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**Effects of ewe age
on offspring development and
performance**

A thesis presented in partial fulfilment of the requirements for the degree of
Doctor of Philosophy in Animal Science
at Massey University, Turitea, Palmerston North, New Zealand.

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Abstract

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In New Zealand only approximately 30% of ewe-lambs are bred each year despite the advantages this practice can offer. Farmers have indicated a reason for not breeding ewe-lambs is that the offspring born to ewe-lambs are typically smaller and lighter to at least weaning. However, there is a lack of information on the post-weaning performance of ewe-lamb progeny in New Zealand. The objective of this thesis was to examine the effects of maternal age (ewe-lamb dams vs. adult ewe dams) on the performance of singleton and twin progeny and also the growth of their progeny to weaning. Progeny born to these two dam age classes were monitored to approximately 3.5 years of age. The results of this thesis have demonstrated that the growth and therefore live weight of offspring born to ewe-lamb dams was in general lower compared with those born to adult ewe dams, especially in twins. However, results also show that there is little impact of maternal age on offspring reproductive and lactational performance. Interestingly, there was a reversal of the influence on grand-offspring birthweight, whereby lambs with ewe-lamb granddams were actually heavier at birth. The lighter live weight of ewe-lamb progeny without negative effects on performance may even suggest these animals are more efficient; however, longer-term studies would be required to confirm this. In conclusion, these results indicate farmers can utilise progeny born to ewe-lamb dams without a negative impact on production, and in fact there may be a positive effect on production efficiency.

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Chapter 1

Introduction

Approximately 80% of New Zealand's agricultural products are exported, including products such as lamb meat, mutton, wool and skins (Cottle, 2010). Since 1989, the number of sheep in New Zealand has decreased from approximately 60 to 31 million (Beef and Lamb New Zealand, 2013). During this period there has been a change in focus from wool to lamb production (Boutonnet, 1999). The increased emphasis on meat production has resulted in an increased lambing percentage from 102% in 1989 to 127% in 2012 (Beef and Lamb New Zealand, 2013). A means to further increase the total number of lambs weaned in the national flock is to breed ewe-lambs (hoggets, aged 7 to 9 months); however, in 2012, only 4% of lambs marked and/or tailed in New Zealand were born to ewe-lambs (ANON, 2013; Beef and Lamb New Zealand, 2013).

Breeding ewe-lambs in their first year of life can increase the number of lambs they wean over their life time (McCall and Hight, 1981; Moore *et al.*, 1983), improve two year old productive performance (McMillan and McDonald, 1983), and be an early recognition of potential fertility (McCall and Hight, 1981; Kenyon *et al.*, 2014). However, the majority of the farmers choose to not breed ewes in their first year of life. One potential reason for the low percentage of ewe-lambs bred in New Zealand is likely due to the fact that little is known about the impact of breeding ewe-lambs on the performance of their progeny. Farmers have indicated that one reason for not breeding ewe-lambs is the resultant small/lighter offspring (Kenyon, 2004). To date, most research has focused on increasing the reproductive performance of ewe-lambs (Dýrmundsson, 1973; Moore *et al.*, 1983) rather than the potential long-term impacts on the offspring born to ewe-lambs.

Therefore the objective of this thesis was to examine the effects of maternal age (ewe-lamb dams vs. adult ewe dams) on the performance of offspring under pastoral-based New Zealand conditions. Specific areas examined include:

- Prenatal development of the singleton fetus (Chapter 3).
- Postnatal growth and body composition of singleton male offspring from birth to 12 months of age (Chapter 4).
- Body composition of singleton female offspring in different physiological stages from 19 to 25 months of age (Chapter 5).
- Postnatal growth, reproductive and lactation performance of singleton female offspring from birth to 40 months of age (Chapter 6).
- Postnatal growth and puberty attainment of singleton and twin-born female offspring from birth to 18 months of age (Chapter 7).
- Growth and reproductive performance of singleton and twin-born female offspring from 18 to 38 months of age (Chapter 8).
- Examination of the development and growth of skeletal muscle of singleton fetuses and singleton and twin-born adult offspring (Chapter 9).

Two cohorts were utilised in this PhD study, the first cohort included singleton offspring born in 2007, while the second cohort included both singleton and twin offspring born in 2009 (**Figure 1**).

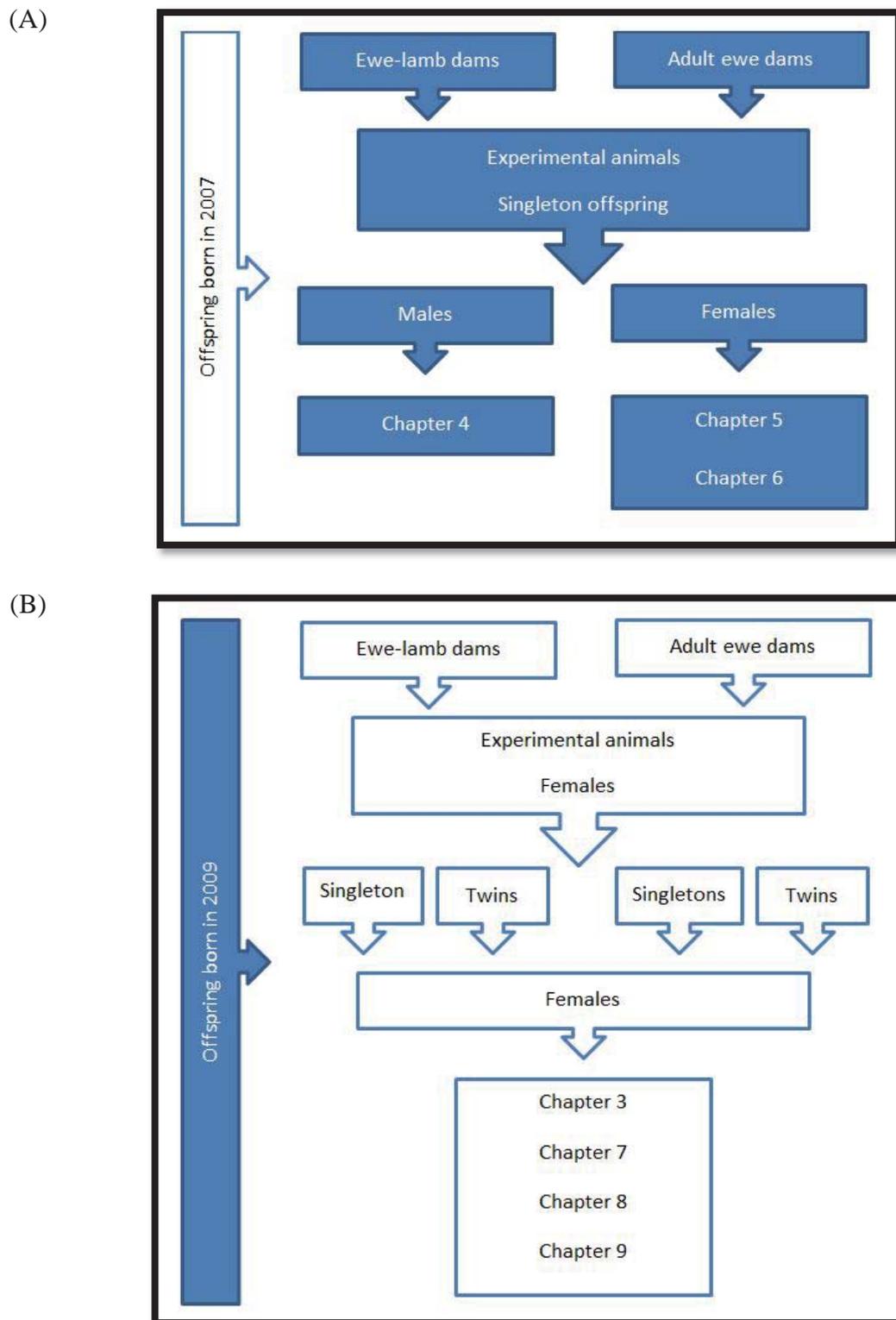


Figure 1. Study designs and generated chapters; (A) illustrates the study with offspring born in 2007, (B) illustrates the study with offspring born in 2009

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Chapter 2

Literature Review

THE SHEEP INDUSTRY – A NEW ZEALAND PERSPECTIVE

New Zealand is a well-established sheep farming country, with sheep numbers that peaked in 1982, at 70.3 million. However, these numbers have reduced dramatically to 31.2 million in 2014 (Beef and Lamb New Zealand, 2014). Currently, sheep and beef farms represent 45% (29,241 of 63,336 farms) of the total farms in New Zealand (ANON, 2013), with the majority of sheep flocks being Romney based (Beef and Lamb New Zealand, 2014). Sixty five percent of the total number of sheep in New Zealand are mature breeding ewes. In 2012, approximately 25,890,000 lambs were born in New Zealand, with 5.6% of this total being born to ewe-lamb dams (7-9 months of age at breeding; Beef and Lamb New Zealand, 2014).

Breeding ewe-lambs has been uncommon in New Zealand and Australia (Tyrrell, 1976; McCall and Hight, 1981; McMillan and McDonald, 1983), with 25-30% of ewe-lambs being bred in New Zealand. It has been reported that figures in Australia are similar to New Zealand, with less than 20% of non-Merino ewe-lambs bred per annum (Kenyon *et al.*, 2014). However, the practice of breeding ewe-lambs has increased in New Zealand recently. Approximately 2,015,897 ewe-lambs (equivalent to 30.2%) were bred in 2011, with this number increasing to 2,380,597 (equivalent to 34%) in 2012 (ANON, 2013; Beef and Lamb New Zealand, 2013). This increase is predominantly driven by higher prices for lamb meat and the need to increase farm revenue (ANON, 2013; Beef and Lamb New Zealand, 2013). Despite the clear increase in numbers bred, there is still room for additional ewe-lambs to be bred, perhaps as high as 50%.

One means to increase total lamb production in New Zealand without increasing total mature ewe numbers is to increase the number of ewe-lambs bred successfully. Breeding ewe-lambs can also reduce the intergenerational interval (Baker *et al.*, 1978),

however, many farmers are reluctant to breed ewe-lambs. One potential reason for this is sparse information on the potential impacts on the young ewe and her offspring. This review will examine aspects of breeding mature ewes and ewe-lambs under New Zealand's and international conditions. Further, the limited information regarding the impacts of breeding ewe-lambs on their lifetime performance and the known positive and negative implications for their progeny will be discussed. Finally, the perspective and hypotheses of this thesis will be outlined.

SHEEP BREEDING PRACTICE

Sheep are generally seasonal animals, displaying oestrus which is driven by hours of daylight (Chemineau *et al.*, 1992; Malpaux *et al.*, 1997), and they are termed “short-day breeders” (Hafez and Hafez, 2000). The breeding season starts generally late in the summer and ends by winter (Chemineau *et al.*, 1992), however this can vary between breeds (Robinson and Karsch, 1984). Commonly lambing occurs during spring (Malpaux *et al.*, 1997; Hafez and Hafez, 2000), to match feed availability and weather conditions. The seasonal nature of their breeding cycle is a limiting factor for the breeding of ewe-lambs at 7-9 months of age, before seasonal anoestrous begins.

Typically in New Zealand and other countries, ewes are first exposed to breeding at 18-20 months of age (Spencer *et al.*, 1942; McMillan and McDonald, 1983; Kenyon, 2004). In New Zealand, as indicated earlier, less than 35% ewes under 12 months are bred each year (ANON, 2013).

Breeding ewe-lambs

Breeding ewe-lambs can increase ewe productivity (Dickerson and Lusted, 1975; McCall and Hight, 1981, Kenyon *et al.*, 2014), by increasing the number and weight of

lambs produced per ewe lifetime (Baker *et al.*, 1978; Dýrmundsson, 1981; Kenyon *et al.*, 2011). Further, it can directly influence farm profitability (Young *et al.*, 2010). Another advantage of breeding ewe-lambs is the early recognition of fertility potential (Keane, 1974). Also, McCall and Hight (1981) reported that breeding ewe-lambs could increase the total number of lambs born, thus increasing the number of ram-lambs available for selection or progeny testing. Breeding ewe-lambs can increase rates of genetic gain by decreasing the generational interval and increase flock efficiency if replacements born to ewe-lambs are kept for replacements (Dýrmundsson, 1981; Kenyon *et al.*, 2004; Gootwine *et al.*, 2007). Additionally, Tyrrell (1976) stated that breeding ewe-lambs at 7-9 months of age dilutes the costs of maintaining a ewe before her first service compared with breeding for the first time at 18 months of age.

Despite the many advantages of breeding ewe-lambs, there are some potential limitations. These include: increasing the total number of ewes bred per season, which can increase work load and feed required (McCall and Hight, 1981; Kenyon, 2004). Additionally, there is a strong belief by farmers that breeding ewes at 7 to 9 months of age can jeopardise their future production (McMillan and McDonald, 1983), including a negative effect on their future reproductive performance (Kenyon, 2004).

Physiological considerations when breeding ewe-lambs

Puberty attainment and live weight

Ewes that attain puberty during their first year of life have the potential to be bred at 7 to 9 months of age. Bichard *et al.* (1974), utilising mating record data over 10 years, showed that 11.6% of all ewe-lambs failed to attain puberty in their first breeding season. A reason that some will not achieve puberty in the first year of life is because of the time of the year that they were born (McCall and Hight, 1981), e.g. if their dam was

bred in early winter, the offspring would be born in early summer, and may not have enough time to achieve puberty before the end of their first potential breeding season. The consequence of not achieving puberty in the first year of life is a delay in potential breeding until the next breeding season, in the following year (Foster *et al.*, 1985). Puberty is related to live weight (Tierney, 1969; Southam *et al.*, 1971; Dickerson and Lasted, 1975), the percentage of body fat (Stephenson *et al.*, 1980) and breed (Tierney, 1969; Southam *et al.*, 1971; Dickerson and Lasted, 1975). Southam *et al.* (1971) utilising five different breeds reported that 96% of ewe-lambs reached puberty at an average live weight of 45.5 kg at an average age of 212 days. In a study by Cedillo *et al.* (1977), utilising four breeds, they reported 90% of ewe-lambs exposed to teaser rams exhibited their first oestrus at an average of 205 days, with the different breeds differing by only a few days to first oestrus. Singleton lambs reach puberty at a younger age than twins and triplets, because they tend to be heavier (McMillan and McDonald, 1983; Alcaraz Romero *et al.*, 2012). It is often suggested that a minimum weight to breed a ewe-lamb is 35 kg (Craig, 1982) or 70% of the maternal weight (Hafez and Hafez, 2000). Craig (1982) reported a positive correlation between live weight and the number of ewe-lambs lambing. Craig (1982) concluded that age combined with live weight has a significant influence on ewe-lamb lambing performance.

Timing of breeding

Reproductive competency tends to be greater in the first breeding season in young ewes that have achieved puberty in their first year (Kenyon *et al.*, 2014). Hare and Bryant (1985) reported an increasing percentage of viable embryos from the first oestrus to second oestrus and also from the first to the third oestrus event. They reported that conception/fertility rates increased by approximately 20% when ewe-lambs were bred at their second oestrus compared with their first oestrus.

Ewe-lambs are generally bred later in the season than mixed-age ewes, due to the requirement for them to reach puberty. Mature ewes in New Zealand are generally bred in March or early April while ewe-lambs are generally bred in late April/early May.

Comparison of ewe-lamb and mature ewe performance

Reproductive performance

In general, ewe-lamb reproductive performance is inferior to that of the mature ewe performance (McMillan and McDonald, 1983; Mulvaney *et al.*, 2013). Ewe-lambs display lower fertility rates compared with mature ewes (Beck *et al.*, 1996; Kenyon, 2012; Mulvaney *et al.*, 2013). Bichard *et al.* (1974) analysing data of approximately 2000 Clun Forest ewe-lambs (6 to 9 months of age) demonstrated that 42% of the young females bred did not produce lambs. While Spencer *et al.* (1942) found a much lower percentage, 29.4% of 119 ewe-lambs, exposed to breeding, failed to conceive, however the age range was 9-10 months. Ewe-lambs that fail to achieve puberty can account for 25 to 30% of empty ewe-lambs at the end of the breeding season (Bichard *et al.*, 1974). McMillan and McDonald (1983) reported that only 58% of ewe-lambs joined weaned lambs. This lower reproductive performance can be explained by the later commencement and earlier cessation of oestrus behaviour during the breeding season and the higher proportion of ewe-lambs that display silent ovulations or that have just one ovulation event during the entire breeding period (Bichard *et al.*, 1974). Hulet *et al.* (1969a) also reported that 62% of ewe-lambs exhibited signs of oestrus just once, with 25% twice, and 8% three times during the breeding season. Ewe-lambs also have a shorter length of standing heat and are relatively inexperienced, resulting in them being less likely to seek out the ram compared with the mature ewe (Dýrmundsson, 1973). Ovulation rates in ewe-lambs are lower compared with mature ewes (Quirke and Hanrahan, 1977; Davies and Beck, 1993; Beck *et al.*, 1996; Mulvaney, 2011 - **Table 1** -

Edey *et al.*, 1977; Mulvaney *et al.*, 2013), while fertilisation losses of ova is higher (McMillan and McDonald, 1983) partially explained by ewe-lamb ova being of inferior quality (Quirke, 1981). Consequently, ewe-lambs display lower pregnancy rates/conception rates compared with mature ewes (Donald *et al.*, 1968; Forrest and Bichard, 1974; Annett and Carson, 2006; Mulvaney, 2011 - **Table 1**). Late embryo death was reported to be a problem in ewe-lambs by Bichard *et al.* (1974). They reported that approximately 50% of ewe-lambs marked by the ram did not lamb. Mulvaney *et al.* (2013) using ultrasound examination reported that pregnancy losses were higher in early gestation (average 69 days) for ewe-lambs compared with mature ewes, and that late gestation (average 110 and 143 days) loss rates did not differ between the two age groups.

Table 1. Comparison of ovulation and pregnancy/conception rates of ewe-lambs and mature ewes.

Comparison between ewe age groups	Ovulation rate	Pregnancy rate/ Conception rate (%)	Reference
Ewe-lamb, multiparous ewes of 3 to 5 yrs of age	1.51 vs. 3.07		Quirke and Hanrahan, 1977
Ewe-lamb, mature multiparous ewes	1.1 vs. 1.5		Davies and Beck, 1993
Ewe-lamb, multiparous ewe of 2 to 5 yrs of age	1.07 vs. 1.25		Beck <i>et al.</i> , 1996
Ewe-lamb, multiparous ewes of 2 to 5 yrs of age	1.14 vs. 1.82	47 vs. 97	Mulvaney, 2011
Ewe-lamb, multiparous		67 vs. 85	Annett and Carson, 2006
Ewe-lamb, 2 and 3 yrs of age ewes		77 vs. 95 vs. 96	Donald <i>et al.</i> , 1968
Ewe-lamb, 2 and 3 yrs of age ewes		56 vs. 93 vs. 95	Forrest and Bichard, 1974

yrs: years; vs.: versus (Adapted from Kenyon *et al.*, 2014)

Performance from lambing to weaning

Offspring born to ewe-lambs are lighter than those born to mature dams from birth to weaning (Donald *et al.*, 1968; Dýrmundsson, 1973; Mulvaney, 2011). Annett and Carson (2006) utilising embryo transfer, also reported lambs born to ewe-lambs were lighter at birth independent of the litter size. Ewe-lambs are also more prone to have dystocia due to a feto-pelvic mismatch as the female is not fully grown (Jackson, 2004). Ewe-lambs display lower number of lambs born per ewe compare to mature ewes (Forrest and Bichard, 1974; Munoz *et al.*, 2009; Mulvaney, 2011). Baker *et al.* (1978) and McCall and Hight (1981) reported that the number of lambs born and alive at weaning were both lower for ewe-lambs compared with adult ewes. The lower weaning rates are at least partly explained by lower survival rates (Donald *et al.*, 1968; Munoz *et al.*, 2009; Morel *et al.*, 2010; Mulvaney, 2011 - **Table 2**) in lambs born to ewe-lambs

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compared with those born to older ewes (McCall and Hight, 1981; Craig, 1982; Atta and El Khidir, 2005; Morel *et al.*, 2010). Corner *et al.* (2013) also reported lower survival rates to weaning for singleton and twin offspring born to ewe-lambs than those born to mature ewes and related it to poor maternal experience behaviour exhibited by the ewe-lamb. Mulvaney (2011) also reported that ewe-lambs display lower maternal ability than mature ewes.

Table 2. Comparison of number of lambs born per ewe, lamb survival rates and lambs weaned per ewe bred between ewe-lamb and mature ewe.

Comparison between ewe age groups	Number of lambs born per ewe	Lamb survival rates (%)	Lambs weaned per ewe bred	Reference
Ewe-lamb, 2 and 3 yrs of age ewes	1.15 vs.1.55 vs.1.73			Forrest and Bichard, 1974
Ewe-lamb, mixed aged ewes		81 vs. 90		Morel <i>et al.</i> , 2010
Ewe-lamb, mature multiparous ewes			0.59 vs.1.50	Annett and Carson, 2006
Ewe-lamb, 2 and 3 yrs of age ewes		78 vs. 88 vs. 98	0.69 vs. 1.28 vs.1.60	Donald <i>et al.</i> , 1968
Ewe-lamb, 2 yrs of age ewes	1.30-1.54 vs. 1.86-2.20	57-78 vs. 68-84	0.25-0.36 vs. 1.18-1.44	Munoz <i>et al.</i> , 2009
Ewe-lamb, multiparous ewes of 2 to 5 yrs of age	0.66 vs. 1.59	79 vs. 89	0.52 vs. 1.42	Mulvaney, 2011
Singleton ewe-lamb, singleton or twin multiparous ewes of 3 to 5 yrs of age (study 1/study 2)		69 vs. 85 vs. 83 / 89 vs. 96 vs. 88		Corner <i>et al.</i> (2013)

yrs: years; vs.: versus (Adapted from Kenyon *et al.*, 2014)

Lactation can be a challenging time for the growing ewe-lamb which can cause retardation in her growth and development (Dýrmundsson, 1973). Baker *et al.* (1978)

reported the average weaning weight for lambs born to ewe-lambs and mature ewes were comparable. Although others have reported the weaning weight of progeny born to ewe-lambs can be lower compared with progeny born to mature ewes (Craig, 1982; Corner *et al.*, 2013).

Long-term effects of breeding ewe-lambs

Lifetime effects on the ewe-lamb of breeding at a young age

Over the last 10 years there has been considerable research examining optimal management of ewe-lambs to maximise their potential. However, there is vastly less information available on the long-term impacts of breeding ewe-lambs. This can be divided into impacts on her and her progeny.

Hulet *et al.* (1969b) stated that the ewe-lambs that achieved puberty in their first year, and were not bred, displayed a higher subsequent reproduction potential compared with females that did not reach puberty during the same period. Ewe-lambs that lamb in their first year of life have been reported to have higher weaning rates and heavier lambs to weaning in later years (Baker *et al.*, 1981). Kenyon *et al.* (2008) reported a minor negative impact on reproductive performance of two-tooth ewes that had lambed as ewe-lambs. Further, Craig (1982) and Kenyon *et al.* (2011) found that two-tooths which lambed as ewe-lambs compared with those that did not lamb, produced more lambs, which had higher survival rates to weaning. While, Cedillo *et al.* (1977) reported that at two years of age, ewes that had lambed as ewe-lambs compared with ewes that did not, gave birth to the same number of lambs per ewe exposed to ram. However, adult ewes that had been bred as a ewe-lamb produced more lambs and weight of lamb per 100 ewes exposed to ram, at weaning.

Lifetime productivity of those ewes that lamb in their first year of life is greater than those that do not (Spencer *et al.*, 1942; Moore *et al.* 1983; Kenyon *et al.*, 2011). Even when the lambs produced at one year of age are not considered, ewes that had been bred as ewe-lambs are more productive throughout their lifetime than those that do not have lambs in the first year (Cedillo *et al.*, 1977). Lambing at one year of age has the potential to negatively affect future live weight (Spencer *et al.*, 1942; Southam *et al.*, 1971; Levine *et al.*, 1978; and McMilland and McDonald, 1983), however this negative effect in live weight is not persistent, and it will be equalised after the second lambing, matching the live weight of ewes that did not lamb at one year of age (Dýrmundsson, 1973; Kenyon *et al.*, 2011). The longevity of ewe-lambs bred in the first year of their life seems to not be affected (Dýrmundsson, 1973; Baker *et al.*, 1981; Kenyon *et al.*, 2011).

Long-term impacts of being born to a ewe-lamb

Human studies suggest negative impacts for the offspring born to very young mothers. They are more likely to be small in size in early life but fatter as they age (Scholl *et al.*, 1994; Lucas *et al.*, 1999). In humans, low birthweight is typically associated with predisposition to obesity, altered metabolic pathways leading to increased risk of coronary heart disease, immune dysfunction and diabetes type 2 later in life (Friede *et al.*, 1987; Geronimus *et al.* 1994; Nowak *et al.*, 2000; Barker, 1998, 2001). If it is considered that the ewe-lamb is equivalent to an adolescent human mother, there is potential for similar long-term effects in the offspring born to ewe-lambs. However as indicated earlier there are few studies which have specifically examined the long-term effects of being born to a ewe-lamb. Craig (1982) stated that progeny born to ewe-lambs compared with mixed-age ewes were not only lighter at weaning they were also lighter at one year of age. They also displayed reduced reproductive performance when bred

themselves as ewe-lambs. This disadvantage suggests that progeny born to ewe-lambs are less suitable to be bred as ewe-lambs than those born to mature ewes (Craig, 1982).

Summary

The potential productive advantages from successfully breeding the ewe-lamb at 7 to 9 months of age have been outlined in this review. Methods to ensure successful breeding are also now well established (Dýrmundsson 1973; Kenyon *et al.*, 2014). Data is also available on the potential long-term lifetime effects on the young ewe from breeding at a early age. It is known that progeny born to ewe-lambs, which are somewhat equivalent to an adolescent human, are lighter to at least weaning. What is less well established is the long-term effects, from both a meat production and reproduction perspective, when selecting progeny born to ewe-lambs as replacements. This will be the focus of this thesis.

Techniques and measurements

The techniques used in this thesis are briefly described in this section. However, it is not meant to be a detailed review of each technique or protocol.

Methods to investigate body composition

Imaging techniques have been utilised to assess body composition in live animals and in the carcass (Lambe *et al.*, 2013). Techniques such as dual-energy X-ray absorptiometry (DXA) and computed tomography (CT scan) have been used to analyse body composition changes in live animals, as these methods can be used to follow the changes across time, as they are non-invasive and non-destructive (Jayo *et al.*, 1991; Mitchell *et al.*, 1996; Bünger *et al.*, 2011).

Dual-energy X-ray absorptiometry (DXA) is largely used as a preferable method for the assessment of bone mass (Kohrt, 1995) and can be extended to analysis of body composition, assessing bone mineral, visceral fat and lean mass (Jensen *et al.*, 1995). However, the differentiation of visceral fat and subcutaneous abdominal fat using DXA is not possible. In contrast, CT scanning can be used to measure with accuracy and distinguish the two locations of fat deposition (Jensen *et al.*, 1995). Both methods utilise radiation to determine which tissue has been analysed. The amount of radiation absorbed or attenuated through the body part that has been scanned define their relative physical densities and consequently the tissues in question (Binkovitz and Henwood, 2007; Bunger *et al.*, 2011).

Computed tomography scanning involves the measurement of the radiodensity of matter. The output is scaled in Hounsfield units (HU) where bone is approximately 1000 HU, water is 0 HU, air is -1024 HU and fat (less dense than water) has an HU range of -10 to -200 (Thompson and Kinghorn, 1992). Applying image analysis

procedures to the greyscale CT scanner output, the areas of equivalent densities can be identified. Stereology, which is the three-dimensional interpretation of cross section images captured in two-dimensions, then permits the quantification of the volume and surface area of the identified tissues and fat depots to determine the body composition (Jopson *et al.*, 1995; Jopson *et al.*, 1997; Campbell *et al.*, 2003), permitting further analysis and comparison.

qPCR Overview

Real time quantitative polymerase chain reaction (qPCR) is a process based on an adaptation of standard PCR, which is used to amplify a targeted DNA molecule. Real time quantitative polymerase chain reaction allows the detection and quantification of target sequences using fluorescent chemistries that provide a correlation between the fluorescence intensity and the abundance of the amplified product (Wang *et al.*, 2006). The fluorescent SYBR[®] Green dye is a DNA binding dye, which binds to the minor groove of any double-stranded DNA and produces a strong fluorescent signal. During the initial phase the fluorescence emission product is below background detection (Bustin, 2002). When the accumulation of PCR product achieves critical threshold, the fluorescence emission rises above background and the PCR product increases exponentially, doubling each cycle. It is the log-linear phase and the information obtained that is used to quantify and calculate the final result. The point at which the fluorescence level is higher than the baseline is the quantification cycle (C_q), and it is used to calculate the relative or absolute levels of transcript. The final phase is the plateau, when the upper limit is reached, and product amplification is no longer proportional to the starting concentration and values cannot be used in the final calculations. Amplification efficiency depends on the efficiency of the primers. The primer should be designed to reach an optimum efficiency value in the range of 1.9 to

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2.1. This efficiency is calculated by measuring the slope of the standard curve produced using a dilution series and plotting the Cq against of the log the starting quantity (Morrison *et al.*, 1998; Bustin, 2002).

Thesis outline

As outlined in the literature review there is sparse information on the long-term effects of selecting progeny born to ewe-lambs. Previous studies have instead been confined to the ewe-lamb development and if the progeny have been examined the studies have ended at weaning. Therefore the objective of this thesis is to examine the effects of being born to either a ewe-lamb or mature ewe on the offsprings growth, body composition, and reproductive and lactational performance. In addition it will follow the growth of the second generation (grand-offspring) to weaning under commercial New Zealand farming conditions.

It is hypothesised that being born to a ewe-lamb compared with a mature adult ewe will negatively affect:

- growth and body composition
- adult live weight and body composition
- female reproductive and lactational performance
- grand-offspring live weight to weaning

A brief overview of the experimental chapters

The experimental animals used in this thesis are from two cohorts born to either ewe-lambs or mature ewes. The first cohort is singletons male followed until 12 months of age and singletons female followed until approximately 3 years of age. The second cohort contained both singleton and twin female offspring and were followed for 3.5 years (**Figure 1**).

Chapter 3: This chapter compares singleton fetuses from ewe-lambs and adult ewes at day 145 of gestation (pre-term). Fetal weights, linear measurements and

organs were measured and some organs were collected for further analysis as described in Chapter 9.

Chapter 4: Singleton male offspring born to either ewe-lambs or adult ewes were followed until 12 months of age and had their growth, body and organs measured, and carcass composition analysed by DXA (offspring born in 2007).

Chapter 5 and Chapter 6: These two chapters analysed the performance of singleton ewes born to either ewe-lambs or mature adult ewes until 3 years of age, comparing their growth trajectory, body composition via CT scanning and reproductive and lactational performance. Furthermore, their offspring's (grand-offspring) development was followed until weaning (offspring born in 2007).

Chapter 7 and Chapter 8: These chapters are similar to Chapter 5 and 6. However, they describe the growth trajectory, morphometric characteristics and reproductive performance of singleton and twin ewes born to either ewe-lambs or adult ewes until approximately 3 years of age and their offspring's development until weaning (offspring born in 2009).

Chapter 9: The microanatomy and gene expression of skeletal muscle tissue were explored in this chapter. Fetal (d145) and adult (2.5 years of age) muscle tissue were analysed and compared in offspring from either ewe-lambs or adult ewes (fetuses collected and offspring born in 2009).

Chapter 10: This chapter includes a discussion of all the results collected in this thesis about maternal age and the long-term implications for the offspring and next generation. Highlighting the important outcomes found in the studies, the implications of these findings and potential limitations of the studies. Future considerations are discussed and finally conclusions are drawn.

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Chapter 3

Do fetuses from primiparous one-year-old ewes differ from those of multiparous mature ewes?

Foreword:

This chapter was published as a brief communication in Proceedings of the New Zealand Society of Animal Production (“Loureiro, M. F. P., Pain, S. J., Kenyon, P. R., Blair, H. T. (2010). Do fetuses from primiparous one-year-old differ from those of multiparous mature ewe? *Proceedings of the New Zealand Society of Animal Production*, 70, 118-120.”) (**Appendix 2**).

For this chapter several measurement and tissues were collected, however just some of the data were selected to be presented in the brief communication above mentioned.

The organs weights for fetuses from both ewe-lambs and adult ewes and male and females fetuses were presented as “unpublished data” in the publication, however it was presented in full detail in “**Appendix 1**”. Some additional parameters were calculated based on morphometric measurements:

Fetal body volume (volume = $3.14 \times [(girth/3.14)/2]^2 \times \text{crown-rump length}$) and the ratios between fetal body volume (FBV) and fore-leg length, hind-leg length and femur length.

Live counterparts of these fetuses will be studied in later chapters to investigate if differences were present at birth and later in life.

ABSTRACT

There is evidence in the literature to suggest that offspring born to young primiparous mothers are smaller and lighter and that this may have health consequences later in life. The aim of this study was to investigate the effect of ewe age and parity on the morphophysiology of singleton fetuses and their placenta at day 145 (d145) of gestation. Nine Romney primiparous ewe-lambs (8 - 9 months of age, 47.0 ± 0.7 kg) and 11 Romney multiparous adult aged ewes (3 to 5 years of age, 64.4 ± 1.5 kg) were managed together under commercial New Zealand grazing conditions from day 1 to d145 of gestation. At d145, the ewes were euthanised. Ewe and fetal organs were weighed and structural measurements were taken. Gravid uteri were collected from each ewe and weighed and placentomes counted. Gravid uterus weight did not differ between the two ewe groups ($P > 0.05$). However, total placentome number was greater ($P < 0.05$) for ewe-lambs compared with adult ewes but their empty caruncle number and resulting occupancy rate did not differ ($P > 0.05$). Fetuses (d145) from ewe-lambs tended ($P = 0.08$) to be lighter than those from mature ewes. Some of the morphophysiological measurements taken (hind-leg lengths, head lengths and brain weights) were smaller ($P < 0.05$) in fetuses from ewe-lambs, when analysed without fetal body weight as a covariate. However, when analysed with fetal body weight as a covariate, these differences were no longer apparent ($P > 0.05$). This indicates that singleton fetuses from primiparous ewe-lamb dams and those from multiparous adult dams are of similar size at d145 of gestation in ewes that were not submitted to nutritional treatments during pregnancy. Live counterparts of these fetuses will be monitored to investigate if differences are present at birth and at later stages of life.

INTRODUCTION

The number of ewe-lambs (8 to 9 months of age) mated in New Zealand has increased to approximately 33% over the last 15 years (Anon, 2009). There are many potential advantages to ewe-lamb breeding including higher net profits, more lambs produced per ewe productive lifetime, better use of spring herbage, increased efficiency, early recognition of fertility potential and increased rates of genetic gain (Hight, 1982). However, little is known about the long-term impacts of selecting progeny born to these young ewes as replacement animals. There is some evidence from human studies to suggest that there may be negative impacts for both the offspring and to the first parity young mother (Lucas *et al.*, 1999). The offspring tend to be lighter (Scholl *et al.*, 1994), have lower rates of survival as well as altered metabolic pathways and a predisposition to obesity and increased risk of coronary heart disease later in life (Barker, 2001). Kenyon *et al.* (2009) using sheep, suggested that offspring born to young primiparous dams are programmed to deposit more abdominal fat, which could have longer-term implications for efficiency of growth and health. Additionally sheep studies have shown that lambs born to adolescent dams had lower birthweights, and smaller head length, crown-rump length and thoracic girth (Annett & Carson, 2006). However, these studies are often confounded by imposed nutritional regimes. Studies with adolescent ewes that were over nourished during early pregnancy show significant placental and fetal growth restriction as assessed during late gestation (Wallace *et al.*, 1996), due to the rapid maternal growth of the adolescent mother driving nutrient partitioning to maternal tissues. Although a similar study by Wallace *et al.* (2005) using mature ewes showed no effect. This indicates that the degree of maturity of the ewe at mating and the

subsequent growth rate of the ewe during pregnancy may be a key factors influencing growth of the fetoplacental unit.

The size and nutrient transfer capacity of the placenta plays an important role in determining the growth trajectory of the fetus (Mellor, 1983; Bell *et al.*, 1999). An indicator of intrauterine growth restriction is an increased brain:liver ratio (Regnault *et al.*, 1999). The growth of the brain is preserved relative to the rest of the body, especially to that of the abdominal organs (Wallace *et al.*, 2005). Therefore it is possible that the reduced fetal size in offspring born to young dams is driven by restricted placental development. There appears to be no sheep fetal studies which compare differences in the morphophysiology of near term fetuses from either primiparous 1-year-old ewe-lambs or mature multiparous ewes managed as one group during pregnancy which had been bred to the same sires. This study was undertaken to examine this point.

MATERIALS AND METHODS

The present study used nine singleton bearing primiparous Romney ewe-lambs (8 - 9 month-old, 47.0 ± 0.7 kg (\pm standard error)) and 11 singleton bearing Romney multiparous mature aged ewes (3 to 5 years old, 64.4 ± 1.5 kg) that had conceived within a 6 day period, d1 was defined as the first day of breeding. Ewes were naturally bred as one cohort with mature, crayon-harnessed, mixed-age, Romney composite rams. Daily mating performance was not recorded. The 20 ewes were managed under commercial New Zealand grazing conditions with a minimum post grazing cover of 1,000 kg DM/ha until maximum day 145 (d145) of gestation. Ewe live weight was measured prior to the mating period, and at d87 and d145. At d145 (range 139-145) ewes were euthanized, the gravid uterus removed, weighed and the fetus removed.

Concomitantly the ewe liver, mammary gland and omental fat were removed and weighed. After removal of the fetus, the remaining uterine membranes were weighed and examined. Total placentomes and empty caruncles were counted to determine caruncle occupancy rate (%). Dressed carcass weight of the ewe and soft tissue depth at the 12th rib (GR) was also recorded. After being removed from the uterus, fetuses were euthanized and immediately identified for sex and measurements of body weight, crown-rump length, thoracic girth, body volume, fore-leg length, hind-leg length, head length, and head width were taken. Fetal organs were weighed; brain, pineal gland, liver, kidneys, kidney capsule fat, heart, pericardial fat, lungs, pancreas, spleen, thymus, thyroid, adrenal glands, semitendinosus muscle, and either ovaries and mammary gland or testes (). Fetal brain:liver ratio was calculated as an indicator of intrauterine fetal undernutrition (Lyon *et al.*, 2004). Data were analysed using a general linear model (Minitab, 2009). This study was conducted with the approval of the Massey University Animal Ethics Committee.

RESULTS

Ewe-lambs were lighter ($P < 0.05$) than the mature ewes; prior to breeding (47.0 ± 0.7 kg vs. 64.4 ± 1.5 kg, respectively), at d87 (48.2 ± 0.7 kg vs. 66.2 ± 2.3 kg, respectively) and at d145 (61.3 ± 1.0 kg vs. 79.2 ± 3.2 kg, respectively). Ewe carcass weight, GR, liver weight, omental fat and mammary weight collected at d145 are detailed in **Table 3**. Not surprisingly mature ewes were significantly ($P < 0.05$) heavier than ewe-lambs for all of these parameters. Gravid uterus weight did not differ between the two ewe groups ($P > 0.05$; **Table 3**). However, total placentome number was significantly ($P < 0.05$) greater for ewe-lambs compared with mature ewes but their empty caruncle number and resulting occupancy rate did not differ ($P > 0.05$; **Table 4**).

Table 3. Mean (\pm standard error) measurements of dressed carcass weight, soft tissue depth at the 12th rib (GR), liver weight, omental fat and mammary weight, gravid uterus weight (analysed with and without fetal body weight as a covariate), total placentome and empty caruncle number and occupancy rate for primiparous ewe-lambs and multiparous mature ewes.

Parameter	Dam type	
	Ewe-lambs	Adult ewes
Carcass weight (kg)	22.8 \pm 0.7 ^a	30.2 \pm 1.4 ^b
GR (mm)	14.9 \pm 2.0 ^a	21.2 \pm 2.3 ^b
Liver weight (kg)	0.87 \pm 0.03 ^a	1.14 \pm 0.03 ^b
Omental fat (kg)	0.95 \pm 0.1 ^a	2.01 \pm 0.3 ^b
Mammary weight (kg)	0.45 \pm 0.06 ^a	0.69 \pm 0.07 ^b
Gravid uterus (kg)	7.8 \pm 0.4	8.5 \pm 0.4
Gravid uterus (kg)*	8.2 \pm 0.2	8.2 \pm 0.2
Total placentomes (number)	105 \pm 5 ^b	89 \pm 5 ^a
Empty caruncles (number)	24.4 \pm 4.2	36.1 \pm 8.2
Occupancy rate (%)	81.5 \pm 2.5	72.8 \pm 5.1

*Analysed with fetal body weight as a covariate

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

Fetuses from ewe-lambs tended to be lighter ($P = 0.08$) than those from mature ewes (**Table 4**). When analysed without fetal body weight as a covariate, fetuses from ewe-lambs had smaller ($P < 0.05$) hind-leg lengths, head lengths and brain weights (**Table 4**). However when analysed with fetal body weight as a covariate, these differences were no longer apparent ($P > 0.05$). The sex of the fetus had no effect on any of the lamb size measurements taken ($P > 0.05$). The organ weights for fetuses from either ewe-lambs or mature ewes did not differ ($P > 0.05$) (**Appendix 1**). No differences were seen for any organ weights between male and female fetuses ($P > 0.05$) (**Appendix 1**), with the one exception being total kidney weight, which was

significantly heavier for males fetuses compared with female fetuses (27.5 ± 0.7 g vs. 24.5 ± 0.7 g, respectively; $P = 0.01$).

Table 4. Mean (\pm standard deviation) measurements of foetal body weight, hind-leg length, head length and brain weight at d145 for foetuses from either primiparous ewe-lambs or multiparous mature ewes.

Parameter	Fetal measurements of progeny born to:	
	Ewe-lambs	Adult ewes
Foetal body weight (kg)	5.1 ± 0.2	5.7 ± 0.2
Hind-leg length (cm)	35.6 ± 0.5^a	37.2 ± 0.5^b
Head length (cm)	17.1 ± 0.2^a	17.5 ± 0.1^b
Brain weight (g)	49.2 ± 0.8^a	52.3 ± 1.1^b
Brain:Liver ratio	0.44 ± 0.02	0.40 ± 0.03

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

DISCUSSION

The d145 singleton fetuses examined here, from primiparous ewe-lamb dams were similar to those from multiparous mature aged dams. This is contrary to expectations as Annett and Carson (2006) showed young primiparous dams gave birth to lighter/smaller lambs. However, this study imposed nutritional treatments and took pregnancy through to term.

The change in total ewe live weight in this study, from breeding to d145 was 14 kg and 15 kg for ewe-lambs and mature ewes respectively. While accurate herbage measurements were not collected ewe live weight changes observed were greater than the gravid uterus weights, 7.8 kg and 8.5 kg for ewe-lambs and mature ewes respectively. These are similar to previously recorded weights of a single-bearing ovine gravid uterus weights (Rattray *et al.*, 1974). This suggests that in the present study ewes

were not under restricted nutritional conditions, although the lack of any herbage measurements mean this cannot be verified. Ewe GR and fetal brain:liver ratio also supports this. Evidence from studies where no nutritional restriction was applied show that lambs born to young primiparous dams did not differ to those from multiparous dams (Trail & Sacker, 1969; Macedo & Hummel, 2006), which supports the findings from this study.

A significant proportion of fetal growth occurs in the last week of gestation and live counterparts of these fetuses will be studied to investigate if differences are present at birth. Performance and growth post birth will also be monitored.

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Chapter 4

**Effect of dam age and on the growth and
body composition of singleton male offspring
to about 12 months of age**

ABSTRACT

Studies from humans suggest that adolescent mothers produce offspring with a later life propensity towards obesity compared with those born to mature mothers. However, there is no indication in the literature that offspring born to ewe-lambs (EL) or multiparous mature adult ewes (AE) have different growth trajectories or differ in body composition in early life. This study utilised 38 singleton-born Romney males born to either ewe-lambs (ELP; n = 17, ram lambs) or adult ewes (AEP; n = 21, ram lambs). Both dam groups were maintained together under commercial grazing conditions during both pregnancy and lactation. After weaning the ram lambs continued to be managed together under commercial conditions. Lambs were weighed at birth and their body dimensions measured. They were subsequently weighed every two months until 11 months of age (d322). At d322, nine ELP and 10 AEP ram lambs were euthanised and several body size and composition measurements were taken. Ram lambs born to EL dams were lighter and smaller ($P < 0.05$) at birth compared with ram lambs born to AE dams and they remained lighter ($P < 0.05$) until d218. After d218 this difference in live weight was no longer apparent ($P > 0.05$). Slaughter data revealed that EL ram lambs tended ($P = 0.07$) to have more visceral fat than AE ram lambs, and were different in total carcass weight ($P < 0.05$). Dual-energy X-ray absorptiometry (DXA) was performed on the left hind-leg. AE ram lambs had greater ($P < 0.05$) lean mass compared with EL ram lambs. Under the conditions of the present study singleton males born to EL dams tended to be lighter than those born to AE dams until 12 months of age only. Despite the live weight differences, carcass weight and dressing out percentage were not affected in either group, indicating that lamb meat production from male progeny is not negatively affected by dam age or parity.

INTRODUCTION

The number of ewe-lambs (hoggets, 8 to 9 months of age) bred in New Zealand has increased to approximately 33% over the last 15 years (ANON, 2013). Little is known about the growth and carcass characteristics of male progeny born to these young ewes. This knowledge is required if farmers are to make informed decisions on whether to breed ewe-lambs or not and will assist farmers regarding market options for the offspring.

Evidence from human studies indicate that offspring born to adolescent mothers tend to be lighter (Scholl *et al.*, 1994), have altered metabolic pathways (Lucas *et al.*, 1999) and have a predisposition to obesity and increased risk of coronary heart disease later in life (Barker, 2001). Studies in sheep have shown that lambs born to ewe-lamb dams are lighter and smaller at birth (Joshi *et al.*, 2005; Annett and Carson, 2006; Gardner *et al.*, 2007) than those born to adult ewes and also at weaning (Safari *et al.*, 2005; Kenyon *et al.*, 2011). In the main, previous comparison studies have bred the two dam age/parity groups at different times, and/or bred them to different rams and/or have fed the two groups differently during pregnancy. All of these differences may be influencing and potentially confounding the results observed.

There is a paucity of information comparing the growth and body composition of offspring born to ewe-lamb (ELP) dams or adult ewes (AEP) when both groups have been bred to the same rams and managed together in pregnancy and lactation. The study of Kenyon *et al.* (2009), comparing singletons males born to young ewes compared with offspring born to mature ewe dams found that male born to young ewes had a tendency to have more abdominal fat than the comparing group. Having this result of Kenyon *et al.* (2009), the aim of this study was to examine the effect of dam age on the growth

trajectory and body composition of singleton male offspring from birth to 12 months of age, with their dams bred and managed together.

MATERIALS AND METHODS

This study was conducted at Massey University's Keeble farm (latitude 41° 10'S, longitude 175° 36'E), 5 km south of Palmerston North, New Zealand. The study occurred between September 2007 to August 2008 and was conducted with the approval of the Massey University Animal Ethics Committee.

Experimental design

The present study utilised 17 and 21 singleton-born Romney male lambs born to either EL-dams or AE-dams, respectively: creating two progeny groups, ewe-lamb progeny (ELP) and adult ewe progeny (AEP). These lambs were part of a larger study, which comprised two-hundred-and-ninety-six Romney EL-dams (8 - 9 months of age) and three-hundred-and-seven Romney AE-dams (3-7 years of age) that had their oestrous synchronised utilising intravaginal progesterone devices (CIDR, Pharmacia & Upjohn, New Zealand) (Mulvaney *et al.*, 2013) (**Figure 1**).

At breeding the 17 EL-dams weighed 40.6 ± 2.14 kg and the 21 AE-dams were 63.6 ± 2.08 kg. All ewes were naturally bred with mixed-age Romney rams during an interval of 22 days at a ram to ewe ratio of 1:12 and were managed as one group under commercial New Zealand grazing conditions with a minimum post-grazing cover of 1000kg DM/ha during gestation and 1200kg DM/ha during lactation (Mulvaney *et al.*, 2013).

In the overall study, of which the present ram progeny were a cohort, the mean birthweight and weaning weights of singleton ELP and AEP were 4.04 ± 0.14 kg vs. 5.3 ± 0.14 kg and 16.8 ± 0.56 kg vs. 21.6 ± 0.57 kg, respectively (Mulvaney *et al.*, 2013).

Experimental measures

Within 12 hours of birth (d1) the ram lambs were weighed and their crown-rump length (CRL), thoracic girth (TG1), fore-leg length (FL), hind-leg length (HL) measurements taken. The ram lambs were weighed again at d24, d72, d128 (weaning), d218, d297 and d322. At d322, nineteen ram lambs (9 ELP and 10 AEP) were euthanised. Post slaughter carcass weight was recorded and dressing out percentage (DO%) calculated (carcass weight/ live weight \times 100%). Thoracic girth (TG322) was recorded, and weights were taken for liver, heart, spleen, kidney (both), lung (both), adrenal (both), visceral fat (omental and mesenteric) and remaining visceral tissue mass. The hindquarter/spine region of each animal was collected and frozen at -20°C until dual-energy X-ray absorptiometry (DXA).

At DXA analysis, the samples were scanned using the Lunar Prodigy (General Electric, Madison, WI), with a dorsoventral projection. The DXA manufacturer's coefficient of variation (CV) was 0.54 % and 1.02 % for bone mineral content (BMC) and lean tissue mass (LM), respectively. Areal bone mineral density (aBMD) was calculated as bone mineral content divided by bone area. Total composition of the hindquarter was determined as the sum of BMC, lean tissue mass and fat tissue mass. Percentage of fat was calculated by dividing fat tissue mass by total composition. The ratio of BMC:Lean mass was calculated as BMC divided by lean tissue mass.

Statistical analysis

All statistical analyses were performed with Minitab[®] (version 16, Minitab Inc, Cary NC, USA) using a General Linear Model (GLM) procedure. Lamb live weights were analysed with dam type (EL vs. AE) as a main effect and date of birth as a covariate. Lamb size measurements at birth were analysed using a GLM procedure with dam type as a fixed effect and date of birth as a covariate, and with, and without, birthweight as an additional covariate. Slaughter data were analysed with dam type as a fixed effect and with, and without, the live weight at slaughter (d322) as a covariate. DXA data were analysed using a GLM procedure with date of birth and carcass weight as covariates and dam type as a fixed effect.

RESULTS

Measurements at birth

ELP ram lambs were lighter ($P < 0.05$) at birth and had smaller ($P < 0.05$) crown-rump length, fore-leg and hind-leg measurements than AEP (**Table 5**). When birthweight was included as a covariate these body dimension differences were no longer apparent (53.6 ± 1.45 cm vs. 55.2 ± 1.55 cm; 39.2 ± 0.78 cm vs. 40.0 ± 0.68 cm; 39.4 ± 0.95 cm vs. 40.5 ± 0.83 cm; respectively for CRL, FL and HL; $P > 0.05$ for ELP and AEP, respectively).

Table 5. Birthweight (kg), crown-rump length (CRL), thoracic girth (TG1), fore-leg length and hind-leg length of ram lambs born to either ewe-lamb dams (ELP) or adult ewes (AEP). Data are presented as least square mean (\pm standard error).

Parameter	Ram lambs born to ewe-lamb dams (ELP)	Ram lambs born to adult ewes (AEP)
n	17	21
Birthweight (kg)	4.2 \pm 0.27 ^a	5.7 \pm 0.24 ^b
CRL (cm)	51.8 \pm 1.06 ^a	56.6 \pm 0.94 ^b
TG1 (mm)	39.4 \pm 0.90 ^a	43.2 \pm 0.79 ^b
Fore-leg (cm)	37.2 \pm 0.87 ^a	41.6 \pm 0.77 ^b
Hind-leg (cm)	37.5 \pm 0.97 ^a	42.0 \pm 0.86 ^b

^{ab} different superscripts within rows indicate values that significantly differ ($P < 0.05$)

Live weight and live weight gain post birth

ELP rams were lighter at d24 (11.0 \pm 0.68 kg vs. 14.4 \pm 0.62 kg; $P < 0.001$), d72 (17.1 \pm 1.14 kg vs. 21.8 \pm 0.98 kg; $P < 0.05$) and d128 (20.4 \pm 0.97 kg vs. 24.9 \pm 0.89 kg; $P < 0.05$) than AEP, respectively. Live weights did not differ ($P > 0.05$) post d218 (**Figure 2**). However at d297 and d322, ELP still tended ($P = 0.06$) to be lighter than AEP. When live weights post birth were analysed with birthweight as a covariate, there were no differences ($P > 0.05$) in live weight between groups at any time points (data not shown).

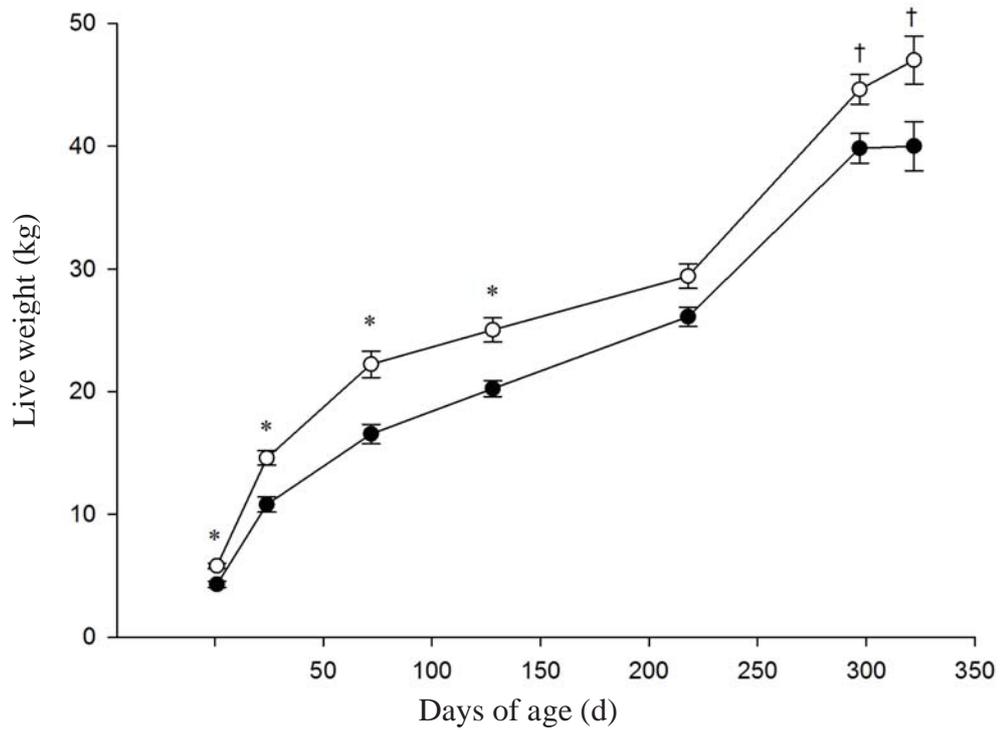


Figure 2. Live weight from birth (d1) to d322 of lambs born to ewe-lambs ● (ELP) or adult ewes ○ (AEP). Data presented are least square means \pm standard error. * indicate values differ significantly ($P < 0.05$) and † indicates a tendency ($P = 0.06$).

ELP grew slower than AEP ($P < 0.05$) during d1-d24 but faster ($P < 0.05$) than AEP between d128-d218 (**Table 6**). ELP displayed a tendency for slower growth during d218-d297 ($P = 0.08$) and d297-d322 ($P = 0.06$) (**Table 6**). When lamb birthweight was included as a covariate no differences ($P > 0.05$) for growth rates were observed (data not shown).

Table 6. The effect of being born to either ewe-lambs (ELP) or adult ewes (AEP) on liveweight gain per day during the period of birth (d1) to d322. Data are presented as least square mean (\pm standard error).

Time period (age in days)	Liveweight gain per day (kg/day)			
	n	Ram lambs born to ewe-lamb dams (ELP)	n	Ram lambs born to adult ewes (AEP)
1 - 24	17	0.28 \pm 0.02 ^a	20	0.35 \pm 0.02 ^b
24 - 72	16	0.12 \pm 0.01	20	0.15 \pm 0.01
72 - 128	16	0.06 \pm 0.01	20	0.05 \pm 0.01
128 - 218	16	0.07 \pm 0.01 ^b	20	0.04 \pm 0.01 ^a
218 - 297	16	0.17 \pm 0.01	20	0.19 \pm 0.01
297 - 322	10	0.04 \pm 0.02	9	0.11 \pm 0.02

^{ab} different superscripts rows indicate values that significantly differ ($P < 0.05$)

Slaughter data

At slaughter (d322) the live weight of the ELP tended to be lighter than AEP (40.6 ± 1.88 kg vs. 46.3 ± 2.0 kg; $P = 0.06$; respectively). There was no difference ($P > 0.05$) in DO%, TG322, heart, lungs, adrenal glands, visceral fat or between groups (**Table 7**), however the carcass weight, spleens, liver and remaining visceral tissue mass weights of ELP were lighter ($P < 0.05$) than AEP (**Table 7**). When slaughter data were re-analysed with live weight at d322 as a covariate, ELP tended ($P = 0.07$) to have greater visceral fat than AEP (563.4 ± 51.43 g vs. 409.5 ± 54.65 g, respectively). There was no difference ($P > 0.05$) in carcass weight, spleens, liver and rumen/visceral weight post adjustment for d322 live weight (data not shown), nor ($P > 0.05$) in any of the other measures.

Table 7. Live weight (kg), carcass weight, thoracic girth (TG322), dressing out percentage (DO%), liver, heart, spleen, kidneys, lungs, adrenal glands, visceral fat and rumen/visceral weights at 322 days of age of ram lambs born to either ewe-lamb dams (ELP) or adult ewes (AEP). Data are presented as least square mean (\pm standard error).

Parameter	Ram lambs born to ewe-lamb dams (ELP)	Ram lambs born to adult ewes (AEP)
n	10	9
Live weight (kg) [†]	40.6 \pm 1.88	46.3 \pm 1.99
Carcass weight (kg)	17.7 \pm 0.78 ^a	20.2 \pm 0.82 ^b
TG322 (mm)	6.8 \pm 0.76	8.1 \pm 0.80
DO %	44.9 \pm 1.54	43.0 \pm 1.62
Liver (g)	689.9 \pm 35.62 ^a	809.3 \pm 37.54 ^b
Heart (g)	236.8 \pm 15.43	250.6 \pm 16.26
Spleen (g)	67.7 \pm 4.49 ^a	84.1 \pm 4.74 ^b
Kidneys (g)	119.4 \pm 4.93	134.3 \pm 5.20
Lungs (g)	485.2 \pm 42.66	472.5 \pm 44.96
Adrenal (g)	3.3 \pm 0.16	3.5 \pm 0.17
Visceral fat (g)	471.8 \pm 70.51	511.3 \pm 74.33
Remaining visceral tissue mass weight (g)	1212.6 \pm 56.56 ^a	1403.2 \pm 59.62 ^b

^{ab} different superscripts within rows indicate values that significantly differ ($P < 0.05$)

[†]Live weight at 322 days of age

DXA data

ELP had lower ($P < 0.05$) lean mass than AEP. There was no difference ($P > 0.05$) between groups for area of hind-leg, BMC, aBMD, fat mass, total composition, percentage of fat and BMC:lean mass ratio (**Table 8**). When the DXA measurements were re-analysed with date of birth and carcass weight as covariates, the lean mass difference became a tendency ($P < 0.09$) (data not shown).

Table 8. Dual-energy X-ray absorptiometry (DXA) measurement of left hind-leg area, bone mineral content (BMC), areal bone mineral density (aBMD), fat mass, lean mass, total composition, fat percentage and BMC:lean ratio at slaughter (d322) of ram lambs born to either ewe-lamb dams (ELP) or adult ewes (AEP). Data are presented as least square mean (\pm standard error).

Parameter	Ram lambs born to ewe-lamb dams (ELP)	Ram lambs born to adult ewes (AEP)
n	10	9
Area (cm ²)	231.6 \pm 11.30	250.0 \pm 11.94
BMC (g)	135.3 \pm 9.65	158.5 \pm 10.20
aBMD (g/cm ²)	0.6 \pm 0.02	0.6 \pm 0.02
Fat mass (g)	810.6 \pm 76.13	877.8 \pm 80.42
Lean mass (g)	2686.2 \pm 113.38 ^a	3152.8 \pm 119.76 ^b
Total composition (g)	3632.1 \pm 180.01	4189.0 \pm 190.15
Percentage Fat (%)	21.9 \pm 1.32	20.6 \pm 1.40
BMC:Lean mass ratio	0.05 \pm 0.0018	0.05 \pm 0.0019

^{ab} different superscripts within rows indicate values that significantly differ ($P < 0.05$)

DISCUSSION

This study was designed to compare development of singleton male lambs born to primiparous ewe-lambs (ELP) or multiparous adult ewes (AEP) managed together under commercial conditions. The present study found that ram lambs born to EL-dams were lighter at birth compared with ram lambs born to AE-dams, which is similar to findings from sheep studies (Dýrmundsson, 1973, Corner *et al.*, 2013, Kenyon *et al.*, 2014) and human studies, where offspring born to adolescent mothers are typically to be lighter than those born to older mothers (Scholl *et al.*, 1994). However, in a study by Muñoz *et al.* (2009) comparing ewes bred in the first year of life as ewe-lambs stated no difference in birthweight of lambs born to primiparous ewe-lambs or themselves as biparous ewes; showing that parity did not affect birthweight.

In the present study ELP remained lighter than AEP until approximately four months of age and tended to be lighter until 12 months of age. These findings support those of Craig (1982) who found that progeny born to ewe-lamb dams were lighter than those born to adult ewes at birth and remained lighter until 11 months of age. The observed differences in live weight to weaning are likely due to the combined influence of smaller birthweight and reduced milk production in young dams (Nicol and Brookes, 2007), both of which affect lamb growth (Kenyon *et al.*, 2004). A lighter weight at weaning may lead to a reduced value at sale or increased feed requirements to achieve desired slaughter weights.

At slaughter there was a tendency for greater levels of visceral fat in ram lambs born to EL-dams compared with ram lambs born to AE-dams, matching the previous results found by Kenyon *et al.* (2009). Additionally, the DXA of the hind-leg indicated that the ram lambs born to EL-dams had less lean mass compared with ram lambs born to

AE-dams, however the amount of fat in the hind-leg did not differ. In the contemporary sheep meat industry there has been a focus on breeding for leaner carcass (McPhee *et al.*, 2008). Afolayan *et al.* (2007) compared the carcass traits of progeny born to ewe-lamb dams and adult ewes. They reported that offspring born to ewe-lamb dams displayed a greater level of carcass fat (fat over the maximum depth of eye muscle) and lower carcass weight compare to lambs born to mature ewes.

The value of a carcass is affected by its level of fatness with the contemporary sheep meat industry breeding for increased lean carcass (McPhee *et al.*, 2008). Whilst the present study provides evidence for increased adiposity in male offspring born to EL-dams, the levels of adiposity observed here are unlikely to affect carcass value.

CONCLUSIONS

Under the conditions of the present study, the live weights of male offspring born to ewe-lamb dams were lighter to four months of age and tended to remain lighter to approximately one year of age. However, this did not appear to dramatically alter carcass weight or carcass characteristics, with only a minor effect on adiposity. Collectively, the results reported here indicate that for the New Zealand sheep meat industry, the growth and meat production performance of male progeny born to ewe-lamb dams is comparable to that of male progeny born to adult ewes. Having examined the growth and carcass composition of male offspring in the present study, the following chapter (Chapter 5) will focus on non-invasively examining the body composition of female progeny born to ewe-lambs and/or adult ewes.

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Chapter 5

Comparison of abdominal and mammary gland composition of females born to ewe-lambs or mature dams

ABSTRACT

Young and mature females have different growth requirements, body composition and size but little is known about how variation in a mother's physiological status affects the body composition of her offspring. This study investigated abdominal adiposity and mammary gland tissue of singleton ewe progeny of ewe-lambs (ELP = ewe-lamb progeny) and adult ewes (AEP = adult ewe progeny), via computed tomography (CT) scanning, over three distinct physiological states: empty (non-pregnant), pregnant and lactating. The results from this study demonstrated that the weight of abdominal adipose tissue (subcutaneous fat, intermuscular fat, internal fat and total fat), during different physiological stages, did not differ ($P > 0.05$) between ELP and AEP nor did ($P > 0.05$) the other carcass components analysed (non-fat visceral component, lean and bone weights) differ between ewe groups at any of the physiological stages investigated. Predicted carcass weights and total tissue weights were heavier ($P < 0.05$) for AEP compared with ELP during pregnancy. Mammary gland architecture did not differ between ewe groups at any of the different physiological stages. In conclusion, abdominal adiposity and mammary gland tissue are comparable for singleton female offspring born to young or mature dams at different physiological states: empty (non-pregnant), pregnant and lactating.

INTRODUCTION

Lamb production is an important aspect of New Zealand agriculture, with New Zealand being the world's largest sheep meat exporter (ANON, 2013). In 2012, export lamb meat production was the single biggest income earner for the New Zealand sheep industry, generating free on board (FOB) earnings of NZ\$ 2,327,297,000 (Beef and Lamb New Zealand, 2013).

Therefore any means which increases the number of lambs weaned would have a significant effect on the total value of the New Zealand sheep industry. A potential means of increasing the national number of lambs weaned per year is by breeding ewe-lambs (hoggets, 8 - 9 months of age) (Moore *et al.*, 1983). Currently only one third of ewe-lambs in New Zealand are bred, with the remainder bred for the first time at 18-19 months (ANON, 2013).

It is well established that young and mature females have different body composition and size; however it is unknown if maternal age affects the body composition of their female offspring. In previous chapters (3 and 4), it has been shown that singleton fetuses from ewe-lambs are potentially exposed to a detrimental *in utero* environment and that at one year of age, male offspring born to ewe-lambs tend to accumulate more visceral fat than their counterparts born to mature adult ewes.

Meat production efficiency depends on the distribution of fat and lean in the carcass (Kempster *et al.*, 1976; Owen *et al.*, 1978). Increased accumulation of internal abdominal fat is a reflection that the animal has begun to convert more energy towards deposition of fat rather than muscle. In males which are predominately used for meat production, this would reduce efficiency. However, this may not be totally undesirable for females. It could be suggested that a greater level of fat stored in a female might be

beneficial in late pregnancy/early lactation as it could be mobilised to meet any energy deficiency in those important periods.

Traditionally, body tissues have been studied through the slaughter of the animals (Jopson *et al.*, 1995). However, this does not allow for comparative results for temporal growth and development. Accurate comparison of temporal development requires data from the same animal to be collected over time (Bünger *et al.*, 2011). The use of non-invasive imaging techniques can be used to assess the same animals at different developmental stages and ages (Wells, 1984). Computed tomography (CT) is a method that can be used to repeatedly measure body composition over time (Jopson *et al.*, 1997; Young *et al.*, 2001; Hopkins *et al.*, 2008) to identify and quantify adipose tissue in different depots (Lambe *et al.*, 2013). Furthermore, CT has been utilised to predict carcass composition (Young *et al.*, 1999; Lambe *et al.*, 2003 and 2006) and to enhance genetic improvement (Kvame and Vangen, 2007), using 3 to 7 cross sectional images per animal (Lambe *et al.*, 2013; Kvame *et al.*, 2004). CT scanning has also been used in cows to examine the composition of the mammary gland and to determine milk yield capacity (Sejrsen *et al.*, 1986).

The aim of the present study was to use CT techniques to compare abdominal adiposity and mammary gland tissue of ewes born to either ewe-lambs or mature ewes, over three different physiological states: empty (non-pregnant), pregnant and lactating. In the previous chapter (Chapter 4) there were indications that being born to ewe-lambs may lead to greater internal abdominal fat in males.

MATERIALS AND METHODS

This study was conducted at Massey University (latitude 41° 10'S, longitude 175° 36'E) Palmerston North, New Zealand. This trial occurred between May 2009 and November 2009 and was conducted with the approval of the Massey University Animal Ethics Committee.

Experimental design

The present study utilised seven 1.5 year old singleton-born Romney ewes that had been born to either ewe-lamb dams or adult ewe dams: ewe-lamb progeny (ELP; n = 4) and adult ewe progeny (AEP; n = 3).

Background

The ewe-lamb and adult ewe dams were part of a larger 2007 study which comprised 296 Romney ewe-lambs (8 - 9 months of age) and 307 mature Romney ewes (3-7 years of age) (Mulvaney, 2011; Mulvaney *et al.*, 2013). In brief, at breeding the ewe-lambs weighed 40.6 ± 2.14 kg and the adult ewes weighed 63.6 ± 2.08 kg. All dams were managed as one group under commercial New Zealand grazing conditions with a minimum post-grazing pasture cover of 1000 kg DM/ha during gestation (Mulvaney, 2011; Mulvaney *et al.*, 2013). The offspring were managed together in a single mob under commercial conditions from birth (d1) to d766. The ewe progeny were first bred at d596 (May, 2009) when their oestrous were synchronised utilising intravaginal progesterone devices (CIDR, Pharmacia & Upjohn, New Zealand) and naturally mated to Romney rams for two days. They were then pregnancy diagnosed via ultrasound 50 days after the end of joining.

Present study

The seven singleton-bearing ewes, taken from the large cohort of ewe progeny, were CT scanned at three different physiological stages in 2009 (**Figure 1A**). The first CT scan occurred seven days prior to breeding (scan 01 - d589; average live weight 51.7 ± 1.31 kg); the second occurred at approximately day 84 of pregnancy, (scan 02 - d680; average live weight 59.5 ± 1.31 kg) and the third occurred during the lactation period, on average 23 days after parturition (scan 03 - d766; average live weight 72.4 ± 1.31 kg).

Procedures for X-ray computed tomography (CT scan)

The seven ewes were managed together under commercial grazing conditions. On the day prior to each CT scanning event the ewes were transported to the Massey University Veterinary Hospital and kept off feed overnight, to reduce rumen and gut contents.

Prior to the CT procedure, ewes were sedated with acepromazine (0.5 mL/ 50kg live weight) to reduce movement and stress during the procedure; and then physically restrained on their back within a CT cradle. The ewes had their fore- and hind-legs fully extended and restrained to avoid movement. Ewes were oriented posterior-anterior within the CT scanner (Brilliance CT 16-slice Scanner, Philips Australia and New Zealand). Cross-sectional images were recorded at 7.5mm intervals along the length of the body and 0.3 seconds of time exposure. After each CT procedure the ewes were returned to commercial grazing conditions.

Image analysis

CT images were analysed using a semi-automated procedure as described below.

Abdominal adiposity

Images were manually selected using a stereological technique (Cavalieri method) to allow prediction of volume and area of irregular shapes (Gundersen *et al.*, 1988). With parallel sections separated by a known distance, counting 10 to 15 images gives an estimation of the true volume value with 95% accuracy (Gundersen *et al.*, 1988; Afonso, 1992; Bünger *et al.*, 2011).

An average of 95, 89 and 85 cross sectional images, with 7.5mm interval between images, were collected per ewe during CT scan 01, 02 and 03, respectively. The average number of images used to predict the volume of abdominal fat was 37, 38, and 39 at scan 01, 02 and 03, respectively, using the Cavalieri method (Gundersen *et al.*, 1988). To be consistent across animals, images were selected starting at the first image of the heart and ending at the ischium. Images were selected manually, excluding every second image, leaving 15mm intervals between images. The Cavalieri method was used to calculate the volume of a tissue: whereby the sum of the area of each tissue from each cross sectional image is multiplied by the distance between images (Gundersen *et al.*, 1988). Images were manually separated into three regions using Adobe Photoshop CS5 for Macintosh software (**Figure 3** and **Figure 4**). The first and second images were of the carcass, the first containing subcutaneous fat and the second intermuscular fat; the third image is the visceral contents (internal fat – omental, mesenteric, kidney and channel fat depots) and the non-fat visceral component (NFVC – internal organs and intestines). Subcutaneous fat was comprised of the depots of fat underneath the skin and the layer on top of the muscle/bone. The contents of rumen, reticulum, omasum and abomasum were excluded from the images and at scan 02 the fetus and uterus was also excluded.

After the images were dissected, stereological analysis was undertaken using the “AutoCAT” image analysis programme (Jopson *et al.*, 1997). Pixel grey scale thresholds were specified to identify the desired tissues (fat, lean tissue and bone). “AutoCAT” sums the areas across the series of CT images within these specified thresholds, and calculates a weighted mean Hounsfield unit. Tissue volume was found by multiplying the summed area by the image interval. Tissue weight was estimated from the volume multiplied by the average density of the given tissue (fat, lean and bone). Density was estimated from the linear relationship between CT Hounsfield units and physical density as described by Fullerton and Zagzebski (1980). Tissue weight estimates included: subcutaneous fat; intermuscular fat; internal fat (adipose tissue in the abdominal and thoracic cavities); total fat (sum of subcutaneous, intermuscular and internal fat depots); carcass lean; NFVC; total lean (carcass lean plus NFVC); bone; carcass weight (sum of subcutaneous fat, intermuscular fat, carcass lean and bone), and; total tissue weight (sum of total fat, total lean and bone) for each animal (Fullerton and Zagzebski, 1980; Jopson *et al.*, 1995) (**Table 9**).

Mammary gland adiposity

A single CT image per animal was used for analysis of mammary gland adiposity. The image selected for each animal was the image that included the teats as this was considered to give the largest sampling of the mammary gland in a single image. The cross-section images were manually manipulated to separate the mammary gland (excluding the mammary fat depot) from body tissue (**Figure 4**), using Adobe Photoshop CS5 for Macintosh software. The images underwent the same analysis process as described for abdominal adipose tissue allowing the calculation of area and average densities of extra-parenchymal tissue (fat), parenchymal tissue (lean).

Statistical analysis

The statistical analyses were performed using Minitab[®] (version 16, Minitab Inc., Cary NC, USA) and SAS (SAS, *Enterprise Guide*[®] 4.2).

The predictions of subcutaneous fat, intermuscular fat, internal fat, total fat, carcass lean, NFVC, total lean, bone, carcass weight, total tissue weight, parenchyma, extra-parenchyma and total mammary gland weight were analysed using Minitab with the GLM procedure and included the fixed effects of dam type (EL vs. AE) for each scan. Live weight and date of birth were included as covariates but found to be not significant ($P > 0.05$) and were removed from the final model.

Predicted abdominal and mammary gland parameters were analysed using an allometric model (Schmidt-Nielsen, 1984) to analyse the relationship of body size to body shape in growing animals; this allows to measurement of relative changes within individual animals. Allometry is the tendency of the size of an organism and its shape to have highly correlated metric characters (Schmidt-Nielsen, 1984; Klingenberg, 1996). The allometric equation is expressed as:

$$y = b \cdot x^a$$

Logarithms can be used to simplify the allometric equation into a linear equation (Schmidt-Nielsen, 1984). The linear form after \log_{10} transformation is expressed as:

$$\log_{10}y = \log_{10}b + a \cdot \log_{10}x$$

where y is the variable (tissue weights) and x is the sum of component weights (total weight); b and a are constants, b is the scaling factor (intercept) and a is the allometry coefficient (slope).

The focus of this study was to compare tissue (fat and parenchyma) deposition relative to changes in other components (i.e. subcutaneous fat, intermuscular fat, internal fat deposition relative to total fat deposition; subcutaneous fat, intermuscular fat, internal fat deposition relative to changes in carcass weight; parenchyma and extra-parenchyma growth relative to live weight) between offspring born to different dam age groups. If the allometry coefficient (a) is equal to one, it indicates isometry indicating that tissue deposition varied in direct proportion to the component weight it was compared with. If a is greater than one there is a lower rate of deposition of one component relative to another (e.g. the fat depot is considered late maturing in that it comprises a lower proportion at lighter weights and a higher proportion at heavier weights), and if a is less than one, there is a higher rate of deposition of one component relative to another (e.g. the fat depot is considered early maturing such that it has a higher relative proportion at lighter weights and a lower relative proportion at heavier weight) (Schmidt-Nielsen, 1984; Shea *et al.*, 1987; Thompson and Kinghorn, 1992; Jopson *et al.*, 1995; Jopson *et al.*, 1997).

The variables (abdominal and mammary gland tissue weights) were \log_{10} transformed and tested utilising the MIXED procedure in SAS, and included the fixed effect of dam type (EL vs. AE) with individual female identification nested with dam type. In addition the model included date of birth and \log_{10} total tissue weight as covariates. If the homogeneity of the variable tested was significantly different ($P < 0.05$), comparison of pairs of allometry coefficients were made using Student's t-test. Analyses of covariance were examined visually by the regression scatters (Williams, 1977).

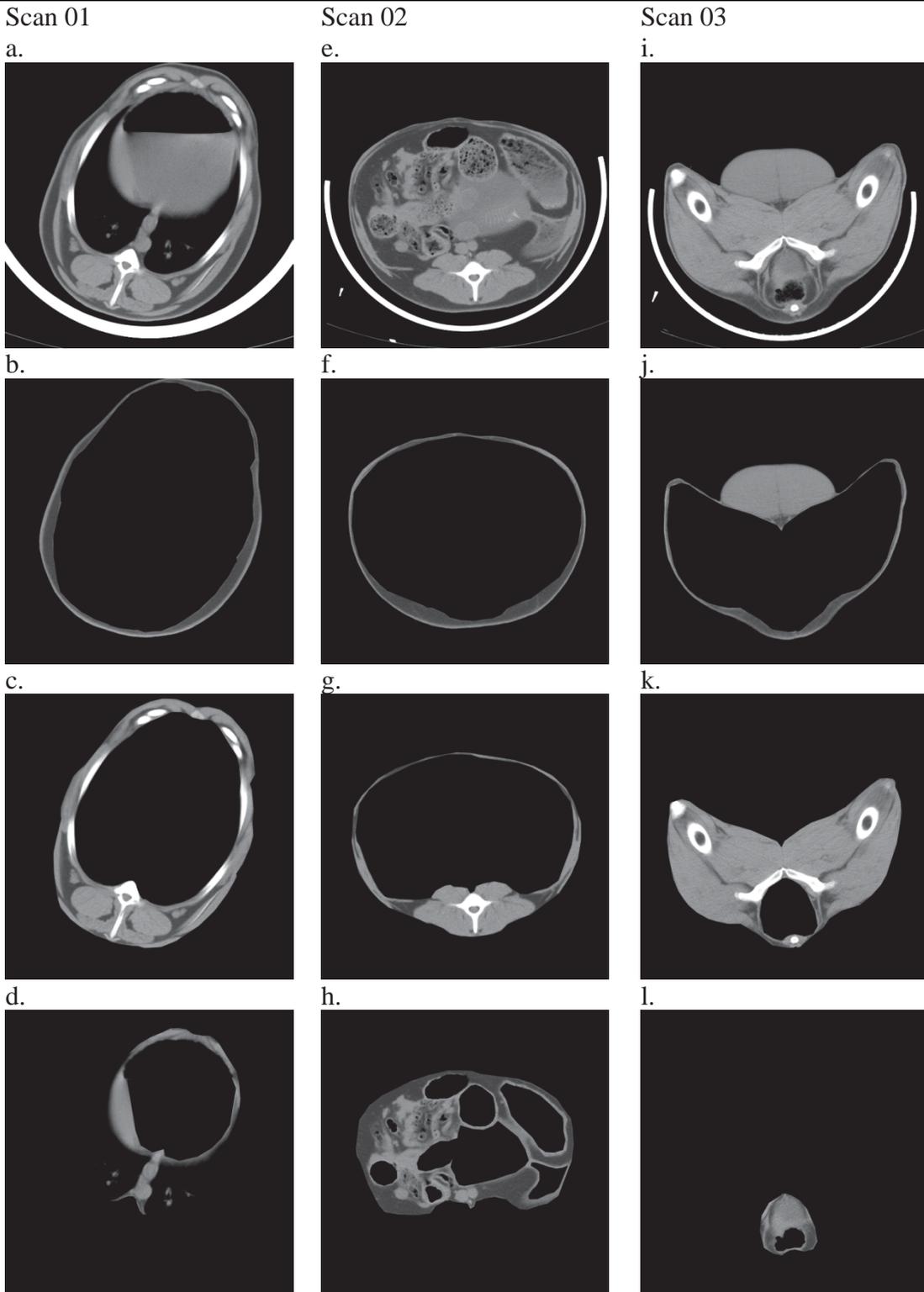


Figure 3. Typical image captured by CT and examples of image dissecting for analysis: Scan 01 = the start point (first image of the heart); Scan 02 = the mid-point of abdomen (approximately between the 3rd and the 4th lumbar vertebrae); Scan 03 = the caudal point (approximately at the 1st caudal vertebrae). Image a, e and i show the original raw CT image; b, f and j are images a, e and i dissected to show subcutaneous fat; c, g and k are images a, e and i dissected to show carcass and intermuscular fat; d, h and l are images a, e and i dissected to show internal fat.

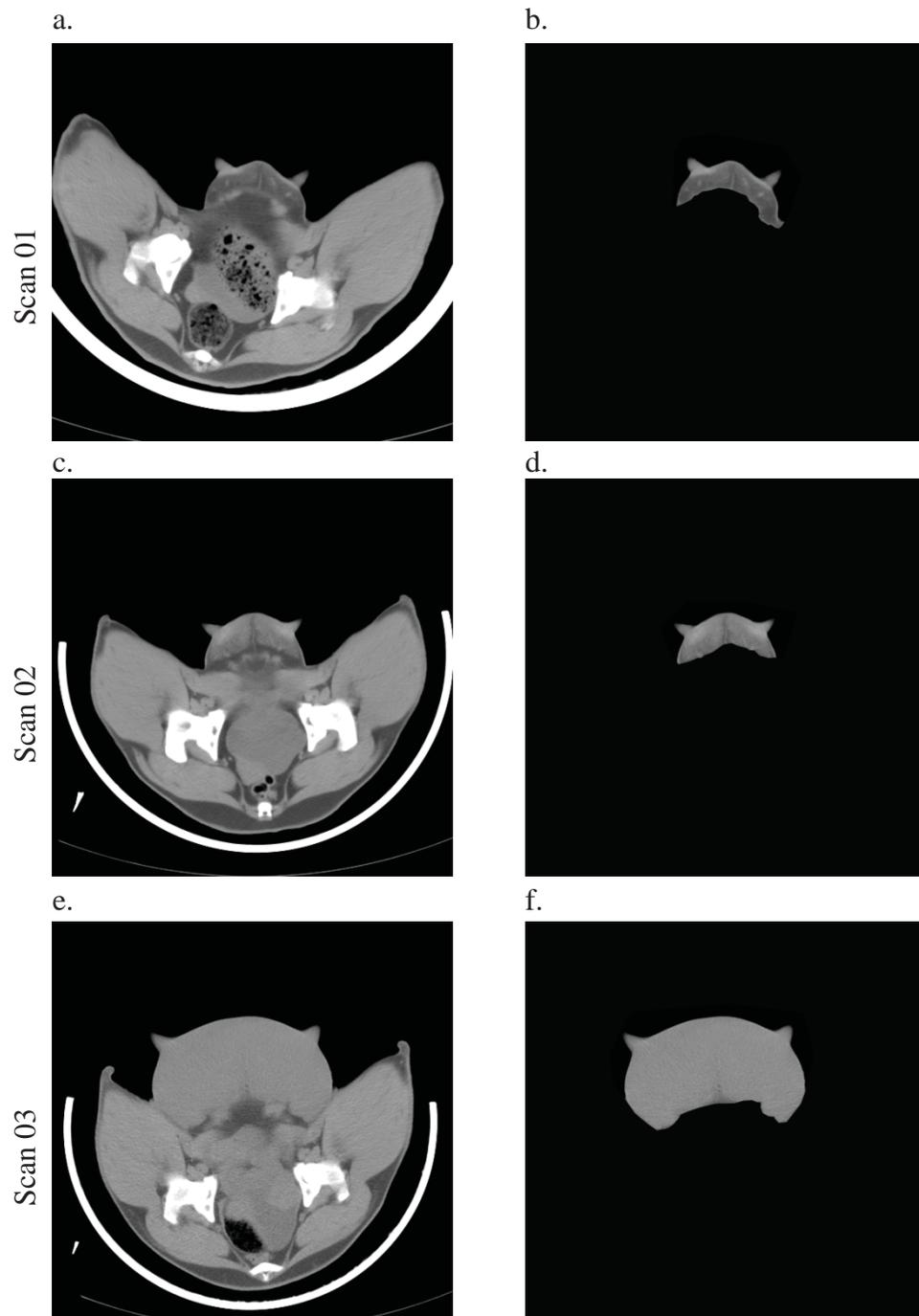


Figure 4. Typical images captured by CT of mammary gland: a. Scan 01 (seven days prior to breeding); b. image a dissected to show only the mammary gland; c. Scan 02 (average day 84 of gestation); d. image c dissected to show only the mammary gland; e. Scan 03 (average day 23 post-lambing); f. image e dissected to show only the mammary gland.

RESULTS

Dam type had no effect on live weight at scan 01 (51.4 ± 1.6 kg vs. 52.0 ± 1.9 kg for ELP and AEP, respectively; $P = 0.81$), scan 02 (57.4 ± 1.4 kg vs. 61.7 ± 1.7 kg for ELP and AEP, respectively; $P = 0.10$) or scan 03 (70.5 ± 2.0 kg vs. 74.3 ± 2.3 kg for ELP and AEP, respectively; $P = 0.27$). The lambs born to these ewes were all singletons (6 males and 1 female) and their birthweight did not differ due to granddam type (EL-granddam vs. AE-granddam, 6.0 ± 0.4 kg and 5.4 ± 0.5 kg, respectively; $P = 0.38$).

There were no significant differences ($P > 0.05$) due to dam type on the predicted weight of the various abdominal tissues at scan 01 or 03 ($P > 0.05$; **Table 9**). However, predicted carcass weight ($P = 0.02$) and total tissue weight ($P = 0.03$) were significantly ($P < 0.05$) greater for AEP compared with ELP during pregnancy (Scan 02; **Table 9**). Dam type had no effect ($P > 0.05$) on mammary gland tissue weights at any of the three scans (**Table 9**).

Table 9. Abdominal and mammary gland CT scan parameters at Scan 01 (seven days prior to breeding), Scan 02 (average day 84 of gestation) and Scan 03 (average day 23 post-lambing) for ewe-lamb progeny (ELP) and adult ewe progeny (AEP). Data presented are least square means \pm standard error.

	Scan 01		Scan 02		Scan 03	
	ELP	AEP	ELP	AEP	ELP	AEP
Abdominal fat						
Subcutaneous fat (kg)	2.2 \pm 0.17	2.0 \pm 0.20	2.8 \pm 0.18	2.9 \pm 0.21	2.2 \pm 0.26	2.6 \pm 0.30
Intermuscular fat (kg)	1.2 \pm 0.11	1.2 \pm 0.13	1.7 \pm 0.09	1.8 \pm 0.10	1.5 \pm 0.19	1.8 \pm 0.22
Internal fat (kg)	3.4 \pm 0.30	3.1 \pm 0.35	3.9 \pm 0.29	4.0 \pm 0.33	3.9 \pm 0.43	4.4 \pm 0.50
Total fat (kg)	6.8 \pm 0.55	6.3 \pm 0.64	8.3 \pm 0.49	8.6 \pm 0.56	7.6 \pm 0.88	8.8 \pm 1.01
Carcass lean (kg)	8.6 \pm 0.41	8.9 \pm 0.47	8.4 \pm 0.41	9.5 \pm 0.47	10.7 \pm 0.28	9.7 \pm 0.32
NFVC (kg)	5.3 \pm 0.27	5.7 \pm 0.31	5.6 \pm 0.34	6.4 \pm 0.39	8.1 \pm 0.29	8.0 \pm 0.33
Total lean (kg)	13.9 \pm 0.31	14.6 \pm 0.70	14.1 \pm 0.72	15.9 \pm 0.84	18.8 \pm 0.50	17.7 \pm 0.58
Bone (kg)	1.6 \pm 0.10	1.8 \pm 0.12	1.6 \pm 0.11	1.8 \pm 0.13	1.8 \pm 0.09	1.7 \pm 0.10
Carcass weight (kg)	13.7 \pm 0.62	13.7 \pm 0.72	14.5 \pm 0.27 ^a	15.9 \pm 0.32 ^b	16.2 \pm 0.61	15.8 \pm 0.71
Total tissue weight (kg)	22.3 \pm 0.98	22.6 \pm 1.13	24.0 \pm 0.51 ^a	26.3 \pm 0.59 ^b	28.2 \pm 1.12	28.2 \pm 1.30
Mammary Gland fat						
Parenchyma (kg)	0.020 \pm 0.003	0.019 \pm 0.004	0.015 \pm 0.004	0.011 \pm 0.005	0.008 \pm 0.001	0.008 \pm 0.001
Extra-parenchyma (kg)	0.007 \pm 0.002	0.007 \pm 0.003	0.031 \pm 0.007	0.032 \pm 0.008	0.231 \pm 0.013	0.211 \pm 0.015

^{ab} different superscripts in rows but within scan indicate values that significantly differ ($P < 0.05$)

Allometric analyses of subcutaneous fat, intermuscular fat and internal fat relative to total fat were not significantly ($P > 0.05$) influenced by dam type (**Table 10**), indicating that proportions of subcutaneous, intermuscular and internal fat deposition relative to total fat was similar between ELP and AEP. In addition, intermuscular and internal fat relative to carcass weight were not significantly ($P > 0.05$) influenced by dam type (**Table 10**), indicating that proportions of intermuscular and internal fat deposition relative to carcass weight was similar between ELP and AEP. Deposition of subcutaneous fat relative to carcass weight showed significant differences ($P < 0.05$) due to dam type (**Table 10**). Further analysis of the slope (a) revealed that AEP increased fat disproportionately to carcass weight ($P < 0.05$). AEP with an a coefficient of 1.90 compared with ELP with an a coefficient of 0.07, indication of relative change, each percentage point increase in carcass weight, subcutaneous fat will increase by 2%. However, when these values were further analysed using Student's t - test, they did not differ from 1, indicating that the allometric difference was due to differences in carcass weight (**Figure 5**). Allometric analyses of mammary gland tissue weights show that parenchyma and extra-parenchyma depots relative to live weight did not differ ($P > 0.05$) due to dam type (**Table 10**).

Table 10. The slope (*a*) and back-transformed intercept (*b*) of \log_{10} transformed allometric for abdominal fat mobilisation relative to total fat and carcass weight and mammary gland fat mobilisation relative to the live weight for ewe-lamb progeny (ELP) and adult ewe progeny (AEP).

	<i>a</i>		<i>b</i>	
	ELP	AEP	ELP	AEP
Relative to total fat				
Subcutaneous fat	0.81	1.11	0.505	0.269
Intermuscular fat	1.24	1.26	0.105	0.104
Internal fat	0.93	0.86	0.571	0.659
Relative to carcass weight				
Subcutaneous fat	0.07 ^a	1.90 ^b	1.966	0.013
Intermuscular fat	1.13	2.46	0.068	0.002
Internal fat	0.91	1.56	0.316	0.053
Relative to live weight				
Parenchyma	1.62	1.46	2.706	2.123
Extra-parenchyma	-0.37	-0.28	0.005	0.005

^{ab} superscripts in rows within unit of measure indicate back-transformed values of \log_{10} transformed allometric that significantly differ ($P < 0.05$)

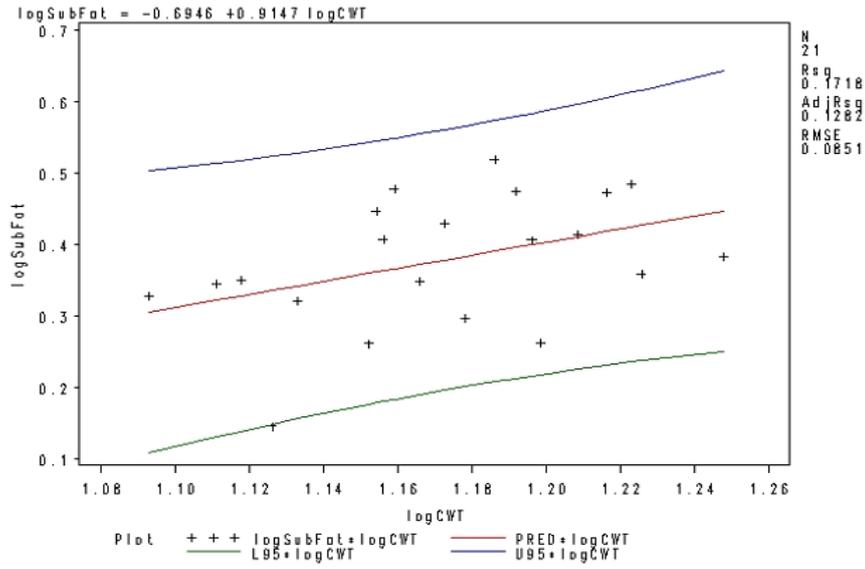


Figure 5. Plot of \log_{10} subcutaneous fat ($\log\text{SubFat}$) and \log_{10} carcass weight ($\log\text{CWT}$).

DISCUSSION

This study was designed to examine the abdominal adiposity and mammary gland tissue of singleton ewes born to either primiparous ewe-lambs or multiparous adult ewes, using CT scanning, over three distinct physiological states: non-pregnant, pregnant and lactating. CT scanning is a highly accurate method to predict lean, fat and bone weight in sheep (Kvame and Vangen, 2006). To date most CT scanning procedures in sheep have been used for genetic improvement programs targeting the enhancement of lean tissue growth based on index selection (Bünger *et al.*, 2011) rather than for scientific research purposes, such as the aim reported here to compare body compositional changes in ewes born to dams differing in age and maturity.

The results from this study demonstrated that the allometric coefficient (a) for subcutaneous fat, intermuscular fat, and internal (visceral) fat relative to either total fat or carcass weight did not differ between the two ewe groups (ELP and AEP) at any of the three different physiological stages. This is contrary to the findings in Chapter 4 and Kenyon *et al.* (2009), where one year old singleton male progeny born to ewe-lamb dams, tended to have more visceral fat than those born to mature adult ewe dams. This disparity could be due to gender and the regulation of fat deposition in males versus females. Typically males are leaner than females (Thornton and Tume, 1987) and have a lower percentage of body fat (Blaak, 2001). If males accumulate fat it is largely as visceral (abdominal) fat compared with females which accumulate fat predominantly in the gluteal-femoral region (Blaak, 2001). Thus the gender difference in generalised fat deposition may explain the lack of difference found in relation to visceral fat accumulation in the ewes examined in this study. The CT scan did not include the complete gluteal-femoral region and thus we are unable to examine fat deposition in this

region. Another potential issue with this study is the sample size; low numbers reduce the statistical power of a study and reduce its ability to detect true differences.

Non-fat visceral component (NFVC), lean and bone weights did not differ between ewe groups during any of the physiological stages analysed. However, predicted carcass weights and total tissue weights were greater for AEP compared with ELP during pregnancy. The reasons for this difference in pregnancy only are unclear. The predicted carcass weights and total weights do not differ before pregnancy and nor do they differ in lactation.

This study also examined mammary gland composition, which can have direct implications for milk yield (McFadden *et al.*, 1990). The architecture of the mammary gland did not differ between the ewe groups. Milk production will be measured in a subsequent study to determine if there are any lactational performance differences between the ewe groups.

CT scanning as a technique to measure the body composition provides accurate estimation (Young *et al.*, 2001), especially with the utilisation of the Cavalieri approach, which is a highly accurate method showing R^2 prediction value ranges of 95% to 97% (Young *et al.*, 2001). In this study, the CT technique was successfully used in young ewes and mature adult ewes and demonstrated that results can be used for tissue weight prediction (lean, adiposity and bone) and comparison between groups of animals in different physiological states. This technique permits a non-lethal, non-invasive whole body examination of tissue depots, mobilisation and identification of tissues (Jopson *et al.*, 1997).

CONCLUSIONS

In this study, CT scanning indicated that ELP and AEP did not differ in abdominal adiposity or mammary gland composition when empty (non-pregnant), pregnant or lactating. These results suggest that dam age is unlikely to greatly alter ewe offspring body composition or deposition/mobilisation of abdominal and mammary fat depots during different phases of the sheep breeding cycle. Further studies with increased animal numbers would be required to more confidently assess the impact of maternal age on ewe offspring body composition.

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Chapter 6

Singleton female offspring born to adult ewes are heavier than those born to ewe-lambs but their reproduction and milk production are unaffected

Foreword:

This chapter was published in *Animal Production Science* based on a presentation at the 2nd Joint Conference of the New Zealand & Australian Societies of Animal Production (“Loureiro, M. F. P., Pain, S. J., Kenyon, P. R., Peterson, S. W., & Blair, H. T. (2012). Single female offspring born to primiparous ewe-lambs are lighter than those born to adult multiparous ewes but their reproduction and milk production are unaffected. *Animal Production Science*, 52(7), 552-556.”) (**Appendix 3**).

In previous chapters we have stated that fetuses, in the last week of gestation (range 139-145 days), from young ewe-lamb dams tend to be lighter than those from adult ewes and singleton male offspring born to ewe-lambs had lighter birthweight and were still lighter until at least one year of age.

Single female offspring born to primiparous ewe-lambs are lighter than those born to adult multiparous ewes but their reproduction and milk production are unaffected

ABSTRACT

Little is known about the long-term impacts of selecting progeny born to ewe-lambs as replacements. This study investigated whether being born to a ewe-lamb affected the live weight, milk production and reproductive performance of the offspring to 3 years of age in comparison with those born to adult multiparous ewes. The study included 27 and 28 singleton-born Romney ewe progeny that were born to either ewe-lambs (EL) or to adult ewes (AE), respectively. Offspring born to ewe-lambs (ELP) were lighter ($P < 0.001$) at birth and up to 12 months of age compared with offspring born to adult ewes (AEP). Reproductive performance of AEP and ELP did not differ in regards to puberty attainment, pregnancy rate and number of fetuses. First lactation milk production, fat, crude protein, total protein, casein, lactose and total solids yield did not differ ($P > 0.05$). The second generation offspring born to ELP and AEP did not differ ($P > 0.05$) in birthweight in the first parity, but it did differ ($P < 0.05$) in the second parity. Those lambs born to ELP were heavier ($P < 0.05$) from birth to weaning compared with those lambs born to AEP. Combined, these results indicate, in the animals used in our study that productive performance of ewes born to ewe-lambs does not differ to 3 years of age to that of ewes born to adult ewe.

INTRODUCTION

Over the last 15 years approximately one-third of ewe-lambs (EL) in New Zealand (7-9 months of age) have been bred each year (Anon. 2011). A potential advantage of breeding EL, is an increase in genetic gain through reducing the generation interval (Kenyon *et al.* 2004) by selecting ewe progeny born to EL. However, little is known about the productive performance of progeny born to young ewes.

Among studies in humans, there is some evidence of negative impacts on both the offspring and the first parity/young mother (Lucas *et al.* 1999). Children tend to be lighter (Scholl *et al.* 1994), have lower survival rates, altered metabolic pathways, a predisposition to obesity and increased risk of coronary heart disease (Friede *et al.* 1987; Geronimus *et al.* 1994; Nowak *et al.* 2000; Barker 2001). The first parity/young mother has an increased risk of premature and/or caesarean delivery, anaemia and maternal death (Scholl *et al.* 1994). In sheep it has been shown that nutrient partitioning can be changed during a young dams first pregnancy (Wallace 2000), which might have implications for the progeny.

Therefore, this study aimed to investigate the effects of being born to either an adult multiparous ewe or an EL on the birth size and live weight, milk production and reproductive performance of the offspring to 3 years of age.

MATERIALS AND METHODS

This study was conducted at Massey University's Keeble farm (latitude 41°10' S, longitude 175°36'E) 5 km south of Palmerston North, New Zealand, during the period September 2007 to January 2011. The study was conducted with the approval of the Massey University Animal Ethics Committee.

Experimental design and measures

Two-hundred and ninety-six Romney primiparous EL (EL, 7-9 months of age) and 307 multiparous Romney adult ewes (AE, 3-5 years of age) had the oestrous synchronised utilising intravaginal progesterone devices (CIDR, Pharmacia & Upjohn, New Zealand) and bred during a 22-day interval to 25 Romney rams. Ewe dams were managed as one group under commercial New Zealand grazing conditions with a minimum post-grazing cover of 1000 kg DM/ha during gestation. At breeding the EL weighed 40.0 ± 0.47 kg and AE 62.2 ± 0.46 kg. The present study utilised 27 and 28 singleton-born Romney ewe progeny born ewe-lambs (EL) or adult ewe (AE), respectively, that were randomly selected at birth (**Figure 1A**).

Within 12 h of birth lambs born to EL and AE were weighed and measurements taken for crown-rump length (CRL), thoracic girth (TG), fore-leg length [from elbow (cubital) joint to toe of the hoof] and hind-leg length [from hip (coxo-femoral) joint to toe of the hoof]. Lambs were born over a 21-day period. The midpoint of lambing was termed d1. The ELP and AEP were weighed again at d30, d74, d130 and a further 18 times during the period to d1198 (**Figure 6**). Body condition score was recorded (scale of 1-5; Jeffries 1961) at d588, d735, d940 and d1080.

Reproductive measures of ewes (AEP and ELP - 2008, 2009 and 2010)

2008

Oestrus activity, an indicator of puberty, during the period d242-d259 (approximately 8 months of age), was assessed using crayon-harnessed vasectomised rams. The presence of any crayon mark on the rump of ewes was indicative of oestrus activity. The oestrus activity was assessed only on a single occasion, progesterone levels were not measured.

2009 and 2010

At d596, ewes born to ewe-lambs (ELP, n = 25) and ewes born to adult ewes (AEP, n = 28) had the oestrous synchronised utilising intravaginal progesterone devices (CIDR, Pharmacia & Upjohn, New Zealand), pregnancy diagnosed 50 days after the end of joining, via transabdominal ultrasound using a real time B-mode scanner with a 5-MHz linear probe (Aloka SSD-500, Aloka Co. Ltd, Tokyo, Japan). In the following year at d945 (n = 25 ELP and 27 AEP) ewes had their oestrous synchronised utilising intravaginal progesterone devices (CIDR, Pharmacia & Upjohn, New Zealand) and were bred with Romney rams for a total of 22 days. Ewes were then pregnancy diagnosed via ultrasound 50 days after the end of joining.

Progeny (G₂ lamb birth and weaning weights) in 2009 and 2010

In 2009, 50 lambs were born (n = 23 ELP-G_{2a} and 27 AEP-G_{2a}). These offspring were weighed and their birth rank and sex determined within 12 h of birth. G_{2a} lambs were re-weighed at average D49 and D91.

In 2010, 70 (n = 27 ELP-G_{2b} and 43 AEP-G_{2b}) lambs were weighed and their birth rank and sex determined and measurements of CRL and TG taken within 12 h after birth. Lambs were re-weighed at average age 28, 50 and 105 days.

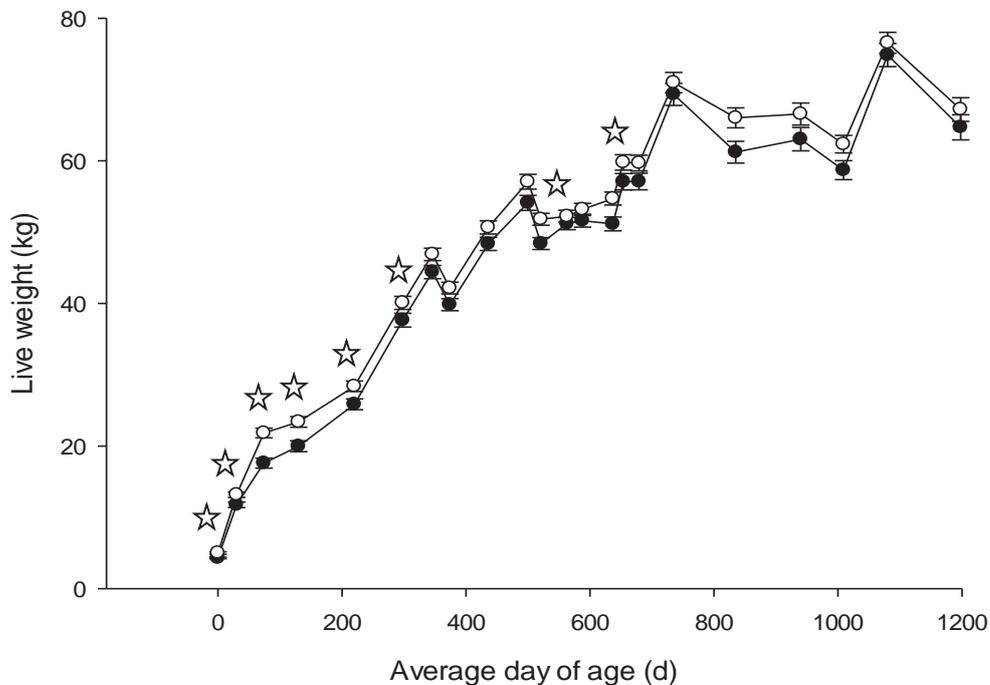


Figure 6. Live weights from birth (d0) to d1198 of offspring born to ewe-lambs (ELP, closed circles) or adult ewes (AEP, open circles). Data presented are least square means \pm standard error. Stars indicate values that significantly differ ($P < 0.05$).

Milk production of ewes (AEP and ELP - 2009)

Single-bearing and rearing ewes ($n = 12$ ELP and 12 AEP) were milked 7 days (average, range of 6-8 days) post-lambing and re-milked once a week for 6 consecutive weeks. Ewes were selected for measurement of milk production according to their lamb survival. Ewes were milked using the 'oxytocin' method by machine followed by hand-stripping once per week during 6 consecutive weeks, as previously described by Morgan *et al.* (2006) and van der Linden *et al.* (2010). Ewes were milked in the

morning and after an interval of ~5 h, in the afternoon, the time and weight of each milking was recorded. Daily milk yield was calculated using the formula:

$$24 \text{ hours} / \text{time between milkings} \times \text{milk yield at 2}^{\text{nd}} \text{ milking}$$

Milk obtained by machine and hand-stripping was mixed and subsampled for analysis of milk composition. Samples were preserved with bronopol and refrigerated at 4°C until analysis (% crude protein, % casein, % fat, % lactose and % true protein and total solids) was determined by infrared spectroscopy using a Milkoscan FT120 (Foss, USA), calibrated for sheep milk (DairyNZ, Hamilton, New Zealand).

Statistical analysis

Statistical analyses were performed with Minitab[®] (version 16, Minitab Inc, Cary NC, USA) using the General Linear Model (GLM) procedure and SAS (SAS, Enterprise Guide 4.2) using the GENMOD procedure, MIXED procedure and Legendre polynomial (details outlined below).

Ewe progeny live weights were analysed with dam type (EL versus AE) as a main effect and date of birth as a covariate. When ewe progeny were themselves pregnant the number of fetuses they carried was included as an additional fixed effect. To account for effects over time (EL, n = 22 and AE, n = 25) ewes that had all live weight data at all points were analysed using repeated-measures via the mixed linear model that included fixed effects of dam type and days of age, and random effect individual with date of birth as a covariate.

The total proportion of ewes that reached puberty up to d259 was analysed with the GENMOD procedure with a linear model that included the fixed effect dam type and the covariate of ewe live weight at d220.

Lamb sizes (AEP and ELP) measured at birth were analysed using a GLM model with date of birth as a covariate and with, and without, birthweight as an additional covariate and dam type as a fixed effect.

In 2009, G_{2a} lamb birthweight and D49 and D91 live weights were analysed with granddam type (EL vs. AE), G_2 birth rank and sex as a fixed effects and two- and three-way interactions were tested with, date of birth as a covariate. There were no significant ($P > 0.05$) two- and three-way interactions between G_2 birth rank, sex and granddam type, therefore they were removed from the final model.

In 2010, G_{2b} lamb birthweight, D28, D50 and D105 live weights and measurements (CRL and TG) were analysed with granddam type, G_2 birth rank and sex as a fixed effects and two- and three-way interactions were tested with date of birth as a covariate. There were no significant ($P > 0.05$) two- and three- way interactions between G_2 birth rank, sex and granddam type, therefore these were removed from the final model.

A third-degree orthogonal polynomial (Legendre model) was fitted to milk yield and composition data for each animal (van der Linden *et al.* 2009):

$$\gamma_i = \alpha_0 \varphi_{0i} + \alpha_1 \varphi_{1i} + \alpha_2 \varphi_{2i} + \alpha_3 \varphi_{3i} + \epsilon_i$$

where γ_i is the record of milk or composition taken at day *ith* (*day of milking*), α_n is the *n* regression, and φ_{ni} is rescaled value of day in milk *i* calculated as:

$$\varphi_{0i} = 1; \varphi_{1i} = \chi; \varphi_{2i} = \frac{(3\chi^2 - 1)}{2}; \varphi_{3i} = \frac{(5\chi^3 - 3\chi)}{2}; \chi = \frac{2[i - (42 + 1)]}{(42 - 1)}$$

and e_i is the error term.

The accumulated yields of milk, fat, crude protein, true protein, casein, lactose and total solids were analysed using a GLM model, which included fixed effects of dam type and sex, date of birth, live weight and G_{2a} birthweight as a covariate. Unrealistic data points (5 of 144 data points) values were excluded.

RESULTS

Measurement at birth to 3 years of age of G_1

ELP lambs had smaller ($P < 0.05$) fore-leg measurements than AEP lambs (**Table 11**). There were no differences ($P > 0.05$) in CRL and hind-leg length between ELP and AEP lambs. When the data was re-analysed with birthweight as a covariate the difference in fore-leg length was no longer apparent (38.2 ± 0.58 cm, 39.0 ± 0.57 cm, ELP and AEP, respectively; $P > 0.05$).

ELP lambs were lighter ($P < 0.001$) within 12 h of birth and up to 12 months of age (d1, 4.2 ± 0.16 versus 5.0 ± 0.16 kg; d30, 11.8 ± 0.38 versus 13.2 ± 0.37 kg; d74, 17.6 ± 0.69 versus 21.8 ± 0.68 kg; d130, 20.0 ± 0.76 versus 23.4 ± 0.75 kg; d222, 25.9 ± 0.75 versus 28.4 ± 0.74 kg) compared with AEP ewes, respectively (**Figure 6**). After 12 months of age there were no differences in live weight except on a single occasion (d521, ELP 48.4 ± 0.84 kg versus AEP 51.8 ± 0.84 kg). When live weights of ewes (ELP and AEP) post birth until d588 were re-analysed with birthweight as a covariate, the only difference in live weight was at d74 ($P < 0.01$, 18.2 ± 0.70 kg versus 21.2 ± 0.70 kg for ELP and AEP, respectively).

During their first pregnancy in 2009 ewe progeny live weight differed in early pregnancy (d637) such that AEP were heavier than ELP ewes (54.8 ± 0.99 versus 51.4 ± 1.09 kg, respectively, $P < 0.05$). In their second pregnancy (2010), 64 days post-breeding (d1009) ELP ewes tended to be lighter than AEP ewes (58.6 ± 1.54 versus 62.2 ± 1.46 kg, respectively; $P < 0.06$). When live weight was analysed via repeated measurement the main effects of dam type and time were significantly different ($P < 0.05$), with ELP ewes always being lighter ($P < 0.05$) than AEP, and there was no interaction ($P > 0.05$) between dam type and time.

Body condition score did not differ between ELP and AEP ewes ($P > 0.05$) at pre-mating (d588) and pre-lambing (d735) in 2009 and at d940 and d1080 in 2010.

Table 11. The effect of dam age [born to either primiparous ewe-lambs (ELP) or multiparous adult ewes (AEP)] on birthweight, crown-rump length (CRL), thoracic girth (TG), fore-leg length and hind-leg length of lambs. Data presented are least square means \pm standard error. Different letters within rows indicate means that significantly differ ($P < 0.05$).

Parameter	ELP	AEP
Birthweight (kg)	4.2 ± 0.16^a	5.0 ± 0.16^b
CRL (cm)	52.8 ± 0.81	54.7 ± 0.80
TG (mm)	39.4 ± 0.73	41.6 ± 0.72
Fore-leg (cm)	37.5 ± 0.61^a	39.60 ± 0.60^b
Hind-leg (cm)	39.0 ± 0.60	40.0 ± 0.59

Reproductive performance of offspring (AEP and ELP)

Puberty attainment

Oestrus activity as indicated by crayon marks on the rumps of ewes (AEP and ELP) between d242 and d259 did not differ ($P > 0.05$) between groups (ELP $15.2\% \pm 0.62$ versus AEP $5.10\% \pm 0.85$, $P > 0.05$). Indicating there was no difference ($P > 0.05$) in the proportion, which had achieved puberty by this age.

Pregnancy rate and number of fetus per ewe

Pregnancy rate in 2009 and 2010 did not differ between groups ($P > 0.05$). Also the number of fetus (es) per ewe presented for breeding did not differ between groups ($P > 0.05$; 2009 = 0.92 ± 0.14 versus 0.93 ± 0.13 ; 2010 = 1.26 ± 0.16 versus 1.56 ± 0.16 , for ELP and AEP, respectively).

G₂ birthweight and live weight to weaning

2009

Singleton G_{2a} offspring were heavier than twins at birth, D49 and D91 (**Table 12**, $P < 0.05$). However, live weights between ELP-G_{2a} and AEP-G_{2a} lambs did not differ ($P > 0.05$) at any of measurement points (**Table 12**).

2010

Singletons were heavier than multiples at all measurement points (**Table 12**, $P < 0.05$). ELP-G_{2b} lambs were heavier ($P < 0.05$) at birth, D28, D50 and D105 than AEP-G_{2b} lambs. Compared with multiple-born lambs, singleton-born lambs had larger CRL (58.4 ± 0.60 versus 55.5 ± 0.37 cm for singleton- or multiple born, $P < 0.05$, respectively) and TG (44.2 ± 0.60 versus 41.5 ± 0.37 cm) for singleton- or multiple born, $P < 0.05$, respectively). There was no difference ($P > 0.05$) in CRL and TG length between AEP-G_{2b} and ELP-G_{2b} lambs. Re-analysis of G_{2b} lambs data with birthweight as a covariate did not change any of the forementioned relationships.

Table 12. The effect of dam age [born to either primiparous ewe-lambs (ELP) or multiparous adult ewes (AEP)] and birth rank on G_{2a} live weight at birth (D1), D49 and D91 and G_{2b} live weight at birth, D50 and D105. Data presented are least square means ± standard error. Different letters within row sections indicate values that significantly differ ($P < 0.05$).

	Birth rank				Granddam			
	n	Singleton	n	Multiples	n	EL	n	AE
A								
G _{2a}								
Birth	28	5.6 ± 0.12 ^b	22	4.2 ± 0.14 ^a	23	4.9 ± 0.14	27	4.9 ± 0.13
d49	27	22.3 ± 0.62 ^b	21	16.1 ± 0.70 ^a	23	19.6 ± 0.68	25	18.8 ± 0.65
d91	26	31.3 ± 0.90 ^b	21	23.6 ± 1.01 ^a	22	28.1 ± 0.99	25	26.8 ± 0.93
B								
G _{2b}								
Birth	16	6.4 ± 0.22 ^b	44	5.7 ± 0.14 ^a	26	6.4 ± 0.19 ^b	34	5.8 ± 0.18 ^a
d50	16	21.8 ± 0.74 ^b	34	19.1 ± 0.52 ^a	20	21.8 ± 0.70 ^b	30	19.2 ± 0.60 ^a
d105	16	36.4 ± 1.10 ^b	34	30.8 ± 0.77 ^a	20	35.7 ± 1.01 ^b	30	31.6 ± 0.90 ^a

G_{2a} offspring born in 2009.

G_{2b} offspring born in 2010.

Milk production of ewes in first lactation (AEP and ELP - 2009)

The accumulated yields of milk, fat, crude protein, true protein, casein, lactose and total solids of AEP and ELP ewes did not differ ($P > 0.05$, **Table 13**).

Table 13. Accumulated milk, lactose, crude protein and fat yield (%) over 42 days of lactation of lambs born to either primiparous ewe-lambs (ELP, n = 12) or multiparous adult ewes (AEP, n = 12). Data presented are least square means \pm standard error.

	ELP	AEP
Milk yield (%)	85.86 \pm 4.30	80.13 \pm 4.33
Lactose yield (%)	5.33 \pm 0.04	5.38 \pm 0.04
Crude protein (%)	5.20 \pm 0.09	5.10 \pm 0.09
Fat yield (%)	6.78 \pm 0.43	7.07 \pm 0.43

DISCUSSION

The aim of this study was to compare the productive performance of offspring born to either primiparous EL or mature multiparous ewes to 3 years of age. The offspring born to primiparous EL were lighter and smaller at birth and consistently remained lighter until 12 months of age and then were lighter on occasion until 3 years of age. This is comparable to previous studies where offspring born to young adolescent mothers were reported to be lighter and smaller at birth (Vesely *et al.* 1970; Al-Shorepy 2001; Annett and Carson 2006; Gootwine *et al.* 2006, 2007) and lighter to 12 months of age (Vesely *et al.* 1970). It is hypothesised that their adolescent dams may not achieved their mature bodyweight, before their pregnancy and consequently they are less able to partition nutrients to their developing fetus and milk production which affected their progenies' early live weights. Interestingly, Wu *et al.* (2006) stated that it was maturity of the dam, and not parity that affected intrauterine growth further suggesting a size/live weight effect. Gootwine *et al.* (2007) found that birthweights of lambs born to primiparous ewes became similar to lambs from mature multiparous ewes only after the primiparous dams were 21 months of age. Gootwine *et al.* (2007) further suggested that it was not parity *per se* that restricted fetal growth but rather the capacity of the uterine

environment and/or the ability of the dam to partition nutrients to the fetus that affected birthweight of the offspring.

The lower live weight of the ewes born to EL did not appear to compromise reproductive performance relative to progeny from AE. Interestingly, ELP ewes were lighter early in their first pregnancy and showed a tendency to be lighter early in their second pregnancy. Although this live weight difference was minimal (~3 kg), it may suggest potential differences in their ability to cope with a pregnancy and which might affect the birthweight of their lambs.

Milk yield at first lactation was not affected by dam age. This matches the lack of difference observed for live weight of the G_{2a} lambs. There appears to be no other studies comparing lactational performance of offspring born to young or mature dams. Therefore, the evidence to date suggests milk production of the progeny is not affected by their dam's age.

G_{2b} lambs born to EL granddams were heavier from birth to weaning compared with those lambs born to mature AE granddams. This was not observed for G_{2a} lambs. Similar results were observed in birthweight by Blair *et al.* (2010) where second parity offspring born to restrictively fed granddams were heavier than those offspring born to granddams that received maintenance levels of feeding during pregnancy, no such result was observed in lambs born to the first parity. It is unknown why differences appear between parities.

In summary, under the conditions of the present study, we have demonstrated that live weight of offspring born to EL were lighter until at least 12 months of age. However, there were no differences in puberty attainment, pregnancy rates and milk production to 3 years of age. The present findings are of importance to farmers as they indicate that

although differences in live weight can occur, there were no longer-term effects on reproductive and lactational performance. Further studies should consider utilising either lighter or/and heavier EL at joining. Also, increased animal numbers would be necessary to more confidently conclude that the productivity and reproductive performance of EL progeny is similar to that of progeny born to mature ewes. In addition it would be of benefit to examine the relationship in twin born progeny. Lifetime performance of progeny born to EL compared with AE is also required.

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Chapter 7

Twin female offspring born to ewe-lambs have lower growth performance compared with those born to adult ewes

Foreword:

This chapter was published in part as brief communication in Proceedings of the New Zealand Society of Animal Production based on a presentation at the 71st Conference of the New Zealand Society of Animal Production (“Loureiro, M. F. P., Paten, A. M., Asmad, K., Pain, S. J., Kenyon, P. R., Pomroy, W. E., Blair, H. T. (2011). The effect of dam age and lamb birth rank on the growth rate, faecal egg count and onset of puberty of single and twin female offspring to 12 month of age. *Proceedings of the New Zealand Society of Animal Production*, 71, 83-85.”) and abstract/oral presentation at the 2011 National Research Centre for Growth and Development Science Symposium (“Loureiro M.F.P., Paten A.M., Asmad K., Pain S.J., Kenyon P.R., Pomroy W.E., Scott I., Blair H.T. (2011). The effect of dam age and parity and lamb birth rank on the growth rate, and onset of puberty of the offspring. *NRCGD Science Symposium, Dunedin, New Zealand.*”) (**Appendix 4**).

ABSTRACT

There is a growing body of evidence that younger ewes give birth to lighter lambs compared with mature ewes and ample evidence that twin-bearing ewes give birth to lighter lambs than singleton-bearing ewes. To date, however, this combination has not been examined in one study. The aim of this study was to investigate the effects of dam age and parity on the growth trajectory of singleton and twin ewe offspring. The study utilised 104 dams (30 singleton-bearing ewe-lambs (EL; 8-9 months at breeding); 18 singleton-bearing mature aged ewes (AE: 3-5 years old); 24 twin-bearing ewe-lambs; 32 twin-bearing mature aged ewes) and their female offspring. The offspring were weighed at birth (d1) and then monthly until 18 months of age (d550) and morphometric measurements taken at d409 and d550. Lambs born to adult ewes (AEP) were heavier at birth than those born to ewe-lamb (ELP) ($P < 0.05$) and singleton-born lambs were heavier than twin-born ($P < 0.05$). At weaning, there was an interaction ($P < 0.05$) between dam age and birth rank for lamb live weight, whereby AEP-singleton were heavier ($P < 0.05$) than all other groups and ELP-twin were lighter than all other groups ($P < 0.05$), while ELP-singleton and AEP-twin did not differ from each other ($P > 0.05$). ELP were lighter ($P < 0.05$) than AEP and twin-born lambs were lighter ($P < 0.05$) than singleton-born lambs from d186 to d550. In addition to being lighter, morphometric data demonstrated that ELP were proportionally smaller ($P < 0.05$) than AEP for fore-leg length and thoracic girth measurements. This study illustrates that the live weight of twin female progeny born to ewe-lambs dams is impaired to at least 18 months of age. If these differences are maintained to adulthood, twin ewes born to ewe-lamb dams may be less suitable for use as replacement ewes, because it may influence reproductive performance if it has a negative influence. This aspect requires further study.

INTRODUCTION

In Chapters 4 and 6 of this thesis, it was demonstrated that singleton female and male offspring born to ewe-lamb dams (8-9 months at breeding) were lighter than offspring born to adult ewes until at least one year of age. Gardner *et al.* (2007) showed that young primiparous ewes gave birth to lighter lambs compared with mature multiparous ewes. Redmer *et al.* (2004) and Wu *et al.* (2006) stated that the lighter weight of offspring born to young dams is due to the dam's stage of maturity rather than her parity. This is thought to be due to competition for nutrients between the growing adolescent mother and her growing conceptus, with nutrient partitioning tending to favor continued growth of the dam at the expense of the fetus (Wallace, 2000). To date, few studies have compared progeny born to either adult ewes (AEP) or ewe-lambs (ELP), when both age groups were bred together. Also there are few studies that have followed those offspring post-weaning. Further, no studies have examined potential effects in twin-born progeny.

Schreurs *et al.* (2010) comparing singleton and twin lambs born to ewe-lambs found that twins lambs were 23% lighter than singleton lambs at birth and 16% lighter at weaning. A similar relationship has been found with mature ewes (Sidwell, 1956; Kenyon *et al.*, 2009). Twins can remain lighter post-weaning into adulthood (Gootwine *et al.*, 2007; Kenyon *et al.*, 2008b).

This study was designed to examine the growth trajectories and morphometric characteristics of singleton and twin offspring born to either adult ewes or ewe-lamb dams. The adult ewes and ewe-lambs dams were maintained together under commercial conditions throughout breeding, pregnancy and lactation. Their progeny were maintained together until 18 months of age under commercial conditions.

MATERIALS AND METHODS

This study was conducted at both Massey University's Riverside Farm (latitude 40° 50'S, longitude 175° 37'E) 11km north of Masterton and Keeble Farm (latitude 41° 10'S, longitude 175° 36'E) 5 km south of Palmerston North, New Zealand. During the period of September 2009 (birth) to January 2010 (weaning) experimental animals were managed on Riverside Farm and post-weaning until April 2011 they were maintained at Keeble Farm. The study was conducted with the approval of the Massey University Animal Ethics Committee.

Experimental design and measures

Background

The study utilised 104 naturally-mated Romney dams, that were selected from a larger mob that originally contained 400 ewe-lambs (EL; 8 to 9 months of age) and 399 adult ewes (AE; 3 to 5 years of age) (Corner *et al.*, 2013). Ewes were naturally bred as one cohort with mature, crayon-harnessed, mixed-age, Romney composite rams for 34 days, at a ram to ewe ratio of 1:40. The ewes were managed together under commercial New Zealand grazing conditions (Corner *at el.*, 2013), with a minimum herbage mass of 1000 kg DM/ha during the breeding and pregnancy period and a minimum of 1200 kg DM/ha pasture mass provided for the lambing and lactation period. The present study utilised a randomly selected sub-set of ewe-lambs and mature adult ewes from this larger cohort (**Figure 1B**).

Present study

The present study utilised 30 singleton-bearing primiparous EL (average live weight (LW) at breeding: 46.2 ± 0.7 kg), 18 singleton-bearing multiparous AE (average LW at breeding: 66.0 ± 0.9 kg); 24 twin-bearing primiparous EL (average LW at breeding:

47.4 ± 0.7 kg) and 32 twin-bearing multiparous AE (average LW at breeding: 67.3 ± 0.9 kg) and their progeny. The female offspring were managed together, as one cohort, from birth to 18 months of age and are the focus of this chapter. In total there were four progeny groups: singleton-born female offspring born to ewe-lamb dams (ELP-S; n = 28), twin-born female offspring born to ewe-lamb dams (ELP-T; n = 29), singleton-born female offspring born to adult ewe dams (AEP-S; n = 17) and twin-born female offspring born to adult ewe dams (AEP-T; n = 42).

Measurements at birth

The midpoint of the lambing period of the dams was defined as day 1 (d1). Within 12 hours of birth, lambs were weighed and their crown-rump length (CRL) and thoracic girth (TG) measured.

Live weights from d38 to d550 and body condition score

All female progeny were weighed approximately monthly from d38 to d550, a total of 22 LW measurements. Weighing occurred within one hour directly off pasture. Body condition score (BCS), to evaluate the level of body fat reserves, was recorded (scale of 1 to 5; Jeffries, 1961) at d367, d410 and d550.

Backfat and eye muscle area

Measurements of backfat and eye muscle area were taken at d410 using an ultrasound device (Aloka SSD-500, Aloka Co. Ltd., Tokyo, Japan) with a linear probe of 5MHz and mineral oil as a conductive medium. Backfat depth, as an indicator of fatness, was measured over the left side of the animals between the 3rd and 4th lumbar vertebrae, 5cm from the midline (vertebral column). Eye muscle area (EMA), as an indicator of muscling, was measured between the 3rd and 4th lumbar vertebrae, 5cm from the midline over the *Longissimus dorsi* muscle (loin). The maximum depth was taken on the long axis of the cross section of the *Longissimus dorsi* and width was the largest

perpendicular measurement of this section (Silva *et al.*, 2005). Depth and width of the muscle were recorded. Eye muscle area was calculated using the formula:

$$EMA = (\text{muscle width} \times \text{muscle depth}) \times 0.0077$$

Puberty attainment

Oestrus activity, as an indicator of puberty attainment, was recorded over a period of 17 days at approximately 8 months of age (d236-d253, May-June 2010; winter solstice 21 June). Puberty attainment was evaluated using crayon-harnessed vasectomised rams. The presence of any crayon mark on the rump of the ewes was indicative of oestrus activity. The oestrus activity was assessed only in a single occasion, progesterone levels were not checked.

Faecal egg count

A single faecal egg count (FEC; *Nematodirus sp.* and Strongylid eggs per gram faeces) was performed at d257. Faecal samples were collected from each ewe and refrigerated until the FEC measurements (maximum 24hrs of refrigeration). The FEC procedure was conducted using 2g of faeces per animal, diluted in a saturated salt solution, sieved and placed in both chambers of a McMaster slide. Eggs were counted under a microscope (Olympus CX41, Japan) at a magnification of $\times 60$ and multiplied by 50 to get the quantity of parasite eggs per gram.

Morphometric measurements

The size of the ewe progeny at d409 (ELP-S, n = 7; ELP-T, n= 3; AEP-S, n = 5; AEP-T, n = 4), and d550 (ELP-S, n = 10; ELP-T, n = 11; AEP-S, n = 11; AEP-T, n = 11) was assessed using morphometric measurement (Brown *et al.*, 1973) of seven different body parts (**Figure 7**): thoracic girth (around the axillae area), fore-leg length (from top of the

humerus bone to the ground level, lined up with the sole of the hoof), hind-leg length (from the coxofemoral joint to the ground level, lined up with the sole of the hoof), body length (cranial part of the top of the humerus to the coxofemoral joint), rump width (distance between the iliac crest), head length (base of the nose to the line of occipital condyles) and head width (external corner between the upper and lower eyelid of each side). Measurements were taken with animals in a stationary standing position. Additional parameters were calculated based on morphometric measurements. These included: body capacity (LW divided by body length; Júnior *et al.*, 2006) and body volume (volume = $3.14 \times [(\text{girth}/3.14)/2]^2 \times \text{body length}$).

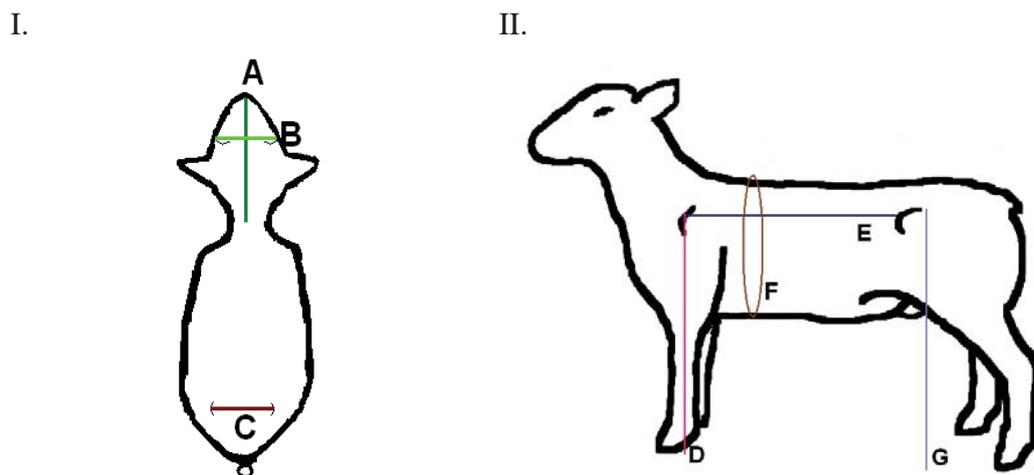


Figure 7. Relative positions of body measures taken on sheep; I. A: head length; B: head width and C: rump width; II. D: fore-leg length; E: body length; F: thoracic girth; G: hind-leg length.

Statistical analysis

Statistical analysis was performed with either Minitab[®] (version 16, Minitab Inc., Cary NC, USA) or SAS (SAS, *Enterprise Guide*[®] 4.2). The study design tested for the main effects of dam age and birth rank and the potential interaction between dam age (EL or AE) and birth rank (singleton vs. twin). Therefore, in all models, even if the interaction was non-significant ($P > 0.05$), the two-way interaction remained in the model to allow for testing of the study design.

Measurements at birth

Birthweight and body dimensions at birth (CRL and TG) were analysed using the GLM procedure in Minitab and included the fixed effects of dam type, birth rank and the two-way interaction between dam type and birth rank. Date of birth was included as a covariate, and the model was fitted with and without birthweight as an additional covariate. The birth rank was classified as being either a singleton or twin, however twin group includes both twin and triplets.

Live weights from d38 to d550

Live weights were analysed using GLM procedure in Minitab and included the fixed effects of dam type and birth rank, as well as their two-way interaction, with date of birth as a covariate. Birthweight was not used as a covariate, as it was only found to be significant at three (d38, d97 and d410) of the 22 LW measurement points.

Offspring LW and liveweight gain were also analysed as a repeat measures ANOVA using the Mixed procedure in SAS. The model included the fixed effects of dam type, birth rank and days of age; the random effect of animal identification; the date of birth as a covariate.

Body condition, backfat and eye muscle area

Body condition score, backfat and EMA were analysed using the Mixed procedure in SAS and included the fixed effects of dam type, birth rank and their two-way interaction. Live weight at the day of the measurement was used as a covariate.

Puberty attainment

The percentage of female offspring displaying oestrus activity between d236 and d253 was analysed using the GENMOD procedure in SAS, with a binomial distribution that included fixed effects of dam type and birth rank and their interaction. Live weight at d236 was used as a covariate. Mean percentages are presented as back-transformed percentages values with the 95% confidence interval in parentheses.

Faecal egg count

Faecal egg count analysis at d257 included the fixed effects of dam type and birth rank, and their interactions, using the GLM procedure in Minitab. *Nematodirus* sp. FEC data were normalised using a \log_{10} (*Nematodirus* sp. count + 25) transformation and Strongylid FEC data were normalised using a square root transformation. Data are presented as back-transformed values for each parameter. Birthweight and date of birth were fitted as covariates.

Morphometric measurements

Morphometric measurements and body volume at d410 and d550 were analysed using the GLM procedure in Minitab. The models included the fixed effects of dam type, birth rank and their two-way interactions. The date of birth and live weight at the day of measurement were used as covariates. Body capacity was also analysed with the fixed effects of dam type, birth rank, and their two-way interaction. The model initially included date of birth as a covariate, however it was removed from the final analysis as it was not significant ($P > 0.05$).

RESULTS

Measurement at birth

AEP had longer CRL and larger TG than ELP ($P < 0.05$). Singletons lambs had longer CRL and larger TG than twins ($P < 0.05$). There was no interaction ($P > 0.05$) between dam age and birth rank for CRL and TG (**Table 14**).

At birth AEP were heavier than ELP ($P < 0.05$) and singletons were heavier than twins lambs ($P < 0.05$; **Table 15**), although ELP-T had the smallest birthweight compared with the other groups. However there was no interaction ($P > 0.05$) between dam age and birth rank for lamb birthweight.

Table 14. Effect of being born to ewe-lamb dams or adult ewe dams on female progeny singleton and twin born on crown-rump length (CRL) and thoracic girth (TG) measurement at birth.

		n	CRL (cm)	TG (cm)
Dam type	Ewe-lamb	57	48.2 ± 0.44 ^a	38.1 ± 0.3 ^a
	Adult ewe	59	50.7 ± 0.50 ^b	40.1 ± 0.36 ^b
Birth rank	Singleton	45	50.4 ± 0.52 ^b	39.5 ± 0.37 ^a
	Twin	71	48.5 ± 0.40 ^a	38.7 ± 0.28 ^a
Dam type* Birth rank ¹	Ewe-lamb singleton	28	48.9 ± 0.65 ^a	38.1 ± 0.46 ^a
	Ewe-lamb twin	29	47.5 ± 0.64 ^a	38.1 ± 0.46 ^a
	Adult ewe singleton	17	51.9 ± 0.84 ^b	40.8 ± 0.60 ^b
	Adult ewe twin	42	49.5 ± 0.51 ^{ab}	39.3 ± 0.37 ^{ab}

^{ab} different superscripts within columns indicate values that significantly differ ($P < 0.05$)

¹ Dam type by birth rank interaction

Live weights from 38 to 550 days

Significant interactions ($P < 0.05$) were found between dam age and birth rank for lamb live weight at d38 and weaning (d97) such that AEP-S were heavier ($P < 0.05$) than all other groups and ELP-T were lighter ($P < 0.05$) than AEP-T and ELP-S (**Table 15** and **Figure 8**). ELP and twins were consistently lighter ($P < 0.05$) than AEP and singletons, respectively, from day 38 to 550 (**Figure 8**; A. and B.). Interactions between dam type and birth rank ($P < 0.05$) were only observed at d102, d131, d160 and d311 (**Figure 8**, C.). On these occasions it was due to twins born to ewe-lambs being lighter than all other groups.

The average daily weight gain did not differ between lambs born to either ELP or AEP or between singleton- and twin-born lambs ($P > 0.05$) during d1 to d550 (data not shown). There was also no interaction ($P > 0.05$) between dam type and birth rank for average daily weight gain between any time points.

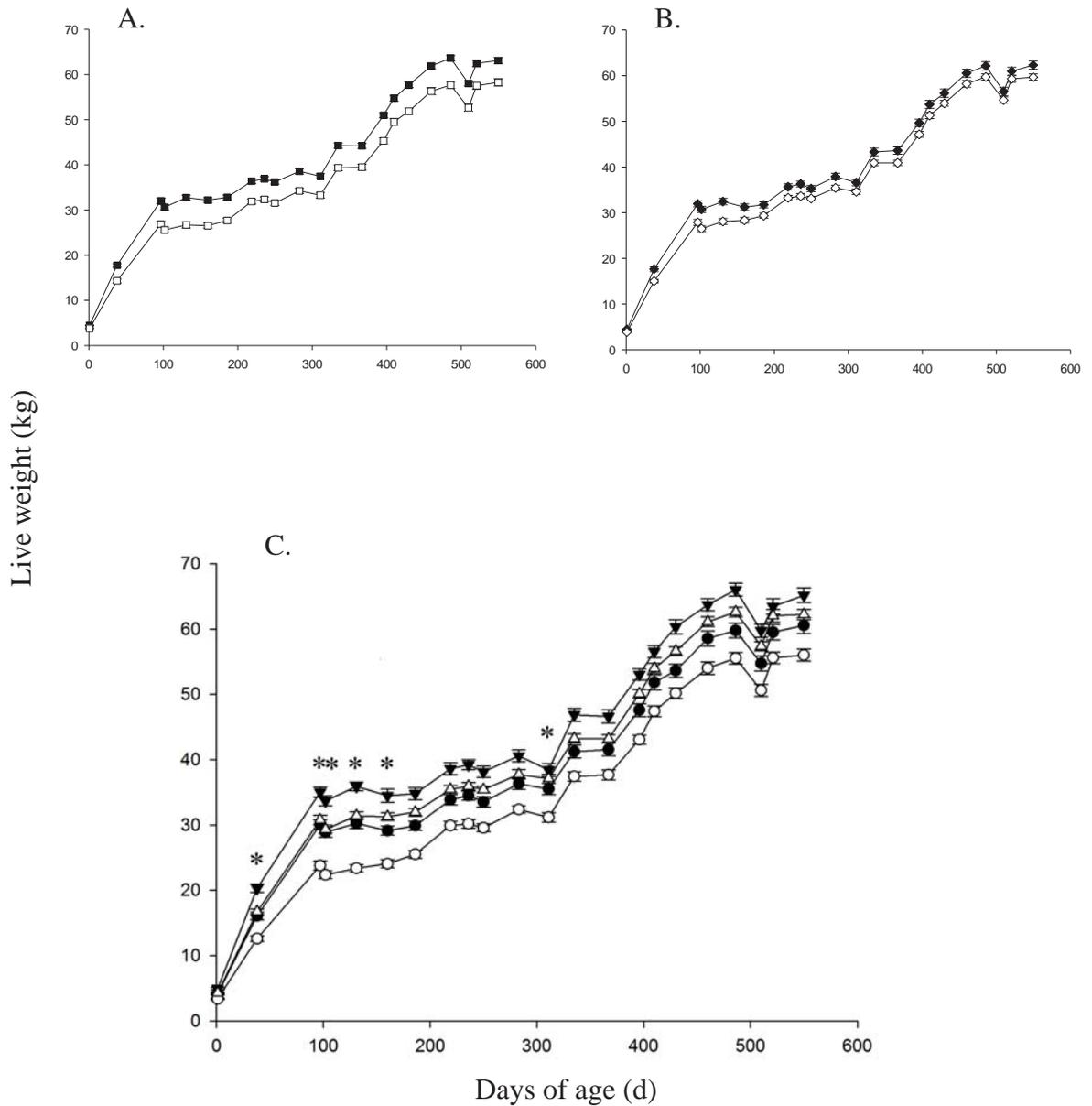


Figure 8. A.: Live weights from birth (d1) to d550 of female offspring born to ewe-lambs (ELP; □) and adult ewes (AEP; ■); B.: Live weights from birth (d1) to d550 of female offspring born as singletons (◆) or twins (◇); C.: Live weights from birth (d1) to d550 of female offspring: ELP-singleton (●), ELP-twins (○), AEP-singleton (▼) or AEP-twins (△). Data presented are Least Square Means \pm standard error. * indicates time points at which the interaction between dam age and birth rank was significantly different ($P < 0.05$).

Table 15. Live weight (kg) from birth (d1) to 550 days of age (d550) of female progeny born to singleton and twin ewe-lamb dams or adult ewe dams. Table shows the least square means \pm standard error and number (n) of female offspring weighed.

	n	d1	n	d97	n	d219	n	d367	n	d550
Dam type										
Ewe-lamb	57	3.7 \pm 0.10 ^a	57	27.2 \pm 0.50 ^a	56	32.1 \pm 0.47 ^a	54	39.7 \pm 0.58 ^a	57	58.3 \pm 0.71 ^a
Adult ewe	59	4.6 \pm 0.11 ^b	58	32.7 \pm 0.54 ^b	59	36.9 \pm 0.51 ^b	58	44.8 \pm 0.61 ^b	58	63.7 \pm 0.77 ^b
Birth rank										
Singleton	45	4.5 \pm 0.12 ^b	45	33.1 \pm 0.59 ^b	45	36.6 \pm 0.56 ^b	42	44.3 \pm 0.67 ^b	45	62.9 \pm 0.83 ^b
Twin	71	3.8 \pm 0.09 ^a	70	26.8 \pm 0.46 ^a	70	32.4 \pm 0.44 ^a	70	40.3 \pm 0.52 ^a	70	59.1 \pm 0.66 ^a
Dam type*										
Birth rank [†]										
Ewe-lamb singleton	28	4.1 \pm 0.15 ^b	28	31.2 \pm 0.75 ^b	28	34.7 \pm 0.72 ^b	25	42.0 \pm 0.89 ^b	28	60.7 \pm 1.07 ^b
Ewe-lamb twin	29	3.4 \pm 0.14 ^a	29	23.2 \pm 0.71 ^a	28	29.5 \pm 0.69 ^a	29	37.5 \pm 0.80 ^a	29	55.9 \pm 1.01 ^a
Adult ewe singleton	17	4.9 \pm 0.18 ^b	17	35.0 \pm 0.91 ^c	17	38.6 \pm 0.86 ^c	17	46.6 \pm 1.02 ^c	17	65.2 \pm 1.30 ^c
Adult ewe twin	42	4.3 \pm 0.12 ^b	41	30.5 \pm 0.59 ^b	42	35.3 \pm 0.55 ^b	41	43.1 \pm 0.66 ^b	41	62.2 \pm 0.84 ^{ab}

^{abc} different superscripts within columns indicate values that significantly differ ($P < 0.05$)

[†] Dam type by birth rank interaction

Body condition, backfat and eye muscle area

Body condition scores at d367, d410, d550 did not differ ($P > 0.05$) between either dam type or birth rank (**Table 16**). There was no interaction ($P > 0.05$) between dam type and birth rank for BCS.

Backfat and EMA at d410 did not differ ($P > 0.05$) due to dam type or lamb birth rank (**Table 16**). There was no interaction ($P > 0.05$) between dam type and birth rank for either backfat or EMA.

Puberty attainment

Despite the differences in live weight observed at d236 (**Table 17**) in female offspring born to EL- or AE-dams and also between singleton- and twin-born there was no difference ($P > 0.05$) in the percentage of female offspring that reached puberty (**Table 17**). There was no interaction ($P > 0.05$) between dam type and birth rank for puberty percentage.

Faecal egg counts

There was an interaction between dam age and birth rank ($P < 0.05$; **Table 18**) for Strongylid sp FEC. Twins born to EL-dams had higher FEC than all other groups. Nematodirus sp. FEC did not differ ($P > 0.05$) between treatment groups and there was no interaction between dam type and birth rank ($P > 0.05$; **Table 18**).

Table 16. Body condition score (BCS), backfat, eye muscle area (EMA) and faecal egg count (FEC) of singleton and twin female progeny born to ewe-lamb dams or adult ewes. Table shows the least square means \pm standard error.

	BCS						Backfat (mm)		EMA (cm ³)	
	n	d367	n	d410	n	d550	n	d410	n	d410
Dam type										
Ewe-lamb	55	2.8 \pm 0.03	55	2.9 \pm 0.04	55	3.7 \pm 0.06	55	4.6 \pm 0.13	55	13.4 \pm 0.20
Adult Ewe	56	2.8 \pm 0.04	56	3.0 \pm 0.04	56	3.7 \pm 0.06	56	4.4 \pm 0.15	56	13.8 \pm 0.23
Birth rank										
Singleton	41	2.8 \pm 0.04	41	2.9 \pm 0.04	41	3.7 \pm 0.06	41	4.4 \pm 0.15	41	13.7 \pm 0.23
Twin	70	2.8 \pm 0.03	70	2.9 \pm 0.03	70	3.7 \pm 0.05	70	4.6 \pm 0.11	70	13.5 \pm 0.18
Dam type* Birth rank [†]										
Ewe-lamb singleton	26	2.8 \pm 0.05	26	2.9 \pm 0.05	26	3.6 \pm 0.08	26	4.4 \pm 0.17	26	13.6 \pm 0.28
Ewe-lamb twin	29	2.8 \pm 0.04	29	2.9 \pm 0.05	29	3.8 \pm 0.08	29	4.9 \pm 0.19	29	13.3 \pm 0.29
Adult ewe singleton	15	2.9 \pm 0.06	15	3.0 \pm 0.07	15	3.8 \pm 0.10	15	4.4 \pm 0.24	15	13.9 \pm 0.38
Adult ewe twin	41	2.8 \pm 0.05	41	2.9 \pm 0.04	41	3.6 \pm 0.06	41	4.4 \pm 0.15	41	13.7 \pm 0.23

^{ab} different superscripts within columns indicate values that significantly differ ($P < 0.05$)

[†] Dam type by birth rank interaction

Table 17. Incidence of puberty attainment at 236 days of age (d236) of singleton and twin female progeny born to ewe-lamb dams or adult ewe dams. Table shows live weight at the day of teaser introduction (d236) as least square means \pm standard error, and the percentage of offspring that reached puberty (\pm 95% confidence interval).

		n	Live weight (kg)	% reaching puberty
Dam type	Ewe-lamb	56	32.5 \pm 0.48 ^a	22.0% (12 – 38)
	Adult Ewe	58	37.5 \pm 0.51 ^b	18.8% (9 – 35)
Birth rank	Singleton	44	37.1 \pm 0.56 ^a	23.8% (12 – 42)
	Twin	70	32.8 \pm 0.44 ^b	17.3% (9 – 30)
Dam type* Birth rank ¹	Ewe-lamb singleton	27	35.1 \pm 0.72 ^b	24.1% (10 – 47)
	Ewe-lamb twin	29	29.9 \pm 0.69 ^a	20.0% (7 – 45)
	Adult ewe singleton	17	39.2 \pm 0.87 ^c	23.5% (8 – 52)
	Adult ewe twin	41	35.8 \pm 0.55 ^b	14.8% (6 – 30)

^{abc} different superscripts within rows indicate values that significantly differ ($P < 0.05$)

¹ Dam type by birth rank interaction

Table 18. Faecal egg count (FEC) at d257 of singleton and twin female progeny born to ewe-lamb dams or adult ewes. FEC values shown are the square root transformation of strongyloides egg/g faeces) and \log_{10} plus 25 of nematodirus egg/g faeces \pm standard error (back transformed values for each are shown in parenthesis).

		n	Strongyloides (egg/g faeces)	n	Nematodirus (egg/g faeces)
Dam type	Ewe-lamb	51	30.4 \pm 1.99 (1093.1 \pm 115.6)	51	1.5 \pm 0.03 (14.0 \pm 5.0)
	Adult Ewe	49	27.5 \pm 2.45 (960.5 \pm 142.1)	49	1.5 \pm 0.04 (10.4 \pm 6.14)
Birth rank	Singleton	38	27.8 \pm 2.57 (1006.2 \pm 148.7)	38	1.5 \pm 0.04 (10.5 \pm 6.42)
	Twin	62	30.2 \pm 1.88 (1047.4 \pm 109.3)	62	1.5 \pm 0.03 (13.9 \pm 4.72)
Dam type* Birth rank ¹	Ewe-lamb singleton	26	26.0 \pm 2.85 ^a (924.1 \pm 165.1)	26	1.5 \pm 0.04 (6.3 \pm 7.13)
	Ewe-lamb twin	25	34.8 \pm 3.10 ^b (1262.0 \pm 179.6)	25	1.6 \pm 0.04 (21.6 \pm 7.75)
	Adult ewe singleton	12	29.6 \pm 4.25 ^a (1088.4 \pm 246.2)	12	1.5 \pm 0.0 (14.6 \pm 10.63)
	Adult ewe twin	37	25.5 \pm 2.25 ^a (832.7 \pm 130.3)	37	1.4 \pm 0.03 (6.2 \pm 5.63)

^{ab} different superscripts within columns indicate values that significantly differ ($P < 0.05$)

¹ Dam type by birth rank interaction

Morphometric measurements

At d409, morphometric measurements revealed that ELP had smaller body length, thoracic girth and rump width ($P < 0.05$; **Table 19**) compared with AEP. There was an interaction between dam type and birth rank for fore-leg length ($P < 0.05$) whereby ELP-twin born offspring were shorter than all other groups (**Table 19**). Twin offspring had smaller fore-leg length and thoracic girth compared with singletons ($P < 0.05$; **Table 19**). Body volume and body capacity did not differ ($P > 0.05$) between groups. There was no interaction ($P > 0.05$) between dam type and birth rank for body length, thoracic girth, hind-leg length, rump width, head length and head width.

At d550, ELP was smaller for fore-leg length, hind-leg length, thoracic girth, head length and head width ($P < 0.05$; **Table 19**) than AEP. Twin born offspring had greater head width than singletons ($P < 0.05$; **Table 19**). Whilst body volume did not differ ($P > 0.05$) between groups; body capacity was greater in AEP compare to ELP (1.3 ± 0.03 vs. 1.2 ± 0.03 , respectively; $P < 0.05$). There was no interaction ($P > 0.05$) between dam type and birth rank for any morphometric measures at d550.

Table 19. Morphometric measurement of singleton and twin female progeny born to ewe-lambs dams or adult ewes at 409 (d409) and 550 days of age (d550). Table shows number (n) of female offspring measured and the least square means \pm standard error.

		n	Body length (cm)	Fore-leg length (cm)	Hind-leg length (cm)	Thoracic girth (cm)	Rump width (cm)	Head length (cm)	Head width (cm)
d409									
Dam type	Ewe-lambs	10	50.0 \pm 1.15 ^a	57.1 \pm 0.86 ^a	60.6 \pm 0.88	94.3 \pm 1.14 ^a	17.7 \pm 0.45 ^a	33.1 \pm 0.40	18.7 \pm 0.31
	Adult ewes	9	54.0 \pm 1.13 ^b	60.2 \pm 0.84 ^b	62.9 \pm 0.87	98.8 \pm 1.12 ^b	20.1 \pm 0.44 ^b	33.6 \pm 0.39	19.5 \pm 0.30
Birth rank	Singleton	12	52.3 \pm 0.93	60.3 \pm 0.70 ^b	62.0 \pm 0.72	98.5 \pm 0.92 ^b	19.3 \pm 0.36	33.4 \pm 0.32	19.2 \pm 0.25
	Twin	7	51.7 \pm 1.27	57.0 \pm 0.95 ^a	61.5 \pm 0.97	94.6 \pm 1.26 ^a	18.5 \pm 0.50	33.2 \pm 0.44	19.0 \pm 0.34
Dam type*	Ewe-lambs singleton	7	51.1 \pm 1.31	60.2 \pm 0.98 ^b	61.9 \pm 1.01	97.2 \pm 1.29 ^{ab}	18.4 \pm 0.51 ^{ab}	33.5 \pm 0.46	19.0 \pm 0.36
	Ewe-lambs twin	3	49.0 \pm 1.99	53.9 \pm 1.48 ^a	59.3 \pm 1.53	91.3 \pm 1.97 ^a	17.1 \pm 0.78 ^a	32.7 \pm 0.70	18.5 \pm 0.54
Birth rank ¹	Adult ewes singleton	5	53.6 \pm 1.46	60.3 \pm 1.09 ^b	62.2 \pm 1.12	99.7 \pm 1.44 ^b	20.2 \pm 0.57 ^{ab}	33.4 \pm 0.51	19.4 \pm 0.40
	Adult ewes twin	4	54.5 \pm 1.64	60.2 \pm 1.22 ^b	63.7 \pm 1.26	97.9 \pm 1.62 ^{ab}	20.0 \pm 0.64 ^b	33.8 \pm 0.57	19.5 \pm 0.45
d550									
Dam type	Ewe-lambs	22	49.4 \pm 0.56	60.2 \pm 0.56 ^a	61.5 \pm 0.54 ^a	100.1 \pm 0.77 ^a	17.4 \pm 0.24	34.8 \pm 0.23 ^a	19.6 \pm 0.17 ^a
	Adult ewes	21	49.8 \pm 0.54	62.2 \pm 0.55 ^b	63.4 \pm 0.53 ^b	104.0 \pm 0.75 ^b	17.9 \pm 0.23	35.6 \pm 0.23 ^b	20.2 \pm 0.17 ^b
Birth rank	Singleton	21	49.6 \pm 0.57	61.7 \pm 0.57	63.1 \pm 0.55	102.4 \pm 0.79	17.4 \pm 0.24	35.0 \pm 0.24	19.6 \pm 0.17 ^a
	Twin	22	49.6 \pm 0.56	60.6 \pm 0.56	61.8 \pm 0.54	101.7 \pm 0.77	17.8 \pm 0.24	35.4 \pm 0.23	20.2 \pm 0.17 ^b
Dam type*	Ewe-lambs singleton	10	49.4 \pm 0.86	61.2 \pm 0.86 ^{ab}	62.6 \pm 0.83 ^{ab}	99.8 \pm 1.18 ^a	17.3 \pm 0.37	34.4 \pm 0.36 ^a	19.4 \pm 0.27 ^a
	Ewe-lambs twin	11	49.4 \pm 0.80	59.3 \pm 0.80 ^a	60.3 \pm 0.77 ^a	100.5 \pm 1.10 ^a	17.4 \pm 0.34	35.2 \pm 0.34 ^{ab}	19.9 \pm 0.25 ^{ab}
Birth rank ¹	Adult ewes singleton	11	49.9 \pm 0.77	62.3 \pm 0.78 ^b	63.5 \pm 0.75 ^b	104.9 \pm 1.07 ^b	17.6 \pm 0.34	35.7 \pm 0.33 ^b	19.9 \pm 0.24 ^{ab}
	Adult ewes twin	11	49.8 \pm 0.78	62.0 \pm 0.79 ^{ab}	63.3 \pm 0.76 ^b	103.0 \pm 1.08 ^{ab}	18.3 \pm 0.34	35.5 \pm 0.33 ^{ab}	20.5 \pm 0.24 ^b

^{ab} different superscripts within rows indicate values that significantly differ ($P < 0.05$)

¹ Dam type by birth rank interaction

DISCUSSION

This study aimed to examine the growth trajectories, puberty attainment, fat depots and morphometric measurements of singleton and twin females born to either ewe-lamb or adult ewe that were continually managed together under the same commercial conditions.

At birth, ELP were 20% lighter than AEP. These results support the previous findings in Chapters 4 and 6, where ELP were lighter than AEP. In the large cohort (Corner *et al.*, 2013) have also found that lambs born to ewe-lamb dams are lighter than those born to mature adult ewe dams to weaning. Further Vesely *et al.* (1970) reported that Romnelets offspring born to young ewes were lighter at birth and remained lighter until 12 months of age compared with lambs born to mature adult ewes. While Gootwine *et al.* (2006) demonstrated that Assaf offspring born ewe-lambs remained lighter until two years of age, after which they did not differ in live weight. This phenomenon has also been observed in humans studies where babies born to teenage mothers are more likely to be of low birthweight (Cogswell *et al.*, 1995).

The present study also found that twins were 15.5% lighter than singletons at birth and remained lighter until 18 months of age. Numerous studies have reported that singletons are heavier at birth and weaning compared with twins (Gootwine *et al.*, 2007; Kenyon *et al.*, 2008). Kenyon *et al.* (2008b) showed that Romney twins were lighter than their singleton counterparts until nine months of age, after which this difference was no longer apparent. In the present study twins remained lighter than singletons until d550.

There are few examples of studies that include both parity and maternal age, and compare the potential interaction of both on offspring live weight. Generally across time in the present study, twins-born to EL-dams were lighter than singletons-born to

EL-dams. This relationship was not observed in progeny born to adult ewes. Similarly Keane (1976) found that singletons born to EL-dams had a live weight comparable to that of twins born to AE-dams which is similar to the findings in the present study. Gardner *et al.* (2007) suggested that dam age is more likely to influence offspring birthweight than dam parity and this can be explained by competition for nutrients during pregnancy between the fetus and the still growing mother (Wu *et al.*, 2004).

In the present study, puberty attainment by females progeny was not affected by dam age, even though differences in LW were observed. However, this study assessed puberty attainment for only one cycle (d 236-d253). This suggests farmers do not need to take these parameters into consideration when selecting ewe progeny for ewe-lamb breeding. Also the birth rank did not have any influence in the puberty attainment. In contrast, Dickerson and Lusted (1975) working with six different breeds of sheep, found that a higher percentage of singletons attained puberty compared with other birth-rearing classes.

Twin offspring born to ewe-lambs demonstrated higher concentration of *Strongyloides* egg counts than the other groups, as it was expected because twins are more susceptible to gastro-intestinal parasites (Wolf *et al.*, 2008). This may suggest that ELP-T are more susceptible to internal parasites. However more frequent sampling regimes need to be undertaken in future studies to confirm this.

CONCLUSIONS

Under the conditions of the present study, ELP were lighter and smaller at birth and remained so until at least 550 days of age (18 months of age). This was especially the case in twins-born to EL-dams in comparison to those born to AE-dams. Future work is required to determine how female offspring born to EL-dams perform if bred as

ewe-lambs themselves. In addition, it would be of interest to determine if these live weight effects persist as the progeny age and whether they affect later adult performance.

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Chapter 8

The impact of having either a ewe-lamb dam or a mature dam on the reproductive performance of singleton and twin female offspring

Foreword:

Aspects of this chapter were presented as a poster at the 2013 Gravida National Centre for Growth and Development Science Symposium (“Loureiro M.F.P., Pain S.J., Kenyon P.R. and Blair H.T. (2013). Reproductive performance of single and twin female offspring born to either young or mature dams. *Gravida Science Symposium, Auckland, New Zealand.*”).

ABSTRACT

This study was undertaken to compare the reproductive and live weight performance of female singleton and twin ewes born to either mature or young dams between 18 to 3.2 years of age. One hundred and fifteen singleton- and twin-born female offspring born to either ewe-lamb (ELP) or adult ewe (AEP) dams were maintained as one cohort under commercial New Zealand grazing conditions. Live weight of ELP ewes were lighter ($P < 0.05$) than AEP during their first and the second pregnancies. Twin-born ewes were lighter ($P < 0.05$) than their singleton-born counterparts. There was no difference in the number of ovulations ($P > 0.05$; 1.49 vs. 1.32 corpora lutea for AEP and ELP, respectively) at 560 days of age. At their second breeding (30 months-of-age), ELP were heavier ($P < 0.05$) than those born to AEP at the first (5.1 ± 0.11 vs. 4.7 ± 0.11 for ELP and AEP, respectively) and second (5.1 ± 0.08 vs. 4.7 ± 0.09 for ELP and AEP, respectively) lambing. However, these differences were no longer apparent at weaning. At their first parity, ELP (1.84 ± 0.11 kg of lamb/kg of ewe) ewes had a tendency to have lower ($P < 0.06$) production efficiency than AEP (2.14 ± 0.12) ewes based on kg of lamb weaned/ewe metabolic at breeding and ewe metabolic weight at weaning (2.07 ± 0.12 vs. 1.77 ± 0.11 kg of lamb/kg of ewe, for AEP and ELP, respectively). There were no differences at the second parity ($P > 0.05$) for production efficiency. In conclusion, ELP were consistently lighter than AEP ewes until 3.2 years of age, especially those ewes that were twin-born. However, while ELP ewes were lighter, they produced a similar number and weight of lambs compared with AEP. This suggests that there maybe no negative impact on the reproductive performance of twin-born ELP. These ewes now need to be studied to obtain lifetime performance.

INTRODUCTION

The majority of ewes are bred for the first time at 18 - 19 months of age (Spencer *et al.*, 1942; McMillan and McDonald, 1983). Breeding ewe-lambs can increase lifetime production efficiency (Kenyon *et al.*, 2014), reduce the cost of lamb production (Dickerson and Lusted, 1975) and increase farm profitability (Young *et al.*, 2010). In addition, selecting progeny born to ewe-lambs as replacements is a means of reducing the generation interval and increasing genetic gain (Dýrmundsson, 1973). However, there is sparse information on the performance of offspring born to ewe-lambs compared with those born to mature adult ewes.

Ewe-lamb progeny are lighter at birth and have lower survival than those born to mature adult ewes (Davies and Beck, 1993; Annett and Carson, 2006; Corner *et al.*, 2013). In Chapter 6 it was demonstrated that singletons born to ewe-lambs were lighter up to one year of age compared with those born to mature ewes, but their reproductive and lactational performance at two years of age did not differ. This suggests singletons born to ewe-lambs appear to be just as suitable as those born to mature ewes as replacement ewes. This may not be the case with twins born to ewe-lambs. Twins are typically lighter at birth and can remain lighter as mature animals in comparison to singletons (Reid and Hinks, 1962; Hight and Jury, 1970). It is therefore possible that twin offspring born to ewe-lamb dams may display an even bigger impact on live weight and show impaired performance; it appears no literature is available on this issue.

This Chapter examines the female progeny investigated in Chapter 7, where it was reported that female progeny born to ewe-lamb dams were consistently lighter to 18 month of age compared with those born to adult ewes dams and also that twin-born offspring were lighter than those born as singletons. The aim of this Chapter was to

extend the work from Chapter 7 and compare the live weights and the reproductive performance from 18 months to 3.2 years of age (two lambing events) of singles and twins born to either ewe-lamb dams or mature adult ewe dams. Both ELP and AEP were first bred at 18 month of age, as the majority of ewes are bred at this age in New Zealand.

MATERIALS AND METHODS

This study was conducted at Massey University's Keeble farm (latitude 41° 10'S, longitude 175° 36'E) 5 km south of Palmerston North, New Zealand, from April 2010 to December 2012. This study was conducted with the approval of the Massey University Animal Ethics Committee.

Experimental design and measures

Background

This study utilised 115 Romney ewes born in September 2009 to either ewe-lamb (EL) or adult ewe (AE) dams, as previously reported in Chapter 7 and Corner *et al.* (2013) (**Figure 1B**). This chapter reports on these offspring from 551 days (d551) of age (18 months) to d1166 (38 months). Four progeny groups were compared: singleton progeny of ewe-lamb dams (ELP-S; n = 28); twin progeny of ewe-lamb dams (ELP-T; n = 29); singleton progeny of adult ewe dams (AEP-S; n = 17) and twin progeny of adult ewe dams (AEP-T; n = 41). All ewe progeny were maintained as one cohort under commercial New Zealand grazing conditions from birth to d1166.

Ewe live weights and body condition score from d551 to d1166

The ewes were weighed within an hour off pasture at 16 time points between d551 and d1166: d573, d605, d626, d677, d690, d754, d800, d880, d902, d914, d999, d1055, d1090, d1124, d1166 (Fig.1). Their body condition score (BCS – Jeffries, 1961; Kenyon

et al., 2014; scale 0 - 5) was also recorded 10 times during this period: at d626, d690, d754, d800, d902, d914, d999, d1055, d1090 and d1166.

The birth rank was classified as being either a singleton or twin, however twin group includes both twin and triplets. Two twins born to adult ewes, one singleton born to a ewe-lamb and three twins born to ewe-lamb dams were removed from the study due to illness or death. Their data were kept for analysis until their removal from the study. All had been removed by the second lambing events.

Reproductive measures (2011 and 2012)

At their first mating, in 2011, ewes had their oestrous synchronised utilising intravaginal progesterone devices (CIDR, Pharmacia & Upjohn, New Zealand) fourteen days prior to being naturally mated with eight Romney rams. Ovarian activity was checked at d560 using a real-time B-mode scanner (Mindray™, DP-6600 Vet, Nanshan, China) with a transrectal probe of 7.5 MHz (Viñoles *et al.*, 2004). The numbers of corpora lutea (CL) present on both ovaries was counted and recorded per ewe. Ewes were categorised as ovulated or had not ovulated, if they presented with at least one CL or not, respectively. During the breeding period (d551 to d573) rams were crayon-harnessed and marks on the rumps of the ewes were recorded for the first 5 days (d551-d556, first cycle), and for a further 17 days (d557-d573; second cycle). These records were used as an indicator of breeding activity. Pregnancy diagnosis was undertaken 54 days after the end of breeding (d627), via transabdominal ultrasound using a real time B-mode scanner with a 5MHz mechanical sector probe (Ovi-Scan 6, BCF Ultrasound Australasia Ltd, Auckland, New Zealand). Number of fetuses (0, 1, 2, or 3) present were recorded.

At their second mating, in 2012, ewes were again progesterone synchronized (CIDR) at d900 for 14 days. Ewes were naturally mated with eight crayon-harnessed Romney

rams for a total of 22 days (first cycle, d914 to d919 and second cycle, d920 to d936). Pregnancy diagnosis, via ultrasound, was carried out at 63 days after the end of joining (d999) and the number of fetuses per ewe was recorded.

Crayon marks (tup marks) on the rumps of ewes were used to indicate breeding activity in the first and second cycle. For both the 2011 and 2012 breeding seasons breeding activity was calculated using the following formula:

$$\text{breeding activity (\%)} = \frac{\text{number of ewes marked in the first cycle}}{\text{total number of ewes exposed to the ram}} \times 100$$

Progeny born in 2011 and 2012

In late August 2011, 178 lambs were born; the numbers in each treatment are shown in **Table 20**. Lambs were weighed within 12 hours of birth and measurements taken for crown-rump length (CRL: distance from the top of the head to the base of the tail), thoracic girth (TG: circumference close to the heart-girth, the smallest chest circumference), fore-leg length (FL: from elbow (cubital) joint to toe of the hoof) and hind-leg length (HL: from hip (coxofemoral) joint to toe of the hoof). Their birth rank and sex was also recorded. Lambs were born over a 23 day period. The midpoint of lambing was termed W1. The lambs were weighed again at W32, W54 and weaning (W100).

In late August 2012 (second lambing), 188 lambs were born (**Table 20**). The lambs were weighed, their birth rank and sex recorded and measurements of CRL, TG, FL and HL taken within 12 hours of birth. The lambing period occurred over 24 days, with the midpoint being termed W1. Additional lamb live weight measurements were taken at W23, W57 and weaning (W99).

Table 20. Number of lambs born and weaned at the first (2011) and second (2012) parity of ewes born to either ewe-lamb or adult ewe dams.

		2011		2012	
		born	weaned	born	weaned
Granddam type	Ewe-lamb	81	65	92	75
	Adult ewe	97	81	96	84
Dam birth rank	Singleton	68	61	77	63
	Twin	110	85	111	96
Granddam type*	Ewe-lamb singleton	39	33	50	39
Dam birth rank ¹	Ewe-lamb twin	42	31	42	36
	Adult ewe singleton	29	28	27	24
	Adult ewe twin	68	53	69	61

¹ Granddam type by dam birth rank interaction

Statistical analysis

The aim of the present study was to examine the productive consequences of being born to either a ewe-lamb dam or adult ewe dam, and to determine if any affects are influenced by birth rank (singleton vs. twin). Therefore, in all models, even if the interaction between dam age and ewe birth rank was non-significant ($P > 0.05$), the two-way interaction remained in the model to allow for testing of the study design. Statistical analysis was performed with Minitab[®] (version 16, Minitab Inc., Cary NC, USA) and SAS (SAS, *Enterprise Guide*[®] 5.2).

Ewe live weights and body condition score

Live weights were analysed using the GLM procedure in Minitab and included the fixed effects of dam type and birth rank, and their interaction, with date of birth as a covariate. During the two gestational periods (2011: d573 to d690 and 2012: d914 to d1055), the number of fetuses carried was included as a fixed effect. The number of fetuses was classified as being either a singleton or multiple, the later including both

twin and triplets. Date of birth was originally used as a covariate but removed from the model because it was not significant ($P > 0.05$).

Body condition score was analysed utilising the MIXED procedure in SAS and included the fixed effects of dam type, birth rank and their two-way interaction. Live weights on the day of each measurement were used as covariates. In addition, the number of fetuses was used as a fixed effect during the gestation period.

Ewe reproductive measures (2011 and 2012)

The percentage of ewes bred in the first cycle of breeding, for both 2011 and 2012, was analysed as a binomial trait after logit transformation using the GENMOD procedure in SAS. The model included dam type, birth rank and their interaction as fixed effects. Live weights at d573 and d914 for the 2011 and 2012 breeding seasons, respectively, were included as covariates; however these were not significant ($P > 0.05$) and removed from the final model.

Ovarian activity was determined for the 2011 breeding season, at d560, based on the presence (or absence) of a CL. This was analysed in SAS as a binomial trait after logit transformation. Additionally, the number of CL was analysed using Poisson regression with a log-linear model, in SAS and back-transformed percentages were presented. In both analyses, dam type and birth rank and their interaction were included as fixed effects. Live weight of the ewes at d560 was initially used as a covariate but removed because it was not significant ($P > 0.05$).

The percentage of lambs born and weaned in both 2011 and 2012 was analysed based on all ewes presented for breeding, in SAS, using GENMOD procedure with a Poisson regression analysis using a log-linear model. Both models included dam type and birth rank and their interactions as fixed effects. Live weights of the ewe at d690 and d1055

were included as a covariate for the respective models, however they were not significant ($P > 0.05$) and removed from the final models. Least square means are presented as back transformed values with the back-transformed 95% confidence limits in parentheses.

Ewe production efficiency in both 2011 and 2012 was calculated using the total lamb weaning weight per ewe (include weight of two lambs if both weaned) divided by ewe metabolic live weight ($LW^{0.75}$) at breeding (d573 and d914; 2011 and 2012, respectively), and also by ewe live weight at weaning (d800 and d1166; for 2011 and 2012, respectively), presented by kg/kg of lamb of ewe. All ewes present at breeding were included (ewes that failed to wean a lamb were given a value of 0). The GLM model in Minitab was used to analyse ewe efficiency and total weight of lamb weaned with fixed effects of dam type and birth rank and their interactions.

Progeny (2011 and 2012)

Lamb size measurements at birth were analysed using the GLM model in Minitab with date of birth as a covariate and with, and without, birthweight as an additional covariate. Granddam type (EL vs. AE), dam birth rank (singleton or twin) and their interaction were included as fixed effects and lamb birth rank (singleton or twin) was also included as a fixed effect.

Lamb birthweight was analysed using the GLM model in Minitab. The model included date of birth as a covariate and lamb birth rank, granddam type, dam birth rank and the granddam type and dam birth rank interaction as fixed effects. Live weights at W32, W54 and weaning (W100) for lambs born in 2011 and W23, W57 and weaning (W99) for lambs born in 2012, were analysed with granddam type, dam birth rank and their

two-way interaction as fixed effects, with and without date of birth and birthweight as covariates. Lamb birth rank was also included as a fixed effect.

RESULTS

Ewe live weights and body condition

Live weights in pregnancy

During their first pregnancy (2011: d573 to d690 - **Figure 9**) ELP were lighter than AEP ($P < 0.05$) and ewes born as singletons were heavier than those born as twins ($P < 0.05$). There was no significant interaction ($P > 0.05$) between dam type and birth rank during this period. The number of fetuses carried by the ewe did not affect live weight during early to mid-gestation (d573 to d626), although at d677 and d690 (gestation day 104 and 117) live weight of pregnant ewes differed ($P < 0.05$) from non pregnant ewes ($P < 0.05$; **Table 21**).

Live weights during their second pregnancy (2012: d999 to d1055 - **Figure 9**) showed that ELP were lighter ($P < 0.05$) than AEP and singleton born ewes were heavier ($P < 0.05$ - **Table 22**) than twin born ewes. There was no significant ($P > 0.05$) interaction between dam type and birth rank during this period. The number of fetuses carried by the ewe did not affect live weight until day 141 of pregnancy (d1055), after which time those carrying multiple fetuses were heavier ($P < 0.05$) than those carrying a singleton fetus.

Live weights outside the gestation period

At d754, d800, d880, d909, d1090, d1124 and d1166 ELP were lighter ($P < 0.05$) than AEP (**Table 23; Figure 9**). At d754, d909, d1090, d1124 and d1166 twin-born ewes were lighter ($P < 0.05$) than those born as singletons. There was no significant interaction ($P > 0.05$) between dam type and birth rank during gestation, however there

was tendency ($P = 0.08$) at d1166 for twins-born ELP to be lighter than all other groups (Table 23).

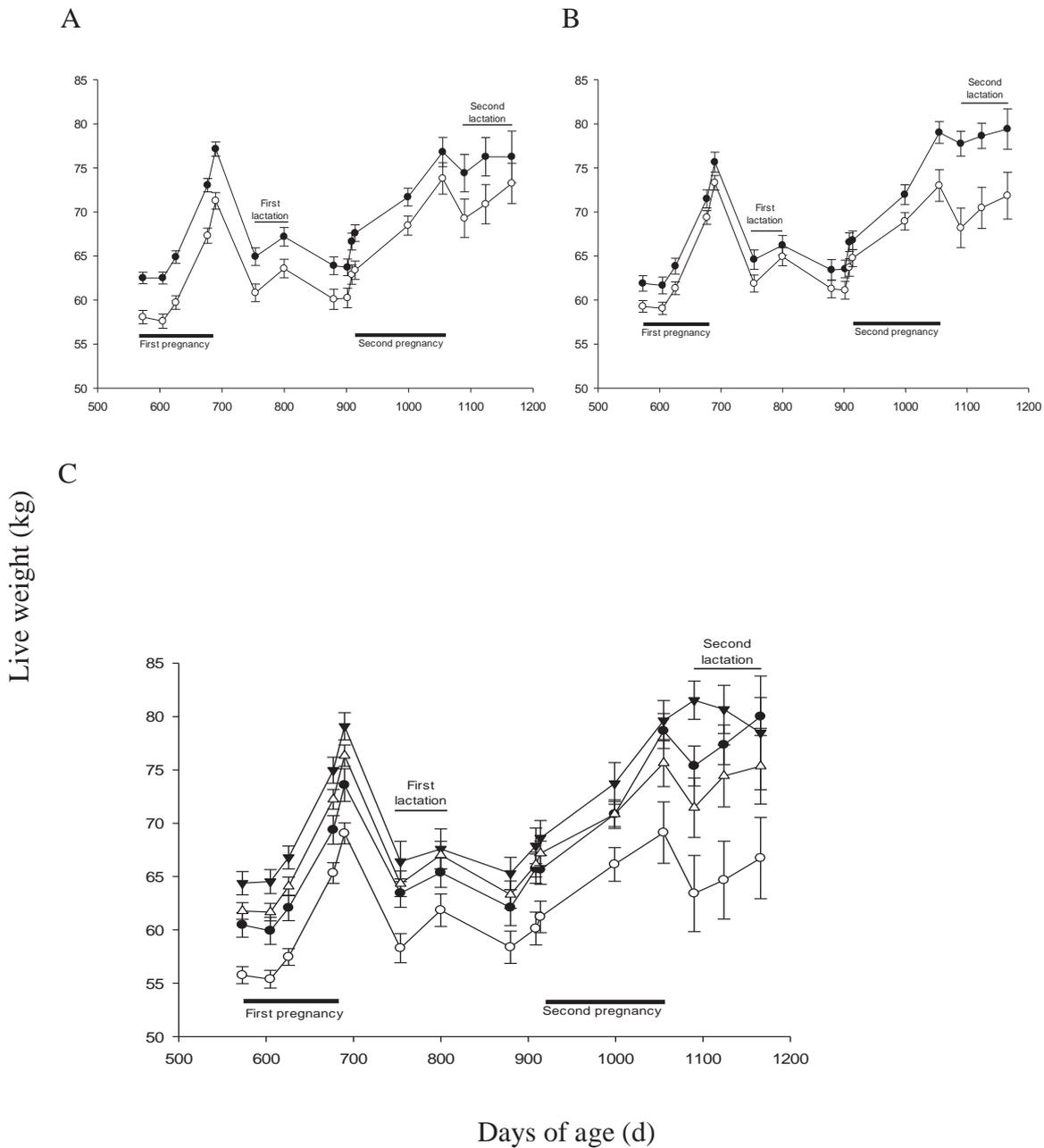


Figure 9. A: Live weights from day 573 of age (d573) to d1166 of ewe-lamb progeny (ELP) (○) and adult ewe progeny (AEP) (●); **B:** Live weights from d573 to d1166 of ewes born as singletons (●) or twin (○); **C:** Live weights from d573 to d1166 of ELP- singleton (●), ELP-twin (○), AEP-singleton (▼) or AEP-twin (△). Data presented are least square means ± standard error.

Table 21. Live weights (kg) of ewes born to ewe-lambs or adult ewe dams, or born as a singleton or twin, carrying 0, 1 or >1 fetus during their first gestation (2011). Data presented are least square means ± standard error.

		Live weight (kg)					
	n	d573 (22 days of gestation)	d605 (54 days of gestation)	d626 (75 days of gestation)	d677 (126 days of gestation)	d690 (139 days of gestation)	
Dam type							
	Ewe-lamb	57	57.1±1.00 ^a	56.5±1.06 ^a	58.7±1.05 ^a	65.7±1.15 ^a	68.7±1.23 ^a
	Adult ewe	58	61.7±1.13 ^b	61.6±1.19 ^b	64.1±1.18 ^b	71.5±1.30 ^b	74.6±1.38 ^b
Birth rank							
	Singleton	45	61.3±1.13 ^b	61.0±1.19 ^b	63.3±1.18 ^b	70.2±1.29 ^b	73.5±1.38 ^b
	Twin	70	57.5±1.01 ^a	57.1±1.08 ^a	59.5±1.06 ^a	66.9±1.17 ^a	69.9±1.25 ^a
Dam type* Birth rank ¹							
	Ewe-lamb singleton	28	59.6±1.23 ^b	59.0±1.32 ^b	61.1±1.30 ^b	67.6±1.43 ^{ab}	71.0±1.53 ^{ab}
	Ewe-lamb twin	29	54.5±1.22 ^a	54.1±1.31 ^a	56.4±1.29 ^a	63.7±1.42 ^a	66.5±1.51 ^a
	Adult ewe singleton	17	63.0±1.53 ^b	63.1±1.61 ^b	65.5±1.58 ^b	72.9±1.74 ^c	75.9±1.86 ^b
	Adult ewe twin	41	60.4±1.15 ^b	60.2±1.22 ^b	62.7±1.20 ^b	70.1±1.33 ^{bc}	73.2±1.42 ^b
Number of fetuses							
	0	4	56.9±2.57	56.4±2.74	59.1±2.70	64.9±2.97	65.9±3.17 ^a
	1 (singleton)	40	60.3±0.81	59.9±0.87	62.0±0.85	69.3±0.94	73.5±1.01 ^{ab}
	> 1 (multiple)	71	61.0±0.64	60.9±0.67	63.1±0.66	71.4±0.73	75.6±0.78 ^b

^{ab} different superscripts within columns indicate values that significantly differ ($P < 0.05$)

¹ Dam type by birth rank interaction

Table 22. Live weight (kg) during their second gestation (2012) of ewes born to ewe-lambs or adult ewe dams, or as singleton or twin, carrying 0, 1 or more than one fetus in their second gestation (2012). Data presented are least square means \pm standard error.

		Live weight (kg)			
		n	d914 (1 days of gestation)	d999 (85days of gestation)	d1055 (141 days of gestation)
Dam type	Ewe-lamb	55	64.6 \pm 1.74 ^a	67.5 \pm 1.83 ^a	72.1 \pm 1.76 ^a
	Adult Ewe	58	68.9 \pm 1.68 ^b	71.5 \pm 1.77 ^b	75.8 \pm 1.70 ^b
Birth rank	Singleton	44	68.2 \pm 1.76	71.3 \pm 1.84 ^b	76.1 \pm 1.78 ^b
	Twin	69	65.3 \pm 1.69	67.7 \pm 1.77 ^a	71.8 \pm 1.71 ^a
Dam type* Birth rank ¹	Ewe-lamb singleton	27	66.8 \pm 2.05 ^{ab}	69.7 \pm 2.16 ^{ab}	75.1 \pm 2.08 ^b
	Ewe-lamb twin	28	62.4 \pm 2.03 ^a	65.3 \pm 2.13 ^a	69.1 \pm 2.07 ^a
	Adult ewe singleton	17	69.6 \pm 2.17 ^b	73.0 \pm 2.2 ^b	77.1 \pm 2.20 ^b
	Adult ewe twin	41	68.2 \pm 1.81 ^b	70.0 \pm 1.89 ^{ab}	74.6 \pm 1.83 ^b
Number of fetus	0	3	69.4 \pm 4.34	67.9 \pm 4.55	69.2 \pm 4.39
	1 (singleton)	29	65.2 \pm 1.39	69.8 \pm 1.46	74.6 \pm 1.40
	>1 (multiples)	81	65.7 \pm 0.86	70.7 \pm 0.91	78.0 \pm 0.88

^{ab} different superscripts within columns indicate values that significantly differ ($P < 0.05$)

¹ Dam type by birth rank interaction

Table 23. Live weights (kg) outside the gestation periods of ewes born to ewe-lamb or adult ewe dams, or born as a singleton or twin. Data presented are least square means \pm standard error.

		Live weight (kg)											
		n	d754	n	d800	n	d909	n	d1090	n	d1124	n	d1166
Dam type	Ewe-lamb	55	60.8 \pm 1.01 ^a	55	63.6 \pm 1.07 ^a	55	62.9 \pm 1.02 ^a	53	71.7 \pm 1.07 ^a	53	73.4 \pm 1.14 ^a	53	75.9 \pm 1.15 ^a
	Adult ewe	58	65.4 \pm 1.07 ^b	58	67.3 \pm 1.14 ^b	58	67.0 \pm 1.09 ^b	56	78.4 \pm 1.13 ^b	56	79.5 \pm 1.21 ^b	54	82.3 \pm 1.24 ^b
Birth rank	Singleton	44	64.9 \pm 1.17 ^b	44	66.4 \pm 1.25	44	66.8 \pm 1.18 ^b	44	78.2 \pm 1.23 ^b	44	78.9 \pm 1.31 ^b	43	81.7 \pm 1.34 ^b
	Twin	69	61.4 \pm 0.93 ^a	69	64.5 \pm 0.99	69	63.1 \pm 0.94 ^a	65	72.0 \pm 1.01 ^a	65	74.1 \pm 1.07 ^a	64	76.6 \pm 1.08 ^a
Dam type*	Ewe-lamb singleton	27	63.3 \pm 1.50 ^{ab}	27	65.2 \pm 1.60	27	65.7 \pm 1.52 ^{ab}	27	74.8 \pm 1.58 ^b	27	77.0 \pm 1.68 ^b	27	80.0 \pm 1.69 ^b
	Ewe-lamb twin	28	58.4 \pm 1.43 ^a	28	62.0 \pm 1.52	28	60.1 \pm 1.44 ^a	26	68.6 \pm 1.54 ^a	26	69.8 \pm 1.65 ^a	26	71.9 \pm 1.66 ^a
Birth rank ¹	Adult ewe singleton	17	66.4 \pm 1.80 ^b	17	67.6 \pm 1.92	17	67.9 \pm 1.82 ^b	17	81.5 \pm 1.88 ^c	17	80.7 \pm 2.00 ^b	16	83.4 \pm 2.08 ^b
	Adult ewe twin	41	64.4 \pm 1.17 ^b	41	67.1 \pm 1.24	41	66.1 \pm 1.18 ^b	39	75.3 \pm 1.25 ^b	39	78.4 \pm 1.34 ^b	38	81.3 \pm 1.36 ^b

^{ab} different superscripts within columns indicate values that significantly differ ($P < 0.05$)

¹ Dam type by birth rank interaction

Body condition score (BCS)

BCS in pregnancy

During the first gestation (2011) neither dam type or birth rank affected ewe BCS ($P > 0.05$ - **Table 24**). In addition there was no significant interaction ($P > 0.05$) between dam type and birth rank during this period.

During the second gestation (2012), at mid-pregnancy (d999), birth rank ($P < 0.05$) affected ewe BCS, whereby twin-born ewes had higher body condition than ewes born as singletons. Although at the end of gestation (d1055), ewe BCS was not affected by dam type or birth rank. There was no interaction between dam type and birth rank ($P > 0.05$; **Table 24**).

BCS outside the gestation periods

During the first lactation period (d754), ELP had higher ($P < 0.05$) BCS than AEP and twin-born ewes had greater ($P < 0.05$) BCS than those born as singletons. At weaning (d800), ELP had higher body condition than AEP ($P < 0.05$) and twin-born ewes had higher body condition than those born as singletons ($P < 0.05$). At d902, ELP still had higher ($P < 0.05$) body conditions than AEP. There was no significant interaction between dam type and dam birth rank.

During their second lactation period (d1090) and at the weaning of their lambs (d1166) neither dam type nor birth rank affected body condition score. There were no significant interactions between dam type and dam birth rank ($P > 0.05$; **Table 24**).

Table 24. Body condition score (BCS) of ewes born to ewe-lamb or adult ewe dams or singletons or twins. Data presented are least square means \pm standard error.

		BCS									
		First pregnancy					Second pregnancy				
Dam type	Birth rank	d626 (75 days of gestation)	d690 (139 days of gestation)	d754	d800	d902	d914 (1 days of gestation)	d999 (85 days of gestation)	d1055 (141 days of gestation)	d1090	d1166
Ewe-lamb		3.7 \pm 0.07	3.0 \pm 0.07	2.4 \pm 0.06 ^b	2.7 \pm 0.06 ^b	2.8 \pm 0.05 ^b	2.9 \pm 0.05	2.9 \pm 0.06	2.9 \pm 0.06	2.8 \pm 0.07	3.3 \pm 0.06
Adult ewe		3.5 \pm 0.07	3.0 \pm 0.07	2.2 \pm 0.07 ^a	2.4 \pm 0.06 ^a	2.6 \pm 0.05 ^a	2.8 \pm 0.06	2.8 \pm 0.06	2.7 \pm 0.06	2.6 \pm 0.08	3.2 \pm 0.07
Singleton		3.5 \pm 0.08	3.0 \pm 0.07	2.2 \pm 0.07 ^a	2.4 \pm 0.07 ^a	2.6 \pm 0.05	2.8 \pm 0.06	2.7 \pm 0.07 ^a	2.8 \pm 0.06	2.6 \pm 0.08	3.2 \pm 0.07
Twin		3.7 \pm 0.06	3.0 \pm 0.06	2.4 \pm 0.06 ^b	2.6 \pm 0.05 ^b	2.7 \pm 0.04	2.9 \pm 0.05	2.9 \pm 0.05 ^b	2.8 \pm 0.05	2.7 \pm 0.06	3.3 \pm 0.06
Dam type*		3.6 \pm 0.09	3.0 \pm 0.09	2.3 \pm 0.09 ^a	2.6 \pm 0.09 ^{bc}	2.7 \pm 0.06 ^{ab}	2.8 \pm 0.07	2.7 \pm 0.08 ^a	2.9 \pm 0.08	2.7 \pm 0.09	3.2 \pm 0.08 ^a
Birth rank ¹		3.8 \pm 0.10	3.0 \pm 0.09	2.5 \pm 0.09 ^b	2.8 \pm 0.09 ^c	2.8 \pm 0.06 ^b	2.9 \pm 0.08	3.0 \pm 0.08 ^b	2.8 \pm 0.08	2.8 \pm 0.10	3.5 \pm 0.09 ^b
Adult ewe singleton		3.5 \pm 0.12	3.0 \pm 0.11	2.1 \pm 0.11 ^a	2.3 \pm 0.10 ^a	2.5 \pm 0.08 ^a	2.8 \pm 0.09	2.8 \pm 0.10 ^{ab}	2.8 \pm 0.10	2.6 \pm 0.12	3.2 \pm 0.11 ^a
Adult ewe twin		3.6 \pm 0.08	2.9 \pm 0.07	2.3 \pm 0.07 ^{ab}	2.5 \pm 0.07 ^b	2.6 \pm 0.05 ^a	2.9 \pm 0.06	2.8 \pm 0.07 ^a	2.7 \pm 0.06	2.6 \pm 0.08	3.2 \pm 0.07 ^a

^{abc} different superscripts within columns indicate values that significantly differ ($P < 0.05$)

¹ Dam type by birth rank interaction

Ewe reproductive measures (2011 and 2012)

In 2011, there were fewer ($P < 0.06$) ELP marked in the first cycle by the crayon-harnessed rams compared with AEP. Neither dam type or birth rank ($P > 0.05$) influenced ovarian activity as measured by ultrasound five days post CIDR removal (**Table 25**). Number of fetuses per ewe, number of lambs born and weaned did not differ ($P > 0.05$) due to dam type or birth rank (**Table 25**).

In 2012, the second year of breeding, a greater percentage of twin-born ewes ($P < 0.05$) were marked by the crayon-harnessed ram compared with those ewes born as singletons in the 5 days period (**Table 26**). The number of fetuses per ewe did not differ ($P > 0.05$) due to dam type or birth rank. Number of lambs born and weaned did not vary ($P > 0.05$) due to dam type or birth rank (**Table 26**). There was no significant ($P > 0.05$) interaction between dam type and birth rank for any reproductive measures taken in either 2011 or 2012.

Table 25. Effect of being born to ewe-lamb or adult ewe dams and as either a singleton or twin on reproductive performance at first parity (2011), the number of corpora lutea (CL) per ewe, back-transformed percentage of ewes displaying at least one CL, breeding activity (percentage of ewes marked at the first cycle - 5 days post CIDR), percentage of fetus at scanning, percentage of lambs born and percentage of lambs weaned (\pm 95% confidence interval).

2011	Dam Age		Birth Rank		Dam type* Birth rank ¹			
	Ewe-lamb	Adult Ewe	Singleton	Twin	Ewe-lambs singleton	Ewe-lambs twin	Adult ewes singleton	Adult ewes twin
Number of CL	1.49 (1.20 – 1.84)	1.32 (1.03 – 1.70)	1.38 (1.06 – 1.79)	1.43 (1.17 – 1.75)	1.54 (1.14 – 2.07)	1.45 (1.07 – 1.96)	1.24 (0.80 – 1.89)	1.41 (1.09 – 1.83)
Ewe with CL	91% (81 – 96)	86% (72 – 93)	91% (78 – 97)	87% (76 – 93)	93% (76 – 98)	90% (72 – 97)	88% (63 – 97)	83% (68 – 92)
Breeding activity	80.50% (68 – 89)	93.40% (81 – 98)	87.10% (69 – 95)	89.70% (80 – 95)	74.0% ^a (55 – 87)	85.7% ^{ab} (67 – 94)	94.1% ^{ab} (67 – 99)	92.7% ^b (80 – 97)
% of fetus	152.60% (132 – 189)	167.70% (135 – 209)	156.20% (122 – 199)	163.80% (136 – 198)	148.10% (108 – 201)	157.10% (117 – 211)	164.70% (113 – 238)	170.70% (135 – 215)
% lambs born [†]	152.60% (123 – 189)	166.50% (134 – 207)	156.20% (123 – 199)	162.60% (135 – 196)	148.10% (108 – 201)	157.10% (116 – 211)	164.70% (113 – 238)	168.30% (133 – 213)
% lambs weaned [†]	118.20% (93 – 150)	143.30% (114 – 180)	139.30% (108 – 180)	121.50% (98 – 151)	122.20% (87 – 172)	114.30% (80 – 161)	158.80% (109 – 231)	129.20% (99 – 169)

^{ab} different superscripts within rows indicate values that significantly differ ($P < 0.05$)

¹ Dam type by birth rank interaction

[†] based on all ewes presented to breeding

Table 26. Effect of being born to ewe-lamb or adult ewe dams and as either a singleton or twin on reproductive performance at second parity (2012). Table presents breeding activity (percentage of ewes marked at the first cycle - 5 days post CIDR), percentage of fetus at scanning, percentage of lambs born and percentage of lambs weaned (\pm 95% confidence interval).

2012	Dam Age		Birth Rank		Dam type* Birth rank ¹			
	Ewe-lamb	Adult Ewe	Singleton	Twin	Ewe-lambs singleton	Ewe-lambs twin	Adult ewes singleton	Adult ewes twin
Breeding activity	69.80% (56 – 80)	60.00% (45 – 73)	53.2% ^a (38 – 68)	75.3% ^b (63 – 84)	59.3% ^{ab} (40 – 75)	78.6% ^b (59 – 90)	47.0% ^a (25 – 69)	71.8% ^{ab} (55 – 83)
% of fetus	169.10% (138 – 207)	165.20% (132 – 206)	164.50% (130 – 208)	169.80% (140 – 205)	170.40% (128 – 227)	167.90% (126 – 223)	158.80% (108 – 232)	171.80% (135 – 218)
% lambs born [†]	159.90% (130 – 197)	163.90% (131 – 205)	164.50% (130 – 209)	159.30% (131 – 193)	170.40% (127 – 227)	150.00% (110 – 202)	158.80% (109 – 232)	169.20% (133 – 215)
% lambs weaned [†]	134.50% (107 – 169)	149.80% (118 – 190)	141.00% (109 – 182)	143.00% (116 – 175)	140.00% (102 – 193)	128.60% (93 – 178)	141.20% (95 – 210)	159.00% (124 – 203)

^{ab} different superscripts within rows indicate values that significantly differ ($P < 0.05$)

¹ Dam type by birth rank interaction

[†]based on all ewes presented to breeding

Progeny (2011 and 2012)

2011

Birthweight (W1) of lambs was affected by the lamb birth rank, with singletons being heavier than ($P < 0.05$) twins (**Table 27**). Lambs with EL-granddams were heavier ($P < 0.05$) than those lambs with AE-granddams, when analysed both with (**Table 27**) and without date of birth as a covariate (data not shown). Dam birth rank did not ($P > 0.05$) affect lamb birthweight. The birth rank of the lambs themselves affected CRL, whereby singletons had greater ($P < 0.05$) CRL than twins (**Table 27**). Additionally, when lamb birth measurements were analysed without date of birth and birthweight as covariates, TG and FL length became significant; lambs with EL-granddam having greater TG and FL length than lambs with AE-granddams ($P < 0.05$; data not shown). Granddam type affected CRL and FL, whereby lambs with EL-granddams had greater CRL and FL than that of lambs with AE-granddams. Lambs with EL-granddams tended ($P = 0.06$) to have greater TG than lambs with AE-granddams (data not shown). However, when those parameters were analysed with date of birth and birthweight as a covariate these differences and tendency were no longer apparent ($P > 0.05$) (**Table 27**). Lambs born to singleton-dams had greater ($P < 0.05$) TG and HL than those lambs born to twins-dams (**Table 27**). When these parameters were analysed with date of birth and birthweight as a covariate, these differences persisted (data not shown). There was no interaction between granddam type and dam birth rank ($P > 0.05$) for any of the above parameters.

The live weight of twin lambs born in 2011 were heavier during the lactation period ($P < 0.05$) than twins (**Table 27**). However, neither granddam type or dam birth rank affected live weight from day 54 (W54; **Table 27**) to weaning (W100; **Table 27**). There was a significant interaction between dam type and dam birth rank at weaning

($P < 0.05$), however pairwise comparison did not show differences between these groups.

2012

At the second lambing (2012), singletons were heavier ($P < 0.05$) than twins at birth (**Table 28**). There was an interaction ($P < 0.05$) between granddam type and dam birth rank, whereby lambs born to ELP-twins were heavier ($P < 0.05$) than lambs born to ewes that were born as singletons from EL-dams and both singleton and twin lambs born to AE ewes.

Lamb birth size measurements were not affected by birth rank when analysed with date of birth and birthweight as covariates (**Table 28**). When lamb birth size measurements were analysed without covariates they become significant (data not shown). Granddam type did not ($P > 0.05$) affect lamb birth size measurements when analysed with date of birth and birthweight as covariate (**Table 28**). When analysed without covariates, the CRL of lambs with EL-granddams were greater ($P < 0.05$) than those with AE-granddams. Additionally lambs with EL-granddams showed a tendency ($P = 0.09$) for longer HL length (data not shown) than those with AE-granddams. Dam birth rank did not affect ($P > 0.05$) lamb birth size measurements. There was no interaction ($P > 0.05$) between granddam type and dam birth rank.

Singletons were heavier ($P < 0.05$) than twins at W57 and weaning (W99). Granddam type and dam birth rank did not affect lamb live weight to weaning ($P > 0.05$). There was no interaction between granddam type and dam birth rank ($P > 0.05$).

Table 27. The effect of granddam age (primiparous ewe-lamb (EL) or multiparous adult ewe (AE)) and dam birth rank on first parity singleton and twin offspring born in 2011: live weight at birth (W1), 54 days post birth (W54) and weaning (W100). Data presented are least square means \pm standard error.

2011	Lamb birth rank		Granddam type		Dam Birth Rank		Granddam type* Dam Birth rank ¹			
	Singleton	Twin	Ewe-lamb	Adult Ewe	Singleton	Twin	Ewe-lambs singleton	Ewe-lambs twin	Adult ewes singleton	Adult ewes twin
CRL (cm)	57.0 \pm 0.44 ^b	55.2 \pm 0.21 ^a	55.7 \pm 0.28	55.5 \pm 0.27	55.7 \pm 0.30	55.5 \pm 0.24	55.9 \pm 0.39	55.5 \pm 0.38	55.6 \pm 0.45	55.4 \pm 0.30
TG (cm)	40.2 \pm 0.45	39.4 \pm 0.21	39.7 \pm 0.27	39.7 \pm 0.26	40.3 \pm 0.29 ^a	39.1 \pm 0.23 ^b	40.3 \pm 0.38	39.0 \pm 0.36	40.3 \pm 0.43	39.2 \pm 0.29
FL (cm)	30.3 \pm 0.26	30.4 \pm 0.13	30.5 \pm 0.16	30.3 \pm 0.16	30.5 \pm 0.17	30.4 \pm 0.14	30.5 \pm 0.22	30.6 \pm 0.21	30.5 \pm 0.26	30.1 \pm 0.17
RL (cm)	36.5 \pm 0.41	36.5 \pm 0.20	36.6 \pm 0.25	36.7 \pm 0.24	37.0 \pm 0.27 ^b	36.3 \pm 0.21 ^a	36.8 \pm 0.35	36.3 \pm 0.34	37.1 \pm 0.40	36.3 \pm 0.27
W1 (kg)	6.0 \pm 0.13 ^b	4.5 \pm 0.07 ^a	5.1 \pm 0.11 ^b	4.7 \pm 0.11 ^a	4.9 \pm 0.12	4.8 \pm 0.10	5.1 \pm 0.16	5.1 \pm 0.17	4.7 \pm 0.19	4.6 \pm 0.12
W54 (kg)	23.0 \pm 0.56 ^b	19.0 \pm 0.28 ^a	19.6 \pm 0.40	20.1 \pm 0.37	20.0 \pm 0.40	19.8 \pm 0.36	20.0 \pm 0.57	19.2 \pm 0.57	19.9 \pm 0.60	20.4 \pm 0.44
W100 (kg)	33.8 \pm 0.77 ^b	29.7 \pm 0.39 ^a	30.4 \pm 0.54	30.6 \pm 0.50	30.4 \pm 0.54	30.5 \pm 0.48	31.1 \pm 0.75	29.7 \pm 0.77	29.8 \pm 0.80	31.4 \pm 0.60

^{ab} different superscripts within row sections indicate values that significantly differ ($P < 0.05$)

¹ Granddam type by dam birth rank interaction

Table 28. The effect of granddam age (primiparous ewe-lambs (EL) or multiparous adult ewes (AE)) and dam birth rank on second parity singleton and twin offspring born in 2012: live weight at birth (W1), 57 days post birth (W57) and weaning (W99). Data presented are least square means \pm standard error.

2012	Lamb birth rank		Granddam type		Dam Birth Rank		Granddam type* Dam Birth rank ¹			
	Singleton	Twin	Ewe-lamb	Adult Ewe	Singleton	Twin	Ewe-lambs singleton	Ewe-lambs twin	Adult ewes singleton	Adult ewes twin
CRL (cm)	56.8 \pm 0.44	56.7 \pm 0.17	56.7 \pm 0.21	56.4 \pm 0.22	56.5 \pm 0.24	56.7 \pm 0.19	56.8 \pm 0.28	56.7 \pm 0.31	56.2 \pm 0.38	56.7 \pm 0.24
TG (cm)	38.6 \pm 0.43	39.4 \pm 0.16	38.9 \pm 0.21	39.5 \pm 0.22	39.3 \pm 0.24	39.1 \pm 0.19	39.0 \pm 0.28	38.7 \pm 0.31	39.5 \pm 0.38	39.4 \pm 0.24
FL (cm)	30.8 \pm 0.27	30.7 \pm 0.10	30.7 \pm 0.13	30.8 \pm 0.14	30.8 \pm 0.15	30.7 \pm 0.12	30.7 \pm 0.17	30.7 \pm 0.20	30.9 \pm 0.24	30.7 \pm 0.15
RL (cm)	36.8 \pm 0.28	36.7 \pm 0.10	36.6 \pm 0.14	36.8 \pm 0.15	36.6 \pm 0.15	36.8 \pm 0.13	36.4 \pm 0.18	36.8 \pm 0.20	36.7 \pm 0.25	36.9 \pm 0.16
W1 (kg)	5.9 \pm 0.14b	4.7 \pm 0.06 ^a	5.1 \pm 0.08 ^b	4.7 \pm 0.09 ^a	4.8 \pm 0.10	5.0 \pm 0.08	4.9 \pm 0.11 ^a	5.4 \pm 0.12 ^b	4.8 \pm 0.16 ^{ab}	4.7 \pm 0.10 ^a
W57 (kg)	24.1 \pm 0.60b	21.7 \pm 0.24 ^a	21.8 \pm 0.31	22.6 \pm 0.33	22.4 \pm 0.36	21.9 \pm 0.29	21.9 \pm 0.43	21.7 \pm 0.47	23.0 \pm 0.56	22.1 \pm 0.35
W99 (kg)	36.7 \pm 0.84	33.3 \pm 0.33	33.7 \pm 0.46	34.3 \pm 0.47	34.3 \pm 0.51	33.7 \pm 0.41	33.8 \pm 0.62	33.6 \pm 0.66	34.8 \pm 0.80	33.9 \pm 0.50

^{ab} different superscripts within row sections indicate values that significantly differ ($P < 0.05$)

¹ Granddam type by dam birth rank interaction

Ewe production efficiency

Ewe production efficiency was calculated as kg of lamb weaned/kg ewe metabolic live weight at breeding (d573 and d914 in 2011 and 2012 lambings respectively) and kg of lamb weaned/kg ewe metabolic live weight at weaning (d800 and d1166 in 2011 and 2012 lambings respectively). Ewe production efficiency at the first parity (2011) showed a tendency ($P = 0.07$; **Table 29**) to be influenced by dam type such that ELP had lower production efficiency than AEP based on breeding weight and weaning weight (**Table 29**). These differences were no longer apparent ($P > 0.05$) at the second parity (**Table 29**). At both the first and the second parities there was no dam type by birth rank interaction for ewe production efficiency (data not shown).

Table 29. First (2011) and second (2012) parity ewe production efficiency of ewes born to ewe-lamb dams or adult ewe dams or born as singletons or twins. Ewe production efficiency calculated as kg of lamb weaned/ewe metabolic weight at breeding (d573 and d914 in 2011 and 2012 lambings respectively) and kg of lamb weaned/ewe metabolic weight at weaning (d800 and d1166 in 2011 and 2012 lambings respectively). Data presented are least square means \pm standard error.

	First parity			Second parity	
	d573 (Breeding)	d800 (Weaning)	d914 (Breeding)	d1166 (Weaning)	
Dam type					
Ewe-lamb	1.84 \pm 0.11 [†]	1.77 \pm 0.11 [†]	2.42 \pm 0.12	2.16 \pm 0.11	
Adult ewe	2.14 \pm 0.12 [*]	2.07 \pm 0.12 [*]	2.51 \pm 0.13	2.21 \pm 0.12	
Birth rank					
Singleton	2.09 \pm 0.13	2.04 \pm 0.12	2.34 \pm 0.14	2.05 \pm 0.13	
Twin	1.88 \pm 0.1	1.81 \pm 0.1	2.34 \pm 0.14	2.32 \pm 0.11	
Dam type* Birth rank					
Ewe-lamb singleton	1.90 \pm 0.16	1.83 \pm 0.16	2.30 \pm 0.16	2.03 \pm 0.15	
Ewe-lamb twin	1.77 \pm 0.15	1.72 \pm 0.15	2.55 \pm 0.18	2.29 \pm 0.17	
Adult ewe singleton	2.29 \pm 0.2	2.25 \pm 0.19	2.39 \pm 0.23	2.08 \pm 0.21	
Adult ewe twin	1.98 \pm 0.13	1.90 \pm 0.13	2.63 \pm 0.14	2.34 \pm 0.13	

^{†*} superscripts indicate values which have a tendency to differ $P < 0.10$

DISCUSSION

The aim of this research was to compare the liveweight trajectory from 18 months of age to 3 years of age, and the reproduction performance of singleton and twin female progeny born to either ewe-lamb dams or adult ewe dams (ELP and AEP respectively). The hypothesis was that maternal age and the ewes birth rank would have a long-term impact on her subsequent growth and reproductive performance.

Females born to primiparous ewe-lamb dams (ELP) were consistently lighter from birth, through their first breeding (at 18 months of age – Chapter 7) and until weaning of their second set of lambs (at approximately 3 years of age). Catch-up or compensatory growth is a physiological process that occurs after a period of restricted development (Hornick, *et al.*, 2000). Although, some compensatory live weight gain might have been expected (Owens *et al.*, 1993), ELP did not display any compensatory growth, as they remained lighter until 3 years of age, particularly those that were ELP-twin. This is supported by similar results in the cohort of animals studied in Chapter 6, whereby EL-singleton ewes, remaining lighter until 12 months of age compared with AE-singleton.

There is a positive relationship between reproductive performance and body condition score (subcutaneous fat in lumbar position) in mature ewes up until a body condition score of 3.0 (Kenyon *et al.*, 2014). The study reported in this Chapter found that whilst body condition score was similar during pregnancy, differences in body condition score were found after their first pregnancy and again after their second pregnancy, whereby ELP-twin appeared to retain/gain more subcutaneous fat. Similarly in Chapter 4, singleton males born to ewe-lamb dams displayed a tendency to have more internal abdominal fat at around one year of age compared with their adult ewe born counterparts. Conversely, the CT scan results in Chapter 5 showed no differences in

total body fat composition between singleton females born to ewe-lamb dams or adult ewe dams during pre-breeding, pregnancy or lactation stages. The observed differences in body condition after pregnancy reported in this Chapter do not appear to be due to differences in lactational demand, as Chapter 6 showed that there was no difference in the milk production or milk composition of ewes born either to ewe-lamb dams or mature ewe dams and in the present study on differences in lamb live weight (a proxy for milk production) in lactation. The greater body condition score in ELP progeny did not result in greater reproductive performance. There were no differences in ovulation rate in 2011 or in the number of fetuses, lambs born or weaned in both 2011 and 2012. Similar result were reported in Chapter 6, whereby reproductive performance in regards to puberty attainment, pregnancy rate or number of fetuses did not differ. Thus, combined these results demonstrate that despite of the generally lighter live weight of daughters born to ewe-lamb dams, there appears to be no negative impact on their reproductive performance.

Progeny born to ewe-lambs (ELP) gave birth to heavier lambs in both their first and second lambing compared with AEP. Chapter 6 reported a similar result where ewes from ewe-lamb dams gave birth to heavier lambs at their second lambing. Blair *et al.* (2010) who applied a maternal pregnancy nutrition treatment found a similar birthweight reversal between generations at the ewe progeny's second lambing. It is unclear what drives this birthweight reversal effect. The differences in birthweight in both 2011 and 2012 were no longer apparent at weaning. Similar results were found in Chapter 6.

Production efficiency (total lamb weaning weight divided by ewe metabolic live weight at breeding and at weaning) of AEP tended to be greater than that of ELP at their first

lambing only, likely due to the wider numerical difference in percentage of lambs weaned between ELP and AEP in 2011. There was no difference in production efficiency between ELP and AEP at their second lambing. It would be of interest to examine these ewes throughout their lifetime to determine lifetime production efficiency.

This study was conducted with singleton and twin offspring born to either adult ewe or ewe-lamb dams. Birth rank of the ewe progeny had an influence on the live weight during both pregnancies (2011 and 2012), whereby ewes born as singletons were heavier than those born as a twin. Interestingly, when the interaction between dam type and birth rank was analysed the twin born progeny of ewe-lamb dams were consistently lighter by around 12% than their singleton adult ewe progeny counterparts. In their first year of breeding (2011), singleton and twin born ewes did not differ in any reproductive measurements. However, in their second year of breeding (2012), a lower percentage of singleton progeny were marked in their first oestrous cycle compared with twin born progeny. This finding supports evidence in the literature that shows ewes born as twins have higher ovulations rates and birth rates compared with those born as singletons (Gonzalez *et al.*, 1986). Over all, birth rank does not appear to have any negative effect on reproductive performance of the offspring studied in this Chapter.

CONCLUSIONS

Under the conditions of present study, ewe-lamb progeny, in particular those that were twin born were lighter from 1.5 years of age and remained lighter than adult ewe progeny until the completion of this study around 3.2 years of age. Despite this apparent live weight disadvantage of ELP, there were little to no differences in any reproductive parameters for their first and second parities. There was no difference in lamb weaning weights between the two groups. The findings presented in this study are important to farmers as the set of data generated here demonstrate that notwithstanding the differences in live weight, ELP are still suitable to keep as replacement breeding ewes without significant negative effects on their performance or that of their progeny to weaning. However, before this can be confirmed, the lifetime performance of these ewes needs to be monitored and the ewe-lambs progeny should be bred as ewe-lambs themselves.

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Chapter 9

The influence of maternal age on skeletal muscle development and growth of female sheep offspring

ABSTRACT

In production animals, such as the sheep, muscle growth is of major importance for producers. Challenges imposed during fetal life can have long-term impacts on the metabolism and growth performance of the offspring. Maternal age during pregnancy is a potential challenge that can impose an influence on a developing fetus. To date there is little known regarding the effect of maternal age on offspring skeletal muscle development. The objective of this study was to compare the microanatomy and gene expression of growth hormone receptor (GH-r), insulin-like growth factor 1 (IGF-I) and 2 (IGF-II), insulin-like growth factor binding proteins 3 (IGFBP3) and 5 (IGFBP5), in skeletal muscle of fetal and adult offspring from ewe-lamb dams and adult ewe dams. Fetal semitendinosus samples were collected at day 145 of gestation from five female and four male singleton fetuses from primiparous Romney ewe-lamb dams (ELF-S) and five female and six male singleton fetuses from Romney adult dams (AEF-S). Adult semitendinosus samples were collected from 40 ewes at 2.5 years of age; 10 singleton-born ewe progeny from primiparous ewe-lamb dams (ELP-S), 10 singleton-born ewe progeny from multiparous mature adult ewe dams (AEP-S), 10 twin-born ewe progeny from primiparous ewe-lamb dams (ELP-T) and 10 twin-born ewe progeny from multiparous mature adult ewe dams (AEP-T). The number of myosin heavy chain (MHC) fast muscle fibres, MHC slow muscle fibres and the total number in fetal muscle tissue did not differ ($P > 0.05$). In adult muscle tissue, there was no effect of dam age on fibre type or total fibre number ($P > 0.05$); however there was a significant effect of progeny birth rank, whereby twin-born progeny had more MHC fast muscle fibres and subsequently a greater total number of muscle fibres ($P < 0.05$). Gene expression in fetal tissue was not affected by dam age. Gene expression in the four adult progeny groups differed ($P < 0.05$) for IGF-I, IGF-II and IGFBP3. Singleton-born ewes

with adult ewe dams had higher expression of IGF-I and IGFBP-3, while singleton with ewe-lamb dams had higher gene expression for IGF-II. Twin-born ewes with ewe-lamb dams tended ($P < 0.10$) to have reduced GH-r. There was no difference found in adult muscle tissue for expression of IGFBP5. Thus further studies are warranted to more conclusively determine the extent and magnitude of those effects on offspring born to either ewe-lambs or mature ewes.

INTRODUCTION

Previous chapters have shown consistently differences in growth and live weight between progeny born to ewe-lamb and those born to adult ewe. This chapter was design to examine, at a molecular level, factors controlling muscle growth that may be contributing to the productive differences observed in previous chapters.

The developmental programming theory suggests that when a fetus developing is exposed to a specific challenge, such as a metabolic insult via maternal nutrient supply, permanent adaptations to skeletal muscle metabolism, postnatal muscle development growth, muscle reparation and energy substrate partitioning can occur (Gluckman and Hanson, 2004; Bowker, 2013). Myogenesis, adipogenesis and fibro-genesis are processes involved in fetal skeletal muscle development (Du *et al.*, 2010). Muscle mass is determined by fibre number which is known to be essential for postnatal growth. Further, fibre number is set during prenatal development (Rehfeldt *et al.*, 2000; Fahey *et al.*, 2005). Luff and Goldspink (1967) demonstrated that live weight was correlated with both the number of muscle fibres and their size.

The majority of leg muscle fibre formation is completed in fetal sheep around day 85 of gestation coinciding with a peak of insulin-like growth factor II (IGF-II) and myogenin (Fahey *et al.*, 2005). While maturation of skeletal muscle in sheep occurs by approximately day 105 of gestation and muscle fibre hypertrophy starts about day 115 of gestation (Brameld *et al.*, 2010). Therefore nutrient restriction in late gestation will not affect muscle fibre number as this is set by the end of the second trimester, but it can affect the degree of fibre hypertrophy (Greenwood *et al.*, 1999). Skeletal muscle development in the adult involves only hypertrophy of existing muscle fibres (Glore and Layman, 1983; Greenwood *et al.*, 2000; Nissen *et al.*, 2003),.

Therefore in meat animals, the fetal stage is an important period to affect future production (Zhu *et al.*, 2006; Du *et al.*, 2010). Primary myofibers are developed during the initial stage of myogenesis at the embryonic stage while secondary myofibres are formed during the fetal stage (Du *et al.*, 2010). In general, primary myofibres will mature into slow-twitch fibres (Type I: slow oxidative) and secondary myofibres will mature into fast-twitch fibres (Type IIa: fast oxidative/glycolytic, Type IIb: fast glycolytic) (Oksbjerg *et al.*, 2004) which both display distinct metabolic and contractile properties (Braun and Gautel, 2011). Secondary fibres account for the majority of skeletal muscle fibres. Type I fibres are less efficient for growth, having greater protein turnover, whilst type II fibres are more efficient with reduced catabolic rates (Garlick *et al.*, 1989; Du *et al.*, 2010).

Studies have linked developmental programming to animal performance in livestock (Du *et al.*, 2010) showing under- and over-nutrition during gestation can influence offspring growth and muscle development (Zhu *et al.*, 2004; Symonds *et al.*, 2007; Quigley *et al.*, 2008). Offspring born to restrictively fed ewes display altered muscle fibre numbers and increased adiposity (Daniel *et al.*, 2007; Ford *et al.*, 2007). Further, maternal under-nutrition can alter the somatotrophic axis during fetal development and these changes can persist into postnatal life (Greenwood *et al.*, 2000b; Wu *et al.*, 2006; Ford *et al.*, 2007). The somatotrophic axis includes growth hormone (GH), insulin-like growth factor I (IGF-I), IGF II and their binding proteins (IGFBP 1 to 6).

Insulin-like-growth-factors have been implicated in skeletal muscle growth, hypertrophy and regeneration. When IGF-I is overexpressed in rodents, they have larger muscle fibres (Barton-Davis *et al.*, 1998). IGF-binding proteins (IGFBPs) modulate the action of IGF and have been implicated in the control of growth in cattle (Rausch *et al.*,

2002). IGFBP3 binds the majority of IGF-I and IGF-II in postnatal life (Frystyk *et al.*, 2002). The level of circulating IGF-I increases from birth to puberty, and is inversely proportional to the protein turnover in myosin heavy chain (MHC) slow and MHC fast muscles (Garlick *et al.*, 1989) and growth of skeletal muscle depends on protein turnover and cell turnover. However, the IGF's are mediated by GH, which is responsible for metabolic process, such as cellular division, cellular growth/apoptosis and protein synthesis involved in growth and these cellular responses are regulated by the growth hormone receptor (GH-r) (Hornick, *et al.*, 2000).

It is known that fetuses from ewe-lamb dams are smaller/lighter than those from mature ewe dams (Chapters 3). In addition lambs born to ewe-lamb dams are lighter than those born to mature ewe dams (Chapters 4, 6 and 7). However, to date it is unknown if fetuses and offspring from young dams have altered muscle development compared with fetuses from mature dams. The objective of this study was to examine the muscle fibre microanatomy and expression of IGF-I, IGF-II, IGFBP3, IGFBP5 and growth hormone receptor (GR-r) in skeletal muscle of fetuses and adult offspring from ewe-lamb dams and mature ewe dams.

MATERIALS AND METHODS

Background

Fetal semitendinosus samples were collected at day 145 (range of 139-145 days) of gestation from five female and four male singleton fetuses from primiparous Romney ewe-lamb dams (47.0 ± 0.7 kg) and five female and six male singleton fetuses from Romney multiparous mature mixed- aged dams (64.4 ± 1.5 kg) that had conceived within a 6 day period. The management of these dams has been previous described in Chapter 3.

Adult semitendinosus samples were collected from 40 ewes at 2.5 years of age born to either ewe-lambs or mature ewes (the management of these ewes has been previously described Chapter 7). These ewes were randomly selected within maternal group and birth rank: 10 singleton-born primiparous ewe-lamb dam progeny (ELP-S), 10 singleton-born multiparous mature adult ewe dam progeny (AEP-S), 10 twin-born primiparous ewe-lamb dam progeny (ELP-T) and 10 twin-born multiparous mature adult ewe dam progeny (AEP-T).

Sample collection

Fetal tissue

Examination and collection of fetal organs and tissue has been previously described in Chapter 3. In brief, at day 145 of pregnancy, 9 ewe-lamb and 11 adult ewe dams were euthanised and their gravid uterus was removed. The uterine wall was incised, the fetus removed, euthanised, and weighed. Fetal left hind-leg muscles were dissected and the semitendinosus was identified, separated from surrounding tissues and removed, with the terminal attachments intact. The semitendinosus muscle was weighed, measured (length) and tissue samples were collected for immunohistochemistry and gene expression.

For each immunohistochemistry sample a cross-sectional area was cut at the middle region of the muscle, width and depth about 1 cm by 1 cm respectively. Each sample was mounted in a disposable clear vinyl mould for frozen sectioning (Cryomold, Tissue Tek, Sakura Finetek, USA, INC) with optimal cutting temperature compound (O.C.T. compound, Tissue Tek, Sakura Finetek, USA, INC). The muscle samples were aligned in the mould to allow cryosections to be cut perpendicular to the direction of the muscle

fibres. Each mould containing a sample was snap-frozen in liquid nitrogen, wrapped in aluminium foil, labelled and stored at -80°C.

Concomitantly, fetal muscle samples were collected for gene expression from the same region, adjacent to the sample taken for immunohistochemistry. Samples were divided in two, with half put in a cryovial containing RNAlater[®] (Ambion, Austin, TX) and the other half wrapped in aluminium foil. Both were snap-frozen in liquid nitrogen and stored at -80° C for later gene expression analysis.

Adult tissue

Surgical muscle biopsies were taken from 40 adult ewes at 2.5 years of age. The samples were taken from the left hind-leg, semitendinosus muscle. Ewes were immobilised in a pen and the wool at the biopsy site was shorn. Alcohol-ibitane solution was used to clean the area. Local anaesthetic (Lignocaine hydrochloride; 3mL) was injected subcutaneously into the biopsy site. An incision was made into the skin horizontally, and muscle was accessed and clasped with Allis clamps and excised. After removal of the muscle sample, pressure was applied to the biopsy site for several minutes and then the skin was closed using adhesive (UltraBonder; Holdfast NZ Ltd, New Zealand). Antibacterial aerosol (Aerotet Forte, Virbac Animal Health, New Zealand) was applied around the incised area and ewes were provided with intermuscular analgesic (Ketoprofen 10%; 3.0 mg/kg of live weight; Kela, Belgium) and antibiotic (Engemycin; oxytetracycline 20mg/kg of live weight; Schering Plough Animal Health Ltd, New Zealand). Ewes were maintained in individual pens for a minimum of 2 hours after the biopsy procedure to ensure bleeding had ceased. After this time they re-joined the rest of the flock in the paddock. After one week, biopsy sites

were checked to ensure appropriate healing and sprayed with antibacterial aerosol if necessary.

Each muscle sample was divided into two parts: one was stored for immunohistochemistry analysis whilst the other was stored for gene expression. For immunohistochemistry, the muscle sample was trimmed to approximately 1 cm³ and the muscle fibre direction identified. The sample was mounted in a cryomold, with O.C.T. compound in such a way as to allow the cryosections to be cut transversal to the direction of the muscle fibres. Each mould containing the sampling was frozen by immersion in isopentane cooled in liquid nitrogen. Frozen samples were then individually wrapped in aluminium foil, labelled and stored at -80°C. Muscle samples for gene expression were immediately snap-frozen in liquid nitrogen and put in labelled cryovials. These samples were stored at -80°C until RNA extraction.

Immunohistochemistry

The procedure described below was applied to both fetal and adult muscle samples.

Immunohistochemistry for myosin heavy chain fibre typing

The O.C.T. embedded samples were removed from the -80°C freezer and placed in the cryostat for approximately 4 hours to permit the muscle to reach -16°C. The sample was then mounted on a cryostat chuck with a few drops of O.C.T. and 10 µm thick cross-sections were cut, with the muscle fibres perpendicular to the microtome (Leica 2020, Wetzlar, Germany) blade. The sections were mounted on Superfrost[®] Plus microscope slides (Menzel-Glaser, Braunschweig, Germany) and stored at -80°C until commencement of the staining procedures.

Sectioned samples of skeletal muscle were removed from the freezer and allowed to air dry for at least 1 hour. Sections were fixed in 100% acetone for 12 minutes, air-dried for 5 minutes, then rehydrated with phosphate buffered saline (PBS), pH 7.4 (0.1 M PBS; Gibco[®] Life Technologies Corporation, New Zealand). Sections were washed in 0.1 M glycine in PBS and rinsed with PBS. The mouse monoclonal antibody against myosin heavy chain slow or type I (WB-MHCs; Vector Laboratories - VP-M667; diluted 1:400 in PBS), or against myosin heavy chain fast or type II (clone MY 32, Sigma-Aldrich New Zealand Co.; M4276; diluted 1:400 in PBS) was applied to tissue sections and incubated in a humid chamber at 4°C overnight. Sections were washed twice in washing buffer (PBS pH 7.4 plus 0.05% Tween 20). To quench endogenous peroxidase activity, sections were incubated for 12 minutes in 3% hydrogen peroxide in methanol and rinsed in PBS. The secondary biotinylated antibody (Kit Histostain SP; Zymed Laboratories; Z95 9943 B) was applied for 15 minutes. The slides were washed with PBS and subsequently incubated with biotin streptavidin peroxidase (Kit Histostain SP) for 10 minutes at room temperature. Slides were rinsed with PBS and the peroxidase substrate 3,3'-diaminobenzidine (DAB; Vector Laboratories, Inc.) applied for 10 minutes or until the reaction could be visualised. Sections were rinsed in running tap water for 5 minutes, dehydrated and cleared using graded ethanols and xylene and mounted using Entellan[®] mounting medium (Merck Millipore, New Zealand).

Estimation of fibre number

Cross-sections were viewed using a light microscope (Axiophot microscope, Carl Zeiss) linked to an image capture system (Olympus DP72). At least 10 fields of view (FOV) for fetal samples and five FOV for adult samples were randomly selected. Photomicrographs were counted using Adobe Photoshop CS5 software for Macintosh,

the total area of the cross-section was calculated by adding a known grid line scale; this grid generated 35 squares of 50 μm x 50 μm .

Fetal samples had a large amount of connective tissue and empty spaces. To avoid these areas only squares containing muscle fibres were selected, and the number of squares selected and counted was recorded for each FOV. The same procedure was applied for counting myosin heavy chain slow fibres, myosin heavy chain fast fibres and the total number of fibres.

Gene expression procedure

The procedures described below were applied to both fetal and adult muscle samples.

RNA extraction

Semitendinosus samples were removed from -80°C storage and transferred to a container containing liquid nitrogen. Approximately 100mg of frozen tissue was placed in a 2mL screw-cap microvial containing one stainless steel bead (5 mm diameter), plus 1mL of TriZol reagent (Invitrogen). The most effective method of cell disruption was with a mini-bead-beater (Biospec Products, Bartlesville, OK, USA), homogenising for a total of two minutes (two times one minute periods of homogenisation separated by a 30 seconds rest period) followed by an incubation period of 5 minutes at room temperature to allow dissociation of nucleoprotein complexes. The homogenised tissue/TriZol mix was transferred to a clean 2mL tube and 200 μL chloroform was added. This was vortex-mixed for 15 seconds followed by an incubation period of 5 minutes at room temperature. The sample was centrifuged at 12000g for 10 minutes at 4°C , to allow separation into two separate phases: an upper (aqueous) and a lower (organic) phase. The aqueous phase, containing RNA, was removed and an equivalent amount of 70% ethanol was added and mixed by pipetting. Purification was performed with the Qiagen

RNeasy kit (Qiagen, Valencia, CA), 800µL of the solution was transferred to an RNeasy column and centrifuged at 12000 rpm for 1 minute. The flow-through was discarded and 350µL RW1 buffer was added to the column and centrifuged at 9500g for 1 minute, again the flow-through was discarded. A mixture of 10µL DNase I stock solution with 70µL RDD buffer was transferred directly to the RNeasy column membrane and incubated in a water bath at 25°C for 15 minutes. The column was washed by adding 350µL RW1 buffer and centrifuged at 9500g for 1 minute. The flow-through was discarded and the washing procedure was repeated once more. The column was transferred to a new collection tube and 500µL RPE buffer added to the RNeasy column and centrifuged at 9500g for 1 minute. Another 500µL RPE buffer was added onto the RNeasy column, and centrifuged for 2 minutes at 9500g. An additional 1 minute of centrifugation was used to dry the membrane. The collection tube was discarded and the column transferred to a 1.5 mL tube, 30µL of nuclease free water was added to the centre of the RNeasy column membrane, and incubated at room temperature for 1 minute, then centrifuged for 1 minute at 9500g to elute the RNA. The collection tube attached to the column was removed, labelled and placed on ice. A 10µL aliquot of RNA was transferred to a new tube for RNA quantification. The remaining RNA was stored at -80°C.

RNA quantification

To determine the concentration of RNA a NanoDrop Spectrophotometer ND-1000 (Nanodrop Technologies, Wilmington, Delaware, USA) was used. The purity (quality) of the sample is assessed by the ratio of absorbance at 260 nm and 280 nm (absorbance wave length of RNA and DNA, respectively). A ratio of 2.0 ng/ µL and 1.8 ng/ µL is accepted as pure RNA and DNA, respectively. A secondary measure assessing the ratio

of absorbance at 260 nm and 230 nm is generally accepted if in the range of 2.0-2.2 ng/ μ L.

RNA agarose gels

Quality control was performed by running 20 μ l of each sample on a 2% E-Gel[®] (Agarose Gel Electrophoresis System, Invitrogen) using E-Gel[®] iBase[™] Power (Invitrogen) to check the integrity of the RNA. The E-Gel[®] 1 Kb Plus DNA ladder (Invitrogen) was run on the gel to give an estimate of RNA size. Images of the gels were captured using E-Gel[™] Imager (Invitrogen) and stored in digital format.

cDNA synthesis

The conversion of RNA to cDNA was done using a SuperScript[®] VILO[™] cDNA Synthesis Kit (Invitrogen), following the protocol specified in the manufacturer's instructions. In a tube on ice, 1 μ g RNA was combined with 4 μ L 5X VILO[™] Reaction Mix, 2 μ L 10X SuperScript[®] Enzyme Mix, 20 μ L nuclease free water and mixed. This was then incubated at 25°C for 10 minutes, followed by 60 minutes at 42°C and for 5 minutes at 85°C to terminate the reaction. The resulting cDNA product was stored at -80°C until qPCR.

DNA purification

The DNeasy blood and tissue kit (Qiagen, Valencia, CA) was used for rapid purification of total DNA following the protocol specified in the manufactures instructions. Tissue was cut into small pieces and placed in 1.5mL tube, 180 μ L ATL buffer was added followed by 20 μ L proteinase K, this was then mixed by vortex and incubated at 56°C until all tissue had been lysed. Total tissue lysate was then vortex mixed for 15 seconds, 200 μ L AL buffer was incorporated and mixed by vortex, 200 μ L of ethanol was then added and mixed by vortex. The mixture was transferred into a DNeasy Mini spin

column and placed in a 2 mL collection tube and centrifuged for one minute at 8000rpm. The column was transferred to a new collection tube and 500µL AW1 buffer added and centrifuged for more one minute. The flow-through was discarded and placed into a new collection tube, 500 µL AW2 buffer was added and centrifuged at 14000rpm for 3 minutes. The collection tube was discarded and the column transferred to a 1.5 mL tube and 200µL of 60°C nuclease free water was added to the centre of the mini spin column membrane, and incubated at room temperature for 1 minute, then centrifuged for 1 minute at 8000 rpm to elute the DNA. The collection tube containing the DNA was removed from the column, labelled and placed on ice. The DNA was stored at -20°C.

Primer design

Primers were designed to ensure that only one PCR product was amplified. The partial sheep genome and coding sequence were obtained for each candidate gene using sheep genome v2.0 (Retrieved August 8, 2012, from <http://www.livestockgenomics.csiro.au/sheep/>). Alignment of the cDNA and genomic DNA sequences was performed using the NCBI Spider (Retrieved August 8, 2012, from <http://www.ncbi.nlm.nih.gov/spidey/>). Following alignment the Primer3 Input program (version 0.4.0. Retrieved August 8, 2012, from: <http://bioinfo.ut.ee/primer3-0.4.0/>) was used to design the primers. The coding sequence of each selected gene was inserted into Primer3 with settings adjusted to design primers with a $T_m > 60$ °C and a nucleotide length between 15 and 30. Primer sets which amplified fragments between 80 and 200 bp were used. Primer3 generates numerous candidate primer sets for the selected genes and these candidate sets were tested for possible secondary structures with Launch Beacon Designer Free Edition (Retrieved August 8, 2012, from <http://www.premierbiosoft.com/qpcr/index.html>). If a primer showed any indication that it may form primer dimers it was discarded. Primers

were designed for insulin-like growth factor 1 (IGF-I), insulin-like growth factor 2 (IGF-II), insulin-like growth factor-binding protein 3 (IGFBP3), insulin-like growth factor-binding protein 5 (IGFBP5) and growth hormone receptor (GR-r), these hormones are involved in the growth and muscle development. The primer sequence and efficiency values are shown in **Table 30**.

Table 30. Selected genes, forward and reverse primer sequences, amplicon length (bp), annealing temperature (°C), qPCR efficiency value and concentration for fetal and adult muscle qPCR analysis.

Gene	Forward primer (5' – 3')	Reverse primer (3' – 5')	Amplicon Length (bp)	Annealing temperature (°C)	Efficiency (%)	Cycles	Concentration
Fetuses samples							
GH-r	ggacagatggggctaatcac	tgfttcaccagcagagacg	117	62	100.97	40	300nm
IGF-I	cagcagtctccaaccaat	gatgcgagaggatgtgact	86	62	98.78	40	100nm
IGF-II	aggacgggggaaccattat	cgacttctctgtgatcc	116	62	98.83	40	200nm
IGFBP3	tccaagcagagacaatacgg	ttatccacacaccagcagaacc	191	64	101.35	40	100nm
IGFBP5	cccgagctgagacaggaat	acagttgggcaggtaacg	117	64	105.47	40	200nm
TBP*	cctaaagaccattgcactcg	cttcacttggctctctgtg	146	62	93.7	40	300nm
SF3A1*	agcccgtgggtggtattt	cgcttcaattcaggctccat	98	62	91.88	40	300nm
Adult samples							
GH-r	ggacagatggggctaatcac	tgfttcaccagcagagacg	117	60	89.993	40	300nm
IGF-I	cagcagtctccaaccaat	gatgcgagaggatgtgact	86	60	108.283	40	300nm
IGF-II	aggacgggggaaccattat	cgacttctctgtgatcc	116	60	94.015	40	300nm
IGFBP3	tccaagcagagacaatacgg	ttatccacacaccagcagaacc	191	60	101.81	40	300nm
IGFBP5	cccgagctgagacaggaat	acagttgggcaggtaacg	117	60	93.526	40	300nm
TBP*	cctaaagaccattgcactcg	cttcacttggctctctgtg	146	62	90	40	300nm
SF3A1*	agcccgtgggtggtattt	cgcttcaattcaggctccat	98	62	91	40	300nm

*references genes - K. Petera pers. comm.

References genes

Real time quantitative polymerase chain reaction (RT-qPCR) relies on normalisation, by one or multiple reference genes after it has been validated (Erkens *et al.*, 2006). High quality reference genes are important for the interpretation of RT-qPCR data (Nygard *et al.*, 2007).

The reference genes were validated in fetal and adult semitendinosus muscle tissue by K. Perera (unpublished data). Eight potential reference genes were tested: succinate dehydrogenase A complex (SDHA), hydroxymethylbilane synthase (HMBS), TATA-box binding protein (TBP), splicing factor 3a subunit 1 (SF3A1), ribosomal protein large P0 (RPLP0), translation elongation factor 1 alpha 2 (EEF1A2), phosphoglycerate kinase (PGK1), Tubulin Beta 2A Class IIa (TUBB2A). Analysis of results was performed using geNorm (Biogazelle). The two most stable genes were identified to be TBP and SF3A1 and analysis showed that use of these two reference genes would be sufficient for normalisation of gene expression data. Splicing factor 3a subunit 1 has been used successfully as a reference gene for bovine muscle (Perez *et al.*, 2008; Castigliego *et al.*, 2010) and TBP has been demonstrated as a stable gene in both pig tissue (Nygard *et al.*, 2007; Perez *et al.*, 2008) and bovine tissue (Perez *et al.*, 2008).

Real time PCR setup

The reactions were setup in a UV sterilised PCR hood and performed using an Applied Biosystems® StepOnePlus™ Real Time PCR (California, USA) machine. Each sample was analysed in triplicate.

Primer specificity and efficiency testing

Primers, obtained from Invitrogen, were diluted to a concentration of 1 mM stock solution. A working solution was made by diluting the stock solution 1:100 with

nuclease free water, making a 10 pmol/ μ L concentration. A standard curve, generated by performing qPCR with a dilution series of cDNA was utilised to test the efficiency, precision, sensitivity and working range of each primer set. Serial dilutions of cDNA that contained the PCR target were prepared from the original solution as a four-fold dilution (original – 50 ng/ μ L; 1:10 – 5 ng/ μ L; 1:100 – 0.5 ng/ μ L; 1:1000 – 0.005 ng/ μ L and 1:10000 – 0.005 ng/ μ L). Each dilution was then analysed in triplicate.

RT-qPCR

A MicroAmp[®] Fast Optical 96-Well Reaction Plate 0.1 mL was used to perform the qPCR. Each well contained of 5 μ L of template (cDNA) and 15 μ L of master-mix. Master-mix was made up for each primer pair according to their optimal concentration. All reactions used SYBR[®] Green FastMix[®] (10 μ L):

- for the 100nM primer concentration of 0.2 μ L of both the forward and reverse primers;
- for the 200nM concentration 0.4 μ L of both the forward and reverse primers;
- for the 300nM concentration 0.6 μ L of the both forward and reverse primers.

Nuclease free water was added to make up a final volume of 15 μ L. Plates were sealed with an optical adhesive cover (Applied Biosystems), centrifuged and placed in the qPCR machine. Standard curves were generated and a melt curve was obtained for each sample. The qPCR programme applied to fetal and adult muscle samples for each of the gene targets are outlined in the tables below (**Table 31**, **Table 32** and **Table 33**).

Table 31. Thermal cycling parameters of RT-qPCR of fetal muscle samples for growth hormone receptor (GR-r), insulin-like growth factor 1 (IGF-I) and insulin-like growth factor 2 (IGF-II).

	qPCR					
	SYBR Green Activation	Cycle (40 cycles)			Melt Curve	
		Hold	Denaturation	Annealing/ Extension	Hold	
Temperature	95 °C	95 °C	62 °C	95 °C	60 °C	95 °C
Time	20 sec	3 sec	30 sec	15 sec	60 sec	15 sec
Volume	20 µL					

Table 32. Thermal cycling parameters of RT-qPCR of fetal muscle samples for insulin-like growth factor-binding protein 3 (IGFBP3) and insulin-like growth factor-binding protein 5 (IGFBP5).

	qPCR					
	SYBR Green Activation	Cycle (40 cycles)			Melt Curve	
		Hold	Denaturation	Annealing/ Extension	Hold	
Temperature	95 °C	95 °C	64 °C	95 °C	60 °C	95 °C
Time	20 sec	3 sec	30 sec	15 sec	60 sec	15 sec
Volume	20 µL					

Table 33. Thermal cycling parameters of RT-qPCR of adult muscle samples for growth hormone receptor (GR-r), insulin-like growth factor 1 (IGF-I), insulin-like growth factor 2 (IGF-II), insulin-like growth factor-binding protein 3 (IGFBP3) and insulin-like growth factor-binding protein 5 (IGFBP5).

	qPCR					
	SYBR Green Activation	Cycle (40 cycles)			Melt Curve	
		Denaturation	Annealing/ Extension			
	Hold	Cycle		Hold		
Temperature	95 °C	95 °C	60 °C	95 °C	60 °C	95 °C
Time	20 sec	3 sec	30 sec	15 sec	60 sec	15 sec
Volume			20 µL			

Statistical analysis

The aim of the present study was to examine at two different physiological stages (fetal and adult life) skeletal muscle from progeny of ewe-lambs or adult ewe dams. Fetal muscle samples were collected from singletons offspring (fetuses from ewe-lamb dams (ELF) and fetuses from adult ewe dams (AEF)). Adult muscle samples were collected from both singleton and twin progeny born either to ewe-lambs or adult ewes (ewe-lamb singleton-born (ELP-S) or ewe-lamb twin-born (ELP-T), adult ewes singleton-born (AEP-S) or adult ewes twin-born (AEP-T)). Statistical analysis was performed in Minitab[®] (version 16, Minitab Inc, Cary NC, USA), SAS (SAS, *Enterprise Guide*[®] 4.2) and Qbase PLUS (Biogazelle).

Fetal semitendinosus weights

Fetal semitendinosus weights were analysed using General Linear Model (GLM) procedure in Minitab, with dam type (ewe-lamb vs. adult ewe) and sex of the fetuses fitted as main effects and fetal weight fitted as a covariate.

Immunohistochemistry

Fetal

The number of muscle fibres per image was analysed using the MIXED procedure in SAS. For fetal muscle tissue, the mean fibre number per image was obtained by dividing the FOV by the number of squares ($50\mu\text{m}^2$ each) that had been counted. The fixed effects of dam age and sex of the fetus and the interaction between dam age and sex of the fetus were analysed. Fetal semitendinosus weight and fetal body weight were included as covariates, but were not significant ($P > 0.05$) and removed from the final model. Marginal means were obtained from the least squares means statement with the standard error adjusted by the standard deviation of the subsampling.

Adult

For adult muscle samples, the mean number of muscle fibres per image was obtained by averaging the number of muscle fibre types per FOV. Dam age and progeny birth rank were included as fixed effects and their interaction was also included in the model. Live weight was included as a covariate, but was not significant ($P > 0.05$) and removed for the final model.

Gene expression

Fetal

Data of fetal and adult muscle samples for gene expression was analysed using Qbase PLUS software (Biogazelle). Data files generated by the StepOnePlus™ Real Time PCR were directly imported in to Qbase PLUS. The software combines the gene expression analysis of the target genes and the reference genes and executes the normalisation. This software does not allow the inclusion of interactions in the models. Therefore the data from fetal muscle samples were analysed only with dam age included

as a fixed effect. Mean values are presented with the 95% confidence interval in parentheses.

Adult

The data from adult progeny muscle samples were also analysed using Qbase PLUS software (Biogazelle) with both dam age and birth rank as fixed effects. An additional model was analysed using the following four groups: ELP-S, ELP-T, AEP-S and AEP-T. Mean values are presented with the 95% confidence interval in parentheses.

RESULTS

Fetal semitendinosus weight

Fetal semitendinosus weight did not differ ($P > 0.05$) due to dam age ($6.8 \pm 0.45\text{g}$ vs. $7.8 \pm 0.60\text{g}$ for ELF and AEF, respectively), nor did ($P > 0.05$) it differ between female and male fetuses ($7.4 \pm 0.68\text{g}$ vs. $7.3 \pm 0.44\text{g}$ for female and male, respectively).

Immunohistochemistry

Fetal

Fetal muscle tissue did not differ ($P > 0.05$) due to either dam type or sex of the fetus for number of myosin heavy chain (MHC) fast, MHC slow or total number of muscle fibres (**Table 34**). There was no interaction ($P > 0.05$) between dam type and sex of the fetus for any parameters measured.

Table 34. Fetal muscle fibre numbers for myosin heavy chain fast (MHC Fast), myosin heavy chain slow (MCH Slow) and total fibre number (Total) of fetuses from ewe-lamb dams and adult ewes. Table shows the least square means \pm standard error.

		Number of muscle fibre (per 50 μm^2)		
		MHC Fast	MHC Slow	Total
Dam type	Ewe-lamb	5.74 \pm 0.70	0.70 \pm 0.07	6.47 \pm 0.67
	Adult Ewe	5.15 \pm 0.94	0.60 \pm 0.10	5.82 \pm 0.94
Sex of the fetus	Male	5.60 \pm 0.76	0.77 \pm 0.08	6.45 \pm 0.74
	Female	4.57 \pm 1.57	0.53 \pm 0.10	5.84 \pm 0.88

Adult

In adult muscle tissue, dam age did not have any affect on the distribution of fibre type or on total fibre number ($P > 0.05$; **Table 35**). However, there was a significant effect of birth rank whereby singleton-born progeny had less ($P < 0.05$; **Table 35**) MHC fast muscle fibres than twin-born progeny. The number of MHC slow muscle fibres did not differ ($P > 0.05$; **Table 35**) due to birth rank and consequently the total number of muscle fibres was less for singleton-born progeny compared with twin-born progeny ($P < 0.05$).

Table 35. Adult muscle fibre numbers for myosin heavy chain fast (MHC Fast), myosin heavy chain slow (MHC Slow) and total fibre number (Total) of male and female fetuses from ewe-lamb dams and adult ewes. Table shows the least square means \pm standard error.

		Number of muscle fibre (per 50 μ m ²)		
		MHC Fast	MHC Slow	Total
Dam type	Ewe-lamb	56.69 \pm 3.49	4.80 \pm 0.51	61.37 \pm 3.76
	Adult Ewe	59.81 \pm 3.23	5.78 \pm 0.45	65.51 \pm 3.43
Birth Rank	Singleton	51.38 \pm 3.62 ^a	4.90 \pm 0.49	55.58 \pm 3.89 ^a
	Twins	65.12 \pm 3.08 ^b	5.68 \pm 0.47	71.30 \pm 3.29 ^b
Dam type* Birth rank	Ewe-lamb singleton	50.46 \pm 5.55	4.43 \pm 0.78	54.01 \pm 5.99
	Ewe-lamb twin	62.94 \pm 4.23	5.16 \pm 0.65	68.73 \pm 4.56
	Adult ewe singleton	52.33 \pm 4.65	5.36 \pm 0.58	57.16 \pm 4.96
	Adult ewe twin	67.30 \pm 4.47	6.20 \pm 0.68	73.87 \pm 4.75

^{ab} different superscripts within columns sections indicate values that significantly differ ($P < 0.05$)

*Gene expression**Fetal*

Dam age did not ($P > 0.05$) affect the expression of any of the genes examined in fetal muscle tissue (**Table 36**).

Table 36. Gene expression values for GH-r, IGF-I, IGF-II, IGFBP3 and IGFBP5, in fetal sheep semitendinosus muscle (\pm 95% confidence interval).

		Selected genes				
	n	GH-r	IGF-I	IGF-II	IGFBP3	IGFBP5
Ewe-lamb	9	1.25 (1.05-1.47)	1.05 (0.89-1.25)	0.95 (0.79-1.15)	1.66 (1.14-2.40)	1.38 (1.16-1.65)
Adult Ewe	11	1.17 (1.06-1.30)	1.24 (1.02-1.52)	1.02 (0.82-1.26)	1.50 (1.06-2.14)	1.28 (1.15-1.44)
<i>P</i> value		0.82	0.18	0.46	0.55	0.30

Adults

In adult muscle tissue, there were no differences ($P > 0.05$) in gene expression due to dam type or birth rank. However, there was a tendency ($P = 0.052$) for gene expression of IGFBP3 to be more highly expressed in AEP compared with ELP (**Table 37**).

The Q-base analysis of the four groups (ELP-S, ELP-T, AEP-S and AEP-T) demonstrated a tendency ($P = 0.079$) for a difference in expression of GH-r between the four groups, with ELP-T having the lowest expression (**Table 37**), compared with the other groups. The expression of IGF-I in AEP-S was higher ($P < 0.05$) than in AEP-T (**Table 37**), with IGF-I expression of ELP-S and ELP-T being intermediate to the aforementioned groups. The expression of IGF-II was higher ($P < 0.05$) in ELP-S compared with ELP-T and AEP-S (**Table 37**). Expression of IGFBP-3 was higher ($P < 0.05$) in AEP-S compared with ELP-S and ELP-T (**Table 37**).

Table 37. Gene expression values for GH-r, IGF-I, IGF-II, IGFBP3 and IGFBP5 in adult sheep semitendinosus muscle (\pm 95% confidence interval).

		Selected genes				
	n	GH-r	IGF-I	IGF-II	IGFBP3	IGFBP5
Dam type	Ewe-lamb	1.16 (0.96-1.41)	0.53 (0.39-0.73)	1.40 (1.04-1.87)	0.69 [*] (0.60-0.79)	1.23 (1.00-1.49)
	Adult ewe	1.33 (1.07-1.65)	0.54 (0.31-0.93)	1.06 (0.71-1.59)	0.95 [†] (0.74-1.24)	1.03 (0.87-1.21)
Birth rank	Singleton	1.29 (1.08-1.53)	0.64 (0.39-1.06)	1.37 (0.96-1.95)	0.89 (0.69-1.15)	1.09 (0.97-1.22)
	Twin	1.20 (0.94-1.52)	0.45 (0.31-0.64)	1.08 (0.76-1.54)	0.74 (0.62-0.88)	1.16 (0.91-1.47)
Dam type* Birth rank [†]	Ewe-lamb singleton	1.42 [†] (1.03-1.96)	0.41 ^{ab} (0.28-0.62)	2.37 ^b (1.88-2.98)	0.65 ^a (0.57-0.74)	1.12 (0.91-1.37)
	Ewe-lamb twin	0.95 [*] (0.79-1.15)	0.69 ^{ab} (0.42-1.13)	0.82 ^a (0.65-1.02)	0.73 ^a (0.56-0.96)	1.34 (0.92-1.96)
Adult ewe singleton	Adult ewe singleton	1.17 [†] (0.97-1.40)	1.01 ^b (0.40-2.52)	0.79 ^a (0.49-1.27)	1.22 ^b (0.79-1.88)	1.06 (0.90-1.24)
	Adult ewe twin	1.50 [†] (0.98-2.32)	0.29 ^a (0.19-0.44)	1.44 ^{ab} (0.73-2.83)	0.75 ^{ab} (0.57-0.99)	1.00 (0.72-1.39)

^{ab} superscript with columns indicate values that significantly differ ($P < 0.05$)^{†*} superscripts indicate values which have a tendency to differ ($P < 0.10$)

DISCUSSION

The objective of this study was to analyse the microanatomy and gene expression of skeletal muscle of fetuses and adult offspring from either ewe-lamb or adult ewe dams. Young mothers give birth to lighter, smaller offspring (Friede *et al.*, 1987; Cogswel *et al.*, 1995; Corner *et al.*, 2013), most likely as a result of competitive nutrient partitioning favouring the young growing mother rather than the fetus. Additionally, twin-born offspring are lighter and smaller at birth than their singleton counterparts (Vesely *et al.* 1970; Al-Shorepy 2001; Annett and Carson 2006; Gootwine *et al.* 2006, 2007). Sales *et al.* (2013) reported that late gestation twin sheep fetuses had lower skeletal muscle weight compared with singleton fetuses, due to muscle hypotrophy as a result of the restricted uterine environment. To date there are no studies in the sheep literature that combine dam age and birth rank and compare muscle fibre microanatomy and gene expression.

The fetal muscle tissue utilised in the present study was derived from singleton fetuses described in Chapter 3. Those results showed that the weight of singleton fetuses in late gestation did not differ due to dam age, similar to results reported here that indicate the total number of muscle fibres did not differ. As muscle fibre numbers are set in fetal life and do not change after birth (Greenwood *et al.*, 2000; Rehfeldt *et al.*, 2000; Nissen *et al.*, 2003; Fahey *et al.*, 2005). However, a difference in muscle fibre numbers was observed in adult ewes of different birth rank, whereby twin-born ewes had more total muscle fibres per unit area than singleton ewes. This was driven largely by twin-born ewes having an increased number of MHC fast (type II) fibres per unit area compared with singleton ewes, which given the uniform unit area measured, suggests that the size/diameter of MHC fast fibres in twin-born ewes were smaller than in singleton born ewes. This apparent reduction in muscle fibre hypertrophy in twin-born ewes may

explain the reduced live weight of twin-born ewes compared with singleton- born ewes as described in Chapter 7 and 8. In support of this, McCoard *et al.* (1997) found that late gestation twin fetuses had a smaller cross sectional area and weight of the semitendinosus compared with singletons. Conversely, in a later experiment, McCoard *et al.* (2000) reported that semitendinosus muscle fibre number in singleton and twin fetuses at 140 days of gestation did not differ. Studies have shown that singleton and twin fetuses have distinct hormone profiles: van der Linden *et al.* (2013) reported lower IGF-I concentrations in twin fetuses compared with singletons fetuses. IGF-I stimulates systemic body growth, including that of skeletal muscle and thus it is feasible that there are may be differences in fibre number or type in fetuses from single/multiple pregnancies. The present study did not have muscle samples from twin fetuses and thus this could not be investigated.

IGF-I is a potent muscle growth factor (Braun and Gautel, 2011) which induces hypertrophy in muscle cells (Vandenburgh *et al.*, 1991), and whose action is modulated by IGFbps (Rausch *et al.*, 2002). In the present study IGF-I and IGFbp3 were more highly expressed in the semitendinosus tissue of singleton adult offspring born to adult ewe dams (AEP-S) compared with singleton and twin offspring born to ewe-lamb dams (ELP-S and ELP-T, respectively) and twin offspring born to adult ewe dams (AEP-T). While GH-r expression tended to be reduced in ELP-T compared with other groups (ELP-S, AEP-S and AEP-T). These differences may explain the reduction in MHC fast muscle fibre hypertrophy observed in twin-born ewes, although, if this was the case it would be expected that the gene expression would be consistently across dam groups. It is also consistent with the growth performance reported in Chapter 7 and 8, where AEP-S were heaviest and ELP-T were the lightest. IGFbp5 was not differently

expressed in adult sheep muscle suggesting that neither maternal age nor birth rank may have any influence on the modulation of this binding protein.

The major role of IGF-II is as a fetal growth promoter during gestation, however, no differential expression of IGF-II was observed in fetal semitendinosus tissue. The lack of a twin fetus meant it was not possible to compare IGF-II expression for the different birth ranks. Little is known about the action and expression of this hormone in sheep muscle during adulthood. The present study showed that semitendinosus tissue of adult singletons born to ewe-lamb dams had greater expression of IGF-II compared with both their twin-born counterparts and singletons born to adult ewe dams. The reason for this differential expression in IGF-II remains unclear.

CONCLUSIONS

Under the conditions of the present study, late gestation singleton fetuses from either ewe-lamb dams or adult ewe dams were comparable in regards to their muscle fibre microanatomy and gene expression. In adult tissue (2.5 years of age), there is an interaction between dam age and progeny birth rank for expression of IGF-I and IGFBP3 which supports findings of differential MHC fast muscle fibre hypertrophy and growth performance. However, to better understand these findings, more studies are needed. The differences in growth factor expression observed in the present study may, in part, explain the differences in live weight of ewe-lamb progeny compared with adult ewe progeny.

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Chapter 10

General Discussion and Conclusion

INTRODUCTION

Approximately 30% of ewe-lambs are bred in New Zealand each year (ANON, 2013). One reason for this relatively low proportion is the limited information available for farmers on the potential short to long-term impacts on offspring born to a ewe-lamb (Kenyon, 2004; Kenyon *et al.*, 2014). Therefore, an objective of this thesis was to examine the effects of being born to a ewe-lamb dam, in terms of progeny growth, development, and reproductive and lactational performance. Further, the growth from birth to weaning of the grand-offspring was also examined. Increased knowledge of the potential short- to long-term impacts on the progeny will provide useful information to farmers and may influence their decision of whether to breed their ewe-lambs or not and also whether or not progeny from those ewe-lambs are suitable to be kept as replacements. In addition, this information regarding the effects of maternal age may be of potential interest to researchers examining other species.

Important outcomes

The following section is a brief summary of the findings detailed in this thesis.

Chapter 3

The hypothesis tested was that late gestation singleton fetuses from primiparous ewe-lamb dams would differ to those from multiparous adult ewe dams. It was observed that fetuses (day 145) from ewe-lambs had similar fetal weights compared with those from adult ewes. Although, fetuses from ewe-lamb dams had some body dimension and organ measurements (hind-leg lengths, head lengths and brain weights) that were smaller than fetuses from adult ewes. Overall, this study indicated that maternal age had little effect on the weight of singleton fetuses in late gestation.

Chapter 4

The effect of maternal age on the growth trajectory, body and carcass composition of singleton male offspring from birth to one year of age was examined. It was hypothesised that singleton male lambs born to ewe-lamb dams would be lighter to one year of age than those ram-lambs born to mature adult ewe dams. It was found that males born to ewe-lamb dams were lighter to four months of age only relative to males born to adult ewe dams. Although, at slaughter, males born to ewe-lamb dams had lighter carcass weights and tended to have more visceral fat than those born to adult ewe dams. Despite these observed differences, it is unlikely that utilising these males for finishing would have a significant negative economic impact for farmers.

Chapter 5 and Chapter 6

These chapters were designed to examine the growth trajectory and body composition of singleton ewe-offspring to three years of age and their reproductive and lactational performance at their first parity. In Chapter 5, it was hypothesised that ewe-offspring born to ewe-lamb dams would display differences in body composition, as measured by computed tomography (CT) scanning, at different physiological stages in adult life compared with ewe-offspring born to mature adult ewe dams. In Chapter 6 it was hypothesised that ewe-offspring born to ewe-lamb dams would display poorer reproductive and lactational performance compared with those born to adult ewe dams.

Ewe-offspring born to ewe-lamb dams were lighter than those born to mature adult ewe dams to 12 months of age. However, there was no effect on their reproductive or lactational performance to the weaning of their first set of lambs born at dam age of 2 years. In their second pregnancy/lactation, offspring with a ewe-lamb granddam were heavier at birth than those with an adult ewe granddam. These findings are important for

the sheep industry, as this demonstrates to farmers that despite the live weight differences between singleton progeny born to ewe-lamb and adult ewe dams, there appears to be no negative effects on their reproductive and lactational performance to at least 3 years of age.

Chapter 7 and Chapter 8

These chapters were designed to analyse the growth trajectory, morphometric characteristics and reproductive performance of both singleton and twin-born ewes from either ewe-lamb dams or adult ewe dams until approximately 3 years of age, as well as their offspring's development (grand-offspring) until weaning. It was hypothesised that singleton and twin ewes born to ewe-lamb dams would display inferior postnatal growth and puberty attainment and reproductive performance compared with singleton and twin ewes born to adult ewe dams from birth to 38 months of age. Ewes born to ewe-lamb dams were lighter from birth to 550 days of age compared with those with adult ewe dams, although body condition score did not differ between the two groups. Maternal age had no effect on ewe-offspring puberty attainment, nor did it affect reproductive performance at their first or second lambing. Second generation lambs (grand-offspring) with ewe-lamb granddams were heavier at birth than those that had adult ewe granddams at both the first and second lambing of the first generation ewe-offspring; however, those differences were not observed at weaning. Combined, these results support the findings of Chapter 6 that farmers can utilise both singleton and twin-born ewe offspring from ewe-lamb dams without being concerned that there will be a negative impact of dam maternal age on their potential production levels to at least 3 years of age.

Chapter 9

The focus of this chapter was to determine if there were differences in the semitendinosus muscle microanatomy and gene expression of IGF-I, IGF-II, GR-r, IGFBP3 and IGFBP5 of singleton fetuses and singleton and twin-born adult ewes from either ewe-lamb dams or adult ewes. It was hypothesised that singleton fetuses and singleton and twin born adult offspring from ewes born to ewe-lamb dams would demonstrate poorer development and growth of skeletal muscle compared with singleton fetuses and singleton and twin born adult offspring from ewes born to adult ewe dams. The results demonstrated no differences in fetal muscle microanatomy. Twin-born adult ewes were found to have a greater number of myosin heavy chain fast muscle fibres per unit area compared with singleton-born adult ewes. Maternal age did not influence gene expression in fetal muscle tissue; however, there were some differences in gene expression of adult muscle tissue. The expression of IGF-I and IGFBP3 was higher in singleton ewes with adult ewe dams, which aligns with the growth differences observed in Chapter 7 and 8. In addition, adult singletons born to ewe-lamb dams had greater IGF-II gene expression compared with both their twin-born counterparts. Combined, these results indicate there are differences evident at the molecular level between progeny of different birth rank and dam age, which may influence their growth patterns. However, further work is required to determine the significance these differences and how they may contribute to the limited production effects observed in the earlier chapters.

IMPLICATIONS OF THESIS FINDINGS

There are very few studies examining the long-term effects of being born to a ewe-lamb dam (Craig, 1982; Corner *et al.*, 2013; Kenyon *et al.*, 2014). The sparse, and often contradictory, information currently available has made it difficult for farmers to make informed decisions. The combined results of this PhD study indicate there are few measureable production differences in singleton and twin-born offspring (to 3 years of age) born to either ewe-lambs or adult ewe dams. Despite generally having relatively lower live weights, ewe progeny born to ewe-lamb dams did not have impaired reproductive or lactational performance. This should indicate to farmers that ewe-offspring born to ewe-lamb dams can be selected as suitable replacement females within a breeding flock. However, farmers may need to be aware that ewes, especially those born as twins, from ewe-lambs dams are typically lighter in their first year of life and that this may cause some difficulty if they are to be bred as a ewe-lamb themselves at 8 months of age. In addition, male progeny born to ewe-lamb dams and reared to slaughter do not appear to be negatively impacted in relation to their carcass characteristics.

POTENTIAL LIMITATIONS OF THE STUDIES

If these studies were to be repeated some alterations might be considered. Future studies may wish to collect serial fetal data compared with only one data collection point and also data from multiple birth ranks, rather than the one late gestation time point and collection of only singleton data described in Chapter 3. The collection of more fetal tissue and twin muscle tissue would allow further gene expression comparisons between prenatal and postnatal life. In that regard, serial tissue collection post-birth to adulthood

should also be considered. The collection of additional tissues (e.g. liver biopsy and plasma collection) to investigate the mechanism(s) leading to the different growth trajectories observed may be useful, as IGF-I is synthesised primarily in the liver as an endocrine hormone responsible for normal skeletal growth and in late gestation IGF-I mRNA levels in fetal liver are positively correlated with IGF-I in plasma (Hannon *et al.*, 1991). It is known that low levels of IGF-I are related to low fetal growth rates (Brameld *et al.*, 2000). Also analysis of growth hormone (GH) would demonstrate if there is a direct change in the level of this hormone being produced by the hypothalamic pituitary axis. Combined analyses of these parameters would allow a clearer understanding of the cascade of events starting with secretion of GH from the pituitary gland, expression of liver IGF-I, and secretion of IGF-I from the liver into circulation for systemic growth.

In hindsight, another limitation was the image collection by CT scanning. The images obtained were deliberately designed to investigate internal abdominal fat based on the findings in Chapter 4 that suggested an increase in the abdominal visceral fat accumulation in male-offspring from ewe-lamb dams. Therefore, when performing CT of the ewe-offspring, images were restricted to the abdominal region and the shoulders and rear limbs of the animals were excluded. Collection of images from a greater area of the body would have allowed for a better prediction of total body weight and total body fat. Additionally, increasing the number of ewes scanned would have improved the power of the statistical analyses.

FUTURE CONSIDERATIONS

This PhD study has uncovered several potential questions that could be addressed in future research. In particular, a longer-term study would be of benefit. In this thesis offspring were followed until 3-3.5 years of age, or until their second parity only. A life time study to 6 years of age to determine longevity and lifetime productivity would provide further information to farmers; however it is necessary to acknowledge that a study of this duration is beyond the scope of a PhD programme. Additionally, the results reported in this thesis indicate some intergenerational effects (i.e. grand-offspring productivity and life-time performance) that could be explored; however it must again be acknowledged that such an undertaking would necessitate long-term study.

An economic modeling study, exploring the effects of maternal age on the profitability of the progeny, would provide valuable information to producers. Dam age had an effect on offspring live weight (Chapter 6, 7 and 8), especially in twins, but did not negatively impact their reproductive and lactational performance. This suggests that those ewes born to ewe-lamb dams may be more efficient in terms of kilogram of product produced per kilogram of dry matter eaten.

The vast majority (70.7%; ANON, 2014) of ewes do not produce their first lamb until 2 years of age. This because the majority of farmers believe that breeding young ewes (ewe-lambs, 7-9 months of age) can have a negative impact on their future reproductive performance, largely because it will disrupt the growth trajectory of those young ewes. Additionally, the specialised pre- and post-breeding management required to successfully breed ewe-lambs would increase on farm workloads and total flock feed requirements. This thesis reports on comparisons between progeny of ewe lamb dams

and mature aged dams (3-5 years of age). The inclusion of progeny of first lambing 2-year-old dams may also be warranted, as this would provide information that reflects the typical age of ewes at first lambing in the majority of New Zealand's sheep breeding programmes.

The body composition assessment by CT scan was performed in ewes carrying singleton fetuses only. More variables could have been analysed if ewes carrying twin fetuses had been included, such as, a comparison between fetus size and dam size, and comparisons of body composition changes between single and twin-bearing ewes that were themselves born to either ewe-lamb dams or adult ewe dams. Additionally, the ewes that underwent CT scanning were all singleton-born ewes. The inclusion of twin-born ewe progeny from ewe-lamb dams or adult ewe dams would have allowed investigation of progeny birth rank, as it was the live weight of twin-born ewe progeny (Chapter 7 and 8) from ewe-lamb dams that was most affected. As indicated earlier, it would be of great value to repeat the CT study with a greater number of animals.

There is opportunity to explore more aspects of the molecular biology underpinning the growth differences observed; there are potential genes that could be explored to complement the work that has been started in Chapter 9. It was found that the expression of IGF-I, IGF-II and GH-r genes in adult ewe skeletal muscle was influenced by dam type and birth rank. Analysis of muscle IGF receptor gene expression may contribute to better understanding the implications of the differences reported here in the expression levels for IGF-I and IGF-II. Analysis of liver tissue GH-r and IGF-I gene expression would also be of interest as the liver is a major target organ for GH and the principal site of IGF-1 synthesis. The release, last year (2013), of the latest ovine genome (Sheep Genome v3.0 - OARv3.0) will allow for greater access

to gene primer sequences with adequate specificity; this was a limitation for some of genes we intended to examine here, such as GH.

CONCLUDING STATEMENT

The studies reported in this thesis are some of the few specifically exploring the performance of ewe-lamb progeny. This thesis provides new information to farmers who may be considering keeping progeny born to ewe-lambs. It has also identified new areas that would benefit from further investigation. In summary, the results showed offspring born to ewe-lamb dams were lighter than those born to mature adult ewe dams until at least one year of age; however, this had no negative impact on later-life offspring reproductive or lactational performance to 3 years of age. The performance to weaning of grand-offspring was also not negatively affected; in fact the grand-offspring of ewe-lambs were found to be heavier at birth. Collectively, the body of work conducted in this thesis suggests to farmers that, if well managed, progeny born to ewe-lamb dams could be retained and have future productive performance similar to ewe-lambs born to mature ewes.

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Appendices

Parameter	Adult Ewe	Ewe-Lamb	P value	Female	Male	P value
Fetal body Volume (FBV)	6311.31±341.93	5659.65±219.38	0.83	6251.26±363.38	5784.87±246.23	0.88
FBV:Fore-leg	201.69±9.29	185.72±5.9	0.84	199.82±10.19	189.19±6.1	0.95
FBV:Hind-leg	169.18±7.92	158.55±5.41	0.77	168.91±8.5	159.87±5.43	0.94
FBV:Femur	590.99±24.2	563.42±20.48	0.79	588.56±29.34	568.61±14.51	0.83
Brain (g)	52.3±1.14	49.28±0.8	0.20	51.21±1.29	50.67±0.97	0.94
Pineal (g)	0.03±0.02	0.01±0	0.33	0.03±0.02	0.01±0	0.27
Brain:Liver	0.4±0.03	0.44±0.02	0.65	0.42±0.03	0.42±0.3	0.30
Liver (g)	137.05±9.86	122.84±5.12	0.45	127.46±9.75	124.85±8.75	0.28
Left Kidney (g)	14.25±0.62	12.05±0.78	0.36	12.99±0.97	13.53±0.5	0.02
Right Kidney (g)	13.56±0.55	11.94±0.68	0.73	12.55±0.84	13.11±0.39	0.02
Total Kidney (g)	27.81±1.14	23.99±1.45	0.49	25.54±1.8	26.64±0.87	0.01
Kidney fat (g)	18.38±0.95	16.85±0.96	0.66	18.29±1.15	17.09±0.75	0.68
Heart (g)	38.41±2.04	36.03±1.92	0.45	39.3±2.2	35.38±1.63	0.59
Heart fat (g)	5.52±0.4	4.8±0.4	0.57	5.52±0.43	4.87±0.38	0.50
Pancreas (g)	4.08±0.22	4.13±0.16	0.78	4.16±0.21	4.04±0.19	0.83
Spleen (g)	7.08±0.43	6.89±0.68	0.75	7.47±0.6	6.52±0.43	0.48
Lungs (g)	189.62±10.51	170.56±9.48	0.84	188±13.13	174.09±6.61	0.86
Thymus (g)	26.18±3.08	22.72±2.54	0.84	23.94±2.35	25.31±3.44	0.29
Thyroid (g)	1.33±0.12	1.17±0.07	0.90	1.28±0.12	1.24±0.09	0.53
Adrenal (g)	0.52±0.04	0.53±0.02	0.16	0.57±0.03	0.48±0.03	0.14
Semitendinosus (g)	7.75±0.6	6.76±0.45	0.89	7.22±0.44	7.39±0.68	0.17

Appendix 1. Mean (\pm standard deviation) measurements of male and female fetuses from either ewe-lambs or adult ewes at d145 for fetus radius, fetus volume, volume:fore-leg, volume:hind-leg, volume:femur, brain, pineal, brain:liver, liver, left kidney, right kidney, total kidney, kidney fat, heart, heart fat, pancreas, spleen, lungs, thymus, thyroid, adrenal, semitendinosus weight.

Appendix 2. Statement of Contribution to Doctoral Thesis Containing Publications

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(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Maria Fernanda Pereira Loureiro

Name/Title of Principal Supervisor: Professor Paul R. Kenyon

Name of Published Research Output and full reference:

LOUREIRO, M. F. P., PAIN, S. J., KENYON, P.R. & BLAIR, H. T. 2010. Do fetuses from primiparous one-year-old differ from those of multiparous mature ewe? Proceedings of the New Zealand Society of Animal Production, 70, 118-120.

In which Chapter is the Published Work: Chapter 3

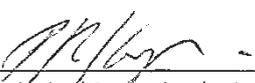
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LOUREIRO, M. F. P., PAIN, S. J., KENYON, P.R., PETERSON, S. W. & BLAIR, H. T. 2012. Single female offspring born to primiparous ewe-lambs are lighter than those born to adult multiparous ewes but their reproduction and milk production are unaffected. *Animal Production Science*, 52, 552-556.

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In which Chapter is the Published Work: Chapter 7

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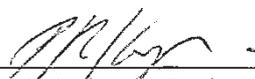
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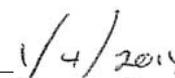
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