alternated between tests. The lamb was given 90 seconds to respond to this audio cue. Responses were recorded using a number of classifications as outlined below.

Classification 1: Lamb responds – Yes/No (may be either vocal or movement)

If yes:

Classification 2: Type of response – Vocal/Movement

If movement:

Classification 3: Sensible or non sensible movement. For example, sensible would be moving towards the audio cue whereas non sensible would be moving away from the sound or up and down the race.

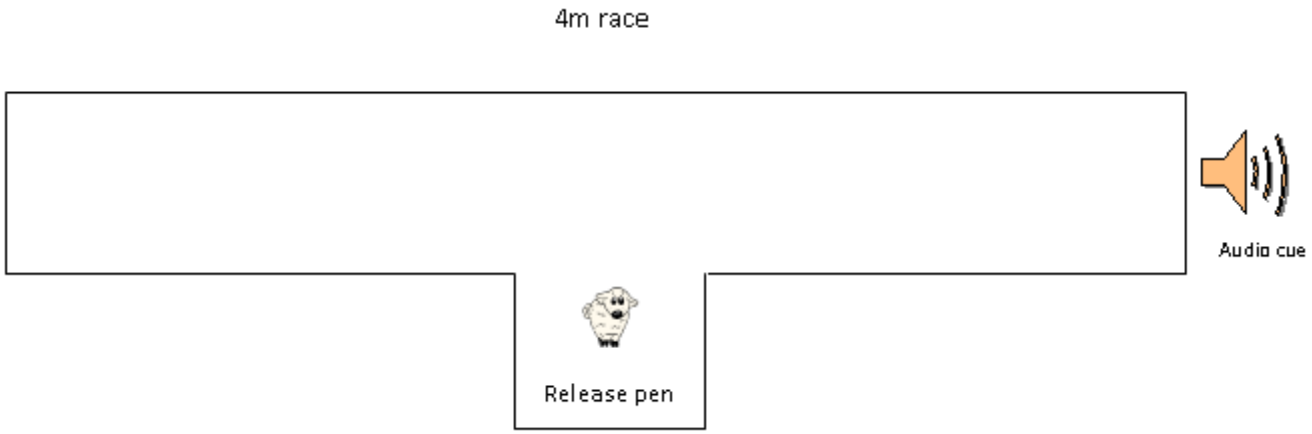

Figure 3.3: Behavioural test arena

3.3.2.3 Statistical Analysis

The rectal temperature (RT) response curves were used to calculate basal RT (30 minutes prior to treatment), peak RT, time to peak RT, area under the curve, length of response (start of ice vest treatment to time to reach two standard deviations above basal RT), slope to peak RT and difference between peak and basal RT. Data were analysed to determine differences between ice vest treatments and differences between ice vest and noradrenaline challenge using PROC GLM in SAS. Two analyses were run, one using treatment (0, 10, 20 or 30 minutes) as a fixed effect to examine differences between ice vest treatments and the effect of prior treatment on response to noradrenaline. The second analysis used treatment and noradrenaline challenge (present or not present) and their interaction as fixed effects to examine the difference between the ice vest challenge and the noradrenaline challenge in

rectal temperature response curves. Birth weight did not differ significantly between treatment groups ($P<0.05$) so was not included in the final model.

Time may be confounded in this analysis as the noradrenaline injection was only administered to lambs at 12 hours rather than at 3 hours of age when the ice vest was used. However, the results from a prior study (Section 3.1, pp. 45) showed that the temperature responses to a noradrenaline challenge at 12 hours of age were similar to those observed at 3 or 6 hours of age. Therefore, an injection at 12 hours only was used.

The behavioural test data were not analysed as the tests were carried out in the same animal house that the ewes were housed in. This resulted in too much vocal interference from the ewes to obtain meaningful information. The behaviour test did however contribute to changes in rectal temperature responses due to handling stress so was accounted for when calculating rectal temperature response curve parameters.

3.3.3 Results

Birth weight did not differ between treatment groups. Table 3.6 shows least square means for the parameters derived from the rectal temperature response curves (Figure 3.4). Basal and peak rectal temperature, slope of the response curve and time to reach peak rectal temperature did not differ significantly ($P>0.05$) due to treatment group. Length of response, area under the curve and difference between peak and basal rectal temperature all differed significantly due to treatment group. The 20 minute treatment group had the longest length of response and greatest area under the curve and differed from the control and 10 minute group. The control group had a significantly lower difference between peak and basal rectal temperature compared to the three cold treatments. There were no differences between treatment groups

for any of the rectal temperature response curves during the noradrenaline challenge (Table 3.6).

When rectal temperature response parameters were compared for the ice vest and the noradrenaline challenges there were significant differences ($P<0.05$) in the length of response, peak rectal temperature, area under the curve, time to peak, slope and difference between peak and basal rectal temperature (Table 3.7). All these parameters except slope were greater for the noradrenaline challenge than the ice vest challenge. The treatment by noradrenaline challenge interaction was not significant for any of the rectal temperature response curve parameters.

3.3.4 Discussion

Using an ice vest to induce a cold response appears to have some merit and overcomes the issue of separation stress and the use of simulated cold exposure. Although some separation stress is still likely while the vest is fitted, this effect is probably much less than other methods of inducing cold stress such as cold water baths. The presence of an ice vest, even for 10 minutes, was enough to create a difference in rectal temperature when compared to control animals. In this study, 20 minutes appeared to be the optimal length of exposure as this was enough time to significantly increase the area under the curve and the length of response. However, the method for estimating the length of the thermogenic response (time to reach temperature > two standard deviations above basal rectal temperature) may have influenced this result. The drop in rectal temperature, by more than two standard deviations below the mean, while the vest was still on the animals in the 30 minute treatment may have been a reflection of continuing cold stress rather than in recovery from cold stress and equilibration back to basal temperature.

Table 3.6: Least square means for rectal temperature response curve parameters for ice vest challenge and noradrenaline challenge across the four ice vest treatments.

	Treatment			
	0 min	**10 min**	**20 min**	**30 min**
	n=10	*n=10*	*n=10*	*n=10*
Birth weight (kg)	4.45±0.29	4.40±0.29	3.97±0.29	4.34±0.29
Ice vest challenge				
Basal RT (°C)	39.17±0.09	39.11±0.09	39.14±0.09	39.07±0.09
Length of response (min)	29.80±3.25^{a}	26.40±3.25^{a}	39.60±3.25^{b}	32.90±3.25^{ab}
Peak RT (°C)	39.63±0.11	39.84±0.11	39.86±0.11	39.84±0.11
Time to peak (min)	8.00±1.42	10.00±1.42	9.40±1.42	10.00±1.42
Area under curve (°Cmin)	1136.54±129.14^{a}	1006.25±129.14^{a}	1530.38±129.14^{b}	1265.35±129.14^{ab}
Slope from basal to peak RT (°C/min)	0.12±0.03	0.13±0.03	0.12±0.03	0.08±0.03
Difference between peak and basal RT (°C)	0.46±0.06^{a}	0.73±0.06^{b}	0.72±0.06^{b}	0.77±0.06^{b}
Noradrenaline challenge	*n=9*	*n=10*	*n=10*	*n=10*
Basal RT (°C)	39.29±0.13	39.13±0.12	39.20±0.12	39.13±0.12
Length of response (min)	57.56±5.17	55.80±4.9	57.50±4.9	68.20±4.9
Peak RT (°C)	40.20±0.13	40.08±0.12	40.13±0.12	40.42±0.12
Time to peak (min)	24.67±2.96	22.00±2.80	28.20±2.80	30.60±2.80
Area under curve (°Cmin)	2254.09±208.85	2181.12±198.13	2252.04±198.13	2688.87±198.13
Slope from basal to peak RT (°C/min)	0.04±0.01	0.05±0.01	0.03±0.01	0.04±0.01
Difference between peak and basal RT (°C)	0.91±0.14	0.95±0.13	0.93±0.13	1.29±0.13

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 3.7: Least square means for rectal temperature response curve parameter for the ice vest challenge and the noradrenaline challenge.

	Ice vest challenge *n=40*	**Noradrenaline challenge** *n=39*
Basal rectal temperature (°C)	39.12±0.05	39.19±0.05
Peak rectal temperature (°C)	39.79±0.06^{a}	40.21±0.06^{b}
Time to peak (min)	9.40±1.10^{a}	26.40±1.10^{b}
Length of response (min)	32.20±2.10^{a}	59.80±2.10^{b}
Area under the rectal temperature response curve (°Cmin)	1234.63±84.70^{a}	2343.50±85.70^{b}
Slope from start of challenge to peak (°C/min)	0.11±0.01^{a}	0.04±0.01^{b}
Difference between peak and basal rectal temperature (°C)	0.67±0.05^{a}	1.02±0.05^{b}

Different superscripts within rows indicate means that differ significantly (P<0.05)

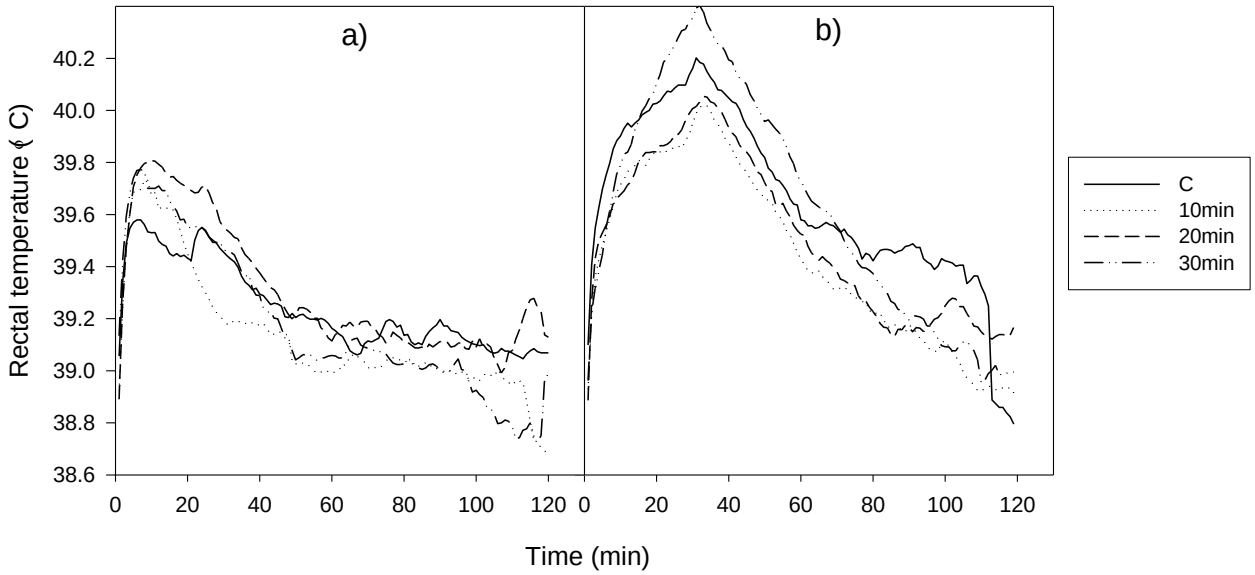

Figure 3.4: Rectal temperature response curve for a) ice vest challenge and b) noradrenaline challenge.

From this experiment, the use of a noradrenaline challenge does not appear to produce the same response as an ice vest with the noradrenaline challenge resulting in a much larger change in rectal temperature. Peak rectal temperature, the length of response and the area under the curve all increased following a noradrenaline challenge compared to the ice vest challenge. This larger response to the noradrenaline challenge may be due to the dosage used which may be higher than that produced by the lamb during cold stress. However, the differences seen between the ice vest and the noradrenaline challenges need to be treated with some caution as there was a 9 h age difference between the cold challenge and the noradrenaline challenge.

It appears as though prior cold exposure may influence the response to a noradrenaline challenge as the lambs in the 30 minute treatment had a higher area under the curve and peak rectal temperature although these were not significant when compared to the other treatments.

However, the lack of an interaction between treatment and noradrenaline challenge for any of the parameters measured suggests that prior treatment does not influence the response to noradrenaline challenge.

The length of time the vest was on may need to be extended to get a true test of a lamb's response to a cold stress. Slee *et al.* (1990) used a cooling water bath to test thermogenic responses to cold challenges and showed a general pattern in rectal temperature response where there was an initial increase to a peak and then a gradual decline. The basal rectal temperature reached plateau for a short time followed by a rapid decline before reaching a critical temperature (<35°C) when the challenge was terminated. In the current experiment, no lamb reached their basal rectal temperature while the ice vest was on suggesting a longer time of exposure was needed to actually test the lamb's ability to respond to a cold stress and to reach critical temperature.

There was no difference seen in the time to reach peak rectal temperature or the slope to peak during the ice vest challenge. This suggests that the initial response to a cold challenge was not a useful way of measuring the capacity of the lamb to respond to a cold challenge. A more useful measure may be how long the lamb can maintain basal rectal temperature under a prolonged cold challenge.

The behavioural test produced a second small peak for all treatment groups. From Figure 3.4, the second peak for the control group is the same as the first peak suggesting that the change in rectal temperature response curve for the control group may be due to the stress of separation from the ewe and the handling required for the behavioural test.

The behavioural test did not appear to produce consistent results probably due to the proximity of the test race to the ewe. Consequently, the behavioural test data was not analysed. The audio cue and the bleating of the lambs often induced a vocal response from the ewes regardless of whether it was their lamb undertaking the test. This caused confusion for the lambs leading to spurious results for this test. The age of the lambs during the test may also have influenced the results of this test. Much of the work on the ability of the lamb to discriminate its mother from an alien ewe has used lambs of 12 hours and older (Nowak *et al.* 1989; Sebe *et al.* 2007) with most lambs performing best at >18 hours (Nowak *et al.* 1989). There does not appear to be any evidence of the successful use of traditional discrimination tests in lambs younger than 12 hours of age. The lambs in this experiment were between 3 and 6 hours of age at the first test and 12 hours of age at the second. The use of a standard audio cue may also have impacted on these results. Some lambs may not have responded to this as readily as that of their own mother bleating or once again may have been able to hear their mother bleating in another part of the animal house so did not respond to the cue.

The use of an ice vest appears to be a valid method of inducing a cold response in neonatal lambs. However, more work is needed on how to prolong this response to measure a lamb's ability to maintain body temperature under prolonged cold stress. The results of the present experiment suggest that the use of noradrenaline to simulate cold exposure may not be testing the same response as a real cold challenge as it produces a different rectal temperature response curve compared to the ice vest. In light of these results, ice vests were used for an extended period of time (1 hour) to induce cold stress in neonatal lambs in the experiment outlined in Chapter 5.

3.4 A modified barrier test can be used to assess breed differences in lamb vigour

3.4.1 Introduction

Various methods of measuring lamb vigour and the ability of lambs to respond to the ewe in the first few days of life have been developed. These include time to progress through a series of critical early behaviours (Dwyer 2003; Slee and Springbett 1986) and behavioural tests such as discrimination tests (Bickell *et al.* 2009; Nowak and Lindsay 1992; Nowak *et al.* 1987; Sebe *et al.* 2007). Measuring early behaviours requires intensive observations over a period of a number of hours which may be difficult or impractical to do in many situations (eg. at night within extensive environments). Discrimination tests are often performed when the lamb is 12 hours of age up until 72 hours of age (Bickell *et al.* 2009; Nowak and Lindsay 1990). Attempts to apply these at younger ages (< 12 hours of age) have generally not been successful (Nowak *et al.* 1987) and for some breeds, such as Suffolks, poor results in the test have been observed up until 24 hours of age (Coombs and Dwyer 2008; Pickup and Dwyer 2001).

Pfister *et al.* (2006b) used a barrier test to determine differences in the performance of lambs from ewes either fed or not fed locoweed during gestation. For this test, lambs were placed behind a mesh barrier 2 m from their penned dam and were allowed 90 seconds to move to her. Pfister *et al.* (2006b) found that lambs from ewes fed locoweed performed poorly in the test compared to control lambs. They were much slower to move past the barrier and reach the dam and were less likely to actually reach the ewe.

An adaptation of this test, where the ewe is replaced by a model ewe and a standardised audio cue, may be an alternative way of measuring early vigour in neonatal lambs. Replacement of

the ewe with a model would have the advantage of providing a standard stimulus for the lamb so that the focus would be on how the lamb responds without the confounding effect of differences in the expression and intensity of ewe behaviours.

Vigour differences between Scottish Blackface and Suffolk lambs have been well documented by Dwyer and her colleagues (Dwyer *et al.* 2005; Dwyer and Lawrence 2000; Dwyer *et al.* 1996). Scottish Blackface lambs are more vigorous than Suffolk lambs as measured by time to perform neonatal behaviours and their performance in discrimination tests in the first few days after birth (Coombs and Dwyer 2008; Dwyer 2003; Pickup and Dwyer 2001). Due to these neonatal differences, these breeds provide an ideal model to assess the usefulness of an adapted barrier test as a measure of early lamb vigour.

The aim of this experiment was to determine if Scottish Blackface lambs and Suffolk lambs differed in their ability to perform a barrier test and whether the age of the lamb influenced this performance. It was hypothesised that Scottish Blackface lambs would perform better than Suffolk lambs and that there would be an improvement in the response of lambs with increasing age.

3.4.2 Materials and Methods

This experiment was conducted at the Scottish Agriculture College's Woodhouselee Farm as part of a broader lamb vigour experiment. Experiments were conducted under a licence granted under the Animal Scientific Procedures (1986) Act, licence no. PPL 60/4081. The entire experimental protocol was reviewed and approved by the SAC Animal Experiments and Ethics Committee.

3.4.2.1 Protocols and measures

Fifty-six Blackface (B) lambs and twenty-seven Suffolk (S) lambs were used in this experiment. Single (B n=10; S n=9), twin (B n=26; S n=12) and triplet lambs (B n=9; S n=6) were used. At 4 and 10 hours of age, each lamb was tested in a modified barrier test as shown in Figure 3.5 and Plate 3.2. Prior to the 4 h test lambs had been under intensive observation to record time to perform early behaviours and had rectal temperature recorded and were moved into an individual pen with their mother. Between the 4 and 10 h tests lambs were generally left undisturbed. The test took place in a separate building to where ewes and lambs were housed so there was no visual or auditory contact with other ewes or lambs. The test consisted of a trapezoid arena with a semi-circular, wire mesh barrier at the narrow end and a model ewe 2.5 m from the barrier, at the wide end. Lambs were placed behind the wire mesh barrier and allowed 3 minutes to reach the model ewe. The audio cue, of a Blackface and Suffolk ewe bleating alternately, was played from the model ewe at a volume equivalent to normal ewe bleating in the shed environment. The ewe bleats used were not the mothers of any lambs in the study and the bleats were high pitched bleats of ewes responding to the separation of their lambs. The floor of the arena was marked with a 0.5 m grid. Video footage of the lambs in the arena was recorded and the following information was obtained:

1. Time to reach the ewe
2. Number of bleats
3. Bleat score – 1. No bleats, 2. Some bleats but usually quiet and 3. Loud continuous bleating
4. Number of grids crossed

5. Linear movement score - 0. Stays behind barrier, 1. Moves past barrier <0.5 m, 2. 0.5 m – 1 m past barrier, 3. 1.5 m past barrier, 4. 1.5 – 2 m past barrier and 5. Reaches ewe
6. Overall score (defined in Table 3.8):

Table 3.8: Definitions for overall score in the modified barrier test.

Score	Description
0	Sits, does not bleat or move, is not alert
1	Standing still, may look around and bleat occasionally
2	Circles behind barrier, bleats occasionally, may move but not towards ewe or in a sensible manner
3	Lamb actively trying to get past barrier, bleating frequently, alert
4	Moves past barrier towards ewe, bleating frequently, alert
5	Reaches ewe, bleating frequently, alert

3.4.2.2 Statistical Analysis

Data were analysed using SAS (version 9.1.3). PROC FREQ was used to determine any differences in the proportion of lambs reaching the ewe due to breed (Suffolk or Blackface), test time (4 or 10 hours) or litter size (single, twin or triplet). PROC GLM was used to determine differences in the time to reach the ewe, number of bleats before reaching the ewe, bleat rate (bleats/s) and the number of grids crossed to reach the ewe with breed, test time and litter size included as fixed effects. PROC GLM was used to determine differences in the total number of bleats in the three minute test period, number of bleats to the end of the test (where the end was defined as the lamb reaching the ewe or 3 minutes whichever occurred first), bleat rate, total number of moves in the three minute test period, number of moves to the end of the test, movement rate, linear movement score and overall score with breed, test, litter size and to-ewe (yes or no if the lamb did or did not reach the ewe, respectively) as fixed effects. Birth weight was included as a covariate. Correlations between overall barrier test score and time to perform early behaviours were calculated using PROC CORR to determine Pearson's correlation coefficients with 95% confidence intervals. Only the

behaviours: time to shake head, reach knees, attempt to stand, stand and reach the udder were used as there was not enough data available on time to unsuccessfully or successfully suckle for calculation of correlations. For more detailed information on how these behaviours were recorded and defined see Dwyer *et al.* (1996; 2008).

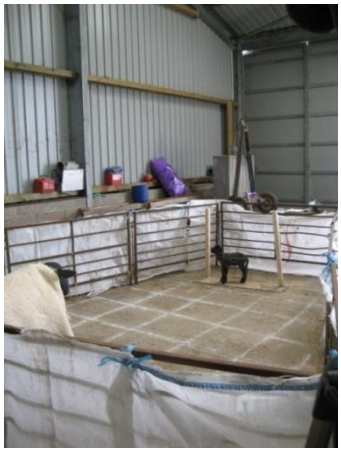

Plate 3.3: Modified barrier test arena with Suffolk lamb undergoing test.

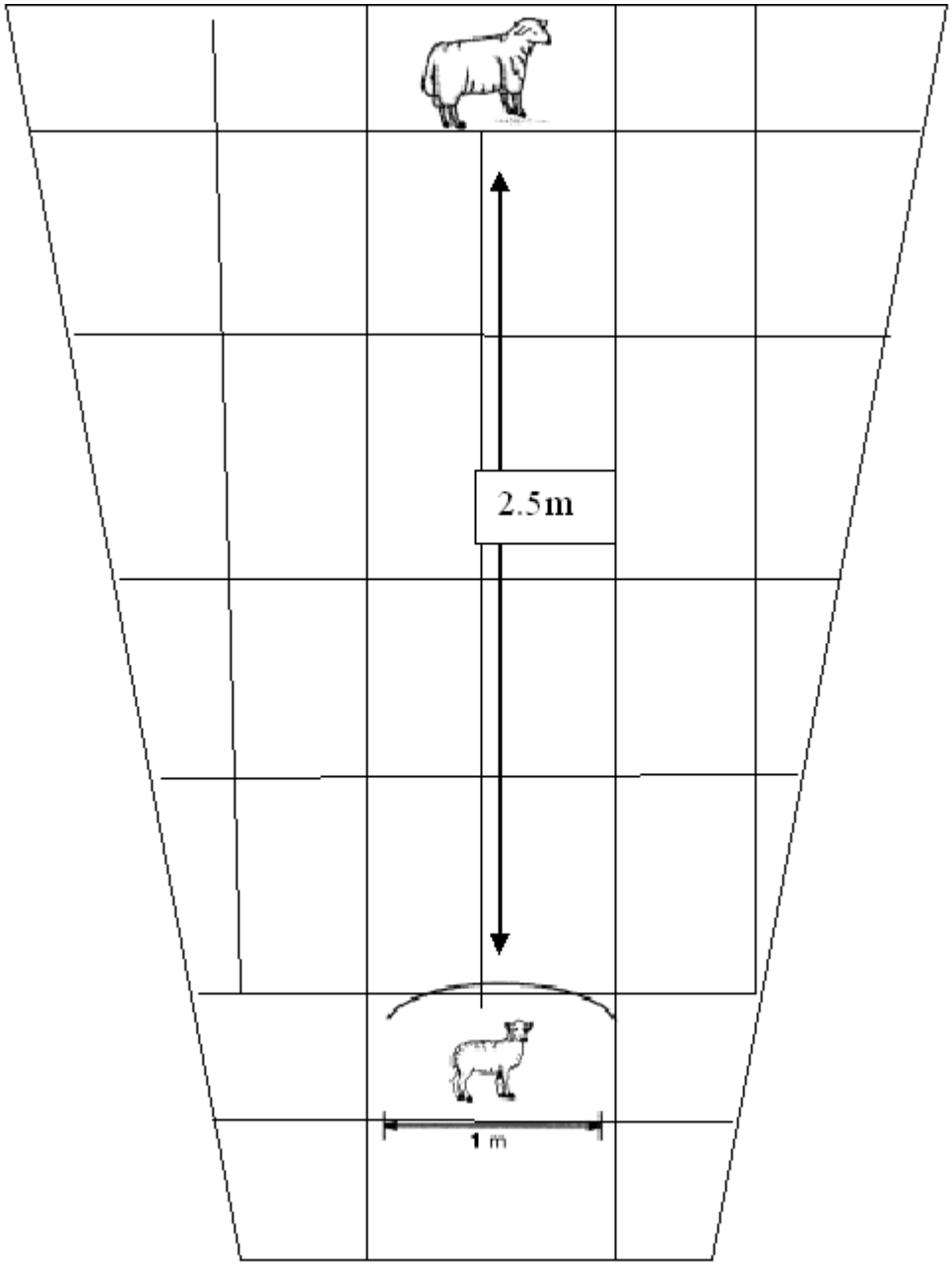

Figure 3.5: Schematic of modified barrier test arena showing position of lamb in relation to model ewe and the grid system.

3.4.3 Results

A significantly (P<0.05) greater proportion of Blackface lambs reached the ewe than Suffolk lambs (25.4% vs. 8.5%, respectively). The proportion of lambs reaching the ewe did not differ due to test time (4 hour: 14.8%, 10 hour: 19.0%, P>0.05) or litter size (single: 9.2%, twin: 19.0%, triplet: 5.6%, P>0.05). Blackface lambs were significantly (P<0.001) lighter than Suffolk lambs (4.3±0.1kg and 5.1±0.2kg, respectively) and birth weight differed across litter sizes with single lambs being heavier than twins and triplets within breeds (Blackface: single: 4.9±0.2kg, twin: 4.1±0.2kg and triplet: 3.6±0.3kg; Suffolk: single: 5.7±0.2 kg, twin: 4.6±0.2 kg and triplet: 5.0±0.3 kg, P<0.001). There was no significant difference in birth weight between twin and triplet lambs.

Of the lambs that reached the ewe, the time taken was significantly faster at 10 h compared to 4 h (P<0.05, Table 3.9). Lambs also bleated less at the 10 h test compared to the earlier test (P<0.01, Table 3.9) and triplet lambs bleated significantly more than single and twin lambs across both tests (P<0.01, Table 3.9). However, there was no significant (P>0.05) difference in the rate of bleats between test times. There was no significant (P>0.05) difference in the number of moves taken to reach the ewe between the two test times and the time taken to reach the ewe, bleat rate and moves to the ewe did not differ significantly due to litter size or breed (P>0.05, Table 3.9). The number of bleats did not differ due to breed.

Table 3.10 and Table 3.11 show the results for all lambs for the other variables measured. The number of bleats to the end of the test tended to differ (P=0.08) due to test time. Lambs that reached the ewe bleated significantly (P<0.01) more than lambs that did not reach the ewe over the three minute test period. The number of grids crossed to the end of the test differed significantly due to breed (P<0.001) and whether or not the lamb reached the ewe;

the Blackface lambs took more moves to reach the ewe. Lambs that did not reach the ewe crossed significantly less grids than those that successfully reached the ewe ($P<0.001$) and there was a significant test time x to-ewe (whether or not the lamb reached the ewe) interaction for the total number of grids crossed in the three minute test period (Figure 3.6, $P<0.001$). The total number of grids crossed also differed significantly due to litter size with single lambs crossing less grids than twin or triplet lambs ($P<0.001$,Table 3.11). Blackface lambs crossed significantly more grids than Suffolk lambs ($P<0.001$, Table 3.10) and more grids were crossed at the 10 h test than the 4 h test ($P<0.001$, Table 3.10). The linear movement score only differed due to whether or not the lamb reached the ewe with lambs reaching the ewe having a higher score ($P<0.001$,Table 3.11). The rate at which the lambs crossed grids differed significantly between breeds, test times and whether the lamb reached the ewe ($P<0.001$, Table 3.10 & Table 3.11). Suffolk lambs moved slower than Blackface lambs. Lambs in the 4 h test moved slower than at the 10 h test and lambs that reached the ewe moved more quickly than those that did not. For the overall score, there was a significant breed x to-ewe interaction (Figure 3.7, $P<0.001$) and overall score also differed due to breed, litter size and whether the lamb reached the ewe ($P<0.001$, Table 3.10 & Table 3.11). Blackface lambs had a higher score than Suffolk (Table 3.10) lambs and triplet lambs had a higher score than single lambs, but twin lambs did not differ from either single or triplet lambs (Table 3.11).

Correlations between overall score and time to perform early critical behaviours (up to time to reach the udder) are shown in Table 3.12. At the 4 h test, there were very low correlations between overall score and behaviours. However, at the 10 h test, there were moderate favourable correlations between overall score and time to stand and reach the udder which

approached significance (P=0.06 and 0.08, respectively). No other correlations were significant.

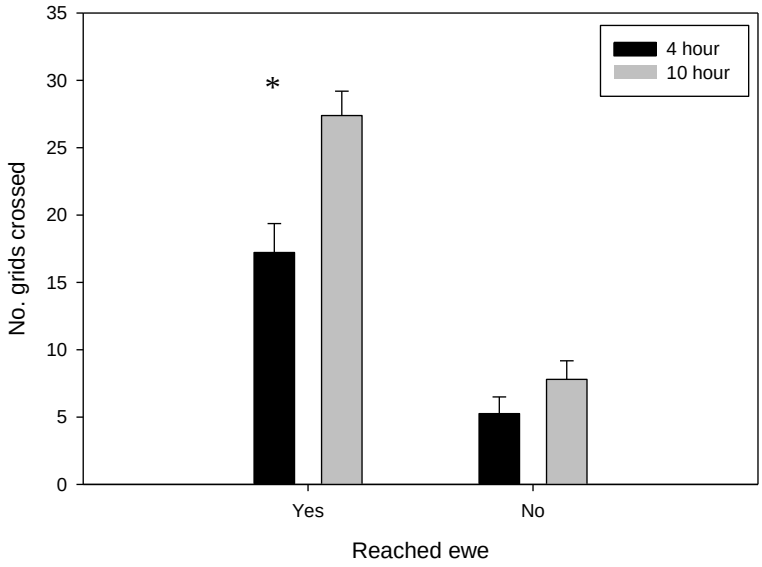

Figure 3.6: Interaction between test time and whether the lamb reached the ewe for the total number of grids crossed in the three minute test period. Error bars represent standard errors and asterisks indicate means that differ significantly (P<0.05).

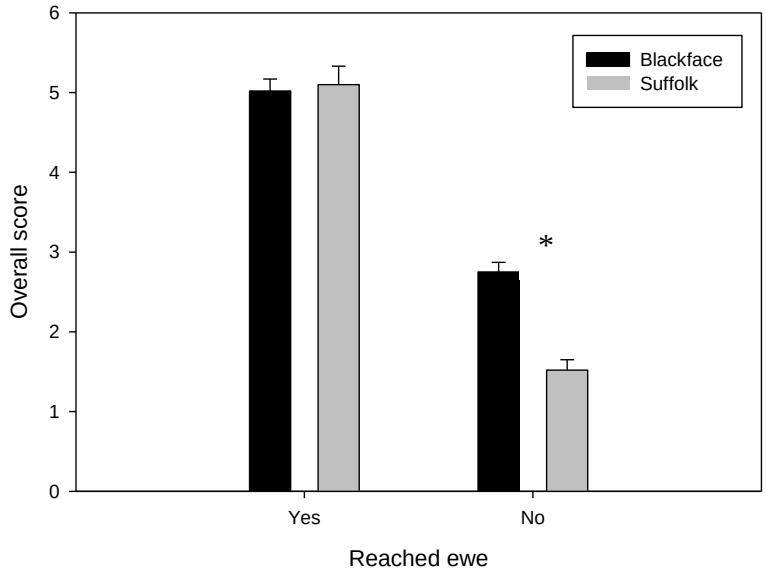

Figure 3.7: Interaction between breed and whether the lamb reached the ewe for the overall test score. Error bars represent standard errors and asterisks indicate means that differ significantly ($P<0.05$).

Table 3.9: Least square means and standard errors (*n*) for time, number of bleats and grids crossed to reach the ewe for those lambs that reached the ewe.

	Breed		Test time		Litter size		
	Blackface	**Suffolk**	**4 hour**	**10 hour**	**Single**	**Twin**	**Triplet**
Time to reach ewe (s)	113.42±11.57 (32)	126.38±17.18 (11)	150.94±15.25^{a} (20)	88.87±13.09^{b} (23)	100.83±17.81 (12)	98.59±12.73 (23)	160.29±27.96 (8)
No. bleats to ewe	37.32±4.08 (33)	46.09±6.09 (11)	51.63±5.59^{a} (17)	31.79±4.41^{b} (26)	33.24±6.27^{a} (11)	32.94±4.22^{a} (25)	58.95±9.78^{b} (8)
Bleat rate (bleats/s)	0.37±0.04 (33)	0.41±0.06 (11)	0.36±0.06 (17)	0.42±0.05 (26)	0.39±0.07 (10)	0.39±0.05 (22)	0.40±0.10 (8)
No. grids crossed to ewe	13.81±1.32 (35)	13.25±2.01 (12)	14.02±1.81 (19)	13.04±1.49 (27)	11.48±2.03 (13)	10.61±1.37 (26)	18.50±3.37 (8)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 3.10: Least square means and standard errors (*n*) for differences in barrier test measures across all lambs for breed and test time.

	Breed		Test time	
	Blackface	**Suffolk**	**4 hour**	**10 hour**
No. bleats (test ends when lamb reaches ewe)	40.36±3.36 (85)	33.93±4.29 (45)	42.67±3.80 (66)	31.62±3.58 (64)
No. bleats in 3 minute period	52.82±3.51 (85)	44.89±4.48 (45)	52.79±3.97 (66)	44.89±3.74 (64)
Bleat rate to end of test (bleats/s)	0.33±0.02 (81)	0.28±0.03 (44)	0.31±0.02 (65)	0.30±0.02 (60)
No. grids crossed (test ends when lamb reaches ewe)	11.33±0.87^{a} (88)	6.64±1.01^{b} (54)	8.19±0.91 (72)	9.79±0.87 (70)
No. grids crossed in 3 min	17.87±1.09^{a} (87)	10.97±1.32^{b} (54)	11.24±1.27^{a} (72)	17.59±1.15^{b} (69)
Rate of movement (no. grids/s)	0.11±0.01^{a} (84)	0.07±0.01^{b} (53)	0.07±0.01^{a} (72)	0.12±0.01^{b} (66)
Linear movement score	2.88±0.13 (88)	2.58±0.16 (54)	2.66±0.15 (72)	2.79±0.14 (70)
Overall score	3.87±0.10^{a} (88)	3.32±0.13^{b} (54)	3.55±0.11 (72)	3.65±0.11 (70)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 3.11: Least square means and standard errors (*n*) for differences in barrier test measures across all lambs for litter size and whether the lamb reached the ewe

	Litter size			Reached ewe	
	Single	**Twin**	**Triplet**	**Yes**	**No**
No. bleats (test ends when lamb reaches ewe)	36.49±4.88 (31)	34.48±3.52 (69)	40.46±5.36 (30)	34.48±4.52 (45)	39.81±2.99 (85)
No. bleats in 3 minute period	46.33±5.09 (31)	48.24±3.67 (69)	51.97±5.59 (30)	58.23 ± 4.72^{a} (45)	39.45 ± 3.12^{b} (85)
Bleat rate to end of test (bleats/s)	0.31±0.03 (30)	0.30±0.02 (65)	0.31±0.03 (30)	0.39 ± 0.03^{a} (40)	0.22 ± 0.02^{b} (85)
No. grids crossed (test ends when lamb reaches ewe)	7.56±1.12 (39)	8.97±0.87 (73)	10.43±1.36 (30)	11.46 ± 1.09^{a} (48)	6.52 ± 0.72^{b} (94)
No. grids crossed in 3 min	10.55 ± 1.47^{a} (39)	15.69 ± 1.14^{b} (73)	17.01 ± 1.77^{b} (30)	22.31 ± 1.44^{a} (48)	6.53 ± 0.94^{b} (93)
Rate of movement (no. grids/s)	0.09±0.01 (38)	0.10±0.01 (69)	0.09±0.01 (30)	0.15 ± 0.01^{a} (43)	0.04 ± 0.01^{b} (94)
Linear movement score	2.67±0.17 (39)	2.84±0.14 (73)	2.67±0.22 (30)	4.89 ± 0.18^{a} (48)	0.56 ± 0.11^{b} (94)
Overall score	3.35 ± 0.14^{a} (39)	3.56 ± 0.11^{ab} (73)	3.89 ± 0.17^{b} (30)	5.07 ± 0.14^{a} (48)	2.13 ± 0.09^{b} (94)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 3.12: Correlations and 95% confidence intervals between overall score and time to perform early behaviours.

	Test time					
	4 hour			10 hour		
	Correlation coefficient	***p* value**	**95% CI**	**Correlation coefficient**	***p* value**	**95% CI**
Shakes head	0.08	0.53	-0.18 – 0.33	0.05	0.70	-0.21 – 0.31
Reached knees	0.01	0.95	-0.25 – 0.27	-0.04	0.75	-0.30 – 0.22
Attempts to stand	0.07	0.64	-0.22 – 0.35	-0.05	0.73	-0.33 – 0.24
Stands	-0.07	0.64	-0.35 – 0.22	-0.28	0.06	-0.52 – 0.02
Reached the udder	-0.08	0.63	-0.39 – 0.25	-0.29	0.08	-0.56 – 0.04

3.4.4 Discussion

The results from this experiment suggest that the modified barrier test using a model ewe and audio cue could be a useful way of measuring vigour at an early age in neonatal lambs. The contrasting performance of Blackface and Suffolk lambs in the test was consistent with the findings of Dwyer *et al.* (2005; 1996) where Blackface lambs were more vigorous than Suffolk lambs. More Blackface lambs successfully reached the model ewe and there was also a significant breed difference in the overall score in the test with Blackface lambs having a higher average score than Suffolk lambs. The number of grids crossed to the end of the test (where the end is defined as reaching the ewe or 3 minutes, whichever occurs first) and across the three minute test period was also greater for Blackface lambs. However, the time taken to reach the ewe did not differ with breed which suggests that Blackface lambs took a less direct route to reach the ewe and once they reached the ewe, they did not remain close to her. This may mean that the lamb was not completely deceived by the model ewe and the audio bleats, but it may also be that the lamb was not detecting appropriate responses having reached the ewe, so it continued moving around the arena in search of its mother. Other authors who have used model ewes and audio bleats of lamb's own or alien dams have found that not all lambs will respond and have suggested that this is because the models are not lifelike enough (Shillito Walser and Walters 1987; Shillito Walser *et al.* 1985; Winfield and Kilgour 1976). However, all of these studies used lambs that were much older (1 day – 30 days) than those in the current study and it would be expected that mutual recognition would have developed at these older ages.

The time the test was performed appears to be important with the performance of lambs generally improving at the later 10 hour test. Other authors have shown that the ability of lambs to successfully negotiate discrimination tests and other behaviour tests improves with age (Nowak *et al.* 1989) but no other authors have studied lambs as young as in the current study. The results suggest that the lamb may be showing an element of learning from one test time to the next or alternatively, that older lambs are more able to respond under these test conditions. A larger experiment using different lambs at the two ages would help to clarify which of these interpretations is correct. The improvement in the correlations between time to perform early behaviours and overall score in the modified barrier test suggests that lambs at 10 h of age are responsive to auditory cues and that this response may reflect lamb vigour as identified in early behaviours.

Litter size did not influence the time taken to reach the ewe, the number of bleats or grids crossed to reach the ewe. However, when looking at the overall test score, triplet lambs appeared to perform better in the test than singles but were not significantly different to twins. This is perhaps surprising as triplet lambs tend to be less mature than single and twin lambs (Barlow *et al.* 1987; Stafford *et al.* 2007) and often are slower to perform critical early behaviours and also have poorer vigour scores (Dwyer *et al.* 2005).

This test may provide an alternative and more practical measure of lamb vigour in terms of the ability of lambs to make contact with the ewe as it can be done at an early age and it does not require the presence of the dam. This means the lamb can be moved easily and assessments made at convenient times. As there was no difference

in the overall score between the 4 hour and the 10 hour tests, this test may be a useful measure of vigour anytime between these ages. However, assessment of older animals might be a more accurate reflection of lamb vigour at birth given the moderate correlations between the overall score in the 10 hour test and time to stand and reach the udder. This test may become particularly important as a measure of lamb vigour around the time the ewe moves away from the birth site, when the lamb has increased responsibility to retain contact with its dam.

Chapter 4: Sub-maintenance nutrition in late pregnancy does not compromise lamb vigour

4.1 Introduction

Nutrition during pregnancy has a well documented impact on lamb survival with better nutrition improving lamb survival rates (Alexander *et al.* 1956; Everett-Hincks *et al.* 2005a; Holst *et al.* 1986; Scales *et al.* 1986; Tygesen *et al.* 2008). Mid to late pregnancy nutrition is particularly important as nutritional restriction during this time can result in reduced lamb birth weights as this is the time when 90% of foetal growth occurs (Redmer *et al.* 2004). Furthermore, late pregnancy nutrition is particularly important for improving lamb survival in multiple litters (Everett-Hincks *et al.* 2005a; Holst 1987) and this appears to be largely associated with birth weight effects.

Lamb birth weight has been shown to have a direct impact on lamb survival (Alexander *et al.* 1956; Everett-Hincks and Dodds 2007; Fogarty *et al.* 1992; Khalaf *et al.* 1979) with lambs at either extreme, i.e. very heavy or very light, at higher risk of mortality. Owens *et al.* (1985) reported that a 1 kg increase in birth weight, resulted in a 28% increase in lamb survival in the large litters of Booroola Merinos. Holst *et al.* (1986) and Corner *et al.* (2008) found that when multiple-bearing ewes were fed on a low plane of nutrition in early pregnancy and subsequently on a high plane of nutrition in late pregnancy, lamb birth weights were higher than those from ewes on a low plane of nutrition in late pregnancy regardless of nutrition level in early pregnancy. This pattern has also been reported by Fogarty *et al.* (1992) who found that mid-pregnancy nutrition (high or low) had no impact on lamb survival when all

ewes were on a high plane of nutrition in late pregnancy. However, condition score/fat score at joining and throughout pregnancy may complicate this effect and a clear positive relationship between condition score at lambing and survival rates has been shown by a number of authors (Ferguson *et al.* 2007; Gibb and Treacher 1982; King *et al.* 1990). The study of Ferguson *et al.* (2007) showed that if condition score was maintained at 3 throughout pregnancy then there was a 5% increase in survival for singles and a 12% increase in survival for twins compared to a condition score of 2.5 in Merino ewes. Even greater improvements were reported when contrasts were made at lower condition scores.

Neonatal lamb behaviour and vigour may be influenced by nutritional restriction during pregnancy (Everett-Hincks *et al.* 2005a) and it is thought that lambs with limited energy reserves due to poor maternal nutrition have a reduced suckling drive and chance of suckling successfully (Nowak and Poindron 2006). Dwyer *et al.* (2003), using Scottish Blackface lambs, did not find a direct impact of maternal undernutrition on lamb behaviour but nutrition did impact on lamb birth weights and low birth weight lambs were generally less vigorous. Cloete (1993) reported that lamb vigour, as measured by time to stand and suckle, improved with increasing birth weight with a reduction of 1.4 minutes per kg birth weight in time to suckle. Owens *et al.* (1985) found that a 1 kg increase in birth weight reduced the time for lambs to suckle by 15.8 minutes for all litters, from singles through to quadruplets for Booroola Merinos. Slee and Springbett (1986) also found that within a breed, lamb activity improved with increasing birth weight. However, between breeds, the smaller breeds such as primitive breeds (Soay and Boreray), Welsh and Scottish Blackface sheep tended to have better lamb vigour than the heavier breeds such as Border Leicesters

(Slee and Springbett 1986). Therefore it appears that low birth weight lambs can still be vigorous suggesting that factors other than birth weight alone, such as maturity at birth, determine lamb vigour.

Nutrition during pregnancy is thought to impact on the ability of the lamb to respond to cold with undernourished ewes producing lambs that are less able to cope with cold stress (Budge *et al.* 2004; Kerslake *et al.* 2009). This can be associated with reductions in birth weight, where smaller lambs have a higher surface area to weight ratio and consequently, more heat loss (Alexander 1962c). Alternatively, it may be due to lower energy reserves in the form of brown adipose tissue (BAT) and therefore, reduced thermoregulatory capacity (Budge *et al.* 2004; Stevens *et al.* 1990; Stott and Slee 1987). However, other environmental influences during pregnancy may also impact on lamb birth weight and BAT development. Chronic cold exposure during pregnancy has been shown to increase BAT volume and function in lambs from ewes fed 60% of maintenance energy requirements (Clarke *et al.* 1997a) but lead to no change in lambs from well fed ewes (Gate *et al.* 1999). Budge *et al.* (2004) found that ewes that had a restricted nutrient intake in late pregnancy (day 115 to parturition) produced lambs with decreased levels of perirenal fat compared to unrestricted controls (19.35 vs. 23.1 g). These lambs also had 2.5 times less uncoupling protein 1 (UCP1) mRNA expression. The protein UCP1 is central to BAT metabolism and non-shivering thermogenesis (Himms-Hagen 1985). Interestingly, this study also reported that lambs from ewes energy restricted prior to conception, and for different lengths of time following conception, did not differ in the amount of fat deposited but did differ in levels of UCP1 mRNA (Budge *et al.* 2004). This

highlights the potential importance of nutrition at different times prior to conception and during gestation for subsequent lamb viability.

The present experiment was designed to evaluate the hypothesis that lambs from ewes fed either above or below maintenance energy requirements in the last 50 days of pregnancy would differ in vigour as defined by their immediate post partum behaviours and also physiological parameters associated with lamb survival.

4.2 Materials and Methods

All procedures in this experiment were conducted with the approval of the CSIRO FD McMaster Laboratory Chiswick, Animal Ethics Committee, AEC No. 08/11.

4.2.1 Animals and Management

Fifty four, multiparous, three and four year old Merino ewes and their lambs were used for this experiment. Ewes were selected from a larger group of 270 ewes. Initially, ewes were allocated to one of three mating groups (approximately 90/group) balanced for liveweight. Each group was oestrus synchronised 10 days apart using 300 mg progesterone via EAZI-BREED® CIDR® (Pfizer) and given a 400 i.u. serum gonadotrophin injection (Pregenocol™, Bioniche Animal Health Pty Ltd) on removal of CIDR®. Six Merino rams were introduced following the injection and remained with the ewes for 10 days until the next mating group (MG) had been injected when they were introduced to the next MG. Therefore the same rams were used for each MG.

At day 60 after joining, ewes were pregnancy scanned. Nine single and nine twin bearing ewes were selected from each of the mating groups with the criteria of liveweight >40kg and a condition score ≥ 3. This resulted in 54 experimental ewes which were then run as a single mob on native pasture until day 100 of pregnancy when the nutritional treatments began sequentially for each mating group.

The nutritional treatments were conducted in outdoor pens where ewes were fed in small groups of four or five ewes and were weighed weekly. Two ewes were removed from the experiment as one did not adapt to the pelleted ration (MG1) and the other developed laminitis (MG2). At day 143 of gestation, the ewes within each mating group were moved into individual pens (1 m x 1 m) in the animal house for lambing. Three more ewes were removed from the experiment after they had entered the animal house due to: a vaginal prolapse (MG1), a breech birth (MG3) and abortion post-scanning (MG3).

4.2.2 Treatments and protocols

The experiment was a two x two factorial design with two nutritional and two litter size treatments balanced across mating groups. Ewes were fed at either 0.8 or 1.2 of maintenance energy requirements for the last 50 days of gestation calculated using the SCA Feeding Standards for Australian Livestock (Ruminants 1990) which was adjusted according to litter size and ewe liveweight. Lucerne based animal house pellets (9.04 MJME/kgDM; 16.2% CP) were used as the feed ration.

Ewes were brought into the animal house and penned individually at day 143 of pregnancy to allow them to become accustomed to the conditions. From day 146 ewes were monitored constantly and at the first sign of lambing, video recording commenced and continued until the lamb was three hours of age. The ewe was allowed two hours from the appearance of membranes to give birth before assistance was provided unless malpresentation was clearly apparent.

Following birth, lambs were removed from the pen at 10 - 15 minutes of age and the following measurements were recorded: rectal temperature, birth weight, crown rump length, neck circumference and sex of the lamb. A 4 ml blood sample was also taken and packed cell volume was measured immediately via haematocrit. The lamb was then put back with the ewe and they were left undisturbed until 3 hours of age.

At 3 hours of age, a second 4 ml blood sample was collected and rectal temperature was recorded. At 5.5 hours of age, an iButton® temperature logger (Alfa-Tek Australia) was inserted rectally to log rectal temperature every minute until 8 hours of age. If the lamb was less than 3.5 kg, rectal temperature was measured every 15 minutes using a digital thermometer until 8 hours of age. At 6 hours of age, the lamb had a third 4 ml blood sample taken. The lamb was then injected with noradrenaline (Levophed®) at a rate of 150 µg/kg birth weight to simulate a cold challenge (Slee *et al.* 1987a). At 6.5 hours of age a final blood sample was taken. The iButton® was removed at 8 hours of age.

Lambs were placed in a cradle for ease of handling at sampling times and to standardise sampling method. All blood samples were collected via jugular venipuncture into blood tubes (Vacutainer®, Becton Dickenson and Company) with a sodium fluoride and potassium oxalate anti coagulant. Blood samples were centrifuged at 3000 rpm for 10 minutes and the plasma was collected and frozen immediately and stored at -20^{o}C for later analysis.

Lambs in MG3 had an image taken using an infra red thermal imaging camera (ThermaCAM^{TM}, FLIR systems) at each blood sampling time point and at 8 hours of age. At the first sampling, two images were taken, one with the lamb wet and one towel dry. This resulted in lambs from MG3 being out of the pen for significantly longer (4.67±0.26, 4.90±0.25 and 5.82±0.29 minutes for MG 1, 2 and 3 respectively) at the first sampling time point than lambs from MG1 or MG2.

Ewes and their lambs remained in the animal house until the lamb was 24 hours old when they were released into the paddock surrounding the animal house.

4.2.3 Lamb behaviour

Lamb behaviour (Table 4.1) was assessed from video recordings. The time taken from birth to perform each behaviour was recorded. A vigour score was also assessed for each lamb according to the criteria detailed in Table 4.2. If a lamb had not

performed all behaviours in 3 hours it was given a time of 180 minutes for that behaviour.

Table 4.1: Lamb behaviour definitions (based on Dwyer *et al.* 2005).

Behaviour	Definition
Shakes head	Lamb raises and shakes head
To knees	Lamb rolls onto chest, gathers legs under it and pushed front half of the body up off the ground
Attempts to stand	Lamb supports body weight on at least one foot
Stands	Lamb stands unsupported on all four feet for >5 s
Reaches udder	Lamb approaches ewe and nudges her in the udder region
Unsuccessful suck	Lamb places head under ewe in contact with the udder but either fails to grasp the teat or releases it without sucking
Sucks	Lamb holds teat in its mouth and appears to be sucking with appropriate mouth and head movements, may be tail-wagging, remains in this position for >5 s

Table 4.2: Lamb vigour score (adapted from Holst 1987).

Score	Description
1	Doesn't stand for at least 40 mins; little or no teat-seeking drive; doesn't appear alert or active
2	Attempts to stand after 30 min; low teat-seeking drive and tendency to follow ewe; shows some alertness but not very active. Does not appear very coordinated in attempts
3	Shakes head within 30 sec; attempts to stand within 15 min; seeking teat within 10 min of standing; follows ewe but distracted by other moving objects; generally alert and active. Coordination may be lacking
4	Attempts to stand within 10 min of birth; seeking teat within 5 min of standing; strong tendency to follow ewe; alert and active and movements well coordinated
5	Attempts to stand within 5 min of birth; follows ewe closely; very alert and active

4.2.4 Blood assays

All blood samples were assayed for glucose, fructose, free triiodothyronine (T3) and thyroxine (T4) and non-esterified fatty acids (NEFA). The 10-15 minute blood sample was also assayed for lactate. Glucose (assay variation <0.53%) and lactate (assay variation <2.3%) were tested using the Dimension clinical chemistry system

(DADE Behring). Fructose (assay variation <3.1%) was assayed using an enzymatic colorimetric method (Ameyama *et al.* 1981). Free T3 (assay variation <7.3%) and free T4 (assay variation <1.6%) were tested using a Siemens competitive analog immunoassay on the Immulite system. NEFA (assay variation <3.9%) were assayed using an *in vitro* enzymatic colorimetric method (Wako NEFA C test kit).

4.2.5 Statistical analyses

The iButton temperature profiles were analysed to derive the following temperature parameters for each lamb: basal rectal temperature, peak rectal temperature, time to peak, time at peak, length of response (time of injection to time the temperature reached two standard deviations above basal), slope and area under the curve. These and all other data were tested for normality prior to analysis. A natural log transformation was used to achieve normality for some variables (time at peak, time out, shake head, reach knees, attempt to stand, stands, reach udder, unsuccessful suckle, suckle).

Analysis of variance (PROC GLM, SAS 9.1.3) was used for iButton and behavioural measures with mating group, actual litter size and nutrition fitted as fixed effects. Four levels of nutrition were used in the analysis based on amount of feed offered to each group of ewes namely, 8.4, 10.2, 12.6 and 15.6 MJME/day rather than 0.8 or 1.2 maintenance energy requirements. This was done due to ultrasound scanning errors for litter size. Fifteen ewes were diagnosed as twin bearing but gave birth to singletons and four ewes were pregnant with twins but were diagnosed as single bearing. Consequently, these ewes were fed incorrect rations and therefore not

receiving 0.8 or 1.2 of maintenance energy requirements due to the misdiagnosed litter size. The power of the experimental design was therefore reduced due to the change in design for analysis.

The blood measures were analysed using PROC MIXED in SAS. Blood samples from birth, 3 and 6 hours were analysed initially followed by a second analysis including only the 6 and 6.5 hours sample to examine the effect of the noradrenaline challenge.

4.3 Results

4.3.1 Morphological data

Birth weight differed significantly ($P<0.001$) due to level of nutrition and litter size with single lambs being heavier than twin lambs (Table 4.3 & Table 4.4). Crown rump length, neck circumference and rectal temperature at 10 minutes and 3 hours did not differ due to level of nutrition, litter size or mating group (Table 4.3 & Table 4.4) although crown rump length and neck circumference both increased with increasing birth weight.

4.3.2 Neonatal behaviours

There were no significant ($P<0.05$) differences in neonatal behaviours or vigour score for level of nutrition, litter size or mating group (Figure 4.1a, b and c) and there were no significant interactions.

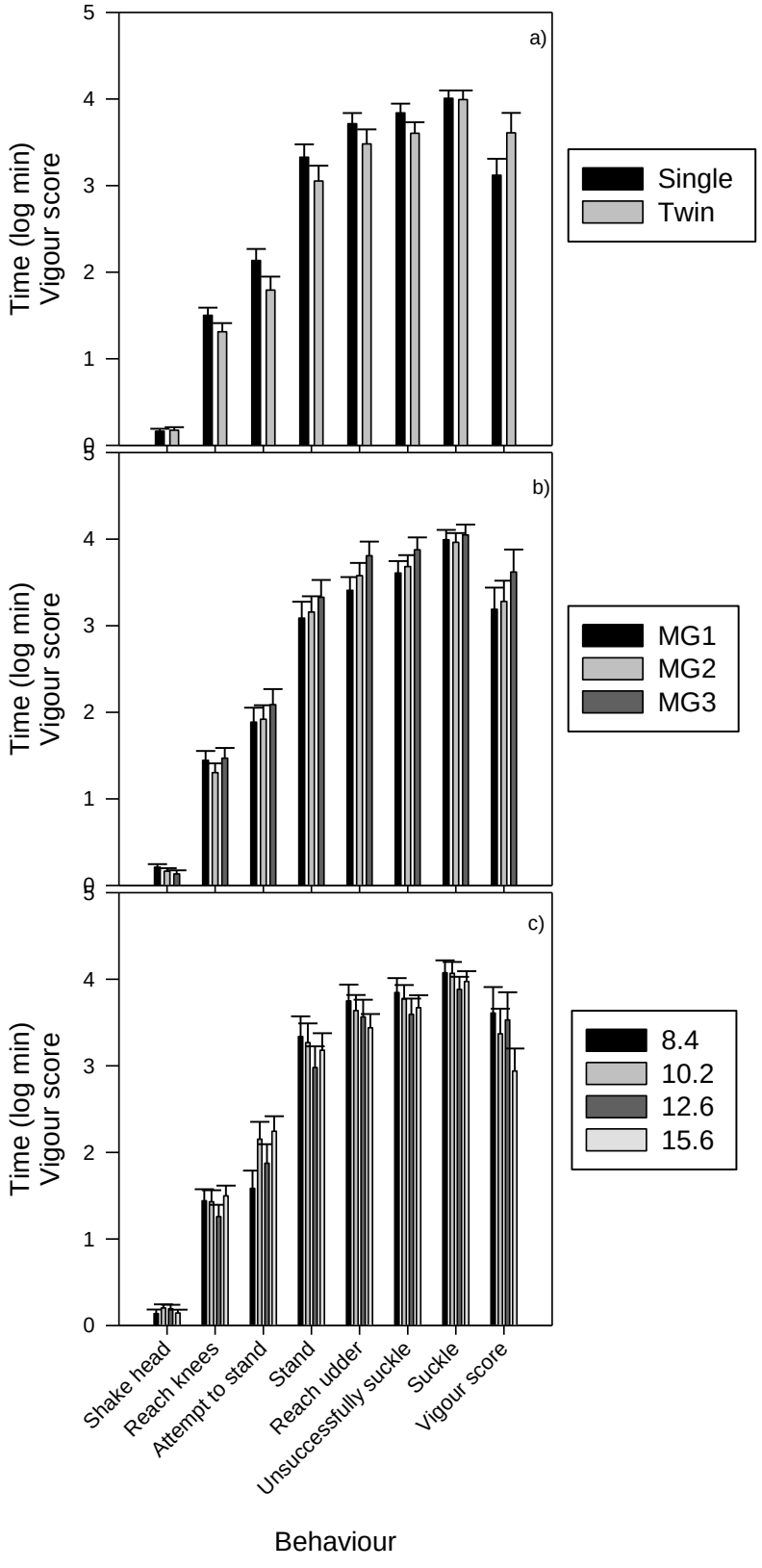

Figure 4.1: Effect of litter size (a), mating group (b) and level of nutrition (c) on time to perform early behaviours and vigour score. Error bars represent standard errors.

4.3.3 Response to noradrenaline challenge

Basal rectal temperature differed significantly (P<0.05, Table 4.5) due to mating group, with MG1 lambs having higher basal rectal temperature than MG2 or MG3. No other rectal temperature measures for response to the noradrenaline challenge differed significantly due to nutrition, litter size or mating group (Table 4.5 & Table 4.6).

Table 4.7 shows the changes in blood metabolite concentrations following the noradrenaline challenge. Plasma NEFA, fructose and glucose concentrations all increased significantly (P<0.001). Free T3 levels tended to increase, although this was not significant (P=0.07) while free T4 levels did not change.

4.3.4 Blood measures

Packed cell volume and lactate did not differ significantly (P>0.05) due to nutrition level (Table 4.9), litter size or mating group (Table 4.8). NEFA, glucose, fructose, and thyroid hormone concentrations were measured at three sample times namely birth, 3 and 6 h of age (Table 4.10). All five of these blood parameters differed significantly across sample times (P<0.001, Table 4.10). NEFA and fructose levels decreased over the sampling period while glucose and free T3 levels increased. Free T4 levels increased from birth to 3 hours but decreased from 3 to 6 h. NEFA levels also differed (P<0.01) due to mating group (Table 4.8) with MG1 and MG2 lambs having significantly higher levels than MG3. Fructose and glucose levels differed significantly (P<0.01) due to litter size with single lambs having higher concentrations of each metabolite than twin lambs (Table 4.8). Free T3 and T4

concentrations did not differ due to litter size or mating group. None of the blood metabolites measured differed (P<0.05) due to nutrition offered (Table 4.9). There was a significant interaction between litter size and sampling time for glucose and free T4 (P<0.01, Figure 4.2a and b) with concentrations increasing with lamb age for single lambs but for twin lambs, the levels increased from birth to 3h but decreased from 3 to 6h.

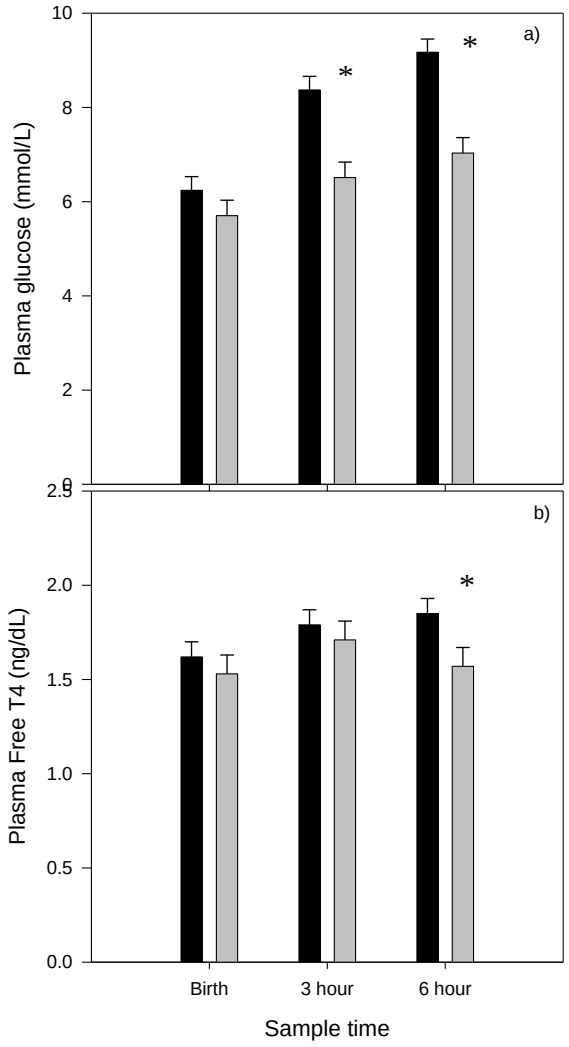

Figure 4.2: Litter size (single – black bars, twin – grey bars) by sample time interactions for a) plasma glucose and b) free T4 concentrations. Error bars represent standard errors and asterisks indicate means that differ significantly (P<0.05).

Table 4.3: Least square means and standard errors (*n*) of morphological measures for litter size and mating group.

	Litter size		**Mating group**		
	Single	**Twin**	**1**	**2**	**3**
Birth weight (kg)	4.41±0.09^{a} (35)	3.90±0.10^{b} (28)	4.14±0.11 (21)	4.05±0.11 (23)	4.29±0.12 (19)
Crown rump length (cm)	45.89±0.59 (34)	44.08±0.81 (28)	44.89±0.83 (20)	44.19±0.79 (23)	45.89±0.81 (19)
Neck circumference (cm)	18.55±0.23 (34)	18.07±0.28 (28)	18.38±0.31 (20)	17.88±0.28 (23)	18.67±0.32 (19)
Rectal temp 10 mins (°C)	39.78±0.12 (33)	39.62±0.14 (27)	39.59±0.15 (20)	39.83±0.14 (23)	39.67±0.16 (17)
Rectal temp 3 hours (°C)	39.59±0.08 (32)	39.60±0.09 (28)	39.64±0.09 (20)	39.51±0.09 (21)	39.64±0.10 (19)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 4.4: Least square means and standard errors (*n*) of morphological measures for nutrition level.

	Nutrition (MJME/day)			
	8.4	**10.2**	**12.6**	**15.6**
Birth weight (kg)	3.84±0.14^{a} (14)	4.41±0.13^{b} (15)	3.96±0.14^{a} (14)	4.41±0.14^{b} (20)
Crown rump length (cm)	44.31±0.94 (14)	45.41±0.89 (15)	44.18±1.34 (13)	46.05±0.83 (20)
Neck circumference (cm)	17.99±0.37 (14)	18.58±0.36 (15)	18.03±0.40 (13)	18.64±0.32 (20)
Rectal temp 10 mins (°C)	39.51±0.18 (14)	39.60±0.17 (15)	39.78±0.21 (12)	39.89±0.16 (19)
Rectal temp 3 hours (°C)	39.62±0.12 (13)	39.55±0.11 (15)	39.62±0.13 (13)	39.58±0.12 (19)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 4.5: Least square means and standard errors (*n*) for rectal temperature response curve parameters for litter size and mating group.

	Litter size		**Mating group**		
	Single	**Twin**	**1**	**2**	**3**
Basal rectal temp (°C)	38.79±0.07 (34)	38.86±0.08 (27)	39.07±0.09^{a} (20)	38.81±0.09^{b} (22)	38.60±0.09^{b} (19)
Cutoff rectal temp (°C)	39.01±0.07 (34)	39.12±0.09 (26)	39.27±0.10 (19)	39.07±0.09 (22)	38.86±0.10 (19)
Length of response (mins)	66.14±3.78 (33)	62.92±4.51 (26)	70.52±5.18 (19)	62.31±4.64 (22)	60.76±5.04 (19)
Peak rectal temp (°C)	40.07±0.10 (34)	40.03±0.12 (27)	40.17±0.14 (20)	40.05±0.13 (22)	39.93±0.14 (19)
Time to reach peak (mins)	25.09±1.89 (34)	21.60±2.45 (23)	23.96±2.48 (20)	25.10±2.67 (18)	20.97±2.55 (19)
Time at peak (log mins)	1.13±0.15 (31)	0.86±0.22 (17)	1.04±0.25 (11)	1.07±0.20 (18)	0.87±0.19 (19)
Slope to peak (°C/min)	0.05±0.01 (33)	0.05±0.01 (27)	0.05±0.01 (19)	0.04±0.01 (22)	0.05±0.01 (19)
Area under the response curve (°Cmin)	2610.78±151.54 (33)	2501.24±180.36 (26)	2789.99±207.41 (18)	2474.23±185.87 (22)	2403.81±201.73 (19)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 4.6: Least square means and standard errors (*n*) for rectal temperature response curve parameters for nutrition level.

	Nutrition (MJME/day)			
	8.4	**10.2**	**12.6**	**15.6**
Basal rectal temp (°C)	38.75±0.11 (14)	38.79±0.11 (15)	39.03±0.12 (13)	38.73±0.09 (19)
Cutoff rectal temp (°C)	38.94±0.12 (14)	39.06±0.11 (15)	39.29±0.13 (13)	38.95±0.10 (18)
Length of response (mins)	69.48±5.86 (14)	63.03±5.57 (15)	59.63±6.61 (12)	65.98±5.15 (18)
Peak rectal temp (°C)	40.24±0.16 (14)	40.00±0.16 (15)	39.99±0.18 (13)	39.96±0.14 (19)
Time to reach peak (mins)	24.93±2.97 (14)	23.66±3.08 (13)	18.74±3.24 (13)	26.04±2.69 (17)
Time at peak (log mins)	1.23±0.27 (10)	0.83±0.25 (12)	0.79±0.26 (12)	1.13±0.22 (14)
Slope to peak (°C/min)	0.06±0.01 (14)	0.05±0.01 (15)	0.04±0.01 (12)	0.05±0.01 (19)
Area under the response curve (°Cmin)	2768.77±234.59 (14)	2499.90±223.3 (15)7	2354.11±264.52 12)	2601.27±206.07 (18)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 4.7: Least square means and standard errors (n) for blood metabolite concentrations before and after the noradrenaline challenge.

	Before	**After**
Glucose (mmol/L)	8.10±0.27^{a} (63)	9.76±0.27^{b} (63)
NEFA (mmol/L)	0.86±0.05^{a} (63)	1.28±0.05^{b} (63)
Fructose (mmol/L)	0.88±0.04^{a} (63)	0.95±0.02^{b} (63)
Free T3 (pg/mL)	5.49±0.19 (61)	5.71±0.19 (61)
Free T4 (ng/dL)	1.71±0.06 (61)	1.70±0.06 (61)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 4.8: Least square means and standard errors (*n*) for blood metabolite concentrations across litter size and mating group.

	Litter size		**Mating group**		
	Single	**Twin**	**1**	**2**	**3**
Packed cell volume (%)	36.79±0.76 (31)	34.73±0.89 (27)	34.82±0.96 (20)	36.41±0.95 (20)	36.04±1.01 9 (18)
Lactate (mmol/L)	6.45±0.48 (33)	7.08±0.57 (27)	7.43±0.61 (20)	6.19±0.58 (22)	6.67±0.65 (18)
Glucose (mmol/L)	7.93±0.22^{a} (33)	6.41±0.26^{b} (27)	7.15±0.28 (20)	7.05±0.27 (22)	7.32±0.30 (17)
NEFA (mmol/L)	1.02±0.04 (33)	1.01±0.05 (27)	1.14±0.05^{a} (20)	1.04±0.05^{a} (22)	0.87±0.05^{b} (18)
Fructose (mmol/L)	1.88±0.09^{a} (33)	1.52±0.11^{b} (27)	1.68±0.12 (20)	1.78±0.11 (22)	1.62±0.12 (17)
Free T3 (pg/mL)	5.28±0.20 (32)	4.88±0.24 (27)	5.15±0.25 (20)	5.05±0.24 (22)	5.04±0.27 (17)
Free T4 (ng/dL)	1.75±0.08 (31)	1.60±0.09 (26)	1.71±0.10 (20)	1.62±0.10 (22)	1.70±0.11 (15)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 4.9: Least square means and standard errors (*n*) for blood metabolite concentrations across nutrition levels

	Nutrition (MJME/day)			
	8.4	**10.2**	**12.6**	**15.6**
Packed cell volume (%)	36.71±1.18^{a} (13)	33.19±1.17^{b} (13)	37.88±1.28^{a} (12)	35.25±0.97^{ab} (20)
Lactate (mmol/L)	7.42±0.76 (13)	6.28±0.73 (14)	5.78±0.79 (13)	7.58±0.62 (20)
Glucose (mmol/L)	7.23±0.35 (13)	7.18±0.33 (14)	7.14±0.36 (13)	7.13±0.29 (20)
NEFA (mmol/L)	1.03±0.06 (13)	1.05±0.06 (14)	0.94±0.06 (13)	1.05±0.05 (20)
Fructose (mmol/L)	1.63±0.14 (13)	1.66±0.14 (14)	1.72±0.15 (13)	1.77±0.12 (20)
Free T3 (pg/mL)	5.13±0.31 (13)	5.30±0.30 (13)	5.00±0.34 (13)	4.88±0.27 (20)
Free T4 (ng/dL)	1.53±0.12 (12)	1.78±0.12 (13)	1.67±0.13 (13)	1.74±0.11 (20)

Table 4.10: Least square means and standard errors (*n*) for blood metabolite concentrations across sample times.

	Sample time		
	Birth	**3 hours**	**6 hours**
Glucose (mmol/L)	5.97±0.22^{a} (60)	7.44±0.22^{b} (60)	8.10±0.21^{c} (63)
NEFA (mmol/L)	1.12±0.04^{a} (60)	1.07±0.04^{a} (60)	0.86±0.04^{b} (63)
Fructose (mmol/L)	2.85±0.09^{a} (60)	1.37±0.09^{b} (60)	0.87±0.09^{c} (63)
Free T3 (pg/mL)	4.22±0.18^{a} (59)	5.50±0.18^{b} (58)	5.51±0.18^{b} (61)
Free T4 (ng/dL)	1.58±0.06^{a} (57)	1.75±0.06^{b} (58)	1.71±0.06^{b} (61)

Different superscripts within rows indicate means that differ significantly (P<0.05)

4.4 Discussion

This study has demonstrated that neonatal lamb vigour traits were not influenced by ewe nutrition in the last trimester of pregnancy, at least within the range of 80% to 120% of maintenance energy requirements and despite birth weights being lower in the sub-maintenance fed groups. However, the ewes in this study were in good body condition (>3) throughout pregnancy and it is possible that there may have been an interaction between feed energy and the energy buffer of ewe condition (Dwyer *et al.* 2003; Gibb and Treacher 1982; McCrabb *et al.* 1992). If this interaction did occur, lambs may not have been nutritionally restricted despite the ewes being feed restricted and this is reflected in the high birth weights measured in this experiment. Further studies of lamb vigour from ewes at lower condition score are needed to clarify this.

4.4.1 Neonatal lamb behaviour

Surprisingly, lamb behaviour did not differ due to litter size. A number of authors have shown that as litter size increases, the time to suckle also increases (Cloete 1993; Dwyer 2003; Dwyer and Morgan 2006). Ewe nutrition has also been shown to affect expression of early lamb behaviours through effects on birth weight (Dwyer *et al.* 2003); however, this was not the case in the present experiment. Birth weight did differ with nutrition offered but after accounting for this difference, it did not change the litter size effects on early lamb behaviours. Greater differences in lamb birth weight may have occurred if the intended nutritional restriction actually took place. As it was, with the errors in pregnancy scanning many ewes were not restricted and therefore twin lambs, which would be expected to be smaller and less vigorous, were not.

4.4.2 Response to noradrenaline challenge

A cold response was simulated in this experiment using a noradrenaline challenge as the response is indicative of response to a true cold challenge based on the change in rectal temperature (Alexander 1969; Cooper *et al.* 1976; Slee *et al.* 1987a). In the present experiment, late pregnancy nutrition did not impact on the ability of lambs to respond to the simulated cold challenge which contrasts with Budge *et al.* (2004) who found that nutritional restriction in late pregnancy resulted in lambs being less able to respond to a cold challenge. In their experiment a much more restrictive nutritional treatment (50% maintenance energy requirements) was used than in the current experiment and the capacity to respond to cold was measured using an *in vitro* assay of UCP-1 activity in brown adipose tissue which was potentially more sensitive to changes than rectal temperature.

Single and twin lambs did not differ in their response to noradrenaline which is surprising as other authors have reported that singletons were able to withstand colder conditions than twins (Stott and Slee 1987) and have linked this to birth weight differences. However, a birth weight effect does not appear to explain the lack of a litter size difference in the current experiment as inclusion of birth weight as a covariate did not change the litter size effect on any of the rectal temperature response parameters.

Plasma glucose, fructose and NEFA concentrations increased following noradrenaline challenge in an expected pattern as noradrenaline stimulates brown adipose tissue metabolism subsequently altering the energy metabolism of the lamb. This pattern is similar to that reported by other workers (Alexander *et al.* 1972; Alexander and Mills 1968). However, thyroid hormone concentrations did not change following noradrenaline challenge which was

unexpected given the role that the thyroid gland has in thermoregulation over the long term. The noradrenaline challenge in this study was an acute challenge mimicking a transient cold exposure and it is possible that responses were therefore limited in the sampling period and blood samples may have been taken before changes were evident. However, Cabello (1983) found that changes in both T3 and T4 levels could be observed during and immediately following a real cold exposure for 1.5 hours which would also be considered to be an acute cold challenge. Therefore, this lack of a change in thyroid hormones may be due to the noradrenaline challenge not inducing the same effect as a true cold challenge.

4.4.3 Blood parameters

Nutritional restriction might be expected to reduce some of the metabolic measurements associated with energy availability but in this experiment nutrition did not account for any difference in the blood measures. Plasma glucose and fructose levels were different due to litter size with single lambs having higher levels than twin lambs which affirm the results of earlier studies in other breeds. In general, if litter size increases, the level of energy metabolites in blood decreases (Barlow *et al.* 1987; Kenyon *et al.* 2007). The lack of difference in plasma lactate levels suggests that the degree of hypoxia was similar across litter sizes. This was *a priori* not surprising given the very low incidence of dystocia in these multiparous ewes and the birth weights of the lambs. There was also no correlation between birth weight and lactate suggesting that chronic hypoxia did not occur (Mellor and Pearson 1977).

The pattern of change in blood parameters from 10 minutes after birth until 6 hours of age are similar to those reported in the literature (De Blasio *et al.* 2006; Mellor and Pearson 1977;

Thompson *et al.* 2006). Fructose concentrations decreased over time while glucose concentrations increased. Thyroid hormone concentrations were low at the first sampling point but had increased to a peak at 3 hours and remained steady to 6 hours which is similar to other studies (Cabello 1983; De Blasio *et al.* 2006; Wrutniak and Cabello 1987b). NEFA concentrations decreased over time which supports the observations of Thompson *et al.* (2006).

These changes in blood measures follow expected patterns suggesting that the lambs in this study were not compromised in terms of their energy metabolism up to 6 hours of age. However, the significant litter size by sample time interaction for free T4, with an increase in T4 from birth to 3 hours for both single and twin lambs and a more rapid decline after 3 hours for twins, may suggest that the thermoregulatory capacity of twin lambs was compromised. A cold challenge would be expected to initiate the conversion of T4 to T3 (Schermer *et al.* 1996) and lower levels of T4 in twins could be a result of T4 conversion to T3 for thermoregulation while single lambs do not have this requirement. This interaction may also suggest that the twin lambs in this study were less metabolically mature than the single lambs (Wrutniak and Cabello 1987b). Cabello (1983) found that preterm lambs, which would not be metabolically mature, had lower levels of T4 than full term lambs to 8 hours of age.

In the last 50 days of pregnancy, nutrition over the range 8.4 to 15.6 MJME/day does not appear to impact on lamb vigour as measured by timed behavioural progression. However, more severe nutritional restriction than was achieved in this experiment may impact on lamb vigour. Further work is needed to determine whether early lamb behaviours provide an accurate assessment of lamb vigour. Lamb genotype may provide a means of exposing

differences in vigour particularly from ewes maintained at a low condition score throughout pregnancy.

Chapter 5: Within breed sire variation has more influence on lamb vigour than sire breed alone

5.1 Introduction

Breed differences in lamb survival and lamb vigour traits have been identified by a number of authors (Alexander *et al.* 1990c; Dwyer and Lawrence 2000; Dwyer *et al.* 1996; Dwyer and Morgan 2006; Kallweit *et al.* 1986; Owens *et al.* 1985; Wassmuth *et al.* 2001), as have differences between purebred and crossbred animals (Fogarty 1972; Fogarty *et al.* 2000; Hall *et al.* 1995; Wassmuth *et al.* 2001). The latter is important in the Australian lamb industry as crossbred lamb production predominates and recent studies, using the Sheep Cooperative Research Centre's information nucleus flock (INF), have confirmed differences in crossbred lamb survival and also identified potentially large within-breed differences in these traits (Brien *et al.* 2009). Within a breed, sires are normally selected for superiority in one or more production traits such as wool production and quality, carcass characteristics and growth traits, but lamb survival is not normally considered.

Crossbred lamb production in Australia consists predominately of a Merino ewe base crossed to maternal or terminal sires such as Border Leicester, Poll Dorset, Texel and White Suffolks (Fogarty *et al.* 2005). Crossbred lambs have been shown to be heavier at birth (Fogarty *et al.* 2000; Fogarty *et al.* 2005; Holst 2002; Nowak and Lindsay 1990) and through to weaning (Fogarty *et al.* 2000) than purebred Merino lambs and sire breed has been shown to be an important determinant of survival differences in crossbred lambs (Fogarty *et al.* 2005). However, ewe breed may also be important in accounting for survival differences between lamb genotypes (Alexander *et al.* 1990c; Fogarty *et al.* 2000; Holst 2002). Fogarty *et al.* (2000) reported that lambs from Border Leicester x Merino ewes had higher survival rates

than lambs from Merino ewes while sire breed had no significant effect. Fogarty (1972) reported that Merino lambs had higher survival rates when compared to Border Leicester or Dorset Horn cross Merino lambs which appears counter-intuitive as higher birth weight lambs would be expected to have improved survival. However, high birth weights can also negatively impact on survival through an increase in the incidence of birth difficulties (Alexander *et al.* 1959; Everett-Hincks and Dodds 2007; Fogarty *et al.* 1992; Hall *et al.* 1995).

Differences in lamb vigour have been reported for Merino lambs when compared to crossbred lambs with crossbred lambs being classified as more vigorous (Nowak and Lindsay 1990; Stevens *et al.* 1984). Purebred versus crossbred differences in vigour traits such as time-to-stand and time-to-suckle, and growth rates have also been reported between other breed crosses (Wassmuth *et al.* 2001). Border Leicester x Merino lambs were able to discriminate their dams at younger ages than purebred Merino lambs and to also spend more time with their dams (Nowak and Lindsay 1990). Purebred Merino twin lambs have also been shown to be separated from their dam more often than Border Leicester x Merino lambs (Stevens *et al.* 1984).

Very little research has been done to examine the relative importance of within breed sire differences in lamb vigour. Some work from the INF has begun studying this using a wide range of sires over eight different flocks across Australia (van der Werf *et al.* 2010). In the INF, lamb vigour is measured using a field scoring system based on the behaviour of the lambs during restraint and release at tagging time, when the lamb is approximately 3 – 12 hours old (Brien *et al.* 2010). The time taken for the lamb to regain contact with the ewe and to suckle is included in this scoring system. Tagging takes place anywhere from birth until

the lamb is 24 hours of age and an estimate of lamb age is accounted for when analysing the lamb vigour data (Brien *et al.* 2010). The accuracy of this estimate and the relevance of the scoring system to more traditional measures of lamb vigour such as time to progress through early behaviours to suckling are still unclear.

The aim of the experiment reported here was to examine breed and sire within breed differences in lamb vigour parameters including early lamb behaviours, physiological measures and cold challenge responses. It was hypothesised that crossbred Border Leicester x Merino lambs would have improved vigour compared to purebred Merino lambs. It was also hypothesised that the field based lamb vigour score, used in the INF, would not be highly correlated with the lamb vigour measures used in the experiment reported here.

5.2 Materials and Methods

This work was carried out with approval from the CSIRO FD McMaster Laboratory, Chiswick, Animal Ethics Committee, AEC No. 08/09.

5.2.1 Ewes and management

One hundred and sixty nine multiparous Merino ewes were oestrus synchronised using 300 mg progesterone via EAZI-BREED® CIDR® (Pfizer) and were injected with 400 i.u. of pregnant mare's serum gonadotrophin (Pregnecol®) on removal of the CIDRs. Two days later, the ewes were artificially inseminated randomly to one of six sires, three Merino (M) and three Border Leicester (BL). All sires had previously been used in the Sheep CRC's INF joining in 2007 and 2008 and their estimated breeding values (EBVs) for birth weight, vigour score and survival were available (Table 5.1). Sixty-one days after insemination, the ewes

were pregnancy scanned for litter size and all multiple-bearing ewes (n=37; Merino n=20, Border Leicester n=17) were retained for the experiment.

Table 5.1: Estimated breeding values from INF data for sires used in the current experiment (M. Hebart, pers. comm.).

Sire	Lamb survival to 3 days	Lamb survival to weaning	Birth weight	Lamb vigour
BL1	0.00582	0.003307	-0.02556	-0.213
BL2	-0.00949	-0.009615	-0.07929	-0.06095
BL3	-0.00409	-0.00916	0.08695	-0.1726
M1	-0.00690	-0.003481	-0.02225	-0.02042
M2	-0.00044	-0.001676	0.3495	0.3716
M3	0.00418	0.00551	-0.2339	0.1264

Ewes were fed on pasture to maintain joining body condition score (BCS) of >3 until day 30 of pregnancy. They were then feed restricted by grazing on low quality pasture so ewes would lose condition to reach a BCS of 2.5 by day 105 of pregnancy. From day 105 to parturition, ewes were fed to maintain this condition by providing access to higher quality pasture compared to that offered during the initial 30 – 105 days of gestation. From day 119, ewes were also supplementary fed with lucerne based animal house pellets (ME=9.04 MJ/kgDM; CP=16.2%) at a rate of 300 g/day/ewe. From day 137, this was increased to 675 g/day/ewe. Ewe body condition scores were monitored monthly from day 30 until day 90 and then fortnightly until lambing. Ewe body weights were monitored fortnightly from day 30.

Ewes were brought into the animal house at day 143 of gestation, penned individually and from day 145 they were constantly monitored. While in the animal house, the ewes were fed a ration of lucerne based animal house pellets (1400 g/ewe/day) and lucerne hay.

5.2.2 Selection of INF sires

EBVs for lamb survival, birth weight and lamb vigour were calculated from lambing data from the INF collected in 2007 and 2008 (M. Hebart, pers. comm.). Lamb survival to 3 days of age and weaning was determined. Birth weight and lamb vigour were measured at tagging time which occurred between birth and 24 hours of age. Lamb vigour was assessed using the following scoring system (Table 5.2) with behaviours during tagging and within 30 seconds of release included. A score of 1 was the most favourable. This score is numerically opposite to the vigour score assessed in the current experiment so negative correlations are favourable. An estimate of lamb age was included in the analysis of this score. For further information on lamb measures in the INF see Brien *et al.* (2009).

Table 5.2: INF lamb vigour score.

Score	Description
1	Constant struggle – bleat in response to ewe – on release reaches ewe quickly and follows
2	Regular struggle while held – moves to the ewe on release – bleating common
3	Some struggle – walking in direction of ewe bleats but no contact – may bleat
4	Some struggle – attempts to walk but aimless – no apparent response to ewe bleats
5	Little movement when held – lies on release

5.2.3 Lambing protocol

Video monitoring began at the first sign of lambing. Lambing assistance was provided if there was no progression two hours after the first appearance of membranes. No further measurements were taken on the ewes. Ten to 15 minutes after birth, a blood sample was taken and rectal temperature was measured for the lamb. Three hours after birth, an infra-red thermal image was taken and birth weight, rectal temperature, crown-rump length and girth circumference were measured. Lambs were also ear tagged at this time. When the oldest twin was 5.5 hours of age all lambs within a litter had a rectal temperature logger (Seastar® Oddi micro-T) inserted. All further time points refer to the oldest lamb's age per litter. At 6

hours of age, the oldest lamb had a blood sample and an infra-red thermal image taken and underwent a behavioural test. The lamb was then cold challenged using an ice vest. The second lamb also wore a vest with an ice pack at room temperature. At 7 hours of age, the vests were swapped on the lambs, an infra red thermal image and blood sample were taken and the behavioural test was performed on all lambs. At 8 hours of age, the vests were removed from all lambs. The second lamb had a blood sample, infra red image taken and a behavioural test completed. At 9 hours, the oldest lamb had an infra red image and blood sample taken and at 10 hours the second lamb had an infra red image and blood sample taken. Temperature loggers were removed from all lambs at this time and video monitoring ceased. Five ewes gave birth to triplet lambs so rather than exclude this data, the third lamb from these litters was randomly allocated to a treatment time schedule either the same as the first or second born lamb.

5.2.4 Behavioural measures

The first three hours of video footage were analysed to determine: the time taken by the lamb to shake its head, to reach its knees, attempt to stand, stand, reach udder and appear to suckle and a lamb vigour score as outlined in Chapter 4, pp. 98. Video footage was also used to determine the effects of the cold challenge and the vest on lamb behaviours and ethograms were determined for the hour; before, during ice vest challenge, during control vest challenge and after the vest was removed. Behaviours were recorded at five minute intervals and scored as follows:

- 0 = sitting/lying
- 1 = standing
- 2 = standing close to ewe and following
- 3 = in position to suckle

The behavioural test, performed immediately before and after the ice vest challenge, was the same as that outlined in Chapter 3 (pp. 76). The lamb was placed behind a curved wire mesh barrier 2.5 m from a model ewe. A standard audio cue of a recently lambed Merino ewe (not the dam) bleating when separated from her lamb was played from behind the model. The lamb was allowed 90 seconds to attempt to get past the mesh barrier and reach the ewe. All behaviour tests were video recorded and the following information obtained:

- Number of vocalisations
- Vocalisation score (1 – no response; 2 – some bleating; 3 – loud, distressed bleating)
- Alert – Yes/No
- Movement score – 0 to 5 based on how far they move from the barrier towards the ewe
- Overall score – 0 to 5 with 5 being the best (see Chapter 3, pp. 79)

5.2.5 Rectal temperature data

Rectal temperatures were logged every minute from half an hour before the first vest was put on the lamb until two hours after vests were removed. The standard deviation of rectal temperature was calculated for the half hour before a vest went on, the last half hour the ice vest was on, the last half hour the warm vest was on and the last half hour of the hour following vest removal. This was used to compare variation in rectal temperature before, during and after the ice vest challenge.

5.2.6 Blood assays

Four blood samples were taken from each lamb at 10-15 minutes of age, immediately prior to and after the cold challenge, and 2 hours after cold challenge. They were collected in 4 ml

potassium oxalate and sodium fluoride anticoagulant tubes (Vacutainer®). Packed cell volume was measured immediately on the first sample via haematocrit and the sample was centrifuged as soon as possible at 3200 rpm for 10 minutes and the plasma taken and frozen immediately at -18°C. Only the first blood sample was assayed for lactate and glucose using the methods outlined in Chapter 4 (pp. 98). Inter assay coefficients of variation were <0.53% and <2.3% for glucose and lactate respectively.

5.2.7 Statistical analysis

Data were analysed using PROC GLM and PROC MIXED in SAS where breed, sire-within breed, litter size and birth order were fitted as fixed effects for early physiological and morphological data and early behaviours. Birth order and the order the vest went on (ice vest then control or vice versa) were confounded as first born lambs always had the ice vest on first followed by the control vest and second born lambs were the opposite. Treatment period (before, ice vest, control vest and after) was an additional fixed effect included for the analysis of the effect of ice vest challenge on behaviour and rectal temperature. Time (before and after ice vest) was also included as a fixed effect for the behavioural test parameters. Birth weight was fitted as a covariate in all models. Correlations between EBV for INF lamb vigour score and early lamb behaviours, lamb vigour score, behavioural test measures, plasma lactate at 10 minutes and rectal temperature at 10 minutes were also determined using PROC CORR to calculate Pearson's correlation coefficients and 95% confidence intervals. Data from infra-red thermal imaging were not included for data analysis due to high ambient temperatures (>30°C) making it difficult to differentiate the lamb surface temperatures from air temperatures.

5.3 Results

Birth order had no significant effect (P>0.05) on any of the measures recorded so it will not be referred to further in these results.

5.3.1 Morphological and early physiological data

Effects of breed, sire within breed and litter size for morphological and early physiological data are outlined in Table 5.3 and Table 5.4. Rectal temperature at 10 minutes was significantly (P<0.05) higher for Border Leicester cross progeny than Merino progeny, but no other parameters differed due to sire breed. PCV was the only measure to differ significantly (P<0.05) due to sire within breed with progeny from M1 sires having lower PCV than M3 sires but M2 sires did not differ to M1 or M3 (Table 5.4). Girth circumference, plasma glucose and plasma lactate did not differ due to litter size. For all other measures, triplet lambs had significantly (P<0.05) lower values than twin lambs (Table 5.3). Plasma lactate and glucose levels did not differ significantly due to breed or sire within breed (P>0.05, Table 5.3 & Table 5.4).

5.3.2 Early behaviours

Time to suckle was the only early behaviour to differ significantly (P<0.05) but only due to sire within breed (Figure 5.1 & Table 5.6). M1 progeny were significantly faster than M2 and M3 progeny to suckle and BL1 and BL3 progeny were significantly faster than BL2 progeny. No other early behaviours differed significantly due to breed, sire within breed or litter size (Table 5.5 & Table 5.6). Vigour score differed significantly (P<0.01) due to sire within breed (Figure 5.1) and tended to differ due to breed (P=0.08) and litter size (P=0.09)

(Table 5.5). M1 progeny had higher vigour scores than M2 and M3 lambs but Border Leicester progeny did not differ between sires (Table 5.6).

5.3.3 Ice vest challenge

Rectal temperature variation did not differ significantly due to the ice vest challenge (Figure 5.2a) or litter size. Rectal temperature variation differed significantly ($P<0.01$) due to sire within breed (Figure 5.2b) and breed. Border Leicester cross lambs had greater variation in rectal temperature than Merino lambs (0.16±0.01 °C and 0.12±0.01 °C, respectively).

Behaviours during the ice vest challenge differed due to the treatment period i.e. before, during or after ice vest (Figure 5.3). The proportion of time spent sitting was greatest while the ice vest and control vest were on. The proportion of time spent standing, moving and suckling was lowest while the ice vest and control vest were on compared to before and after the presence of the vests. There was no significant ($P>0.05$) effect of breed, sire within breed or litter size on these behaviours.

5.3.4 Behaviour test

Table 5.7 and Table 5.8 show the differences in behavioural test scores due to breed, sire within breed, litter size and time (before and after challenge). Overall score differed significantly ($P<0.05$) due to breed with Border Leicester cross lambs scoring more highly than Merino lambs (Table 5.7). Movement and overall score differed significantly ($P<0.05$) due to sire group. The number of vocalisations tended to differ due to breed with Border Leicester progeny making more vocalisations than Merino progeny ($P=0.08$). There were no significant ($P>0.05$) differences in any measurements due to litter size or timing of the test (Table 5.8).

5.3.5 Correlations to INF vigour score

The correlation coefficients between EBVs for INF lamb vigour score and time to suckle, vigour score, overall score in the behaviour test, plasma lactate and rectal temperature at 10 minutes were 0.77, -0.67, -0.29, 0.68 and -0.71, respectively (see Table 5.9).

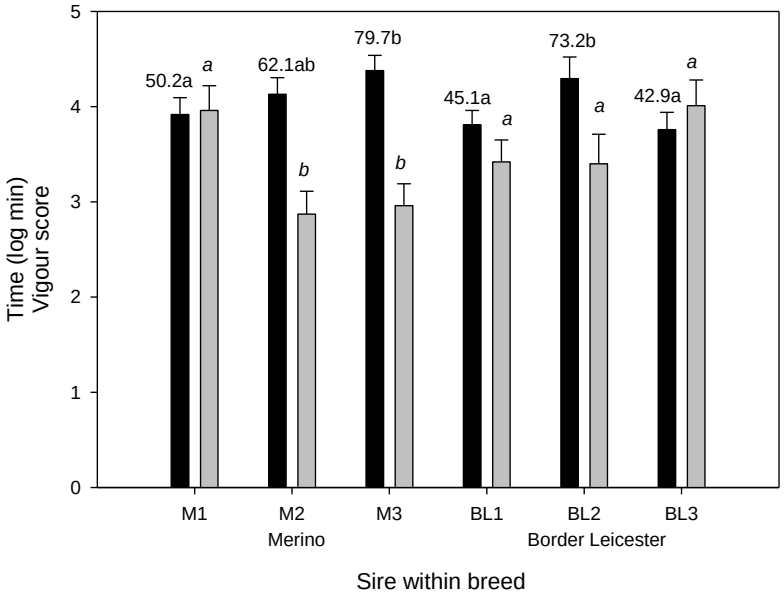

Figure 5.1: Sire within breed differences in time-to-suckle (black bars) and vigour score (grey bars. Data labels are back transformed means and letters indicate means that differ within breed and within variable. Error bars represent standard errors.

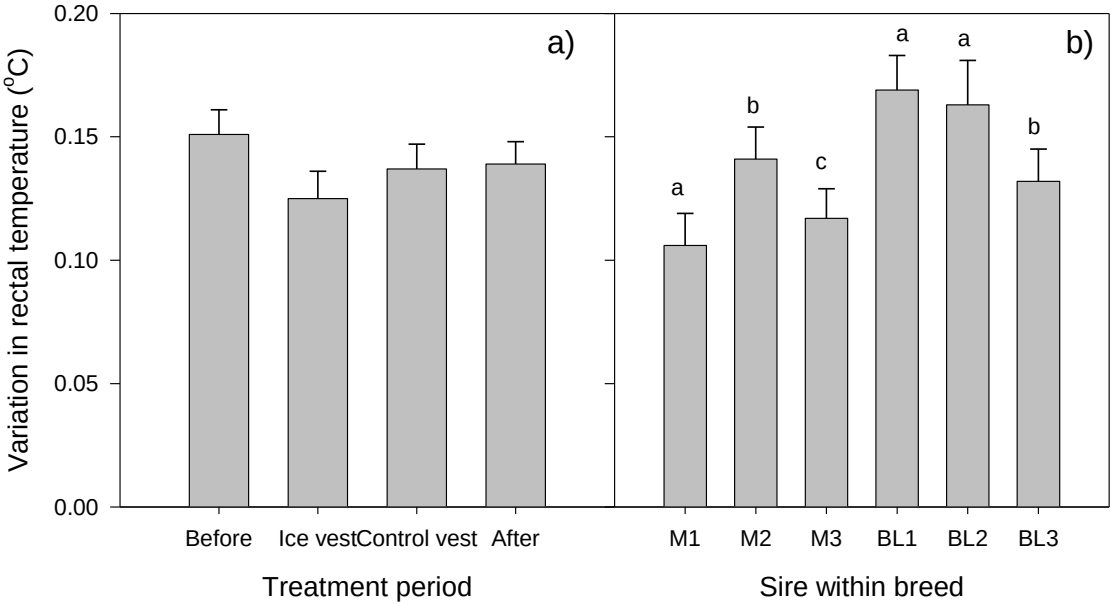

Figure 5.2: Mean variation in rectal temperature for a) treatment period and b) sire within breed. Letters indicate means that differ within breed. Error bars represent standard errors.

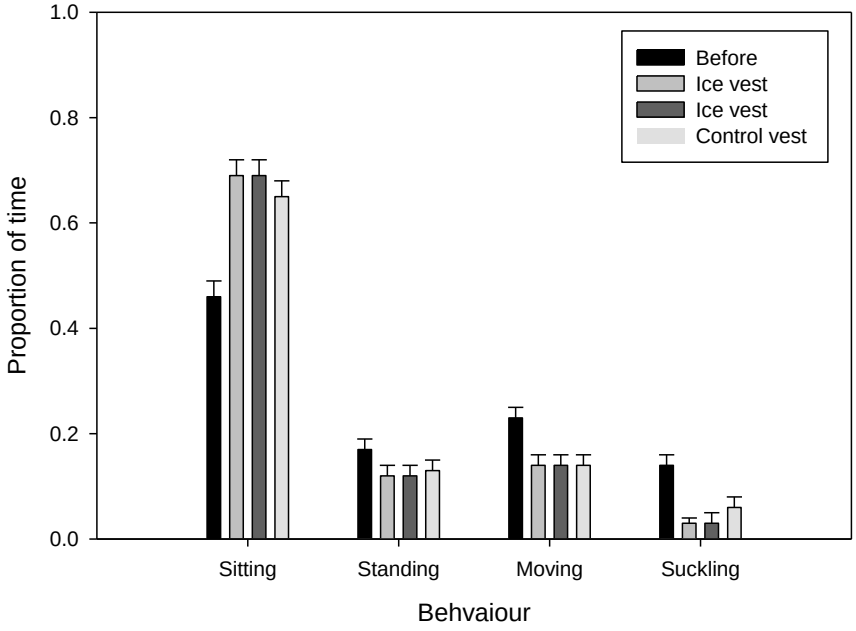

Figure 5.3: Proportion of time spent performing each behaviour during each treatment period. Error bars represent standard errors.

Table 5.3: Least square means and standard errors (*n*) for morphological and physiological parameters according to breed and litter size.

	Breed		**Litter size**	
	Border Leicester	**Merino**	**Twin**	**Triplet**
Birth weight (kg)	3.29±0.12 (35)	3.07±0.11 (38)	3.53±0.13^{a} (58)	2.84±0.16^{b} (15)
Rectal temp 10 mins (°C)	39.88±0.13^{a} (35)	39.55±0.12^{b} (37)	39.92±0.13^{a} (57)	39.50±0.17^{b} (15)
Rectal temp 3 hours (°C)	39.51±0.13 (35)	39.45±0.09 (38)	39.62±0.10^{a} (58)	39.32±0.12^{b} (15)
Packed cell volume (%)	33.68±1.10 (33)	35.14±1.04 (38)	37.14±1.18^{a} (56)	31.69±1.49^{b} (15)
Lactate (mmol/L)	6.19±0.56 (34)	7.22±0.54 (37)	6.72±0.61 (56)	6.69±0.77 (15)
Glucose (mmol/L)	1.67±0.45 (34)	2.72±0.43 (37)	2.31±0.48 (56)	2.07±0.61 (15)
Crown rump length (cm)	40.39±0.49 (35)	40.91±0.46 (38)	41.55±0.52^{a} (58)	39.75±0.66^{b} (15)
Girth circumference (cm)	35.02±0.32 (35)	34.78±0.31 (38)	34.96±0.35 (58)	34.84±0.44 (15)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 5.4: Least square means and standard errors (*n*) for morphological and physiological parameters for sire-within breed.

	Sire within breed					
	M1	**M2**	**M3**	**BL1**	**BL2**	**BL3**
Birth weight (kg)	3.19±0.18 (14)	2.92±0.18 (11)	3.10±0.17 (13)	3.21±0.16 (15)	3.12±0.22 (7)	3.54±0.19 (13)
Rectal temp 10 mins (°C)	39.75±0.19 (14)	39.47±0.18 (11)	39.42±0.18 (12)	39.63±0.16 (15)	39.97±0.22 (7)	40.04±0.19 (13)
Rectal temp 3 hours (°C)	39.51±0.13 (14)	39.36±0.13 (11)	39.47±0.13 (13)	39.33±0.12 (15)	39.53±0.16 (7)	39.62±0.14 (13)
Packed cell volume (%)	31.58±1.62^{a} (14)	35.66±1.61^{ab} (11)	38.19±1.52^{b} (13)	35.34±1.50 (13)	30.09±1.93 (7)	35.61±1.67 (13)
Lactate (mmol/L)	6.69±0.86 (13)	7.71±0.83 (11)	7.27±0.77 (13)	5.27±0.76 (14)	6.37±0.99 (7)	6.96±0.86 (13)
Glucose (mmol/L)	2.39±0.69 (13)	2.52±0.66 (11)	3.26±0.63 (13)	1.56±0.60 (14)	1.62±0.79 (7)	1.83±0.69 (13)
Crown rump length (cm)	41.68±0.72 (14)	39.77±0.71 (11)	41.27±0.67 (13)	40.42±0.64 (15)	39.73±0.86 (7)	41.03±0.74 (13)
Girth circumference (cm)	34.35±0.49 (14)	35.08±0.48 (11)	34.91±0.46 (13)	35.93±0.43 (15)	34.01±0.58 (7)	35.12±0.50 (13)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 5.5: Least square means and standard errors (*n*) for early behaviours for breed and litter size.

	Breed		**Litter size**	
	Border Leicester	**Merino**	**Twin**	**Triplet**
Shake head (log min+1)	0.82±0.14 (16)	0.48±0.11 (21)	0.75±0.15 (31)	0.55±0.22 (6)
Reach knees (log min)	1.71±0.13 (22)	1.43±0.14 (21)	1.67±0.16 (35)	1.48±0.20 (8)
Attempt to stand (log min)	2.31±0.14 (28)	2.24±0.13 (31)	2.51±0.15 (47)	2.03±0.21 (12)
Stand (log min)	3.06±0.13 (29)	3.12±0.12 (31)	3.22±0.14 (48)	2.97±0.19 (12)
Reach udder (log min)	3.42±0.13 (29)	3.65±0.1 (31)	3.62±0.13 (48)	3.45±0.18 (12)
Suckle (log min)	3.95±0.12 (28)	4.14±0.10 (31)	4.05±0.12 (48)	4.05±0.17 (11)
Vigour score	3.61 ± 0.17^{a} (30)	3.26 ± 0.16^{b} (35)	3.16±0.18 (51)	3.71±0.23 (14)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 5.6: Least square means and standard errors (*n*) for early behaviours for sire-within breed.

	Sire within breed					
	M1	**M2**	**M3**	**BL1**	**BL2**	**BL3**
Shake head (log min+1)	0.42±0.18 (8)	0.68±0.18 (7)	0.34±0.18 (6)	0.84±0.19 (6)	0.82±0.24 (4)	0.80±0.21 (6)
Reach knees (log min)	1.21±0.21 (8)	1.59±0.19 (8)	1.49±0.25 (5)	1.72±0.18 (10)	1.66±0.23 (5)	1.75±0.22 (7)
Attempt to stand (log	1.92±0.22 (11)	2.28±0.22 (9)	2.51±0.20 (11)	2.52±0.19 (12)	2.19±0.26 (6)	2.22±0.23 (10)
Stand (log min)	2.79±0.20 (11)	3.25±0.20 (9)	3.30±0.18 (11)	2.95±0.17 (13)	3.32±0.24 (6)	2.94±0.21 (10)
Reach udder (log min)	3.44±0.19 (11)	3.77±0.19 (9)	3.74±0.18 (11)	3.31±0.17 (13)	3.68±0.23 (6)	3.27±0.20 (10)
Suckle (log min)	3.92 ± 0.17^{a} (11)	4.13 ± 0.17^{ab} (9)	4.38 ± 0.15^{b} (11)	3.81 ± 0.15^{ab} (13)	4.29 ± 0.22^{a} (5)	3.76 ± 0.18^{b} (10)
Vigour score	3.96 ± 0.26^{a} (11)	2.87 ± 0.24^{b} (11)	2.96 ± 0.23^{b} (13)	3.42±0.23 (13)	3.40±0.31 (6)	4.01±0.27 (11)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 5.7: Least square means and standard errors for breed and sire group differences in behavioural test scores.

	Breed		**Sire within breed**					
	Border	**Merino**	**M1**	**M2**	**M3**	**BL1**	**BL2**	**BL3**
	n=35	*n=38*	*n=14*	*n=11*	*n=13*	*n=15*	*n=7*	*n=13*
Number of vocalisations	15.1±1.5	11.6±1.4	13.3±2.1	8.4±2.1	13.1±1.9	13.5±1.9	15.4±2.5	16.5±2.2
Vocalisation score	2.1±0.1	1.9±0.1	1.9±0.2	1.7±0.2	2.0±0.1	2.0±0.1	2.3±0.2	1.9±0.2
Movement score	1.2±0.3	0.8±0.3	1.7 ± 0.4^{a}	0.1 ± 0.4^{b}	0.8 ± 0.4^{b}	0.9±0.4	1.7±0.5	1.1±0.4
Overall score	2.7 ± 0.2^{a}	2.1 ± 0.2^{b}	2.8 ± 0.3^{a}	1.3 ± 0.3^{b}	2.3 ± 0.3^{a}	2.3 ± 0.3^{a}	3.3 ± 0.4^{b}	2.5 ± 0.3^{a}

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 5.8: Least square means and standard errors for litter size and time differences in behavioural test scores.

	Litter size		**Time**	
	Twin	**Triplet**	**Before IV challenge**	**After IV challenge**
	n=58	*n=15*	*n=73*	*n=72*
Number of vocalisations	13.9±1.5	12.8±1.9	15.1±1.4	11.7±1.4
Vocalisation score	2.0±0.1	1.9±0.1	2.0±0.1	1.9±0.1
Movement score	0.7±0.3	1.3±0.4	1.0±0.3	1.0±0.2
Overall score	2.2±0.2	2.6±0.3	2.5±0.2	2.3±0.2

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 5.9: Correlations between Sheep CRC Information Nucleus Flock estimated breeding values for vigour score and behaviour and physiological traits.

	Pearson's correlation coefficient	***p* value**	**95% Confidence intervals**
Time to suckle	0.77	0.07	-0.19 – 0.97
Vigour score	-0.67	0.14	-0.96 – 0.36
Overall score	-0.29	0.58	-0.88 – 0.70
Rectal temperature at 10 mins	-0.71	0.12	-0.96 – 0.32
Lactate	0.68	0.14	-0.36 – 0.96

5.4 Discussion

The results from this experiment indicate that within-breed sire variation may be more important than variation between breeds for lamb vigour. Very few vigour measures differed due to sire breed but time to suckle, vigour score and various behavioural test measures all differed due to sire within breed. However, in the experiment reported here, crossbred lambs were being compared to purebred lambs rather than comparing two completely separate breeds of lambs. In several studies (Dwyer 2003; 2005; Dwyer and Lawrence 2000; 2003) comparing Scottish Blackface lambs to Suffolk lambs, distinct breed differences in lamb vigour measures have been observed with the Blackface lambs being more vigorous than the Suffolk lambs in terms of time to stand and suckle, play activity and subsequent survival. This work did not consider the effect of within breed sire variation on lamb vigour.

Crossbred lambs have been reported to have improved vigour and survival when compared to purebred lambs (Fogarty 1972; Fogarty *et al.* 2000; Nowak *et al.* 1987; Wassmuth *et al.* 2001). In this experiment it was found that vigour, as measured by time to stand and suckle, did not vary due to sire breed but rather sire variation within breed was important particularly for Merino sires. The fact that sire differences within breed exist for lamb vigour suggests that selection for improved vigour may be a useful indirect selection criteria to improve lamb survival (Sawalha *et al.* 2007). Low favourable genetic correlations (-0.22) between lamb survival and lamb vigour have been reported (Brien *et al.* 2009; Brien *et al.* 2010) supporting this suggestion although, genetic improvement in either vigour or survival would be slow due to the low heritabilities reported for these traits (Brien *et al.* 2009).

The observation that crossbred lambs tended to make more vocalisations when separated from their dam has also been reported by Nowak (1989) who found that crossbred lambs

bleated twice as much as Merino lambs. Crossbred lambs in that study were also better able to perform a discrimination test than purebred Merino lambs although data on whether this translated into survival differences was not reported. However, work from the Sheep CRC INF has shown genetic associations between vocalisations at tagging and lamb survival (Brien *et al.* 2010).

Behavioural differences shown between when the ice vest was on and before and after the vest suggest that the use of the vest, rather than the cold *per se*, was influencing lamb behaviour. The vest appeared to suppress activity in the lamb as shown by a greater proportion of time spent sitting or lying while the vest was on compared to when it was removed. Suckling bouts were higher when the vest was not present. Behaviour while the warm vest was on was similar to that observed with the cold vest which tends to reinforce that it was the vest rather than the temperature affecting lamb behaviour. The lack of difference in recorded behaviours following the cold challenge may be more a reflection of the inadequacy of the challenge in this study than in the utility of the test. In this experiment, ambient maximum temperatures were often over 30°C and it may be that the vests helped to minimise difficulties due to excessive warmth rather than inducing cold stress. However, the use of an ice vest for a cold challenge needs to be revised or an alternative is necessary in order to elicit a large effect without the confounding effect on behaviour.

The correlations between sire EBVs for INF lamb vigour and time to suckle, lamb vigour score, plasma lactate and rectal temperature at 10 minutes in the current experiment confirm the utility of lamb vigour score as a trait to target for genetic improvement in lamb survival. Despite the wide age range of lambs used, this score, based on restraint and release behaviour, appears to provide a correlated measure of lamb vigour as determined by a more

detailed assessment in the current experiment where the time of birth is known. This suggests that the field based measure of vigour is providing information on the metabolic status of the lamb and key behavioural measures and therefore the likelihood of survival.

This experiment has shown that sire variation in key lamb vigour measures exist and that there may be potential for genetic improvement based on selection for lamb vigour. The high correlations between EBV for vigour and various behavioural and physiological measures support the use of a vigour scoring system when selecting for lambs with improved vigour. It would therefore be useful in a production system where lambs are tagged and/or handled soon after birth to potentially be used for selection. Further work is required to determine the extent of the variation in lamb vigour measures and the heritability of these measures to establish how useful they would be for use in a selection program.

Chapter 6: Sire effects on neonatal lamb vigour and following behaviour

6.1 Introduction

Merino ewes are renowned for their gregariousness which, although it may diminish at lambing, may impact on the time the ewe spends at the birth site before returning to the flock (Alexander *et al.* 1983). Time spent at the birth site is central to the formation of a strong ewe-lamb bond which in turn leads to improved lamb survival (Murphy *et al.* 1994a; Putu *et al.* 1988a). Ideally, ewes should remain at the birth site for at least 6 hours (Kilgour *et al.* 1982; Murphy *et al.* 1994a; Nowak 1996). However, in Australian studies, Merino ewes tend to spend much less time at the birth site with reports ranging from 1.3 – 3.5 hours (Bickell *et al.* 2010; Murphy *et al.* 1994a; Stevens *et al.* 1982). Other breeds such as Romneys and Dorset Horns have been shown to spend more time at the birth site (up to 11 hours) than Merino ewes (Alexander *et al.* 1984; Alexander *et al.* 1983; Kilgour *et al.* 1982). Due to the relatively short amount of time the Merino ewe and her lambs spend alone at the birth site, there is increased pressure on Merino lambs to stand and suckle quickly so as to access colostrum and establish an appropriate bond with their dams. Lambs then need to be mobile enough to maintain contact with the ewe as she moves away from the birth site to join the flock.

Stevens *et al.* (1982) found that 29% of twin lambs became permanently separated from the ewe after leaving the birth site and of these lambs, 88% subsequently did not survive. Alexander *et al.* (1983) have shown breed differences in the proportion of lambs becoming separated from the ewe early in life with Merino ewes having a higher proportion of lamb separation and mortality than Romney or cross bred ewes. This breed difference was thought to be due to Merino ewes being more mobile and inherently more gregarious than Romney

ewes (Alexander *et al.* 1983). Therefore, they were more likely to move away from the birth site to rejoin the flock before the lambs were mobile or an adequate bond had been established.

An extensive amount of information is available on the ability of ewes and lambs to identify each other from an alien ewe or lamb; however, most of these data refer to lambs that are at least 12 hours of age (Cloete and Scholtz 1998; Cloete *et al.* 2005; Nowak 1990; Nowak and Lindsay 1990; Nowak *et al.* 1989). This information has been used to assess the strength of the ewe lamb bond and therefore, the ability of the ewe-lamb unit to maintain contact. Data on the discriminative ability, following behaviour and ability to respond to the ewe in lambs less than 12 hours of age is lacking. Aside from the birth process, lambs may be exposed to other physiological stressors such as exposure to cold, which may also impact on their ability to perform critical behaviours associated with retaining contact with their mother during the first few hours of life. The ability of the lamb to continue to respond to and maintain contact with the ewe when exposed to additional stressors may be a significant determinant of the probability of starvation-mismothering losses occurring.

There seems to be no studies that have examined the variation in the capacity of lambs to retain following activity under stressful conditions although there is evidence of genetic differences in the incidence of starvation-mismothering losses (Everett-Hincks and Dodds 2007; Gudex *et al.* 2005). Gudex *et al.* (2005) reported sire and breed differences in neonatal lamb losses due to starvation exposure and recent data from the Sheep CRC information nucleus flock (Brien *et al.* 2010) has shown sire differences in lamb survival within breeds and differences in the causes of loss in the first 3-7 days post partum. In the latter, there is preliminary evidence to show some sires appear to have a higher proportion of lamb mortality

due to starvation-mismothering compared to others (F. Brien pers. comm.). In the experiment reported here, it was hypothesised that sires with high losses due to starvation-mismothering would produce lambs that were less vigorous in terms of the time taken to stand and suckle and who were also less responsive to the ewe during the period they would normally be on the birth site compared to lambs from sires that had no lamb losses. It was also hypothesised that these differences would be accentuated when the lambs were under the physiological stress of cold.

6.2 Materials and Methods

This experiment was conducted with the approval of the CSRIO FD McMaster Laboratory Animal Ethics Committee, AEC no. 10/04.

6.2.1 Animals and Management

Three hundred multiparous Merino ewes were oestrus synchronised (CIDR®), given a 400 i.u. injection of PMSG (Pregnecol®) and joined randomly to one of four Merino sires via artificial insemination. Ewes were joined in four groups (n=75) each a week apart. Sires were selected from data obtained in the 2009 Sheep CRC information nucleus flock (Brien *et al.* 2010) lambing with two sires having progeny losses to three days predominately due to starvation/mismothering, and the other two sires had no lamb losses to three days (Table 6.1, M. Hebart pers. comm.). The sire progeny were combined to result in two groups of lambs, namely high-loss and no-loss. Ewes were pregnancy scanned for litter size between day 60 and 81 of pregnancy and only twin bearing ewes were selected for this experiment. Ewes were maintained on pasture until three weeks before expected lambing when they were fed supplementary lucerne based animal house pellets (200 g/ewe/day) to prepare them for the

animal house. Ewes were weighed and body condition scored every two weeks from scanning until entry into the animal house.

Table 6.1: Sire information from 2009 Sheep CRC INF lambing (M. Hebart, pers. comm.).

Sire	Loss to 3 days	EBV survival to 3 days
High loss 1	10.2%	-0.043
High loss 2	6.3%	-0.026
Low loss 1	0%	0.031
Low loss 2	0%	-0.007

6.2.2 Treatments and protocols

At day 143 of pregnancy, ewes were allocated randomly to individual pens (1.2 m x 1.2 m) where they were fed *ad libitum* lucerne based animal house pellets (ME=9.04 MJ/kgDM, CP=16.2%) and lucerne hay. Ewes remained in the animal house until their lambs were at least 24 hours of age. From day 146 of pregnancy, ewes were under constant monitoring and video recording began at the first sign of lambing up until the lamb was 3 hours of age. Average daily minimum and maximum temperatures in the animal house were 5.1°C and 14.8°C, respectively.

Ten to 15 minutes after birth, lambs had a blood sample taken and rectal temperature measured. At 3 hours of age, lambs were removed from the pen to measure birth weight, crown rump length, girth circumference and rectal temperature. Lambs were also tagged at this time and had a temperature logger (Seastar®) inserted rectally. Time to perform early behaviours and a vigour score were assessed from video of the first three hours following birth. For definitions of behaviours and vigour score see Chapter 4, pp. 98.

Between 4 and 6 hours of age, the ewe and lambs were moved to another pen for treatment. Lambs were allocated to either the control group (remain at ambient temperature) or the cold challenge group with birth order randomised across treatment groups. Cold challenged lambs were first wetted with ice water and were kept wet by using a spray pack to apply cold water every 15 minutes. Refrigerated air (6°C) was blown across the lamb at a rate of 5 m/s for an hour. Control lambs were not wetted and were kept out of the wind and the ewe remained in close proximity to both lambs throughout the treatment time. The cold challenge was designed to produce a cold stress index (CSI) of at least 1250 kJ/m^2/h (average 1257 kJ/m^2/h) while the control lambs had a CSI of less than 900 kJ/m^2/h (average 832 kJ/m^2/h). CSI was calculated according to Donnelly (1984) who reported that lamb survival decreased significantly at a chill index greater than 1000 kJ/m^2/h. This was based on the work by Nixon-Smith (1972) who reported a high chill risk at a CSI greater than 963 kJ/m^2/h and an extremely high risk at 1256 kJ/m^2/h. Each lamb was in a small cage so they could not suckle the ewe during the one hour treatment period.

Prior to treatment, a 4 ml blood sample was taken from each lamb via jugular venipuncture. A second blood sample was taken following the treatment and a modified barrier test performed on the lamb. The modified barrier test was the same as that detailed in Chapter 3 (pp. 78) with a Merino ewe bleating used as the audio cue. The following measures were taken during the behaviour test:

- Number of vocalisations
- Number of grids crossed
- Vocalisation score (1 – no response; 2 – some bleating; 3 – loud, distressed bleating)
- Linear movement score – 0 to 5 based on how far they move from the barrier towards the ewe

- Overall score (See Table 3.8 pp. 79)

Two hours after the end of the treatment, the temperature logger was removed from the lamb. Ewes and lambs were left undisturbed until 24 hours postpartum when another rectal temperature was measured on the lambs. The ewe and lambs were then moved into a small paddock surrounding the animal house and no further measurements were taken.

6.2.3 Blood assays

Packed cell volume (PCV) was measured on the initial blood sample (10 – 15 min post partum) prior to centrifugation. Samples were centrifuged at 3200 rpm for 10 minutes and the plasma collected and frozen immediately for later analysis. Glucose was assayed at all sampling times but lactate was only assayed in the 10 – 15 min sample. Methods used for the blood analyses were the same as those detailed in Chapter 4 (pp. 98) with coefficient of variation for glucose and lactate being <0.53% and <2.3% respectively.

6.2.4 Statistical analyses

Data were analysed using PROC GLM and PROC MIXED in SAS. The model included the fixed effects of sire group (high- or no-loss) and birth order for early behaviours, rectal temperatures, crown rump length, girth circumference, PCV, and lactate and glucose at the 10 – 15 minute sample. An additional fixed effect of treatment (cold or control) was included for measures taken during or after the cold challenge. An additional fixed effect of sample time (birth and 3 hour, or before and after cold challenge) was used for plasma glucose levels. Birth weight differed due to sire group so was subsequently included as a covariate. Rectal temperature response curves were plotted over the cold challenge. From these, the area under the response curve, range, standard deviation, peak rectal temperature during cold and during

the behaviour test and the difference between peaks were calculated for analysis. Early behaviour data were transformed using a natural logarithmic scale prior to analysis.

6.3 Results

6.3.1 Early morphological and physiological measures

Birth weight differed significantly due to sire group with high loss progeny being significantly ($P<0.05$) heavier than no-loss progeny. Rectal temperature at 10 – 15 minutes of age was significantly ($P<0.001$) lower in the no-loss sire group compared to the high-loss group. However, this difference was not apparent by three hours of age and similarly at 24 hours of age (Table 6.2). Birth order was not significant ($P>0.05$) for any of the early life progeny measures as shown in Table 6.2. There was a significant interaction ($P<0.05$) between sire group and birth order for rectal temp at 10 mins (Figure 6.1). Girth circumference, crown rump length, packed cell volume, lactate and glucose at the 10 – 15 minute sample did not differ significantly due to sire group or birth order (Table 6.2). However, at the 3 hour sample, glucose levels had increased and there was a significant sire group by sample time interaction (Figure 6.2).

Table 6.2: Least square means and standard errors for early life measures.

	Sire group		**Birth order**	
	No-loss	**High-loss**	**1**	**2**
	n=33	*n=30*	*n=32*	*n=31*
Birth weight (kg)	3.69±0.09^a	3.98±0.10^b	3.93±0.10	3.74±0.10
Rectal temp 10mins (°C)	38.98±0.13^a	39.50±0.13^b	39.35±0.13	39.13±0.13
Rectal temp 3 hours (°C)	39.79±0.06	39.85±0.07	39.83±0.06	39.81±0.06
Rectal temp 24 hours (°C)	39.51±0.08	39.55±0.08	39.58±0.08	39.48±0.08
Crown rump length (cm)	44.03±0.49	43.90±0.51	44.42±0.49	43.52±0.50
Girth circumference (cm)	36.51±0.26	36.81±0.27	36.70±0.26	36.62±0.26
Packed cell volume (%)	31.9±0.7	33.8±0.7	33.2±0.7	32.7±0.7
Lactate (mmol/L)	7.6±0.4	6.8±0.4	7.4±0.4	7.0±0.4
Glucose (mmol/L)	3.3±0.3	3.1±0.3	4.9±0.3	4.3±0.3

Different superscripts within rows indicate means that differ significantly (P<0.05)

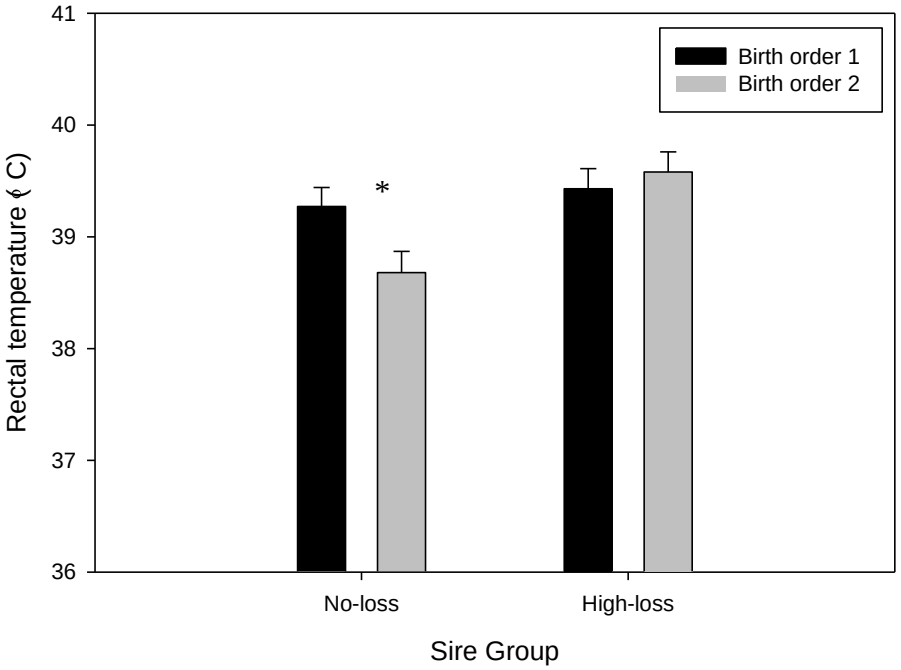

Figure 6.1: Interaction (P<0.05) between sire group and birth order for rectal temperature at 10 minutes. Error bars represent standard errors and asterisks indicate means that differ significantly (P<0.05).

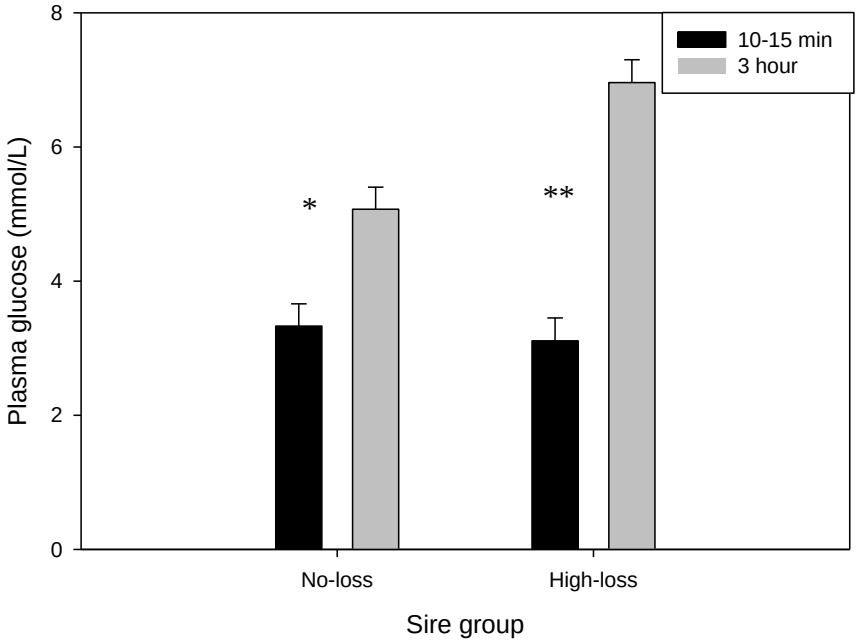

Figure 6.2: Interaction (P<0.05) between sire group and sample time for plasma glucose concentration. Error bars represent standard errors and asterisks indicate means that differ significantly (P<0.05).

6.3.2 Early lamb behaviours

Figure 6.3 shows sire group differences in early lamb behaviours and vigour score. Lambs from the no-loss sire group were significantly (P<0.05) faster to attempt to stand and to stand, and tended (P=0.07) to reach the udder and suckle faster than lambs from the high-loss group. Lambs from the no-loss group also had a significantly (P<0.01) higher vigour score compared to the high-loss group. Time to shake head and reach knees did not differ (P>0.05) between sire groups. There were no significant differences (P>0.05) in early behaviours due to birth order.

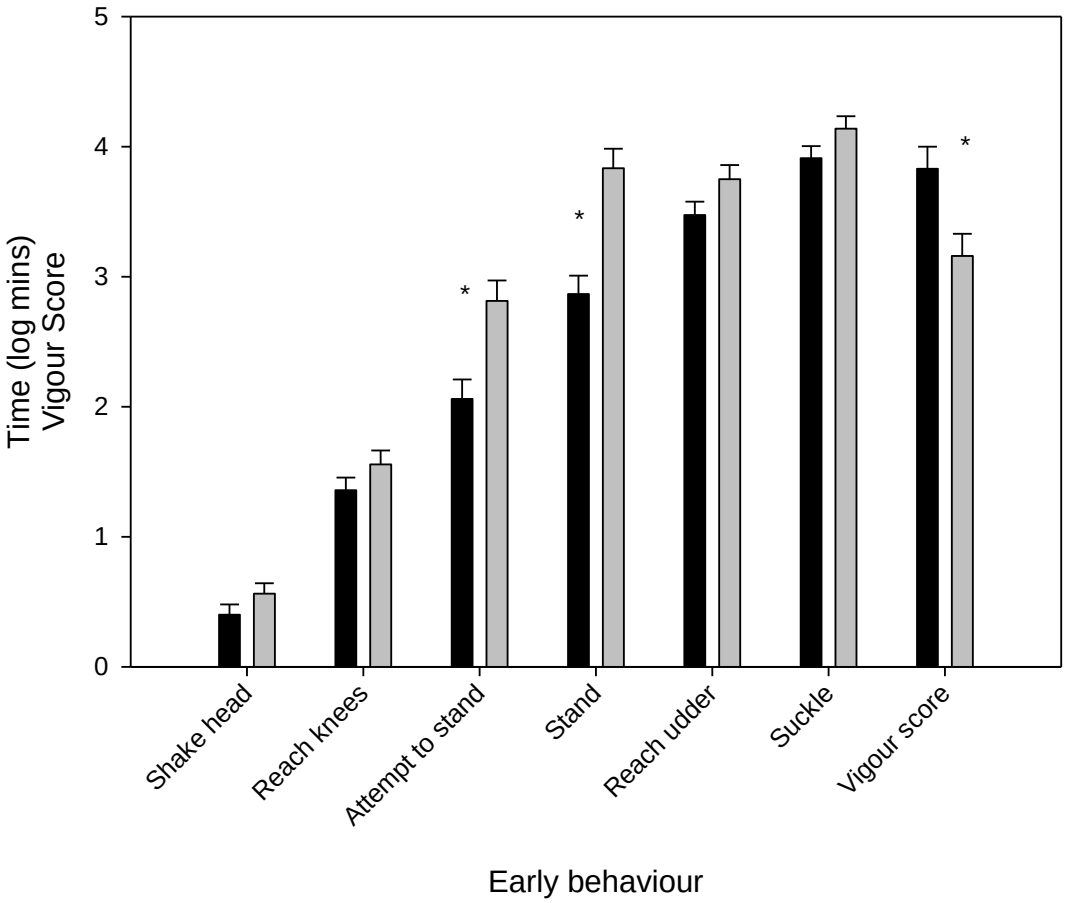

Figure 6.3: Differences in time to perform early behaviour between no-loss (black bars) and high-loss (grey bars) sires. Asterisks indicate behaviours that differ significantly ($P<0.05$) and error bars represent standard errors.

6.3.3 Rectal temperature response to cold challenge

Rectal temperature data during the 60 minute cold challenge is shown in Table 6.3. The area under the curve was the only temperature parameter to differ significantly ($P<0.001$) due to sire group. A significant ($P<0.01$) sire group x treatment interaction was found for the standard deviation and the range of rectal temperature during the cold challenge (Figure 6.4). There was a greater difference between treatments for the no-loss sire group compared to the high-loss group for both measures. All measures except peak rectal temperature during the behaviour test differed significantly ($P<0.01$) between treatment groups. Cold treated lambs

had a significantly greater area under the rectal temperature response curve, standard deviation, peak during treatment, range during treatment and difference between peaks than control lambs. There was no effect (P>0.05) of treatment on plasma glucose concentration (Table 6.3) however, concentrations increased following the treatment period (before: 6.0±0.5 mmol/L; after: 8.5±0.5 mmol/L) regardless of treatment. No parameters differed (P>0.05) due to birth order.

6.3.4 Modified barrier test performance

Performance in the modified barrier test differed significantly (P<0.01) between treatment groups for all parameters (

Table 6.4). Control lambs were more likely to reach the ewe than cold lambs (26% compared to 0%, Chi^2=9.5 P=0.002). However, there was no difference (P>0.05) in the number of lambs reaching the ewe due to sire group. Control lambs had significantly (P<0.01) higher scores than cold lambs for linear movement, bleat, alertness and overall behaviour scores. The number of bleats was the only behaviour that differed significantly (P<0.001) due to sire group with high-loss lambs bleating more than no-loss lambs. First born lambs had higher linear movement and overall behaviour scores in the behaviour test than second born lambs. There was a significant (P<0.01) birth order by treatment interaction for all measures except the number of grids crossed during the test. There were no differences (P>0.05) between birth order for cold treated lambs, but control lambs differed in behavioural responses due to birth order (Figure 6.5). Of the lambs that reached the ewe, there were no significant (P>0.05) differences in the time to reach the ewe, bleat rate or the number of grids crossed for sire group or birth order (Table 6.5).

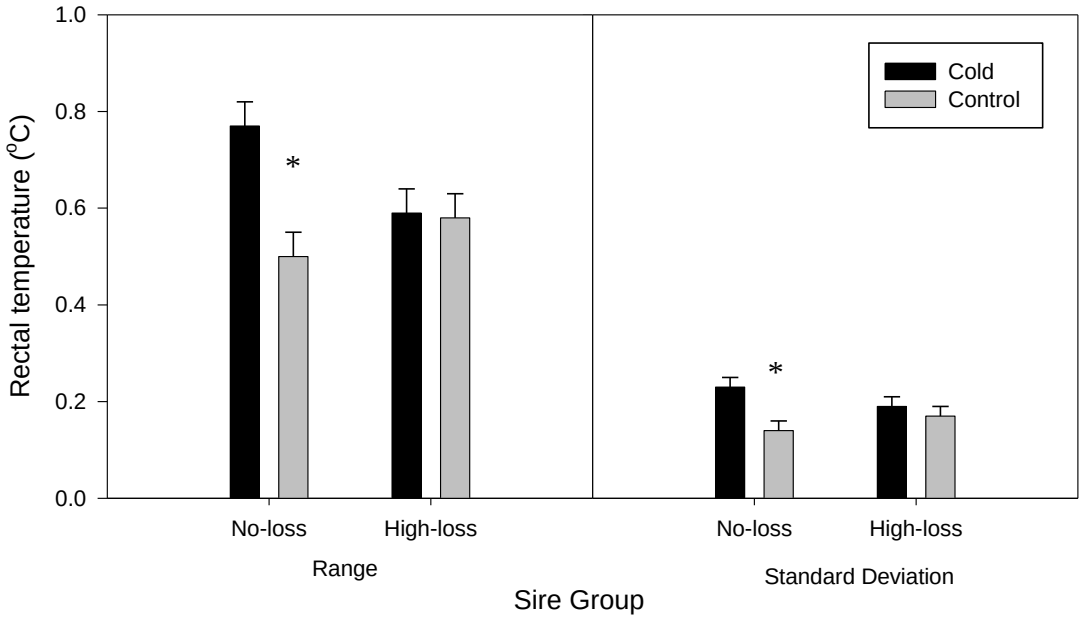

Figure 6.4: Interaction between sire group and treatment group for the range and standard deviation of rectal temperature during the cold challenge. Error bars represent standard errors and asterisks indicate means that differ significantly (P<0.05).

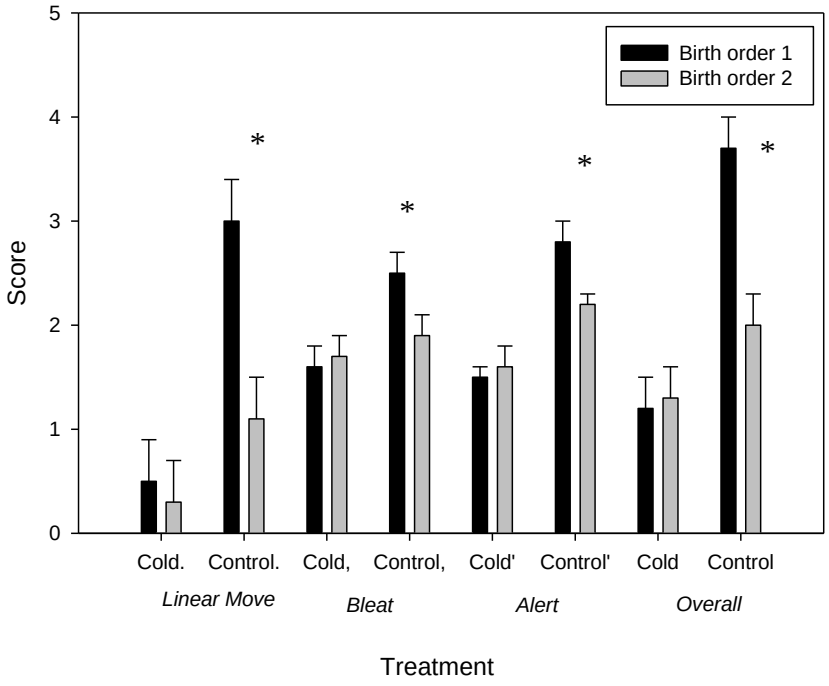

Figure 6.5: Interactions between treatment and birth order for behaviour test measures. Error bars represent standard errors and asterisks indicate means that differ significantly (P<0.05).

Table 6.3: Least square means and standard errors (*n*) for rectal temperature parameters for sire group and treatment.

	Sire Group		Treatment		Birth Order	
	No-loss	**High-loss**	**Cold**	**Control**	**1**	**2**
Area under the curve (°Cmin)	2349.91±3.76^a (27)	2337.09±3.99^b (24)	2354.46±4.01^a (23)	2332.55±3.62^b (28)	2341.49±3.79 (26)	2345.52±3.89 (25)
Standard deviation (°C)	0.19±0.01 (27)	0.19±0.01 (24)	0.21±0.01^a (23)	0.16±0.01^b (28)	0.19±0.01 (26)	0.18±0.01 (25)
Peak during cold challenge (°C)	40.05±0.07 (29)	39.89±0.07 (24)	40.15±0.07^a (25)	39.80±0.07^b (28)	39.93±0.07 (28)	40.02±0.07 (25)
Peak during behaviour test (°C)	39.79±0.07 (27)	39.69±0.08 (24)	39.68±0.08 (23)	39.79±0.07 (28)	39.74±0.07 (27)	39.74±0.07 (24)
Difference between peaks (°C)	0.31±0.05 (27)	0.21±0.05 (24)	0.51±0.06^a (23)	0.01±0.05^b (28)	0.23±0.05 (27)	0.29±0.05 (24)
Range of rectal temp during cold challenge (°C)	0.63±0.03 (27)	0.59±0.03 (24)	0.68±0.04^a (23)	0.54±0.03^b (28)	0.62±0.04 (26)	0.60±0.04 (25)
Plasma glucose (mmol/L)	6.63±0.55 (33)	7.89±0.57 (30)	7.26±0.55 (32)	7.27±0.55 (31)	7.86±0.55 (32)	6.67±0.56 (31)

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 6.4: Least square means and standard errors for sire group, treatment and birth order for behaviour test measurements.

	Sire Group		Treatment		Birth Order	
	No-loss	**High-loss**	**Cold**	**Control**	**1**	**2**
	n=33	*n=29*	*n=31*	*n=31*	*n=31*	*n=31*
No. of bleats	12.3±2.7^a	26.4±2.8^b	14.4±2.7^a	24.4±2.7^b	21.4±2.7	17.3±2.7
No. of grids crossed	6.2±1.4	6.7±1.4	3.1±1.4^a	9.7±1.4^b	6.9±1.4	5.9±1.4
Linear movement score	1.0±0.3	1.4±0.3	0.4±0.3^a	2.0±0.3^b	1.7±0.3^a	0.7±0.3^b
Bleat score	1.8±0.1	2.0±0.1	1.6±0.1^a	2.2±0.1^b	2.0±0.1	1.8±0.1
Alert score	1.9±0.1	2.1±0.1	1.5±0.1^a	2.5±0.1^b	2.2±0.1	1.9±0.1
Overall behaviour score	1.9±0.2	2.2±0.2	1.2±0.2^a	2.8±0.2^b	2.5±0.2^a	1.6±0.2^b

Different superscripts within rows indicate means that differ significantly (P<0.05)

Table 6.5: Least square means and standard errors for behaviour test measurements for those lambs that reached the ewe.

	Sire Group		**Birth Order**	
	No-loss	**High-loss**	**1**	**2**
	n=2	*n=6*	*n=7*	*n=1*
Time to reach ewe (s)	21.8±34.7	71.2±20.4	90.2±14.7	2.8±44.2
Bleat rate (bleats/s)	0.2±0.1	0.3±0.1	0.3±0.1	0.2±0.1
No. grids crossed	7.6±2.4	10.5±1.4	9.7±1.0	8.5±3.1

Different superscripts within rows indicate means that differ significantly (P<0.05)

6.4 Discussion

The ability of the ewe and lamb to maintain contact with each other during the early neonatal period is essential for the development of mutual recognition and the maintenance of a food supply for the lamb. Separation during this time (<12 hours of age) can often be permanent resulting in lamb mortality due to starvation/mismothering. The results of the current experiment suggest that there may be sire differences (at least in the Merino breed) in early lamb behaviours and vigour score that have been associated with subsequent survival (Brien *et al.* 2009; Brien *et al.* 2010). Sires that had no lamb losses recorded in the previous year produced progeny that were quicker to stand and suckle and had higher vigour scores. However, these differences in neonatal behaviour were not necessarily associated with differences in the behavioural and physiological response of these lambs during a cold challenge. Very few of the lamb responses measured during the cold challenge differed significantly due to sire group.

As expected, cold treated lambs showed a greater change in their rectal temperature response compared to control lambs. However, this test could be considered as a short-term (acute) cold stress as the lambs were not forced to maintain body temperature for an extended period and to the point where body temperature begins to decrease rapidly (Slee *et al.* 1990) and the

lamb's survival is threatened. The lack of a sire group difference in rectal temperature suggests that during an acute cold stress, lambs from both groups responded in a similar fashion, mounting a response to maintain body temperature. However, there were significant sire group by treatment interactions with the no-loss cold lambs having a greater temperature range and a higher standard deviation than control lambs. Such differences were not seen in the high-loss lambs suggesting that the high-loss lambs may be less physiologically responsive to a cold challenge than the no-loss lambs and thus more susceptible to cold exposure. Sire differences in cold "resistance" have previously been reported in Merinos by Slee *et al.* (1991) and it would seem that the potential interactions between response to cold and following-behaviour may warrant further investigation using a wide range of sires.

Responses in the modified barrier test following cold treatment also did not vary due to sire group. Cold stressed lambs performed poorly in the test regardless of sire group compared to control lambs. Only control lambs successfully reached the ewe during the modified barrier test suggesting that the cold stress did impact on the behavioural responses of the lambs. The significant difference in the overall barrier test score also supports this. The significant interaction between birth order and treatment for some of the barrier test measures suggests that for control lambs, first born lambs performed better than second born lambs. This may be a reflection of the first born lambs having had a longer time (average of 20 mins) to adjust to the extra-uterine environment than second born lambs, and if true indicates that rapid developmental changes are occurring at this time. However, for cold animals, first and second born lambs both performed poorly suggesting that the cold suppressed any differences that may exist affirming the severe effects that cold exposure can have on lamb behaviour (Alexander and Williams 1966b).

The timing of the cold stress was designed to correspond to a time when the ewe may be moving away from the birth site. Therefore, it indicates whether the lamb would be able to maintain body temperature and also produce an appropriate behavioural response to maintain contact with the ewe. The fact that no lambs from the cold treatment were able to reach the ewe in the test protocol suggests that this ability is inhibited when lambs are cold. The lack of a sire group difference or interaction suggests that the impact of cold on neonatal behaviour may be independent of any genetic differences in vigour although this warrants further investigation. Also, the modified barrier test may not accurately reflect a field based situation as a model ewe rather than the dam was used and there was no movement/responsiveness of the ewe which may have influenced lamb responsiveness while under a physiological stress. However, it may reflect situations where the expression of maternal behaviour is low or poor due to low gestational nutrition or if the ewe is primiparous (Alexander *et al.* 1993; Dwyer and Smith 2008; Putu *et al.* 1988b).

Sire group differences in early behaviours reported here may provide information on why the sires used in this experiment differed in survival in a previous year's data collection (M. Hebart pers. comm.). The high-loss sires produced progeny that were slower to stand and suckle. These are critical neonatal behaviours that are demonstrably associated with subsequent lamb survival (Cloete 1993; Dwyer *et al.* 2001; Owens *et al.* 1985). Lambs that are quicker to suckle successfully are more likely to survive possibly not due just to earlier access to colostrum, but also to the earlier development of a robust bond with the ewe and therefore, maintenance of access to their food supply and improved survival.

Many studies have focused on the physiological response of neonatal lambs to stressors such as cold but there is very little data available on the behavioural responses of lambs while

under physiological stress early in the neonatal period. The closest is possibly the study of Pfister *et al.* (2006a; 2006b) who examined behavioural responsiveness of lambs affected by locoweed poisoning. In a barrier test, similar to the modified barrier test used in the current experiment, locoweed affected lambs were significantly slower to reach their dams than normal lambs and performed other behaviour tests poorly, indicating impaired cognitive ability. This pattern corresponds to the cold treated lambs in this experiment of which none were able to reach the ewe. Perhaps a discrimination test while lambs are cold stressed may have been beneficial in determining the cognitive abilities of the lamb; however, such tests to date, have been found to be ineffective in lambs less than 12 hours of age (Nowak *et al.* 1987). It must also be pointed out that the cold stress used in this experiment was acute rather than chronic and many lambs born are exposed to continuing cold conditions throughout the first day or two of life.

The sire group differences observed in birth weight and rectal temperature at 10-15 minutes of age show that the no-loss lambs had lower birth weights and rectal temperatures than the high-loss lambs. However, when considered alongside normal or optimal values these differences are possibly of little consequence. Birth weights in both groups of lambs were in the range (3.5 – 5kg) that would be considered optimal for lamb survival on the U-shaped curve (Alexander *et al.* 1959) and rectal temperatures (39 – 40 C) for both groups were also within a normal range.

This experiment provides evidence that differences in lamb vigour exist for groups of lambs sired by high starvation/mismothering loss or no lamb loss sires and that the effects of cold may impact negatively on vigour and "following" behaviours. Further studies under field conditions are needed to determine whether these early vigour differences translate to

behavioural differences under stressful conditions at the time the ewe begins to move away from the birth site and also whether such measures may have useful applications for indirect selection for improved lamb survival.

Chapter 7: General Discussion and Conclusion

The aim of this thesis was to develop methods for assessing lamb vigour to provide information on the ability of the lamb to contribute to its own survival. This information could potentially be used to identify traits for genetic improvement of lamb survival. Currently, a single, simple definition of lamb vigour is lacking however it is clear that there are two elements important for defining lamb vigour. These are the critical early behaviours of standing and suckling and the ability of the lamb to maintain contact with the ewe as she moves from the birth site. In this thesis, methods for assessing vigour were expanded from the time taken to stand and suckle to other measures that enabled an assessment of the ability of the lamb to respond to the ewe while under a physiological stress and at a time when the ewe would most likely be moving away from the birth site.

The ability of the newborn lamb to progress through a series of critical early lamb behaviours, especially to stand and suckle, is paramount in the subsequent survival of the lamb (Arnold and Morgan 1975b; Cloete and Scholtz 1998). To date, the standard method of assessing lamb vigour in an objective way is to measure the time it takes for the lamb to perform these critical behaviours (Alexander 1958a; O'Connor and Lawrence 1992). Under intensive production systems, such as those used in Europe, this appears to provide an accurate assessment of the likelihood of lamb survival (Dwyer 2003; Dwyer *et al.* 2005; Wassmuth *et al.* 2001). However, under more extensive conditions, such as those experienced in Australia, it has been suggested that time to perform early behaviours does not provide an accurate reflection of lamb vigour and subsequent survival (Lindsay *et al.* 1990). In this thesis, it was found that the time to stand and suckle did not differ due to level of nutrition during late pregnancy or litter size (Chapter 4), however, time to suckle did differ

due to sire within breed (Chapter 5 and Chapter 6) and there were high correlations between EBVs for the field score of vigour with the phenotypic measure of time to suckle.

Despite differences due to sire in the time it takes for lambs to perform these critical early behaviours, lambs still progress through these behaviours in the time (generally < 2 hours) it would be expected that the ewe will remain on the birth site. However, once these behaviours have been performed, it is still critical for the lamb to maintain contact with the ewe when she is ready to move away from the birth site. Ideally, the ewe would spend at least six hours (Kilgour *et al.* 1982; Murphy *et al.* 1994a) on the birth site to allow the lamb time to adjust to the extra-uterine environment and to establish a strong ewe-lamb bond. However, in practice, particularly under Australian conditions, the Merino ewe often moves away from the birth site less than two hours after birth (Bickell *et al.* 2010; Murphy *et al.* 1994b; Putu *et al.* 1988a) and may not express strong maternal behaviours to retain the link with her lamb/s (Stevens *et al.* 1982; Stevens *et al.* 1984). It is therefore important for the lamb/s to be ready to respond as she moves away from the birth site and to attempt to maintain contact with her for maintenance of their food supply. In Chapter 5 and Chapter 6, the ability of lambs to do this while under a physiological stress was tested using a modified barrier test. It was found that cold stress reduced the ability of lambs to respond to a model ewe and audio cue of a ewe bleating when compared to control lambs at four hours of age, a time when according to the literature (Bickell *et al.* 2010; Murphy *et al.* 1994b) Merino ewes would have moved from the birth site.

Sire selection in industry is based on superiority in one or more production traits such as those related to meat and wool quality. Selection for improved lamb survival is often ignored

as it is thought that heritabilities are too low to make worthwhile progress (Morris *et al.* 2000; Safari *et al.* 2005; Sawalha *et al.* 2007; Shelton and Menzies 1970). However, from this thesis it is evident that sire differences in lamb vigour exist (Chapter 5 and Chapter 6) and therefore, it may be important to consider this when selecting sires. Data from the Sheep CRCs information nucleus flock (INF) has shown that lamb vigour has moderate, favourable correlations with lamb survival suggesting that selecting for improved lamb vigour would improve lamb survival outcomes (Brien *et al.* 2009; Brien *et al.* 2008; Brien *et al.* 2010). Work from this thesis has also shown high correlations between sire EBVs for lamb vigour score in the INF with phenotypic measures including time to suckle, vigour score and rectal temperature at 10 minutes of age of their progeny. This provides some validation of the lamb vigour score used in the INF despite it being used to assess lambs varying in age from one to 24 hours of age. This suggests that the INF vigour score may be a practical means of assessing early lamb vigour in a farm situation and highlights the potential for targeting this trait in genetic selection programs. To test this, vigour scores needed to be used more widely in farm situations particularly in stud breeding where lambs are being handled within the first day of life. Vigour scores could easily be incorporated into the tagging procedure and could therefore be used to provide information on the likelihood of survival of the lamb.

A secondary aim of this thesis was to develop novel methods for inducing and measuring cold stress in neonatal lambs. To date most methods require the lamb to be separated from the ewe (Alexander 1962d; Eales and Small 1980; Stott and Slee 1987) or are a simulated cold stress (Slee *et al.* 1987a), producing a different physiological response when compared to actual cold. One novel method for inducing cold stress was the use of ice vests (Chapter 3 and Chapter 5); however, it was found that the efficacy of this method was dependent on ambient temperatures and it can also cause some disruption to the ewe-lamb bonding process,

presumably due to the introduction of an unfamiliar scent (Vince *et al.* 1985). Of the methods evaluated, the use of cold air and water provided the best method of inducing cold stress in neonatal lambs. Measurement of the physiological response to cold stress was done using small rectal temperature loggers so the lambs could remain with the ewe. This proved to be a useful way of continuously logging body temperatures changes and did not appear to cause too much discomfort for the lamb. Behavioural responses to cold stress were also measured using a modified barrier test to assess lamb responsiveness. Cold stress appeared to suppress lamb behaviours with cold stressed lambs being less likely to move towards a model ewe when compared to control lambs (Chapter 6).

Further work on the ability of lambs to maintain an appropriate behavioural response to the ewe while under physiological stress is needed. From this thesis it appears that even a short duration of cold stress affects the behaviour of lambs. They become less mobile and also appear less alert than control animals. As lambs are often under more prolonged physiological stress in the early neonatal period due to prolonged or difficult births, cold stress or reduced energy reserves, the implications for their subsequent behaviour and ultimately survival is of interest. However, the development of appropriate methods of testing this behaviour may also be needed. The modified barrier test used in this thesis may not be the best method as other authors have shown that lambs of older ages do not respond well to model ewes (Shillito Walser *et al.* 1985; Winfield and Kilgour 1976). Perhaps more detailed work on the following ability of the lamb or the ability to negotiate a maze to reach their mother may be more useful. Ideally this work would be done under field conditions to provide data on lamb responses under conditions they would be exposed to in normal paddock situations. This would also provide information on lambs under a wide range of

physiological stresses not just cold stress. However, controlling the degree of stress and the maternal influence would be difficult.

To date, very little work has been done on the behavioural development of the lamb from the time it successfully suckles until about 12 hours of age when lambs begin to consistently recognise their mother. Work is needed to determine if this is an important determinant of how the lamb responds to the ewe as she moves from the birth site and whether it is related to the mobility of the lambs, coordination, cognition or neuromuscular development.

7.1 Conclusion

From this thesis it can be concluded that the use of intensive recordings of time to perform critical early behaviours is to date providing the most robust assessment of lamb vigour. However, other behavioural measures such as field-based assessments of vigour score and the modified barrier test are providing a valid assessment of lamb vigour. Rectal temperature at 10 minutes of age appears to be providing some physiological information on the vigour of the lamb however more work is needed to determine the physiological basis of lamb vigour. Selection for improved lamb survival through selection on lamb vigour traits also appears promising as seen by the differences between sires in their lambs' time to perform early behaviours. The field-based measure of lamb vigour may be a useful tool in genetic improvement programs for selecting for vigorous lambs and therefore improved survival.

Further work on the concept of lamb vigour and its importance at a time point when the ewe is moving away from the birth site is needed. This thesis began looking at how the lamb was

prepared in a behavioural sense to follow and maintain contact with the ewe at a time when it would be expected she would be moving from the birth site; however, more work on the physiological basis of this behaviour is required. This thesis showed that cold stress can suppress the behaviour of the lamb under an animal house based assessment. However, more work under field conditions is required to determine the importance of the ability of the lamb to maintain appropriate behavioural responses to the ewe particularly while under a physiological stress.

While this thesis has particularly focused on the lamb, it must be remembered that the ewe has an important role to play in the survival of her lamb/s. Although it is clear that the lamb also contributes to its survival, it is impossible to quantify from this work who (the ewe or the lamb) has the greater responsibility for survival and this responsibility may differ markedly with changes in management, environment and nutrition.

References

Adams AK, Nelson RA, Bell EF, Egoavil CA (2000) Use of infrared thermographic calorimetry to determine energy expenditure in preterm infants. *American Journal of Clinical Nutrition* **71**, 969 - 977.

Alexander G (1958a) Behaviour of newly born lambs. *Proceedings of the Australian Society of Animal Production* **2**, 123 - 125.

Alexander G (1958b) Heat production of newborn lambs in relation to type of birth coat. *Proceedings of the Australian Society of Animal Production* **2**, 10 - 14.

Alexander G (1960) Maternal behaviour in the Merino ewe. *Proceedings of the Australian Society of Animal Production* **3**, 105 - 114.

Alexander G (1961a) Energy exchanges in new-born lambs. In 'Symposium on energy metabolism, 2nd Proceedings'. Wageningen, Netherlands pp. 60 - 71.

Alexander G (1961b) Energy expenditure and mortality in new-born lambs. In 'International Congress on Animal Reproduction, 4th'. The Hague pp. 630 - 637.

Alexander G (1961c) Shelter for new-born lambs. In 'International Congress on Animal Husbandry, 8th'. Hamburg p. 2.

Alexander G (1961d) Temperature regulation in the new-born lamb 3. Effect of environmental temperature on metabolic rate, body temperatures and respiratory quotient. *Australian Journal of Agricultural Research* **12**, 1152 - 1174.

Alexander G (1962a) Energy metabolism in the starved newborn lamb. *Australian Journal of Agricultural Research* **13**, 144 - 164.

Alexander G (1962b) Summit metabolism in young lambs. *Journal of Physiology* **162**, 31 - 32.

Alexander G (1962c) Temperature regulation in the new-born lamb 4. The effect of wind and evaporation o f water from the coat on metabolic rate and body temperature. *Australian Journal of Agricultural Research* **13**, 82 - 99.

Alexander G (1962d) Temperature regulation in the new-born lamb 5. Summit metabolism. *Australian Journal of Agricultural Research* **13** 100 - 121.

Alexander G (1969) The effect of adrenaline and noradrenaline on metabolic rate in young lambs. *Biologia Neonatorum* **14**, 97 - 106.

Alexander G (1970) Summit metabolism in young lambs: effect of hormones and drugs that affect mobilization and utilization of substrate for cold-induced thermogenesis. *Biology of the Neonate* **15**, 37 - 48.

Alexander G (1978a) Factors regulating the growth of the placenta: with comments on the relationship between placental weight and fetal weight. In 'Abnormal Fetal Growth: Biological Bases and Consequences'. (Ed. F Naftolin) pp. 149 - 164. (Dahlem Konferenzen: Berlin).

Alexander G (1978b) Quantitative development of adipose tissue in foetal sheep. *Australian Journal of Biological Science* **31**, 489 - 503.

Alexander G (1979) Cold thermogenesis. In 'Environmental Physiology III'. (Ed. D Robertshaw) pp. 43 - 156. (University Park Press: Baltimore).

Alexander G (1984) Constraints to lamb survival. In 'Reproduction in sheep'. (Eds DR Lindsay, DT Pearce) pp. 199 - 209. (Australian Academy of Science: Canberra).

Alexander G, Bell AW (1975) Quantity and calculated oxygen consumption during summit metabolism of brown adipose tissue in newborn lambs. *Biology of the Neonate* **26**, 214 - 220.

Alexander G, Bell AW, Hales JRS (1972) The effect of cold exposure on the plasma levels of glucose, lactates, free fatty acids and glycerol and the blood gas and acid-base status in young lambs. *Biology of the Neonate* **20**, 9 - 21.

Alexander G, Bell AW, Williams D (1970) Metabolic responses of lambs to cold: effects of prolonged treatment with thyroxine and of acclimation to low temperatures. *Biology of the Neonate* **15**, 198 - 210.

Alexander G, Bradley LR, Stevens D (1993) Effect of age and parity on maternal behaviour in single-bearing Merino ewes. *Australian Journal of Experimental Agriculture* **33**, 721 - 728.

Alexander G, Kilgour R, Stevens D, Bradley LR (1984) The effect of experience on twin-care in New Zealand Romney sheep. *Applied Animal Behaviour Science* **12**, 363 - 372.

Alexander G, Lloyd Davies H (1959) Relationship of milk production to number of lambs born or suckled. *Australian Journal of Agricultural Research* **10**, 720 - 724.

Alexander G, Lynch JJ (1976) Phalaris windbreaks for shorn and fleeced lambing ewes. *Proceedings of the Australian Society of Animal Production* **11**, 161 - 164.

Alexander G, Lynch JJ, Mottershead BW (1979) Use of shelter and slection of lambing sites by shorn and unshorn ewes in paddocks with closely or widely spaced shelters. *Applied Animal Ethology* **5**, 51 - 69.

Alexander G, Lynch JJ, Mottershead BW, Donnelly JB (1980) Reduction in lamb mortality due to grass wind-breaks: results of a five-year study. *Proceedings of the Australian Society of Animal Production* **13**, 329 - 332.

Alexander G, McCance I, Watson RH (1956) The relation of maternal nutrition to neonatal mortality in Merino lambs. *International Congress on Animal Reproduction III* **Proceedings Section 1**, 5 - 7.

Alexander G, Mill SC, Scott TW (1968) Changes in plasma glucose, lactate and free fatty acids in lambs during summit metabolism and treatment with catecholamines. *Journal of Physiology* **198**, 277 - 289.

Alexander G, Mills SC (1968) Free fatty acids and glucose in the plasma of newly-born lambs: effects of environmental temperature. *Biology of the Neonate* **13**, 53 - 61.

Alexander G, Peterson JE, Watson RH (1959) Neonatal mortality in lambs: Intensive observations during lambing in a Corriedale flock with a history of high lamb mortality. *Australian Veterinary Journal* **35**, 433 - 441.

Alexander G, Reid RL, Schinckel PG, Ferguson K (1962) Nutrition of the pregnant ewe. *International Congress on Animal Production, 8th, Hamburg, Proceedings* **3**, 269 - 273.

Alexander G, Shillito-Walser EE (1978) Visual discrimination between ewes by lambs. *Applied Animal Ethology* **4**, 81 - 85.

Alexander G, Stevens D, Bradley LR (1990a) Distribution of field birth-sites of lambing ewes. *Australian Journal of Experimental Agriculture* **30**, 759 - 767.

Alexander G, Stevens D, Bradley LR (1990b) Intensive observations during lambing in a goitre-affected flock, with special reference to behaviour of lambs. *Australian Journal of Experimental Agriculture* **30**, 749 - 757.

Alexander G, Stevens D, Bradley LR, Barwick SA (1990c) Maternal behaviour in Border Leicester, Glen Vale (Border Leicester derived) and Merino sheep. *Australian Journal of Experimental Agriculture* **30**, 27 - 38.

Alexander G, Stevens D, Kilgour R, de Langen H, Mottershead BE, Lynch JJ (1983) Separation of ewes from twin lambs: incidence in several sheep breeds. *Applied Animal Ethology* **10**, 301- 317.

Alexander G, Williams D (1966a) Teat seeking activity in lambs during the first hours of life. *Animal Behaviour* **14**, 166 - 176.

Alexander G, Williams D (1966b) Teat seeking activity in new born lambs: the effects of cold. *Journal of Agricultural Science* **67**.

Alexander G, Williams D (1968) Shivering and non-shivering thermogenesis during summit metabolism in young lambs. *Journal of Physiology* **198**, 251 - 276.

Alexander G, Williams D (1970) Summit metabolism and cardiovascular function in young lambs during hyperoxia and hypoxia. *Journal of Physiology* **208**, 85 - 97.

Ameyama M, Shinagawa E, Matsushita K, Adachi O (1981) D-Fructose dehydrogenase of *Gluconobacter industrius*: Purification, characterization and application to enzymatic microdetermination of D-Fructose. *Journal of Bacteriology* **145**, 814 - 823.

Arnold GW, Morgan CA (1975a) Behaviour of the ewe and lamb at lambing and its relation ot lamb mortality. *Applied Animal Ethology* **2**, 25 - 46.

Arnold GW, Morgan PD (1975b) Behaviour of the ewe and lamb at lambing and its relationship to lamb mortality. *Applied Animal Ethology* **2**, 25 - 46.

Atkins KD (1980) The comparative productivity of five ewe breeds 1. Lamb growth and survival. *Australian Journal of Experimental Agriculture and Animal Husbandry* **20**, 272 - 279.

Banchero GE, Quintans G, Martin GB, Lindsay DR, Milton JTB (2004a) Nutrition and colostrum production in sheep. 1. Metabolic and hormonal responses to a high-energy supplement in the final stages of pregnancy. *Reproduction, Fertility, Development* **16**, 633 - 643.

Banchero GE, Quintans G, Martin GB, Milton JTB, Lindsay DR (2004b) Nutrition and colostrum production in sheep. 2. Metabolic and hormonal responses to different energy sources in the final stages of pregnancy. *Reproduction, Fertility, Development* **16**, 645 - 653.

Barlow RM, Gardiner AC, Angus KW, Gilmour JS, Mellor DJ, Cuthbertson JC, Newlands G, Thompson R (1987) Clinical, biochemical and pathological study of perinatal lambs in a commercial flock. *Veterinary Record* **120**, 357 - 362.

Beetson BR (1984) Influence of plane of nutrition during late pregnanct and lactation on the survival and growth of Merino and first cross lambs. In 'Reproduction in Sheep'. (Eds DR Lindsay, DT Pearce) pp. 213 - 215. (Australian Academy of Science: Canberra).

Bickell SL, Nowak R, Poindron P, Ferguson D, Blache D (2010) Maternal behaviour at parturition in outdoor conditions differs only moderately between single-bearing ewes selected for their calm or nervous temperament. *Animal Production Science* **50**, 675-682.

Bickell SL, Nowak R, Poindron P, Sebe F, Chadwick A, Ferguson D, Blache D (2009) Temperament does not affect the overall establishment of mutual preference between the mother and her young in sheep measured in a choice test. *Developmental Psychobiology* **51**, 429 - 437.

Bleker OP, Breur W, Huidekoper BL (1979) A study of birth weight, placental weight and mortality of twins as compared to singletons. *British Journal of Obstetrics and Gynaecology* **86**, 111 - 118.

Brien FD, Hebart ML, *et al.* (2009) Genetics of lamb survival: Preliminary studies of the information nucleus flock. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **18**, 108 - 111.

Brien FD, Hebart ML, Jaensch KS, Smith DH, Grimson RJ (2008) Genetics of lamb survival: A study of Merino resource flocks in South Australia.

Brien FD, Hebart ML, *et al.* (2010) Opportunities for genetic improvement of lamb survival. *Animal Production Science* **50**, 1017 - 1025.

Budge H, Edwards LJ, McMillen IC, Bryce A, Warnes K, Pearce S, Stephenson T, Symonds ME (2004) Nutritional manipulation of fetal adipose tissue deposition and uncoupling protein

1 messenger RNA abundance in the sheep: Differential effects of timing and duration. *Biology of Reproduction* **71**, 359 - 365.

Cabello G (1983) Endocrine reactivity (T_3, T_4, cortisol) during cold exposure in preterm and full-term lambs. *Biology of the Neonate* **44**, 224 - 233.

Cannon B, Nedergaard J (2004) Brown adipose tissue:Function and physiological significance. *Physiological Reviews* **84**, 277 - 359.

Caple IW, Nugent GF, Azuolas JK, Foot JZ, Thompson RL (1982) Effects of iodine supplementation of ewes on survival and growth of lambs. *Animal Production in Australia* **15**, 535 - 538.

Clark MR, McCann DM, Forde MC (2003) Application of infrared thermography to the non-destructive testing of concrete and masonry bridges. *NDT&E International* **36**, 265 - 275.

Clarke L, Bryant MJ, Lomax MA, Symonds ME (1997a) Maternal manipulation of brown adipose tissue and liver development in the ovine fetus during late gestation. *British Journal of Nutrition* **77**, 871 - 883.

Clarke L, Buss DS, Juniper DT, Lomax MA, Symonds ME (1997b) Adipose tissue development during early postnatal life in ewe-reared lambs. *Experimental Physiology* **82**, 1015 - 1027.

Clarke L, Symonds ME (1998) Thermoregulation in newborn lambs: Influence of feeding and ambient temperature on brown adipose tissue. *Experimental Physiology* **83**, 651 - 657.

Cloete SWP (1993) Observations on neonatal progress of Dormer and South African Mutton Merino lambs. *South African Journal of Animal Science* **23**, 38 - 42.

Cloete SWP, Scholtz AJ (1998) Lamb survival in relation to lambing and neonatal behaviour in medium wool Merino lines divergently selected for multiple rearing ability. *Australian Journal of Experimental Agriculture* **38**, 801 - 811.

Cloete SWP, Scholtz AJ, Cloete JJE, van Wyk JB (2005) The ability of Merino ewes and lambs to reunite after separation as affected by divergent selection for ewe multiple rearing capacity. *Australian Journal of Experimental Agriculture* **45**, 1131 - 1137.

Cock ML, Camm EJ, Louey S, Joyce BJ, Harding R (2001) Postnatal outcomes in term and preterm lambs following fetal growth restriction. *Clinical and Experimental Pharmacology and Physiology* **28**, 931 - 937.

Comline RS, Silver M (1972) The composition of foetal and maternal blood during parturition in the ewe. *Journal of Physiology* **222**, 233 - 256.

Coombs T, Dwyer CM (2008) Effects of prenatal undernutrition in early gestation on the ewe-lamb bond and lamb behaviour in Suffolk and Scottish Blackface sheep. *Proceedings of the 42nd International Congress of the International Society for Applied Ethology*, 3 - 9 August, Dublin, Eire, p. 138.

Cooper KE, Pittman QJ, Veale WL (1976) The effect of noradrenaline and 5-hydroxytryptamine injected into a lateral cerebral ventricle on thermoregulation in the new-born lamb. *Journal of Physiology* **261**, 223 - 234.

Corner RA, Kenyon PR, Stafford KJ, West DM, Lopez-Villalobos N, Morris ST, Oliver MH (2008) Effect of nutrition from mid to late pregnancy on the performance of twin- and triplet-bearing ewes and their lambs. *Australian Journal of Experimental Agriculture* **48**, 666 - 671.

Cumming IA, Rizzoli DJ, Clarke JD, McPhee SR (1978) Fecundity of the fat ewe. *Proceedings of the Australian Society of Animal Production* **12**, 261.

Daniels LB, Perkins JL, Krieder D, Tugwell D, Carpenter D (1974) Blood glucose and fructose in the newborn ruminant. *Journal of Dairy Science* **57**, 1196 - 1200.

Davies HL (1964) Lamb losses in south-western Australia. *Proceedings of the Australian Society of Animal Production* **5**, 107 - 112.

De Blasio MJ, Gatford KL, Robinson JS, Owens JA (2006) Placental restriction alters circulating thyroid hormone in the young lamb postnatally. *Am J Physiol Regul Integr Comp Physiol* **291**, R1016 - R1024.

Dennis SM (1972) Perinatal lamb mortality. *Cornell Veterinary* **62**, 253 - 263.

Donnelly JR (1984) The productivity of breeding ewes grazing on lucerne or grass and clover pastures on the tablelands of southern Australia. III Lamb mortality and weaning percentage. *Australian Journal of Agricultural Research* **35**, 709 - 721.

Duff XJ, McCutcheon SN, McDonald MF (1982) Central nervous system injury as a determinant of lamb mortality. *Proceedings of the New Zealand Society of Animal Production* **42**, 15 - 17.

Dwyer CM (2003) Behavioural development in the neonatal lamb: effect of maternal and birth-related factors. *Theriogenology* **59**, 1027 - 1050.

Dwyer CM (2008a) Genetic and physiological determinants of maternal behaviour and lamb survival: Implication for low-input sheep management. *Journal of Animal Science* **86**, E246 - E258.

Dwyer CM (2008b) The welfare of the neonatal lamb. *Small Ruminant Research* **76**, 31- 41.

Dwyer CM, Calvert SK, Farish M, Donbavand J, Pickup HE (2005) Breed, litter and parity effects on placental weight and placentome number, and consequences for the neonatal behaviour of the lamb. *Theriogenology* **63**, 1092 - 1110.

Dwyer CM, Lawrence AB (1999) Does the behaviour of the neonate influence the expression of maternal behaviour in sheep? *Behaviour* **136**, 367 - 389.

Dwyer CM, Lawrence AB (2000) Effects of maternal genotype and behaviour on the behavioural development of their offspring in sheep. *Behaviour* **137**, 1629 - 1654.

Dwyer CM, Lawrence AB (2005) A review of the behavioural and physiological adaptations of hill and lowland breeds of sheep that favour lamb survival. *Applied Animal Behaviour Science* **92**, 235 - 260.

Dwyer CM, Lawrence AB, Bishop SC (2001) The effects of selection for lean tissue content on maternal and neonatal lamb behaviours in Scottish Blackface sheep. *Animal Science* **72**, 555 - 571.

Dwyer CM, Lawrence AB, Bishop SC, Lewis M (2003) Ewe-lamb bonding behaviours at birth are affected by maternal undernutrition in pregnancy. *British Journal of Nutrition* **89**, 123-136.

Dwyer CM, Lawrence AB, Brown HE, Simm G (1996) Effect of ewe and lamb genotype on gestation length, lambing ease and neonatal behaviour in lambs. *Reproduction Fertility Development* **8**, 1123 - 1129.

Dwyer CM, McLean KA, Deans LA, Chirnside J, Calvert SK, Lawrence AB (1998) Vocalisations between mother and young in sheep: effects of breed and maternal experience. *Applied Animal Behaviour Science* **58**, 105 - 119.

Dwyer CM, Morgan CA (2006) Maintenance of body temperature in the neonatal lamb: Effects of breed, birth weight, and litter size. *Journal of Animal Science* **84**, 1093 - 1101.

Dwyer CM, Smith LA (2008) Parity effects on maternal behaviour are not related to circulating oestradiol concentrations in two breeds of sheep. *Physiology and Behaviour* **93**, 148 - 154.

Eales FA, Small J (1980) Summit metabolism in newborn lambs. *Research in Veterinary Science* **29**, 211 - 218.

Eales FA, Small J (1985) Determinants of heat production capacity in newborn lambs and their effects on survival: Substrates for heat production. In 'Agriculture: Factors affecting the survival of newborn lambs'. Brussels. (Ed. JDBaJS G. Alexander). (Commission of the European Communities).

Egan JK, McLaughlin JW, Thompson RL, McIntyre JS (1972) The importance of shelter in reducing neonatal lamb deaths. *Australian Journal of Experimental Agriculture and Animal Husbandry* **12**, 470 - 472.

Egan JK, Thompson RL, McIntyre JS (1977) Stocking rate, joining time, fodder conservation and the productivity of Merino ewes 2. Birth weight, survival and growth of lambs *Australian Journal of Experimental Agriculture and Animal Husbandry* **17**, 909 - 914.

Everett-Hincks JM, Blair HT, Stafford KJ, Lopez-Villalobos N, Kenyon PR, Morris ST (2005a) The effect of pasture allowance fed to twin- and triplet-bearing ewes in late pregnancy on ewe and lamb behaviour and performance to weaning. *Livestock Production Science* **97**, 253 - 266.

Everett-Hincks JM, Dodds KG (2007) Management of maternal-offspring behaviour to improve lamb survival in easy care sheep systems. *Journal of Animal Science* **86**, E259.

Everett-Hincks JM, Duncan SJ (2008) Lamb post-mortem protocol for use on farm: To diagnose primary cause of lamb death from birth to 3 days of age. *The Open Veterinary Science Journal* **2**, 55 - 62.

Everett-Hincks JM, Lopez-Villalobos N, Blair HT, Stafford KJ (2005b) The effect of ewe maternal behaviour score on lamb and litter survival. *Livestock Production Science* **93**, 51 - 61.

Ferguson M, Thompson AN, Gordon D (2007) 'Ewe Management Handbook.'

Fogarty NM (1972) Crossbreeding for lamb production 1. Survival and growth of first cross lambs. *Australian Journal of Experimental Agriculture and Animal Husbandry* **12**, 234 - 239.

Fogarty NM, Hall DG, Holst PJ (1992) The effect of nutrition in mid pregnancy and ewe liveweight change on birth weight and management for lamb survival in highly fecund ewes. *Australian Journal of Experimental Agriculture* **32**, 1 - 10.

Fogarty NM, Hopkins DL, van de Ven R (2000) Lamb production from diverse genotypes 1. Lamb growth and survival and ewe performance. *Animal Science* **70**, 135 - 145.

Fogarty NM, Ingham VM, Gilmour AR, Cummins LJ, Gaunt GM, Stafford J, Hocking Edwards JE, Banks RG (2005) Genetic evaluation of crossbred lamb production. 1. Breed and fixed effects for birth and weaning weight of first-cross lambs, gestation length and reproduction of base ewes. *Australian Journal of Agricultural Research* **56**, 443 - 453.

Fowler DG (2007) Lamb marking performance for ultrasound scanned ewes in Australian sheep flocks. Final Report AHW: 131 Meat & Livestock Australia.

Gardner DS, Buttery PJ, Daniel Z, Symonds ME (2007) Factors affecting birth weight in sheep: maternal environment. *Reproduction* **133**, 297 - 307.

Gate JJ, Clarke L, Lomax MA, Symonds ME (1999) Chronic cold exposure has no effect on brown adipose tissue in newborn lambs born to well-fed ewes. *Reproduction, Fertility, Development* **11**, 415 - 418.

Geenty KG (1997) 'A guide to improved lambing percentage for farmers and advisors.' (Wools of New Zealand and The New Zealand Meat Producers Board: Palmerston North).

Geist V (1971) Behaviour of Ewes and Lambs. In 'Mountain Sheep' pp. 239 - 255. (The University of Chicago Press: Chicago).

Gemmell RT, Bell AW, Alexander G (1972) Morphology of adipose cells in lambs at birth and during subsequent transition of brown to white adipose tissue in cold and in warm conditions. *American Journal of Anatomy* **133**, 143 - 163.

Gibb MJ, Treacher TT (1982) The effect of body condition and nutrition during late pregnancy on the performance of grazing ewes during lactation. *Animal Production* **34**, 123 - 129.

Grandinson K (2005) Genetic background of maternal behaviour and its relation to offspring survival. *Livestock Production Science* **93**, 43 - 50.

Greenwood PL, Bell AW (2003) Consequences of intra-uterine growth retardation for postnatal growth, metabolism and pathophysiology. *Reproduction Supplement* **61**, 195 - 206.

Greenwood PL, Hunt AS, Slepetis RM, Finnerty KD, Alston C, Beermann DH, Bell AW (2002) Effects of birth weight and postnatal nutrition on neonatal sheep: III. Regulation of energy metabolism. *Journal of Animal Science* **80**, 2850 - 2861.

Gudex BW (2001) Sireline variation in neonatal lamb cold tolerance. Lincoln University.

Gudex BW, Hickford JGH, Frampton CM (2005) Sire-line differences in lamb losses from starvation-exposure. *Proceedings of the New Zealand Society of Animal Production* **65**, 186-190.

Hall DG, Egan AR, Foot JZ, Parr RA (1990) The effect of litter size on colostrum production in crossbred ewes. *Proceedings of the Australian Society of Animal Production* **18**, 240 - 243.

Hall DG, Fogarty NM, Gilmour AR (1995) Performance of crossbred progeny of Trangie Fertility Merino and Booroola Merino rams and Poll Dorset ewes 1. Lamb birth weight, survival and growth. *Australian Journal of Experimental Agriculture* **35**, 1069 - 1074.

Hall DG, Holst PJ, Shutt DA (1992a) The effect of nutritional supplements in late pregnancy on ewe colostrum production plasma progesterone and IGF-1 concentrations. *Australian Journal of Agricultural Research* **43**, 325 - 337.

Hall DG, Piper LR, Egan AR, Bindon BM (1992b) Lamb and milk production from Booroola ewes supplemented in late pregnancy. *Australian Journal of Experimental Agriculture* **32**, 587 - 593.

Hatcher S (2007) Ewe nutrition during late pregnancy - vital for ewe and lamb survival. *Lifetime Wool - Maximising the genetic potential of your flock* **3**

Haughey KG (1980a) The effect of birth injury to the foetal nervous system on the survival and feeding behaviour of lambs. In 'Reviews in Rural Science No. 4'. (Eds M Wodzicka-Tomasczewska, TN Edey, JJ Lynch) pp. 109 - 111. (University of New England, Armidale.

Haughey KG (1980b) The role of birth in the pathogenesis of meningeal haemorrhage and congestion in newborn lambs. *Australian Veterinary Journal* **56**, 49 - 56.

Haughey KG (1981) Perinatal lamb mortality. *Refresher course for Veterinarians Proceedings* **58**, 657 - 673.

Haughey KG (1982) Postnatal mortality, bodyweight gain and wool production to two years of age of lambs surviving Caesarean or stressful vaginal birth. *Australian Veterinary Journal* **58**, 173 - 180.

Himms-Hagen J (1985) Brown adipose tissue metabolism and thermogenesis. *Annual Review of Nutrition* **5**, 69 - 94.

Hinch GN (2008) Lamb survival in Australian flocks: A review. Report to the CRC for Sheep Industry Innovation, Armidale, NSW.

Hinch GN, Crosbie SF, Kelly RW, Owens JL, Davis GH (1985) Influence of birth weight and litter size on lamb survival in high fecundity Booroola-Merino crossbred flocks. *New Zealand Journal of Agricultural Research* **28**, 31 - 38.

Holst PJ (1987) Supplementary feeding of oat grain or lucerne hay to crossbred ewes at lambing. *Australian Journal of Experimental Agriculture* **27**, 211 - 216.

Holst PJ, Fogarty, N.M. and Stanley, D.M. (2002) Birth weights, meningeal lesions, and survival of diverse genotypes of lambs from Merino and crossbred ewes. *Australian Journal of Agricultural Research* **53**, 175 - 181.

Holst PJ, Hall DG, Allan CJ (1996) Ewe colostrum and subsequent lamb suckling behaviour. *Australian Journal of Experimental Agriculture* **36**, 637 - 640.

Holst PJ, Killeen ID, Cullis BR (1986) Nutrition of the pregnant ewe and its effect on gestation length, lamb birth weight and lamb survival. *Australian Journal of Agricultural Research* **37**, 647 - 655.

Jackson DM, Hambly C, Trayhurn P, Speakman JR (2001) Can non-shivering thermogenesis in brown adipose tissue following NA injection be quantified by changes in overlying surface temperatures using infrared thermography? *Journal of Thermal Biology* **26**, 85 - 93.

Jordan DJ, Le Feuvre AS (1989) The extent and cause of perinatal lamb mortality in 3 flocks of Merino sheep. *Australian Veterinary Journal* **66**, 198 - 201.

Kallweit E, Smidt D, Profittlich CH (1986) Relationship between breed, litter size , birth weight and mortality in newborn lambs. In 'Factors affecting the survival of newborn lambs'. (Eds G Alexander, JD Barker, J Slee) pp. pp. 123 - 133. (Commission of the European Communitites: Luxembourg).

Kelly RW (1992) Lamb mortality and growth to weaning in commercial Merino flocks in Western Australia. *Australian Journal of Agricultural Research* **43**, 1399 - 1416.

Kenyon PR, Stafford KJ, Jenkinson CMC, Morris ST, West DM (2007) The body composition and metabolic status of twin- and triplet-bearing ewes and their fetuses in late pregnancy. *Livestock Science* **107**, 103 - 112.

Kerslake JI, Everett-Hincks JM, Campbell AW (2005) Lamb survival: a new examination of an old problem. *Proceedings of the New Zealand Society of Animal Production* **65**, 13 - 18.

Kerslake JI, Kenyon PR, Stafford KJ, Morris ST, Morel PCH (2009) The effect of offering concentrate supplement to twin- and triplet-bearing ewes grazing a 60 mm herbage sward height on lamb birth weight, heat production and post-natal growth. *Journal of Agricultural Science* **147**, 613 - 624.

Khalaf AM, Doxey DL, Baxter JT, Black WJM, Fitzsimmons J, Ferguson JA (1979) Late pregnancy ewe feeding and lamb performance in early life: 2. Factors associated with perinatal lamb mortality. *Animal Production* **29**, 401 - 410.

Kilgour RJ (1992) Lamb potential and mortality in Merino sheep as ascertained by ultrasonography. *Australian Journal of Experimental Agriculture* **32**, 311 - 313.

Kilgour RJ, Alexander G, Stevens D (1982) A field study of factors interrupting bonding of ewes and twins. *Proceedings of the 2nd Asia-Oceania Congress of Perinatology, Auckland* **February 1982**, 118 - 123.

King JM, Fisher JS, Murphy PM (1990) Threshold condition scores of Merino ewes for improved autumn lambing performance in Western Australia. *Proceedings of the Australian Society of Animal Production* **18**, 272 - 275.

Kleeman DO, Walker SK (2005) Fertility in South Australian commercial Merino flocks: sources of reproductive wastage. *Theriogenology* **63**, 2075 - 2088.

Kleeman DO, Walker SK, Grosser TI, Grimson RJ, Smith DH (1991) Reproductive loss in commercial Merino flocks in South Australia. *Proceedings of the 23rd Annual Conference of the Australian Society for Reproductive Biology, University of Sydney* **30 September - 2 October 1991 Australian Society for Reproductive Biology** 58.

Knight TW, Oldham CM, Smith JF, Lindsay DR (1975) Studies in ovine infertility in agricultural regions in Western Australia: analysis of reproductive wastage. *Australian Journal of Experimental Agriculture and Animal Husbandry* **15**, 183 - 188.

Lancaster WC, Thomson SC, Speakman JR (1997) Wing temperature in flying bats measured by infrared thermography. *Journal of Thermal Biology* **22**, 109 - 116.

Langlands JP, Sutherland HAM (1968) An estimate of the nutrients utilized for pregnancy by Merino sheep. *British Journal of Nutrition* **22**, 217 - 227.

Lax J, Newton-Turner H (1965) The influence of various factors on survival rate to weaning of Merino lambs 1. Sex, strain. location and age of ewe for single-born lambs. *Australian Journal of Agricultural Research* **16**, 981 - 995.

Lindsay DR, Nowak R, Putu IG, McNeill DM (1990) Behavioural interactions between the ewe and her young at parturition: A vital step for the lamb. In 'Reproductive Physiology of Merino Sheep: Concepts and Consequences'. (Eds CM Oldham, GB Martin, IW Purvis) pp. 191 - 205. (School of Agriculture (Animal Science) The University of Western Australia: Perth).

Lynch JJ, Alexander G (1977) Sheltering behaviour of lambing Merino sheep in relation to grass hedges and artificial windbreaks. *Australian Journal of Agricultural Research* **28**.

Lynch JJ, Alexander G (1980) Selection of shelter by Merino sheep. Fourth Review in Rural Science. *Proceedings of a Symposium on "Reproductive behaviour, productivity and wlefare of farm animals" held at Armidale, NSW in September 1979, University of New England*, 119 - 121.

Lynch JJ, Hinch GN, Adams DB (1992) 'The Behaviour of Sheep: Biological Principles and Implications for Production.' (CAB International and CSIRO Australia: Melbourne).

Macfarlane JM, Matheson SM, Dwyer CM (2010) Genetic parameters for birth difficulty, lamb vigour and lamb sucking ability in Suffolk sheep. *Animal Welfare* **19**, 99 - 105.

Matheson SM, Roden J, Haresign W, Bunger L, Dwyer CM (2010) Selection of sires with good lambing and lamb vigour characteristics within three Suffolk strains. *Proceedings of the British Society of Animal Science and the Agricultural Research Forum* **1**, 170.

McCafferty DJ, Moncrieff JB, Taylor IR, Boddie GF (1998) The use of IR thermography to measure the radiative temperature and heat loss of a barn owl (*Tyto alba*). *Journal of Thermal Biology* **23**, 311 - 318.

McCance I, Alexander G (1959) Onset of lactation in the Merino ewe and its modification by nutritional factors. *Australian Journal of Agricultural Research* **10**, 699 - 719.

McCrabb GJ, Egan AR, Hosking BJ (1992) Maternal undernutrition during mid-pregnancy in sheep: variable effects on placental growth. *Journal of Agricultural Science, Cambridge* **118**, 127 - 132.

McCutcheon SN, Holmes CW, McDonald MF (1981) The starvation-exposure syndrome and neonatal lamb mortality: A review. *Proceedings of the New Zealand Society of Animal Production* **41**, 209 - 217.

McNeill DM, Murphy PM, Lindsay DR (1998) Blood lactose *v.* milk lactose as a monitor of lactogenesis and colostrum production in Merino ewes. *Australian Journal of Agricultural Research* **49**, 581 - 587.

Mellor DJ (1983) Nutritional and placental determinants of foetal growth rate in sheep and consequences for the newborn lamb. *British Veterinary Journal* **139**, 307 - 324.

Mellor DJ (1988) Integration of perinatal events, pathophysiological changes and consequences for the newborn lamb. *British Veterinary Journal* **144**, 552 - 569.

Mellor DJ, Murray L (1981) Effects of placental weight and maternal nutrition on the growth rates of individual fetuses in single and twin bearing ewes during late pregnancy. *Research in Veterinary Science* **30**, 198 - 204.

Mellor DJ, Murray L (1986) Making the most of colostrum at lambing. *Veterinary Record* **118**, 351 - 353.

Mellor DJ, Pearson RA (1977) Some changes in the composition of blood during the first 24 hours after birth in normal and growth retarded lambs. *Ann. Rech. Vet.* **8**, 460 - 467.

Miller DR, Blache D, Jackson RB, Downie EF, Roche JR (2010) Metabolic maturity at birth and neonate lamb survival: Association among maternal factors, litter size, lamb birth weigh and plasma metabolic and endocrine factors on survival and behaviour. *Journal of Animal Science* **88**, 581 - 593.

Mills CS, Scott TW, Alexander G (1967) Influence of catecholamines on plasma levels of free fatty acids, glucose and lactate in new-born lambs. *Proceedings of the Australian Biochemistry Society*, 23.

Morris CA, Hickey SM, Clarke JN (2000) Genetic and environmental factors affecting lamb survival at birth and through to weaning. *New Zealand Journal of Agricultural Research* **43**, 515 - 524.

Mottershead BE, Alexander G, Lynch JJ (1982) Sheltering behaviour of shorn and unshorn sheep in mixed or separate flocks. *Applied Animal Ethology* **8**, 127 - 136.

Mullaney PD (1969) Birth weight and survival of Merino, Corriedale and Polwarth lambs. *Australian Journal of Experimental Agriculture and Animal Husbandry* **9**, 157 - 163.

Murphy PM, Lindsay DR, Purvis IW (1994a) The importance of the birthsite on the survival of Merino lambs. *Proceedings of the Australian Society of Animal Production* **20**, 251-254.

Murphy PM, Purvis IW, Lindsay DR, Le Neindre LE, Orgeur P, Poindron P (1994b) Measures of temperament are highly repeatable in Merino sheep and some are related to maternal behaviour. *Proceedings of the Australian Society of Animal Production* **20**, 247 - 250.

Nash ML, Hungerford LL, Nash TG, Zinn GM (1996) Risk factors for perinatal and postnatal mortality in lambs. *The Veterinary Record* **139**, 64 - 67.

Nixon-Smith WF (1972) The forecasting of chill risk ratings for new born lambs and off-shears sheep by the use of a cooling factor derived from synoptic data. *Bureau of Meteorology Working Paper* **150**.

Nowak R (1990) Mother and sibling discrimination at a distance by three- to seven-day-old lambs. *Developmental Psychobiology* **23**, 285 - 295.

Nowak R (1996) Neonatal survival: contributions from behavioural studies in sheep. *Applied Animal Behaviour Science* **49**, 61 - 72.

Nowak R, Lindsay DR (1990) Effect of genotype and litter size on discrimination of mothers by their twelve-hour-old lambs. *Behaviour* **115**, 1 - 13.

Nowak R, Lindsay DR (1992) Discrimination of Merino ewes by their newborn lambs: important for survival? *Applied Animal Behaviour Science* **34**, 61 - 74.

Nowak R, Murphy PM, Lindsay DR, Alster P, Andersson R, Uvnas-Moberg K (1997) Development of a preferential relationship with the mother by the newborn lamb: Importance of the sucking activity. *Physiology and Behaviour* **62**, 681 - 688.

Nowak R, Poindron P (2006) From birth to colostrum: early steps leading to lamb survival. *Reproduction, Nutrition, Development* **46**, 431 - 446.

Nowak R, Poindron P, Le Neindre LE, Putu IG (1987) Ability of 12-hour-old Merino and Crossbred lambs to recognise their mothers. *Applied Animal Behaviour Science* **17**, 263 - 271.

Nowak R, Poindron P, Putu IG (1989) Development of mother discrimination by single and multiple newborn lambs. *Developmental Psychobiology* **22**, 833 - 845.

Nowak R, Porter RH, Blache D, Dwyer CM (2008) Behaviour and the welfare of the sheep. In 'The Welfare of Sheep'. (Ed. CM Dwyer).

Nowak R, Porter RH, Blache D, Dwyer CM (2009) Behaviour and the welfare of the sheep.

O'Connor CE, Lawrence AB (1992) Relationship between lamb vigour and ewe behaviour at parturition. *Animal Production* **54**, 361 - 366.

Oppong-Anane K (1991) The following (walking) ability of the neonatal lamb. University of Adelaide.

Owens JL, Bindon BM, Edey TN, Piper LR (1985) Behaviour at parturition and lamb survival of Booroola Merino sheep. *Livestock Production Science* **13**, 359 - 372.

Pfister JA, Astorga JB, Panter KE, Stegelmeier BL, Molyneux RJ (2006a) Maternal ingestion of locoweed I. Effects on ewe-lamb bonding and behaviour. *Small Ruminant Research* **65**, 51 - 63.

Pfister JA, Davidson TW, Panter KE, Cheney CD, Molyneux RJ (2006b) Maternal ingestion of locoweed III. Effects on lamb behaviour at birth. *Small Ruminant Research* **65**, 70 - 78.

Pickup HE, Dwyer CM (2001) Does variation in the onset of maternal behaviour affect the strength of association between ewes and their lambs? *Proceedings of the 35th International Congress of the International Society for Applied Ethology*, 4 - 8 August, University of California, Davis, USA.

Poindron P, Le Neindre P, Levy F (1984) Maternal behaviour in sheep and its physiological control. In 'Reproduction in Sheep'. (Ed. DRaP Lindsay, D.T.) pp. 191 - 198. (Australian Academy of Science: Canberra).

Porter WP, Gates DM (1969) Thermodynamic equilibria of animals with environment. *Ecological Monographs* **39**, 227 - 244.

Prior RL, Christenson RK (1976) Influence of dietary energy during gestation on lambing performance and glucose metabolism in Finn-cross ewes. *Journal of Animal Science* **43**, 1114 - 1124.

Purser AF, Young GB (1964) Mortality among twin and single lambs. *Animal Production* **6**, 321 - 329.

Putu IG (1990) Maternal behaviour in Merino ewes during the first two days after parturition and lamb survival. *PhD Thesis, University of Western Australia*.

Putu IG, Poindron P, Lindsay DR (1988a) Early disturbance of Merino ewes from the birth site increases lamb separations and mortality. *Proceedings of the Australian Society of Animal Production* **17**, 298 - 301.

Putu IG, Poindron P, Lindsay DR (1988b) A high level of nutrition during late pregnancy improves subsequent maternal behaviour of Merino ewes. *Proceedings of the Australian Society of Animal Production* **17**, 294 - 297.

Redmer DA, Wallace JM, Reynolds LP (2004) Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. *Domestic Animal Endocrinology* **27**, 199 - 217.

Reynolds LP, Borowicz PP, Vonnahme KA, Johnson ML, Grazul-Bilska AT, Redmer DA, Caton JS (2005) Placental angiogenesis in sheep models of compromised pregnancy. *Journal of Physiology* **565**, 43 - 58.

Ruminants S (1990) 'Feeding standards for Australian livestock: Ruminants.' (CSIRO Publishing: Melbourne).

Sackett D, Holmes P, Abbot K, Jephcott S, Barber M (2006) Project AHW.087. Report to MLA. Assessing the economic cost of endemic disease on the profitability of Australian beef cattle and sheep producers. Meat and Livestock Australia Ltd., North Sydney.

Safari E, Atkins KD, Fogarty NM, Gilmour AR (2005) Analysis of lamb survival in Australian Merino. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **16**, 28 - 31.

Samson DE, Slee J (1981) Factors affecting resistance to induced body cooling in newborn lambs of 10 breeds. *Animal Production* **33**, 59 - 65.

Sawalha RM, Conington J, Brotherstone S, Villanueva B (2007) Analyses of lamb survival of Scottish Blackface sheep. *Animal* **1**, 151 - 157.

Scales GH, Burton RN, Moss RA (1986) Lamb mortality, birthweight and nutrition in late pregnancy. *New Zealand Journal of Agricultural Research* **29**, 75 - 82.

Schermer SJ, Bird JA, Lomax MA, Shepherd DAL, Symonds ME (1996) Effect of fetal thyroidectomy on brown adipose tissue and thermoregulation in newborn lambs. *Reproduction Fertility Development* **8**, 995 - 1002.

Sebe F, Nowak R, Poindron P, Aubin T (2007) Establishment of vocal communication and discrimination between ewes and their lamb in the first two days after parturition. *Developmental Psychobiology* **49**, 375 - 386.

Shelley L (1970) Interrelationships between the duration of parturition, post-natal behaviour of ewes and lambs and the incidence of neonatal mortality. *Proceedings of the Australian Society of Animal Production* **8**, 348 - 352.

Shelton M, Menzies JW (1970) Repeatability and heritability of components of reproductive efficiency in fine-wool sheep. *Journal of Animal Science* **30**, 1 - 5.

Shillito EE, Hoyland VE (1971) Observations on parturition and maternal care in Soay sheep. *Journal of Zoology* **165**, 509 - 512.

Shillito Walser E, Walters DE (1987) Vocal responses of lamb to sound recordings of ewe bleats. *Behaviour* **100**, 50 - 60.

Shillito Walser E, Walters E, Hague P, Williams T (1985) Responses of lambs to model ewes. *Behaviour* **95**, 110 - 120.

Shubber AH, Doxey DL, Black WJM, Fitzsimmons J (1979) Colostrum production by ewes and the amounts ingested by lambs. *Research in Veterinary Science* **27**, 280 - 282.

Shuran M, Nelson RA (1991) Quantitation of energy expenditure by infrared thermography. *The American Journal of Clinical Nutrition* **53**, 1361 - 1367.

Simpson SP, Slee J (1988) The inheritance of non-response to noradrenaline in newborn Scottish Blackface lambs. *Genetical Research* **51**, 65 - 69.

Slee J (1981) A review of genetic aspects of survival and resistance to cold in newborn lambs. *Livestock Production Science* **8**, 419 - 429.

Slee J, Alexander G, Bradley LR, Jackson N, Stevens D (1991) Genetic aspects of cold resistance and related characters in newborn Merino lambs. *Australian Journal of Experimental Agriculture* **31**.

Slee J, Griffiths RG, Samson DE (1980) Hypothermia in newborn lambs induced by experimental immersion in a water bath and by natural exposure outdoors. *Research in Veterinary Science* **28**, 275 - 280.

Slee J, Simpson SP (1991) Description of the effects of a single gene which inhibits the normal metabolic response of newborn lambs to exogenous noradrenaline. *Research in Veterinary Science* **51**, 34 - 39.

Slee J, Simpson SP, Stott AW, Williams JC, Samson DE (1990) An improved water-bath test to study effects of age and previous sucking on metabolic rate and resistance to cold in newborn lambs. *Animal Production* **50**, 319 - 331.

Slee J, Simpson SP, Wilson SB (1987a) Comparative methods for inducing and measuring non-shivering thermogenesis in newborn lambs. *Animal Production* **45**, 61 - 67.

Slee J, Simpson SP, Woolliams JA (1987b) Metabolic rate responses to cold and to exogenous noradrenaline in newborn Scottish Blackface lambs genetically selected for high or low resistance to cold. *Animal Production* **45**, 69 - 74.

Slee J, Springbett A (1986) Early post-natal behaviour in lambs of ten breeds. *Applied Animal Behaviour Science* **15**, 229 - 240.

Slee J, Stott AW (1986) Genetic selection for cold resistance in Scottish Blackface lambs. *Animal Production* **43**, 397 - 404.

Sokoloff G, Blumberg MS (2001) Competition and cooperation among huddling infant rats. *Developmental Psychobiology* **39**, 65 - 75.

Stafford KJ, Kenyon PR, Morris ST, West DM (2007) The physical state and metabolic status of lambs of different birth rank soon after birth. *Livestock Science* **111**, 10 - 15.

Stevens D, Alexander G, Bell AW (1990) Effect of prolonged glucose infusion into fetal sheep on body growth, fat deposition and gestation length. *Journal of Developmental Physiology* **13**, 277 - 281.

Stevens D, Alexander G, Lynch JJ (1981) Do Merino ewes seek isolation or shelter at lambing? *Applied Animal Ethology* **7**, 149 - 155.

Stevens D, Alexander G, Lynch JJ (1982) Lamb mortality due to inadequate care of twins by Merino ewes. *Applied Animal Ethology* **8**, 243 - 252.

Stevens D, Alexander G, Mottershead B, Lynch JJ (1984) Role of the lamb in post-partum separation of ewes from twin lambs. *Animal Production in Australia* **15**, 751.

Stott AW, Slee J (1985) The effect of environmental temperature during pregnancy on thermoregulation in the newborn lamb. *Animal Production* **41**, 341 - 347.

Stott AW, Slee J (1987) The effects of litter size, sex, age, body weight, dam age and genetic selection for cold resistance on the physiological responses to cold exposure of Scottish Blackface lambs in a progressively cooled water bath. *Animal Production* **45**, 477 - 491.

Symonds ME, Bird JA, Sullivan C, Wilson V, Clarke L, Stephenson T (2000) Effect of delivery temperature on endocrine stimulation of thermoregulation in lambs born by cesarean section. *Journal of Applied Physiology* **88**, 47 - 53.

Symonds ME, Lomax MA (1992) Maternal and environmental influences and thermoregulation in the neonate. *Proceedings of the Nutrition Society* **51**, 165 - 172.

Theriez M, Villette Y (1985) Relationship between lamb survival and behaviour during the first hour after birth. In 'Factors affecting the survival of newborn lambs'. (Eds G Alexander, JD Barker, J Slee) pp. 135 - 144. (Commission of the European Communities: Brussels).

Thompson GE (1983) The intake of milk by suckled, newborn lambs and the effects of twinning and cold exposure. *British Journal of Nutrition* **50**, 151 - 156.

Thompson GE, Jenkinson DM (1968) Nonshivering thermogenesis in the newborn lamb. *Canadian Journal of Physiology and Pharmacology* **47**, 249 - 253.

Thompson MJ, Briegel JR, Thompson AN, Adams NR (2006) Differences in survival and neonatal metabolism in lambs from flocks selected for or against staple strength. *Australian Journal of Agricultural Research* **57**, 1221 - 1228.

Thomson AM, Thomson W (1949) Lambing in relation to diet in the pregnant ewe. *British Journal of Nutrition* **2**, 290 - 305.

Thomson BC, Muir PD, Smith NB (2004) Litter size, lamb survival, birth and twelve week weight in lambs born to cross-bred ewes. *Proceedings of the New Zealand Grassland Association* **66**, 233 - 237.

Trayhurn P (1993) Brown adipose tissue: from thermal physiology to bioenergetics. *Journal of Bioscience* **18**, 161 - 173.

Trayhurn P, Thomas MEA, Duncan JS, Nicol F, Arthur JR (1993) Presence of the brown fat-specific mitochondrial uncoupling protein and iodothyronine 5'-deiodinase activity in subcutaneous adipose tissue of neonatal lambs. *Federation of European Biochemical Societies Letters* **322**, 76 - 78.

Tygesen MP, A.H. T, Blache D, Husted SM, Nielsin MO (2008) Late foetal life nutrient restricition and sire genotype affect postnatal performance of lambs. *Animal* **2**, 574 - 581.

van der Werf JHJ, Kinghorn BP, Banks RG (2010) Design and role of an information nucleus in sheep breeding programs. *Animal Production Science* **50**, 998 - 1003.

Varith J, Hyde GM, Baritelle AL, Fellman JK, Sattabongkot T (2003) Non-contact bruise detection in apples by thermal imaging. *Innovative Food Science & Emerging Technologies* **4**, 211 - 218.

Vince MA, Billing AE (1986) Infancy in the sheep: The part played by sensory stimulation in bonding between the ewe and lamb. In 'Advances in Infancy Research'. (Eds LP Lipsitt, C Rovee-Collier) pp. 1 - 39. (ABLEX Publishing Corporation: New Jersey).

Vince MA, Lynch JJ, Mottershead B, Green G, Elwin R (1985) Sensory factors involved in immediately postnatal ewe/lamb bonding. *Behaviour* **94**, 60 - 84.

Vincent IC, Williams HL, Hill R (1985) The influence of a low nutrient intake after mating on gestation and perinatal survival of lambs. *British Veterinary Journal* **141**, 611 - 617.

Volpe JJ (2008) 'Neurology of the Newborn.' (Saunders Elsevier: Philadelphia).

Wassmuth R, Loer A, Langholz HJ (2001) Vigour of lambs newly born to outdoor wintering ewes. *Animal Science* **72**, 169 - 178.

Watson RH, Alexander G, Cumming IA, McDonald JW, McLaughlin JW, Rizzoli DJ, Williams D (1968) Reduction of perinatal loss of lambs in winter in Western Victoria by lambing in sheltered individual pens. *Proceedings of the Australian Society of Animal Production* **7**, 243 - 249.

Wiener G, Deeble FK, Broadbent JS, Talbot M (1973) Breed variation in lambing performance and lamb mortality in commercial sheep flocks. *Animal Production* **17**, 229 - 243.

Winfield CG, Kilgour R (1976) A study of following behaviour in young lambs. *Applied Animal Ethology* **2**, 235 - 243.

Woolliams C, Wiener G, Macleod NSM (1983) The effects of breed, breeding system and other factors on lamb mortality 3. Factors influencing the incidence of weakly lambs as a cause of death. *Journal of Agricultural Science* **100**, 563 - 570.

Wrutniak C, Cabello G (1987a) Effects of food restriction on cortisol, TSH and iodothyronine concentrations in the plasma of the newborn lamb. *Reproduction Nutrition Development* **27**, 721 - 732.

Wrutniak C, Cabello G (1987b) Neonatal changes in plasma cortisol, free and total iodothyronine levels in control and hypotrophic lambs. *Reproduction Nutrition Development* **27**, 945 - 953.

Wrutniak C, Cabello G (1989) Influence of tri-iodothyronine or lipid administration on the response of the pituitary-thyroid axis to exposure to cold in the newborn lamb. *Journal of Endocrinology* **121**, 361 - 365.