Effect of heavier live weight of ewe lambs at breeding on reproductive performance, mammary gland development and subsequent live weight

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Abstract

Breeding ewe lambs at seven to eight months of age can increase farm profitability and ewe lifetime performance. In New Zealand, 30 to 40% of ewe lambs are bred each year with a minimum recommended pre-breeding live weight of 40 kg. Ewe lamb reproductive performance increases with breeding live weight, therefore, some farmers aim to breed ewe lambs heavier than 40 kg. Increasing ewe-lamb growth rates prior to puberty, to achieve a heavier breeding live weight could, however, impair ewe lamb mammary gland development and lactational performance. Currently, little is known about the impact of breeding heavier ewe lambs on their subsequent performance, live weight, and efficiency. The aim of this thesis was to investigate the effect of breeding heavier ewe lambs on their reproductive performance, mammary gland development and live weight over their first three breeding seasons. Ewe lambs were managed from weaning to breeding and achieved an average live weight of 47.9 ± 0.38 or 44.9 ± 0.49 kg at breeding. A growth rate of 150 g/d prior to the first breeding did not affect ewe lamb mammary gland development to the weaning of their second litter, as measured using ultrasonography. Positive relationships were found between ewe lamb mammary ultrasound measures at one year of age and the growth of their progeny to weaning. The associations between ultrasound measurements and growth of the progeny indicate that ultrasound scanning has the potential to be used as a selection technique for heavier lamb live weight at weaning. Although the live weight difference between treatments was limited to three kilograms, compared with lighter ewes, heavier ewe lambs at their first breeding showed greater fertility rate, litter size and lambing percentage but did not differ in the second and third breeding seasons. Over the first three breeding seasons, heavier ewe lambs had greater lamb production than their lighter counterparts. Ewe lamb live weight treatment had no effect on progeny performance to weaning, nor ewe efficiency over the three-year period. A positive association was found between ewe lamb breeding live weight and their mature live weight at 39 months of age. Farmers should aim to breed their ewe lambs at heavier live weights to maximise their reproductive performance as a ewe lamb and, if well managed, they can achieve increased ewe performance over the first three breeding seasons, although there would be no impact on efficiency. Before firm recommendations can be made to farmers, lifetime performance and longevity of the heavier ewe lambs at breeding needs to be examined.

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List of abbreviations

ADF	Acid detergent fibre
BCS	Body condition score
BWT	Birthweight
CP	Crude protein
d*n*	The *nth* day of age of the ewe
DM	Dry matter
FP	Mammary fat pad depth
GC	Mammary gland cistern depth
GnRH	Gonadotrophin-releasing hormone
IGF-1	Insulin-like growth factor I
IU	International unit
LH	Luteinizing hormone
L*n*	The *nth* day of lactation, from the first day of lambing
LW	Live weight
MBS	Maternal behaviour score
ME	Metabolizable energy
MEC	Mammary epithelial cell
MTc	Total depth of mammary gland conservative
MY	Milk yield
MTg	Total depth of mammary gland generous
NIR	Near infrared reflectance
NLB	Number of lambs born
NLW	Number of lambs weaned
NDF	Neutral detergent fibre
NE	Net energy
NR	No relationship
N/S	Not stated
P*n*	The *nth* day of pregnancy, from the first day of breeding
PAR	Mammary parenchyma depth

R	Radius
ROI	Region of interest
SSS	Soluble sugars and starch
UC	Udder circumference
UH	Udder height
UV	Udder volume
W*n*	Week *n* of lactation
WWT	Weaning weight

General introduction

Breeding ewe lambs at seven months of age can increase the number of lambs born per farm per year and the ewe's lifetime performance (McCall and Hight, 1981, Kenyon et al., 2014c). In New Zealand, only 30 to 40% of ewe lambs are bred each year (Beef + Lamb New Zealand, 2020a,b) due to the perceived negative effects of breeding ewe lambs on two-year-old performance, their poor reproductive performance compared to older ewes and the need to achieve good growth rates prior to breeding (Kenyon et al., 2004b). To be successfully bred, ewe lambs should reach between 50 and 70% of their expected mature weight by their first breeding (Dýrmundsson, 1973, Rosales Nieto et al., 2018b). Greater ewe lamb reproductive performance has been reported when ewe live weight at breeding was above 40 kg for Romney-type ewe lambs (Kenyon et al., 2014c, Corner-Thomas et al., 2015b). Based on available industry evidence, it appears that some farmers are growing their ewe lambs to weights well in excess of 40 kg, in order to improve ewe lamb reproductive performance. Increased growth rates before and during puberty can, however, affect the mammary gland development and lactational performance in ewe lambs by altering the development of the milk secretory tissue (Johnsson and Hart, 1985, Umberger et al., 1985, Villeneuve et al., 2010a). Carry-over effects of increased growth rates prior to puberty have been reported in non-dairy ewes into their second lactation (Villeneuve et al., 2010b). Currently, research has focused on comparing the impacts of breeding ewe lambs with those bred at 18 months of age on subsequent and lifetime performance. There is little information on the potential consequences of a heavier ewe lamb live weight at breeding on their subsequent performance and live weight.

The objective of this thesis was to investigate the effects of a heavier live weight of ewe lambs at breeding on subsequent reproductive performance, mammary gland development, live weight and efficiency over their first three breeding seasons under New Zealand pastoral conditions.

Two groups of Romney ewe lambs were managed from weaning to achieve average live weights of 47.9 kg (Heavy) and 44.9 kg (Control) at ewe lamb breeding. Mammary development of a subset of ewe lambs from each treatment was compared during their first pregnancy and lactation using ultrasonography (Chapter 2). Indicators of lamb growth were identified among the ultrasound measures and the relationships between one-year-old ewe milk yield, mammary ultrasound measures during pregnancy and lactation and growth of their progeny were examined (Chapter 3). Potential carry-over effects of increased growth rates prior to the first breeding on the mammary gland of two-year-old ewes were also investigated using ultrasonography (Chapter 4). Ewe reproductive performance, live weight, body condition score and growth of their progeny to weaning was compared between ewe lamb treatments during their first (Chapter 5), second and third (Chapter 6) breeding seasons. Production and feed efficiency of the two treatments were compared over the three breeding seasons and the relationship between ewe lamb breeding and mature live weight was established (Chapter 7).

The intended outcome of this research was to provide new information on the potential consequences of breeding heavier ewe lambs on reproductive performance, mammary development, and live weight, and provide guidelines for breeding ewe lambs. It will also provide a basis for future research on the potential use of ultrasound to select ewes that wean heavier lambs.

Chapter 1

Literature review

1.1. Effects of live weight and BCS on mature ewe and ewe lamb performance

1.1.1. Puberty onset in ewe lambs

Puberty is the result of a chain reaction within the animal resulting in the establishment and development of the communication between the hypothalamo-pituitary gland, ovary and uterus, which leads to the start of the reproductive cycle. This process has been reviewed elsewhere by Valasi et al. (2012) and Edwards and Juengel (2017). When puberty approaches, there is a reduction of steroid negative feedback on GnRH cells and the pituitary gland allows an increase in GnRH and LH pulse frequencies. These increases in GnRH and LH stimulate the maturation of ovarian follicles that produce oestradiol in high enough concentrations to induce behavioural oestrus and then ovulation (Edwards and Juengel, 2017, Valasi et al., 2012).

The live weight and the size of the young animal have a greater influence than age on the attainment of puberty (Foster and Nagatani, 1999). Indeed, females with rapid growth rates (increased rate of muscle and fat accumulation) are more likely to reach the minimum live weight required for puberty and so will be younger at puberty than those with average growth rates (Rosales Nieto et al., 2013a,b, 2018b, Elaref et al., 2021). Rapid live weight gain, particularly fat accumulation, is associated with an attenuation of oestradiol negative feedback and increased pulsatile release of LH (Duittoz et al., 2016). Mechanisms responsible for the timing of puberty are sensitive to a critical size and live weight, to metabolic cues, and hormones such as melatonin, leptin or insulin (Foster and Nagatani, 1999, Pool et al., 2020). The GnRH secretions of fast and slow growing ewe lambs were compared and showed that the frequency of GnRH pulses was higher in fast growing females (Foster and Nagatani, 1999). The GnRH pulse frequency can be changed by increasing the level of nutrition (Foster and Nagatani, 1999). Metabolic and hormonal cues such as glucose, insulin and leptin have been the most commonly studied factors, and are potential indicators to the brain that the somatic growth and energy stores of the female are sufficient to tolerate pregnancy and lactation (Duittoz et al., 2016). Nevertheless, little is known about the key event(s) that lead to the progressive activation of GnRH at attainment of puberty (Duittoz et al., 2016).

There is a positive relationship between live weight and attainment of puberty in ewe lambs, whereby heavier ewe lambs are more likely to attain puberty in their first year of life than lighter lambs (Dýrmundsson, 1973, Kenyon et al., 2014c, Elaref et al., 2021). For example, Paganoni et al. (2014a) showed that an increase of 5 kg in Merino ewe lamb live weight between weaning and the introduction of vasectomised rams increased the probability of attaining puberty by 3 to 9% by 197 days of age. In sheep, a female will attain puberty when weighing between 50 and 70% of mature live weight (Hafez, 1952, Dýrmundsson, 1973, Valasi et al., 2012, Rosales Nieto et al., 2018b, Lozano et al., 2020). The attainment of a minimum live weight, therefore, is the main determinant for achieving puberty (Kenyon et al., 2014c). In the literature, the average live weight at puberty of Romney ewe lambs, the main breed in New Zealand (Cranston et al., 2017), ranged between 31.3 to 38.0 kg (Table 1.1).

Table 1.1. Summary of the average live weight at puberty of Romney ewes

Source	n	Average live weight at puberty	Range (kg)
Hafez (1952)	7	34.9 kg	31.8 – 39.9
Ch'ang and Raeside (1957)	48	34.0 kg	24.0 – 41.7
Lewis (1959)	179	37.6 kg	25.9 – 43.1
Lang and Hight (1967)		33.1 kg	
Hight and Jury (1973)			30.0 – 34.0
Kenyon et al. (2005)	34	38.0 kg	34.6 – 41.4
Kenyon et al. (2012)	62	34.5 kg	37.2 – 31.7
Kenyon et al. (2012)	75	37.0 kg	35.5 – 38.5
Lozano et al. (2020)	6	31.3 kg	27.6 – 35.0

n: number of ewes

In summary, these studies showed that puberty depends on the attainment of a minimum live weight and that faster growing ewe lambs are more likely to attain puberty at a younger age than slower growing light ewe lambs. The key event(s) and the metabolic cues responsible for the onset of puberty are not fully understood.

1.1.2. Reproductive performance in ewe lambs

The impacts of live weight and BCS on mature ewe performance have been reviewed elsewhere (Scaramuzzi et al., 2006, Kenyon et al., 2014c, Brown et al., 2015), therefore, the focus of the following sections is on the impact of live weight and BCS on ewe lamb performance.

1.1.2.1. Impacts of ewe lamb live weight on reproductive performance

Live weight of ewe lambs at breeding influences their breeding performance and ovulation rate. Ewe lambs that were heavier at the start of breeding were more likely to be mated in the first oestrus cycle (first 17 days) than lighter ewe lambs (Kenyon et al., 2005, 2006, 2014c, Brown et al., 2015). Further, a greater ewe lamb live weight at breeding was positively related to ovulation rate (Morley et al., 1978, Bizelis et al., 1990, Scaramuzzi et al., 2006, Paganoni et al., 2014a, Shorten et al., 2021).

1.1.2.1.1. Fertility rate and reproductive rate

Fertility rate (ewes pregnant per ewes presented for breeding) and ewe lamb live weight at breeding are positively correlated (Ferguson et al., 2011, Paganoni et al., 2014a, Corner-Thomas et al., 2015b; Table 1.2). In Romney ewe lambs, greater fertility rates were reported as live weight increased from 32.5 to 47.5 kg (Figure 1.1). Live weight greater than 47.5 kg, however, showed no additional increase in fertility rate (Corner-Thomas et al., 2015b). Similarly, reproductive rate (foetus per ewe bred) was positively associated with ewe lamb live weight at breeding (Rosales Nieto et al., 2013a,b, Corner-Thomas et al., 2015b, Thompson et al., 2019, Shorten et al., 2021; Table 1.2). Reproductive rate also showed a plateauing relationship with live weight (Rosales Nieto et al., 2013a,b, Paganoni et al., 2014a, Corner-Thomas et al., 2015b; “fecundity” in Figure 1.1). For example, Romney ewe lambs had greater reproductive rate as breeding live weight increased from 32.5 to 47.5 kg, but did not differ between the groups of ewe lambs weighing between 47.5 and 52.4 kg and those heavier than 52.4 (Corner-Thomas et al., 2015b).

There is a positive relationship between ewe lamb live weight at breeding and the probability of a ewe lamb carrying two or more foetuses (Drew et al., 1973, Bichard et al., 1974, Gaskins et al., 2005, Kenyon et al., 2005, Schreurs et al., 2010a, b, Brown et al., 2015). For example, Drew et al. (1973) showed that every 1 kg increase in breeding live weight was associated 1.6% increase in number of twin-bearing Romney ewe lambs per ewe lambing.

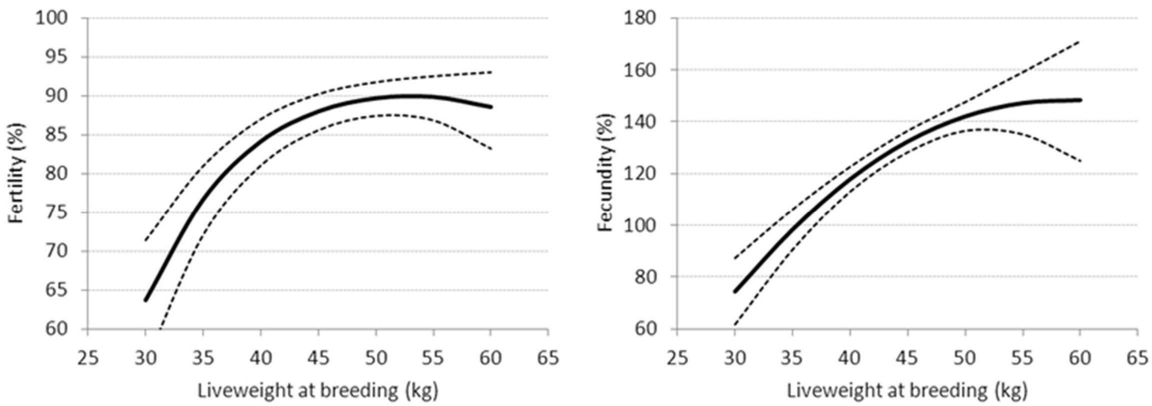


Figure 1.1. The fertility (number of ewe lamb pregnant per ewe lamb bred; left) and fecundity (number of foetuses identified per ewe lamb bred; right) of Romney ewe lambs in relation to their live weight at breeding (logit predictions and 95% confidence intervals shown) (Corner-Thomas et al., 2014b).

1.1.2.1.2. Lambing percentage

Increasing ewe lamb live weight at breeding has been linked to increased lambing percentage (lambs weaned per ewe bred; Kenyon et al., 2004c, 2014c) and the probability of weaning a lamb (Griffiths et al., 2016, Keady and Hanrahan, 2021). Ewe lambs that weighed more than 49 kg at breeding had a lambing percentage 25.5% greater than ewe lambs that weighed less than 36 kg (Kenyon et al., 2004c). In the same study, every additional kilogram of live weight at breeding above 36 kg increased the number of lambs weaned per ewe lamb by approximately 2%. This percentage is lower than the percentage of increase in the reproductive rate reported by Corner-Thomas et al. (2014b), which may be explained by greater mortality rate of ewe lambs compared with mature ewes (Pettigrew et al., 2018). Pettigrew et al. (2018) reported similar mortality rates between single and twin lambs born to ewe lambs at birth (11.7% and 13.6%, respectively) and to weaning (21.4% and 33.6%, respectively).

Table 1.2. Summary of changes in reproductive performance of mature ewes and ewe lambs per kg increase in live weight at breeding

Parameter	Age	Breed	Changes in parameter per kg increase in breeding live weight	Reference
Ovulation rate [b]	Mature ewes	Merino	+ 2.7%	Morley et al. (1978)
	Mature ewes	Four breeds	+ 2 – 2.5%	Morley et al. (1978)
	Ewe lambs	Columbia Hampshire	+ 1.6%	Morley et al. (1978)
	Ewe lambs	Merino	+ 2.3%	Paganoni et al. (2014a)
Fertility rate [c]	Ewe lambs	Merino	+ 1.2%	Paganoni et al. (2014a)
	Ewe lambs	Romney	+ 2%	Corner-Thomas et al. (2014b)
Reproductive rate [d]	Mature ewe	Merino	+ 1.7 – 2.4%	Ferguson et al. (2011)
	Ewe lambs	Merino	+ 4.8%	Rosales Nieto et al. (2013a)
	Ewe lambs	Merino	+ 4.5%	Rosales Nieto et al. (2013b)
	Ewe lambs	Merino	+ 4.0 – 4.5%	Thompson et al. (2019)

[b] Ovulation rate (%): number of ova/ewe mated; [c] Fertility rate (%): ewes pregnant/ewes bred; [d] Reproductive rate (%): foetuses/ewes bred

1.1.2.2. Impacts of BCS of ewe lambs on reproductive performance

Body condition score (BCS) is a technique used to subjectively assess the degree of fatness or condition of a live animal (Jefferies, 1961, Kenyon et al., 2014b). To assess the BCS of a sheep , the lumbar region is palpated, specifically on and around the backbone in the loin area (Jefferies, 1961, Russel et al., 1969, Kenyon et al., 2014b). The scoring was originally based on a 0 to 5 scale (Jefferies, 1961; Table 1.3), but 0.5 and 0.25 units were introduced by Russel et al. (1969).

Table 1.3. Description of the Body condition score (BCS) technique and an illustration of the vertebra and ribs and approximate muscle and fat distribution (Kenyon et al., 2014b).

1.1.2.2.1. Breeding pattern

Among ewe lambs, BCS has a positive impact on their reproductive performance (Kenyon et al., 2009, 2010b; 2014a, Cave et al., 2012, Corner-Thomas et al., 2014a, 2015b, Thompson et al., 2019; Table 1.4). Romney composite ewe lambs with BCS of 2.5 or greater at breeding were more likely to be mated during the first oestrus cycle (67% *vs.* 46%) and were more likely to be pregnant than those with a BCS of 1.5 or 2.0 (Kenyon et al., 2010b).

1.1.2.2.2. Fertility rate and reproductive rate

Corner-Thomas et al. (2015b) reported that compared to Romney ewe lambs with a BCS of 2.5, 3.0, 3.5 and ≥ 4.0, ewes with BCS ≤ 2.0 at breeding had lower fertility rate (85%, 88%, 88% and 89% *vs.* 78%, respectively) and reproductive rate (1.22, 1.27, 1.27 and 1.31 *vs.* 1.09, respectively). Fertility and reproductive rates did not differ between ewe lambs with BCS of 2.5 or greater, which suggested a plateau similar to that seen in mature ewes (Corner-Thomas et al., 2015b). Thompson et al. (2019) reported, however, that BCS at breeding had no effect on ewe lamb fertility and reproductive rates (Table 1.4). Kenyon et al. (2010b) reported that BCS prior to breeding had no effect on the percentage of ewe lambs identified as bearing twin foetuses. Thompson et al. (2019) used different breeds (Table 1.4) and the time of measurement than Kenyon et al. (2010b) and Corner-Thomas et al. (2015b) which may explain the differences between studies. The absence of an impact reported by Kenyon et al. (2010b) may be explained by the time of measurement, as the BCS was measured 17 days before breeding, whereas Corner-Thomas et al. (2015b) measured BCS within seven days of breeding.

1.1.2.3. Summary of the impacts of ewe lamb live weight and BCS on reproductive performance

In summary, ewe lamb live weight at breeding has an important positive impact on ovulation, fertility rate, reproductive rate, twinning, and lambing percentage. The presence of a plateau in the relationship of breeding live weight reproductive performance has been suggested, but little is known about this plateau in live weight and its specificity. This plateau could be dependent of the breed, of each individual, driven by a physiological element or be a physiological limit. The relationships between ewe lamb BCS at breeding and fertility and reproductive rates show variability in the literature and it appears to be a curvilinear relationship, suggesting an optimal BCS at breeding at 2.5 and above (Kenyon et al., 2014c).

1.1.3. Ewe lactation performance

Mature ewes show correlations between ewe live weight and milk production which are inconsistent. Ewe live weight at breeding was either not correlated (Mavrogenis and Papachristoforou, 2000, Danso et al., 2016), or positively correlated (Ángeles Hernández et al., 2018) with milk yield. During pregnancy and lactation, however, ewe live weight was positively associated with milk production (Reynolds and Brown, 1991, Paten et al., 2017, Ángeles Hernández et al., 2018).

1.1.3.1. Impacts of ewe lamb live weight on lactation performance

Ewe lambs are consistently reported to have lower milk production than older ewes (Knight et al., 1995, Gootwine and Goot, 1996, Cardellino and Benson, 2002, Pollott and Gootwine, 2004, Morgan et al., 2006). Knight et al. (1995) reported positive correlations between ewe lamb live weight pre-lambing with both total milk yield ($r = 0.57$) and daily milk yield ($r = 0.38$). Newman and Stieffel (1999), however, did not find any significant correlations between ewe lamb live weight and their milk production. Previous studies have also examined the impact of liveweight change and growth rates on ewe lamb milk production, but reported contrasting results. Morgan et al. (2006) reported a negative relationship between ewe lamb liveweight gain from mid-pregnancy to weaning of their progeny and milk yield. Conversely, Rosales Nieto et al. (2018a) reported that liveweight change during pregnancy in Merino ewe lambs did not influence their milk production. In addition, they found no association between accelerated growth in pregnancy and milk production in Merino ewe lambs during their first lactation. These differences may be explained by the frequency of milking as Rosales Nieto et al. (2018a) milked ewe lambs once during the lactation peak period (week three of lactation), whereas Morgan et al. (2006) milked ewe lambs on three occasions, in week three, four and 12 of lactation.

Increased growth rates prior to first breeding have been associated with an impairment of the mammary gland development of ewe lambs (Johnsson and Hart, 1985, McCann et al., 1989, Umberger et al., 1985, Villeneuve et al., 2010a). These effects will be presented in another section of this Chapter (see section 1.3.3.).

Table 1.4. Summary of studies that examined the relationship between ewe lamb BCS and fertility rate, number of foetuses per ewe, the number of lambs born, lamb survival, lamb birthweight (Lamb BWT) and lamb weaning weight (Lamb WWT).

Reference	Timing & BCS range	Breed	Nutritional treatment(s) [a]	Relationship between BCS and each outcome variable						
				Fertility rate	Foetus/ewe	Lambs born	Lamb BWT	Lamb growth	Lamb survival	Lamb WWT
Kenyon et al. (2009)	Breeding N/S	Romney	Commercial conditions		NR					
Kenyon et al. (2010b)	Breeding 1.5, 2.0, 2.5+	Romney	Commercial conditions	BCS 2.5+ >1.5	NR					
Cave et al. (2012)	Breeding 1.5, 2.0, 2.5+	Romney	N/S	NR or +	BCS 2.5+ >1.5					
Corner-Thomas et al. (2014a)	Breeding ≤2.5, 3.0, 3.5, ≥4.0	Highland composite	Commercial conditions				NR	BCS ≤2.5 <3.5		NR
Corner-Thomas et al. (2014a)	Day 85 of pregnancy ≤2.5, 3.0, 3.5 ≥4.0	Highland composite	Commercial conditions			+	NR	NR	-	NR
Corner-Thomas et al. (2014a)	Day 135 of pregnancy ≤2.5, 3.0, 3.5 ≥4.0	Highland composite	Commercial conditions			+	+	-	-	NR
Corner-Thomas et al. (2015b)	Breeding ≤2.0, 2.5, 3.0, 3.5 ≥4.0	Highland composite Romney Coopworth	Commercial conditions	BCS ≤2.0 <2.5, 3.0, 3.5 & ≥4.0	BCS ≤2.0 <2.5, 3.0, 3.5 & ≥4.0					

Table 1.4 continued

Reference	Timing & BCS range	Breed	Nutritional treatment(s) [a]	Relationship between BCS and each outcome variable						
				Fertility rate	Foetus/ewe	Lambs born	Lamb BWT	Lamb growth	Lamb survival	Lamb WWT
Kochewad et al. (2018)	Pregnancy & 48H pre-lambing 1.0 to 5.0	Deccani	Intensive Semi-extensive Extensive				+			
Thompson et al. (2019)	Breeding 1.0 to 5.0	Merino	During breeding period: target 100 or 200 g/d	NR	NR					

[a] Unless otherwise stated there are no interactions between nutritional treatments and BCS; NR: no relationship or no effect; N/S: not stated; +: positive relationship; - : negative relationship; blank cells indicate that the outcome variable was not reported in the studies.

1.1.3.2. Impacts of BCS of ewes on lactation performance

No effect of BCS at breeding on subsequent milk yield have been reported in the literature (Kenyon et al., 2014b, Danso et al., 2016). Similarly, among other breeds, there was no effect of ewe BCS in mid- or late-pregnancy on milk yield, for example Scottish Halfbred (Gibb and Treacher, 1982, Hossamo et al., 1986) and Latxa breed ewes (Oregui et al., 2004). There was, however, a positive effect of ewe BCS in late-pregnancy on the milk yield of Awassi (Hossamo et al., 1986) and Scottish Halfbred ewes (Gibb and Treacher, 1980). These different results could be explained by litter size which is known to affect the milk yield (Snowder and Glimp, 1991), the different breeds or the feeding conditions (Kenyon et al., 2014b). No studies have investigated the impacts of BCS of ewe lambs on their milk production.

1.1.3.3. Summary of the impacts of ewe live weight and BCS on lactation performance

Combined, these studies suggest that heavier mature ewes in pregnancy and lactation produced more milk than lighter ewes. These studies also suggest that ewe lambs produce less milk than older ewes. Few studies have investigated the effect of ewe lamb live weight and liveweight change on milk production. These studies showed a positive effect of pre-lambing ewe lamb live weight on milk yield and no effect of liveweight change during pregnancy on ewe lamb milk yield and milk composition. The effects of ewe lamb live weight and BCS at breeding on milk production are still unknown.

1.1.4. Lamb live weights

Live weight of mature ewes at breeding has either no effect (Cam et al., 2018) or a small positive effect (Oldham et al., 2011, Paganoni et al., 2014b) on lamb birthweight. An increase in ewe live weight during early and late pregnancy, however, resulted in heavier lambs at birth (Thompson et al., 2011, Schreurs et al., 2012, Kenyon et al., 2011a, Cam et al., 2018) and at weaning (Thompson et al., 2011, van der Linden et al., 2011, Paganoni et al., 2014b). Lamb weaning weight was also positively correlated with ewe live weight at breeding (Brown et al., 2015).

1.1.4.1. Impacts of ewe lamb live weight on lamb live weight

1.1.4.1.1. Lamb birthweight

The association between ewe lamb breeding live weight and the birthweight of their lambs is not clear and the effect appears to be minor. Mulvaney et al. (2010) and Thompson et al. (2019) reported there was no effect of ewe lamb live weight at breeding on lamb birthweight, whereas Kenyon et al. (2006) and Schreurs et al. (2010a,b) reported a positive relationship. The increase in lamb birthweight reported by Kenyon et al. (2006) was 46 g for each one kilogram increase in ewe lamb breeding live weight. It should be noted, however, that Mulvaney et al. (2010) indicated that the number of ewes in their study may have been insufficient to allow small differences to be detected. The variance in results among these studies may be due to differences in the study designs: for example during the breeding period, Mulvaney et al. (2010) had a medium and *ad libitum* nutritional treatments, whereas Thompson et al. (2019) had differing liveweight gain targets. Kenyon et al. (2006) did not include feeding treatments. The association between ewe lamb liveweight change during pregnancy and lamb birthweight was also inconsistent between studies. Several studies have shown no association between liveweight change in pregnancy and the birthweight of their lamb (Morris et al., 2005, Rosales Nieto et al., 2018a, Thompson et al., 2019), whereas others have reported either a positive

(Schreurs et al., 2010b, Paganoni et al., 2014b) or a negative relationship (Corner-Thomas et al., 2014a). In general, the lamb birthweight response was less than 100 g for each additional kilogram of ewe live weight gained during pregnancy (Schreurs et al., 2010b, Corner-Thomas et al., 2014a, Paganoni et al., 2014b). It is difficult to determine the cause of the variation between studies, but it could be due to breed, nutritional or environmental effects. Indeed, in the United Kingdom, Wallace (2000) reported that ewe lambs offered high levels of concentrate feed during pregnancy, gaining approximately 301 g/d, showed reduced lamb birthweight, whereas Morris et al. (2005) in New Zealand showed that the similar liveweight gain achieved on pasture had no effect on lamb birthweight.

1.1.4.1.2. Lamb weaning weight

There is a positive relationship between ewe lamb live weight at breeding and in pregnancy with lamb weaning weight (Kenyon et al., 2014c, Corner-Thomas et al., 2014a, Thompson et al., 2019). Thompson et al. (2019) reported an increase of 0.13 kg in lamb weaning weight for each additional kilogram of ewe lamb live weight at breeding. Ewe lambs that had increased live weights in early or late pregnancy had heavier lambs at weaning than those that had no increase in live weight in pregnancy (Morris et al., 2005, Corner-Thomas et al., 2014a). Corner-Thomas et al. (2014a) reported that for every additional kilogram of ewe lamb live weight in late pregnancy, lambs were 77 g heavier at weaning.

1.1.4.2. Impacts of BCS of ewe lambs on lamb live weight

The effect of BCS of mature ewes on lamb birthweight, survival to weaning, growth rates to weaning and weaning weight has been reviewed elsewhere (Kenyon et al., 2014b), therefore, the following sections focus on the effect of ewe lamb BCS on lamb performance.

1.1.4.2.1. Lamb birthweight

Ewe lamb BCS at breeding had no impact on lamb birthweight (Corner-Thomas et al., 2014a; Table 1.4), however, BCS during pregnancy was positively associated with lamb birthweight (Corner-Thomas et al., 2014a, Kochewad et al., 2018; Table 1.4). Among Romney ewe lambs, Corner-Thomas et al. (2014a) showed that those with a BCS in late-pregnancy of either ≤ 2.5 or 3.0 gave birth to heavier lambs than ewe lambs with a BCS of 3.5 or ≥ 4.0. Kochewad et al. (2018) reported, in Deccani ewe lambs, that those with an average BCS of 2.8 and 2.6 in pregnancy gave birth to heavier lambs than ewe lambs with an average BCS of 2.4 in pregnancy.

1.1.4.2.2. Lamb growth

Between birth and weaning, lambs born to ewe lambs with a BCS ≤ 2.5 at breeding tended to be lighter than lambs born to ewes with a BCS of 3.5 (Corner-Thomas et al., 2014a; Table 1.4). In the same study, ewe lamb BCS recorded in mid-pregnancy influenced lamb growth such that ewe lambs with BCS 2.5 tended to have lighter lambs at 18 days of lactation compared to ewe lambs with BCS 3.0, however, no effect of BCS in late pregnancy was found on lamb growth and lamb weaning weight (Corner-Thomas et al., 2014a).

1.1.4.2.3. Lamb weaning weight

No effects of BCS at breeding and late-pregnancy on lamb live weight at weaning have been reported in the literature (Corner-Thomas et al., 2014a, Kochewad et al., 2018; Table 1.4). Ewe lambs with BCS ≤ 2.5 in mid-pregnancy, however, tended to have lighter lambs at weaning than ewes with BCS 3.0 and ≥ 4.0 (Corner-Thomas et al., 2014a).

1.1.4.3. Summary of the impact of ewe lamb live weight and BCS on lamb live weight

In summary, the relationship between ewe lamb live weight and the birthweight of the progeny seems positive, but minor. There is, however, a positive association between ewe lamb live weight during pregnancy and the lamb weaning weight, suggesting that heavier ewe lambs weaned heavier lambs. Few studies have investigated the impact of BCS of ewe lambs at breeding or during pregnancy on lamb performance, and they reported a positive association between BCS during pregnancy and lamb performance.

1.2. Lifetime impacts of breeding ewe lambs

Breeding ewe lambs at eight months of age can have potential disadvantages, such as a low and variable reproductive performance and, impairment of future live weight and productivity if not well managed (for review: Kenyon et al., 2014c).

1.2.1. Impacts of breeding ewe lambs on subsequent live weight and BCS

1.2.1.1. Live weight as two-year-old ewes

Ewe lamb breeding can have a negative impact on ewe live weight at the weaning of her first lamb (Keane, 1974, Baker et al., 1978, McMillan and McDonald, 1983, Moore et al., 1983, Kenyon et al., 2008b). Breeding live weight as a two-year-old ewe can either be negatively impacted (Cannon and Bath, 1969, Keane, 1974, Kenyon et al., 2008b, 2011b, Thomson et al., 2020, Keady and Hanrahan, 2021) or not impacted (Moore et al., 1983; Table 1.5). In the study of Moore et al. (1983), ewe lambs that had lambed were preferentially fed which may have limited the potential negative consequences on live weight. Kenyon et al. (2008b) reported that the differences observed in live weight between two-year-old ewes that lambed as a ewe lamb and those that did not, were still present in two-year-old ewes in late-pregnancy and at weaning. Keane (1974), however, observed that the differences in live weight identified at two-year-old breeding decreased during pregnancy and lactation and did not exist by two-year-old weaning. The live weight difference found by Kenyon et al. (2008b) at weaning of two-year-old ewes was relatively small (less than 2 kg).

1.2.1.2. Ewe live weight from two to seven years of age

A number of studies have reported negative impacts on live weight at two-year-old breeding of ewes that lambed as a ewe lamb, although those impacts did not persist in subsequent years (Dýrmundsson, 1973, Keane, 1974, Baker et al., 1978, 1981, Thomas and Berger, 2009, Kenyon et al., 2011b, Thomson et al., 2020). Kenyon et al. (2011b), however, reported that Romney ewes that had been pregnant as a ewe lamb were lighter at breeding and pre-lambing at three years of age than ewes that had not been pregnant, but differences were no longer observed reported by the three-year-old weaning. Interestingly, at four years of age, ewes that lambed as a ewe lamb and those not mated were heavier at breeding than ewes that were mated but failed to conceive. At five years of age, no differences were observed between groups on live weight at breeding and pre-lambing, but at weaning, ewes that lambed as a ewe lamb were heavier than non-mated ewes. Thomson et al. (2020) also reported that ewes that lambed as a ewe lamb were lighter at breeding at two and three years of age than ewes that did not, but differences were no longer reported at breeding at four years of age.

1.2.1.3. Ewe BCS from two to seven years of age

Kenyon et al. (2008b) and Thomson et al. (2020) reported that ewes that lambed as a ewe lamb had lower BCS at two-year-old breeding than ewes that did not. Kenyon et al. (2011b) showed that BCS did not differ at three years of age between ewes that lambed as a ewe lamb and those that did not. At four years of age, however, ewes that lambed had a lower BCS at breeding and pre-lambing than those that did not and, at five years of age, BCS at breeding and pre-lambing did not differ, but was greater at weaning for ewes that lambed as a ewe lamb compare to ewes that did not. Thomson et al. (2020) reported that BCS at breeding did not differ between ewes that lambed as a ewe lamb and ewes that did not from three to seven years of age.

1.2.2. Impacts of breeding ewe lambs on reproductive performance at two years of age

1.2.2.1. Attaining puberty in the first year of life on two-year-old performance

Edwards et al. (2015) reported that ewes that attained puberty in their first year of life without being presented for breeding as ewe lambs had higher reproductive performance in subsequent years than ewes that did not attain puberty. Ewes that attained puberty in their first year of life were on average 4.2 ± 0.47 kg heavier at breeding at two, three and four years of age than those that did not attain puberty (Edwards et al., 2015). Ewes that attained puberty in their first year of life also had a greater ovulation rate, greater fertility, more lambs identified at pregnancy diagnosis and gave birth to more lambs as a two-year-old ewe than those that did not attain puberty. These differences, however, were not evident at three or four years of age (Edwards et al., 2015). The first reproductive cycles that a ewe experiences may set the ewe up for future reproductive success. If the first cycles are only experienced at two years of age, the reproductive system may not be in optimal condition and this might reduce performance (Edwards et al., 2015).

1.2.2.2. Fertility rate and reproductive rate

The reported impacts of breeding ewe lambs on their subsequent reproductive performance have been inconsistent. Ponzoni et al. (1979), Kenyon et al. (2008b) and Morel et al. (2010) reported no impact of breeding ewe lambs on fertility as a two-year-old ewe when compared to ewes that were not bred as a ewe lamb (Table 1.5). McCall and Hight (1981), however, found an increase in fertility of two-year-old ewes that lambed as a ewe lamb compared to those that did not (Table 1.5). These differences may be explained by reduced live weight at two-year-old breeding

(Keane, 1974, Kenyon et al., 2008b, 2011b), which may then negatively affect their reproductive performance at two years of age.

Two-year-old ewes mated as a ewe lamb were more likely to be single-bearing compared to those that were not mated (Baker et al., 1978, Kenyon et al., 2008b; Table 1.5). Keane (1974) reported that ewe lamb breeding resulted in decreased litter sizes, however, this difference was not significant (ewe first mated as a two-year-old ewe 1.56 *vs.* 1.34 for ewes first mated as a ewe lamb). While, Moore et al. (1983) found no difference in the percentage of multiple lambs born between ewes that lambed as a ewe lamb and ewes that did not. The reported differences in the effect of ewe lamb breeding on reproduction between studies were likely due to the reduced live weight of ewes at two-year-old breeding. This reduction of ewe live weight may affect the ovulation and the reproductive rate (Scaramuzzi et al., 2006, Brown et al., 2015). Improved feeding during ewe lamb pregnancy can, however, limit the reduction of live weight at the second breeding and thus limit the potential negative effects on litter size (Moore et al., 1983).

1.2.2.3. Milk production

McGloughlin and Crowley (1971) reported no significant difference in milk production between ewes that lambed as a ewe lamb and those that did not. Ewes that lambed as a ewe lamb, however, tended to produce more milk as two-year-old ewes than those that did not lamb. In the same study, no association was found between ewe live weight pre-lambing and during lactation with milk production at two years of age. Knight et al. (1995) reported that total and daily milk yield of two-year-old ewes not mated as a ewe lamb was lower than those that were mated as a ewe lamb, however, ewes that lambed as a ewe lamb did not differ from ewes that were mated as a ewe lamb but did not lamb. Ewe lamb daily milk yield was positively corelated with daily milk yield at two years of age (r = 0.47; Knight et al., 1995). In dairy ewes, the effects of age at first lambing are contrasted. Hernandez et al. (2011) reported that Lacaune ewes that lambed younger than 390 day of age produced less milk over their lifetime than ewes that lambed at older ages. Kasap et al. (2021), however, reported no effect of an early age at first lambing on milk production. The different breeds considered (Istrian and Lacaune) and the type of management (intensive and semi-intensive) may explain these differences. Collectively, these studies suggest that lambing as a ewe lamb has no effect on milk yield at two-years of age, however, being mated as a ewe lamb seemed to have a positive effect on milk yield at two years of age in non-dairy ewes.

1.2.2.4. Lambing percentage

Breeding ewe lambs has been reported to have either a positive impact (Baker et al., 1981, McCall and Hight, 1981, McMillan and McDonald, 1983, Moore et al., 1983) or no impact (Baker et al., 1978, Ponzoni et al., 1979) on the number of lambs weaned in subsequent years (Table 1.5). Two-year-old ewes that lambed as a ewe lamb weaned 15% more lambs than ewes that had not lambed (McMillan and McDonald, 1983). The differences may be explained by the study design as some studies compared ewe lambs mated with entire or vasectomised rams (Ponzoni et al., 1979, McMillan and McDonald, 1983, Moore et al., 1983), while other studies compared ewes mated as a ewe lamb and those mated as a two-year-old (Baker et al., 1978, 1981, McCall and Hight, 1981). The different breeds used in the studies could also explain the variations (Baker et al., 1978; 1981, Ponzoni et al., 1979, McCall and Hight, 1981, McMillan and McDonald, 1983, Moore et al., 1983; Table 1.5).

Table 1.5. Summary of studies that examined the impact of breeding and/or lambing ewe lambs on two-year-old ewe reproductive performance

Reference	Breed	Ewe LWT at two years of age	Fertility	Foetus/ ewe	Lamb BWT	Lamb survival	Lambs weaned	Lamb WWT
Cannon and Bath (1969)	Border Leicester x Merino	Affected at breeding		NR	NR	NR		
McGloughlin and Crowley (1971)	Greyface				NR			NR
Keane (1974)	Suffolk x Galway	Affected at breeding Unaffected at weaning		NR	NR			NR
Baker et al. (1978)	Finnish Landrace Steigar Dala	Affected at weaning		-			NR	
Levine et al. (1978)	Columbia Targee	N/S		NR			NR	
Ponzoni et al. (1979)	Corriedale	Unaffected	NR				NR	
Baker et al. (1981)	Romney	Affected					+	+
McCall and Hight (1981)	Romney	N/S	+			+	+	
McMillan and McDonald (1983)	Romney & Romney x Border Leicester	Affected at breeding	NR	NR	NR	+	+	NR

Table 1.5. continued

Reference	Breed	Ewe LWT at two years of age	Fertility	Foetus/ ewe	Lamb BWT	Lamb survival	Lambs weaned	Lamb WWT
Moore et al. (1983)	Romney Coopworth Perendale	Unaffected	+	NR			+	
Afolayan et al. (2008)	Merino crosses	N/S	+	+		-	+	
Kenyon et al. (2008b)	Romney	Affected at breeding, late pregnancy & weaning	NR	-	NR			NR
Morel et al. (2010)	N/S	N/S	NR	NR		-	+	
Kenyon et al. (2011b)	Romney	Affected at breeding				NR		
Thomson et al. (2020)	East Friesian, Romney, and Kelso composite	Affected at breeding			+	NR		NR
Keady and Hanrahan (2021)	Belclare Suffolk x Belclare Suffolk	Affected at breeding						

NR: no relationship or no effect; N/S: not stated; +: positive relationship; -: negative relationship; BWT: birthweight; WWT: weaning weight; blanc cells indicate that the outcome variable was not investigated in the studies.

1.2.3. Impacts of breeding ewe lambs on live weight of progeny

1.2.3.1. Lamb birthweight and survival

Lamb birthweight was not affected by whether a ewe was first bred as a ewe lamb or as a two-year-old ewe (McMillan and McDonald, 1983, Kenyon et al., 2008b; Table 1.5). Three-year-old ewes that lambed as a ewe lamb, however, gave birth to lighter lambs than those that did not (Kenyon et al., 2011b). The greater proportion of multiple-bearing ewes in ewes that lambed as a ewe lamb compared to those that did not at three years of age likely explained this difference (Kenyon et al., 2011b).

Lambs born to ewes that lambed as a ewe lamb had either greater (McCall and Hight, 1981, McMillan and McDonald, 1983) or similar (Kenyon et al., 2011b, Thomson et al., 2020) survival rates than lambs born to ewes that did not lamb. These differences in survival rates may be due to a greater proportion of single lambs born to two-year-old ewes (Baker et al., 1978, Kenyon et al., 2008b), as lamb mortality is higher in twins compared to single lambs (Sawalha et al., 2007, Dwyer et al., 2015).

1.2.3.2. Lamb growth and lamb weaning weight

Ewes that lambed as a ewe lamb had either no effect on lamb growth rates and weaning weights in subsequent years (Keane, 1974, McMillan and McDonald, 1983, Kenyon et al., 2008b, 2011b, Thomson et al., 2020), or a positive relationship (Baker et al., 1981; Table 1.5). McGloughlin and Crowley (1971) reported a tendency for a positive effect of ewes that had lambed as a ewe lamb on the milk yield of two-year-old ewes in their second lactation compared to those in their first lactation, which may explain the positive effect found by Baker et al. (1981).

1.2.4. Lifetime performance and longevity of ewes first bred as a ewe lamb

1.2.4.1. Reproductive performance

Breeding ewe lambs did not affect the fertility rate of ewes from two to six years of age (Cannon and Bath, 1969, Baker et al., 1978, 1981, Ponzoni et al., 1979, Kenyon et al., 2011b). Fogarty et al. (2007), however, reported that ewes that weaned a lamb as a ewe lamb had a greater fertility rate at two and three years of age than ewes that were not pregnant as a ewe lamb. Kenyon et al. (2011b) reported that breeding ewe lambs had a positive effect on the total number of foetuses from one to five years of age, whereas other studies reported no effects on the number of lambs born or weaned (Cannon and Bath, 1969, Baker et al., 1978, Ponzoni et al., 1979). Afolayan et al. (2008) and Morel et al. (2010) also showed that breeding ewe lambs increased litter size between two to four years of age, whereas Kenyon et al. (2011b) reported that ewes that lambed as a ewe lamb had a greater litter size at three years of age and were not different from those that did not at four and five years of age.

Ewes that lambed as a ewe lamb have been reported to wean a greater number of lambs (Baker et al., 1981, Thomson et al., 2020) and produced heavier lambs (Levine et al., 1978, Thomson et al., 2020) in their lifetime than ewes that did not. Although other studies showed no effect of lambing as a ewe lamb on the number of lambs weaned to four years of age (Baker et al., 1978, Ponzoni et al., 1979), differences between these studies may be explained by the ewe categories considered in each study. Fogarty et al. (2007) compared ewes that either “weaned at least one lamb”, “lambed but lost their entire litter” or “were not pregnant as a ewe lamb”, whereas Levine et al. (1978) and Thomson et al. (2020) compared ewes that lambed or not as a ewe lamb, Baker et al. (1978), (1981) and Kenyon et al. (2011b) combined ewes that were mated and/or lambed as a ewe lamb.

1.2.4.2. Longevity

A number of studies have reported that breeding ewe lambs had no negative impact on ewe longevity (proportion of ewes still present in the flock over six years; Cannon and Bath, 1969, Baker et al., 1978, 1981, Ponzoni et al., 1979). Furthermore, Kenyon et al. (2011b) showed that at four years of age, a greater proportion of ewes that had lambed as a ewe lamb were still present compared with ewes that failed to get pregnant as a ewe lamb. Thomson et al. (2020), however, reported that lambing as a ewe lamb negatively affected longevity of ewe to two years of age but had no effect between two and seven years of age. These results were likely to be due to the high mortality rate reported during the ewe lamb lambing period resulting in fewer ewes present at two-year-old breeding (Thomson et al., 2020).

1.2.5. Summary of the lifetime impacts of breeding ewe lambs

Combined, these studies suggest that there is the potential for impaired reproductive performance and live weight of two-year-old ewes first mated as ewe lambs; however, the impairment did not persist after three years of age (Dýrmundsson, 1973, Baker et al., 1978, 1981, Kenyon et al., 2011b, 2014c, Thomson et al., 2020). This impairment may due to the reduction of live weight at two-year-old breeding (Keane, 1974, Baker et al., 1978, McMillan and McDonald, 1983, Kenyon et al., 2008b, Thomson et al., 2020). It is likely that with appropriate feeding management, longevity is not impaired and the lifetime productivity of ewes that lambed as a ewe lamb is improved compared to those that did not lamb (Dýrmundsson, 1973, Baker et al., 1978, 1981, Kenyon et al., 2011b, 2014c).

The majority of studies have compared ewes that either first lambed as a ewe lamb or as a two-year-old ewe over several years (Cannon and Bath, 1969, Keane, 1974, Baker et al., 1978, 1981, McMillan and McDonald, 1983, Moore et al., 1983, Kenyon et al., 2008b, 2011b, Thomson et al., 2020). Other studies, however, have investigated different feeding treatments on ewe lambs and their subsequent performance (Moore et al., 1978, Moore and Smeaton, 1980). Few studies have investigated the effect of increased growth rates between weaning and the first breeding of ewe lambs on their subsequent performance (Thomas and Berger, 2009, Villeneuve et al., 2010b). Thomas and Berger (2009) compared the performance of dairy ewe lambs fed either *ad libitum* or restricted to grow at 70% of the *ad libitum* group resulting in different live weight at breeding (61.2 and 59.4 kg respectively) over three breeding seasons. Fertility, number of lambs born per ewe lambing and milk yield did not differ between groups at any time (Thomas and Berger, 2009). Villeneuve et al. (2010b) investigated the effects of restricted diets of pre-pubertal ewe lambs on the first and second lactations. Breeding live weight, fertility, number of lambs born, weaned and lamb live weight at birth and weaning did not differ between treatments at one and two years of age (Villeneuve et al., 2010b).

In summary, higher growth rates between four and eleven months of age led to an increased incidence of ewe lamb oestrus and increased the number of multiple lambs born at the two-year-old lambing. No studies were found that investigated the impact of different breeding live weights as ewe lambs on their subsequent live weight, reproductive performance, and performance of their progeny to weaning.

1.3. Mammary gland development and function in sheep

Lactation is an important component of production since it provides the first and only source of nutrients to the new-born lamb during their first weeks of life (Capuco and Akers, 2009, Lérias et al., 2014). Indeed, milk enhances the survival of the progeny by providing passive immunity, behavioural stimulation by appeasing the lamb through suckling and nutrients essential to its growth until weaning (Nowak and Poindron, 2006).

1.3.1. Mammary gland structure and function

Two main tissues compose the mammary gland: the parenchyma and the stroma (Akers, 2002a, Nickerson and Akers, 2011, Lérias et al., 2014). The parenchyma includes the secretory and ductal tissues and is comprised of lobes that contain lobules and alveoli (Figure 1.2a). The secretory units, the alveoli, produce and secrete milk into small alveolar ducts. The alveoli are arranged in groups (lobules) around alveolar ducts. The alveolar ducts join to form larger ducts, which join to form larger ducts and so on until the gland cistern (Colville, 2007; Figure 1.2a). Each alveolus is comprised of a lumen (alveolar light), a single layer of mammary epithelial cells (MECs) that are surrounded by myoepithelial cells (Colville, 2007, Reese et al., 2009; Figure 1.2b). The MECs are the primarily responsible for the synthesis of milk components (lipids, proteins, and lactose) which are secreted into the lumen of alveoli. The MEC number and secretory activity determine the ewe milk production (Capuco et al., 2001, Boutinaud and Guinard-Flament, 2004, Capuco and Ellis, 2013). When the milk letdown begins, oxytocin is secreted and causes myoepithelial cells to contract, which ejects the alveolar milk into the ductal system (Colville, 2007, Reese et al., 2009, Nickerson and Akers, 2011).

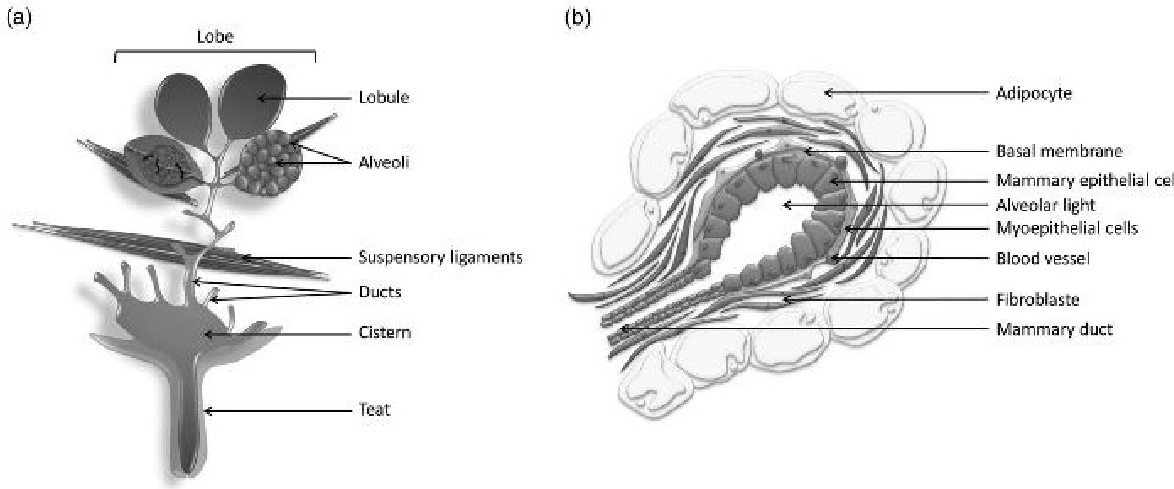


Figure 1.2. Anatomy of ruminant mammary gland (a) and structure of an alveolus (b) (Yart et al., 2014)

The mammary stroma, also called mammary fat pad, is composed of fibroblasts, adipocytes, lymph and blood vessel (Hovey and Aimo, 2010). The fat pad plays an important role in the proliferation, differentiation and regulation of the MEC growth, lipid storage (Hovey and Aimo, 2010) and structural support of the mammary gland (Hovey et al., 1999). Its role during mammary gland development will be detailed in the following sections.

In nulliparous ewe lambs, the gland is mainly composed of epithelium associated with connective tissue, with few lipid-filled adipocytes (Hovey et al., 1999). The MECs are surrounded by several layers of connective tissue and fibroblasts (Figure 1.3), but have no direct contact with adipocytes as is seen in rodents (Hovey et al., 1999). The origin of these fibroblasts is unclear, they may proliferate in parallel with the parenchyma or some of these fibroblasts may be depleted adipocytes (Neville et al., 1998, Hovey et al., 1999).

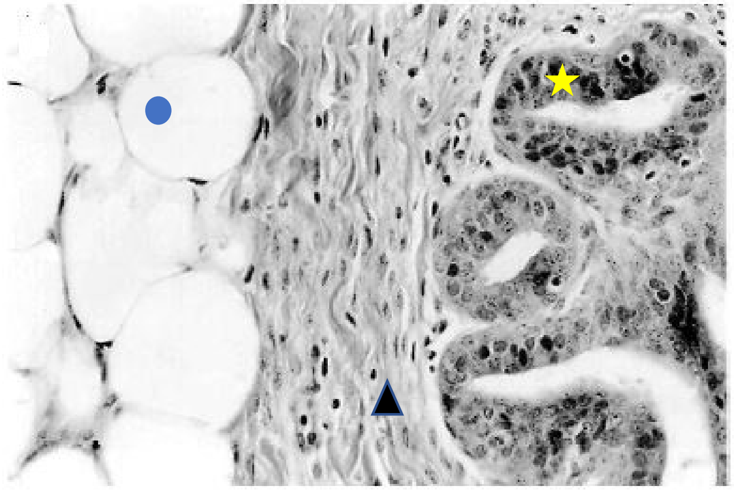


Figure 1.3. High magnification photomicrographs of mammary parenchymal tissue from a pre-pubertal ewe lamb. Rapidly dividing epithelial cells (yellow star) of ducts are separated from adipocytes (blue circle) by multiple layers of fibroblastic connective tissue (black triangle; Hovey et al., 1999).

1.3.2. Mammary gland development in sheep

1.3.2.1. Foetal development

In the ovine mammary gland, organogenesis starts at approximately day 20 of foetal life (Jenkinson, 2003). Early mammary structures form during embryogenesis by the invagination of ectodermal structures and proliferation of mesenchymal tissue to form teat canals, gland cisterns and primary ducts (Jenkinson, 2003, Hurley and Loor, 2011, Rowson et al., 2012, Yart et al., 2014). Secondary ducts start to develop from the primary ducts at the end of the first trimester of pregnancy. The formation of the primary and secondary ducts during foetal life determines the basic structure of the mammary duct network (Jenkinson, 2003, Hurley and Loor, 2011, Rowson et al., 2012, Yart et al., 2014). The mammary fat pad primarily develops during embryogenesis and is the essential environment for the MECs proliferation, differentiation and control for ductal elongation (Neville et al., 1998, Hovey et al., 1999, Hovey and Aimo, 2010). By birth, the mammary fat pad is well developed with fully differentiated white adipocytes (Ellis et al., 1998, Hovey et al., 2000).

1.3.2.2. Pre-pubertal development

At birth, the mammary parenchyma consists of a teat, a primary duct that extends from the teat, and few secondary ducts (Hovey et al., 1999, Hovey and Aimo, 2010, Berryhill et al., 2017). This primitive ductal network develops isometrically (i.e. body parts grow similarly to the body; Huxley and Teissier, 1936) into the mammary fat pad until the start of the allometric phase (i.e. accelerated growth of a body part compared to body growth; Huxley and Teissier, 1936) (Hovey et al., 2002, Hovey and Aimo, 2010).

It is now well established that ewe lambs have a period of accelerated growth of parenchymal tissue starting at about 8-10 weeks of age and ending at approximately 20 weeks of age (Anderson, 1975, Johnsson and Hart, 1985, Hovey et al., 2000, Villeneuve et al., 2010a; Figure 1.4). Recent work has indicated that in the bovine mammary gland, the allometric phase begins at birth instead of three months of age (Geiger, 2019). During this phase, the duct extremities lengthen and extensively ramify into the fat pad (Villeneuve et al., 2010a). This allometric phase is critical for the mammary gland development, as the ductal network development determines the extent of alveolar development (Villeneuve et al., 2010a, Berryhill et al., 2017). An impairment of this phase, through nutrition, for instance, may reduce future milk production by limiting the secretory capacity of the gland (Capuco and Akers, 2010, Berryhill et al., 2017). During this phase, the fat pad produces and secrets IGF-1 which mediates the action of the growth hormone on the mammary gland

(Kleinberg and Ruan, 2008). IGF-1 is critical in the stimulation of parenchymal development, and in particular, ductal morphogenesis (Kleinberg and Ruan, 2008).

With the onset of puberty and regular reproductive cycles, alveolar development begins in response to secretion of progesterone and oestrogen, which leads to the establishment and expansion of alveoli (Capuco and Ellis, 2013). Oestrogen binds with growth hormone receptors in the fat pad, which leads to the production of IGF-1 in the fat pad which stimulates the development of the alveoli (Kleinberg and Ruan, 2008, Colleluori et al., 2021). Following puberty, the mammary gland development is regulated by interactions between the stroma and the mammary epithelium (Hue-Beauvais et al., 2021). The alveolar development mainly occurs during pregnancy and is induced by the greater progesterone and oestrogen secretions (Capuco and Ellis, 2013).

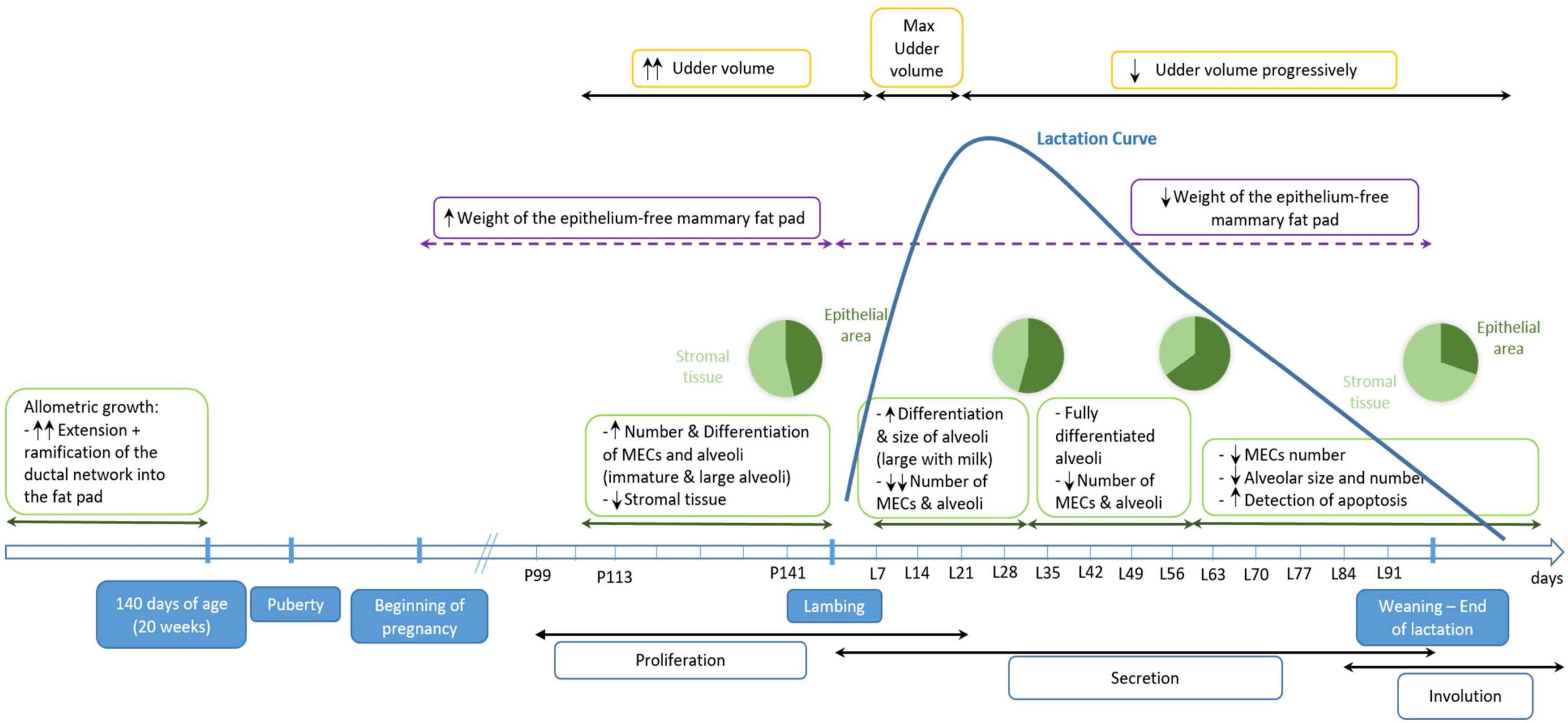
1.3.2.3. Mammary development during pregnancy

During early pregnancy, elongation of the mammary ducts and alveolar development continues (Capuco and Ellis, 2013). Smith et al. (1989) reported that at 50 days of pregnancy, the epithelial tissue (excluding lumen area) represented 14% of the mammary gland, and 19% at 80 days of pregnancy, whereas the mammary fat pad represented 86% and 78% at 50 and 80 days of pregnancy in mature ewes. In dairy ewes, between 100 and 115 days of pregnancy, the developing MECs start to synthesise fat and protein (Castañares et al., 2013, Lérias et al., 2014).

The majority of the development of the mammary gland occurs in late pregnancy with the most extensive epithelial proliferation, and before parturition, with the functional differentiation of the epithelium (Anderson, 1975, Hurley and Loor, 2011, Lérias et al., 2014; Figure 1.4). Development in late pregnancy is crucial for lactation performance as the MECs proliferating and differentiating are responsible for milk production and secretion (Wall and McFadden, 2012). The amount of parenchyma and its histological composition have their major development between day 109 and day 141 days of pregnancy (Nørgaard et al., 2008, Lérias et al., 2014). Closer to parturition (137 days of pregnancy), the mammary gland is composed of immature alveoli with small lumen and large alveoli filled with milk (Colitti and Farinacci, 2009). Castañares et al. (2013) reported that, at day 137 of pregnancy, the number of alveoli and MECs was greater than during lactation. The major changes in mammary volume occur in late pregnancy, as a result of the proliferation and differentiation of the parenchyma and the parallel growth of the fat pad (Hovey et al., 1999, 2000). Davis et al. (1980) showed there was an exponential growth of the mammary gland volume until parturition.

The mammary fat pad size increases in parallel of the epithelium during pregnancy, even though the proportion of the fat pad in the mammary gland decreases (Hovey et al., 1996, 1999, 2000). Hovey et al. (2000) reported that, during late gestation of ewe lambs, when parenchyma increased, the proportion of mammary gland area occupied by parenchyma remained unchanged until parturition, indicating an increase in the size of the fat pad (Hovey et al., 2000). Recent research suggests that, in mice, mature adipocytes in pregnancy have the capacity to dedifferentiate into preadipocytes, similar to fibroblast cells, while the secretory tissue is developing in preparation for lactation and would stay a preadipocyte until the start of involution (Wang et al., 2018, Colleluori et al., 2021). Evidence of possible trans-differentiation between adipocytes and MECs in mice have been observed. It has been theorised that, in pregnancy a portion of adipocytes would transdifferentiate and become secreting epithelial cells and contribute to milk production until the start of involution (Morroni et al., 2004, Giordano et al., 2017, Colleluori et al., 2021). During involution, the secreting epithelial cells have the potential to trans-differentiate into adipocytes (Giordano et al., 2017, Colleluori et al., 2021). The cell changes and remodelling in the fat pad during pregnancy and lactation are not fully understood and require further research.

The function of the adipocytes in the fat pad during pregnancy is site-specific and epithelium-dependent as they produce a number of adipokines such as IGF-1, leptin and adiponectin which regulate MEC proliferation and differentiation (Hovey and Aimo, 2010, Palin et al., 2017, Colleluori et al., 2021). IGF-1 is locally produced by adipocytes to promote the development of the alveoli (Hovey and Aimo, 2010). Adipocytes also produce and secrete leptin in pregnancy, which affect cell proliferation and differentiation (Palin et al., 2017, Colleluori et al., 2021). High levels of leptin transcripts were found in dairy ewes at day 15 and 80 of pregnancy followed by a decrease in leptin transcripts at day 106 and 112 (Palin et al., 2017). The decrease in leptin transcripts could reflect the reduction of adipocytes as pregnancy progresses (Palin et al., 2017).



MECs: Mammary epithelial cell

Figure 1.4. Overview of the mammary development of ewes over the first reproductive period

1.3.2.4. Mammary development during lactation and involution

After parturition, mammary gland volume reached its maximum during week one of lactation in dairy ewes and thereafter, decreases progressively (Rovai et al., 1999, Lérias et al., 2014; Figure 1.4). In sheep, 98% of mammary growth occurs during pregnancy and only 2% occurs during lactation, based on DNA content (Anderson, 1975). The peak of lactation in ewes occurs during week three and four of lactation (Cadar et al., 2012, Lérias et al., 2014). The description of the structural changes in the mammary gland during lactation vary between studies. Some studies have indicated that during early to mid-lactation, the number, size and differentiation of alveoli and MECs continue to increase (Colitti and Farinacci, 2009, Cadar et al., 2012, Lérias et al., 2014). Cadar et al. (2012) reported the presence of both mature and immature alveoli during week three and four of lactation, which corresponds to the peak of lactation. Colitti and Farinacci (2009) reported that among dairy ewes, there is a proliferation index of 58% on day 30 and 46% on day 60 of lactation, indicating an increase in number and differentiation of alveoli and MECs. In the same study, however, there was a reduction in the number of alveoli and MECs from late pregnancy (day 137) to early lactation (day 30). Castañares et al. (2013) also reported a reduction of the number of MECs and alveoli throughout lactation. The difference between these results may be due to the time of measurement, as early lactation measurements by Colitti and Farinacci (2009) and Castañares et al. (2013) were taken on day 30, which is after the peak of lactation in sheep (Lérias et al., 2014), whereas, Cadar et al. (2012) took measurements at the peak of lactation. Boutinaud and Guinard-Flament (2004) reported that there was progressive decrease in milk production after the peak of lactation which was due to the reduction in the number of MECs, which may explain the reduction of MECs and alveoli in early lactation.

In lactation, mature alveoli increase in size, particularly the lumen area (Figure 1.2), where the milk is stored before going into the ductal network to the gland cistern. Colitti and Farinacci (2009) reported a maximum percentage of the secretory area (epithelial and lumen areas) in mid-lactation (day 60) with 66% of the mammary tissue, and a minimum percentage of the stromal area (35%) in mid-lactation (Figure 1.4). Indeed, as the alveoli differentiation progresses, the alveoli increase in size and the stromal area is progressively replace until mid-lactation by the lumen area (Colitti and Farinacci, 2009). Adipose tissue of the mammary gland changed significantly during lactation to support milk production (Hovey and Aimo, 2010). Adipocytes that are close to alveoli have a faster depletion of their lipid storage than adipocytes that are further in mammary tissue (Hovey and Aimo, 2010). Zwick et al. (2018) showed that after the transfer of their lipids for milk production, adipocytes became "slim" but kept the gene expression profile of mature adipocytes until the end of lactation. These results contrasted with the theory of trans-differentiation of adipocytes into MECs and a reverse trans-differentiation from MECs to adipocytes at the end lactation (Colleluori et al., 2021). Both explanations, however, do not exclude one another and both may potentially occur in the mammary gland.

After the end of milk removal, the mammary gland undergoes involution (Rowson et al., 2012, Jena et al., 2018). The involution period is essential for the mammary gland as it has been shown to maximise milk production for cows and goats (Capuco and Ellis, 2013). Involution allows the replacement of senescent MECs and progenitor cells, which have a limited life span and are responsible for maintaining the number of MECs in subsequent lactations (Capuco and Ellis, 2013). Without these progenitor cells, the number of apoptotic MECs (i.e. programmed cell death (Akers 2002a)) increase and their activity is reduced, which reduces milk production (Capuco and Akers, 1999, Capuco and Ellis, 2013).

During involution, extensive tissue remodelling occurs, during which the alveoli regresses and the interalveolar connective tissue, including adipocytes from the fat pad, increases (Colitti et al., 2005, Colitti and Farinacci, 2009, Capuco and Ellis, 2013, Jena et al., 2018). On day one and two after the end of lactation, the secretive tissue is distended due to milk accumulation and occupies most of the tissue area, the percentage of apoptotic cell is low and there is a small amount of connective tissue (Tatarczuch et al., 1997, Colitti, 1999, Colitti and Farinacci, 2009). From four days after the end of lactation, alveoli are less distended and the proportion of MECs undergoing apoptosis increases (Tatarczuch et al., 1997). After eight days of involution, the connective tissue is thicker and the number of cells in apoptosis greatly increase (Tatarczuch et al., 1997, Colitti, 1999, Colitti et al., 2005, Colitti and Farinacci, 2009). Colitti and Farinacci (2009) reported that the highest percentage of stromal area (fat pad area) occurred at eight days of involution (70%; Figure 1.4). After weaning (24 to 72h), milk fat was reported to be transferred from the alveoli to the close adipocytes and start the process of adipocyte hypertrophy continues throughout involution (Zwick et al., 2018). The mammary fat pad is also essential for tissue remodelling during early involution (Zwick et al., 2018). A recent theory explained that during involution, in rodents, some MECs would trans-differentiate into adipocytes (Colleluori et al., 2021), which would be coherent with the increase of the fat pad area. Colitti (1999) and Castañares et al. (2013) also reported that at eight days of involution, alveoli collapsed, interalveolar tissue was filled with large bundles of connective tissue and the percentage of apoptotic cells increases (4.05 ± 2.75% and 1.79 ± 0.87%, respectively). Several studies showed that the greatest percentage of apoptotic cells occurred eight days after the end of lactation (Colitti et al. 1999, Colitti and Farinacci 2009), however, these studies did not examine the percentage of apoptotic cells further than eight days of involution. Tatarczuch et al. (1997) examined this percentage from the end of lactation to day 60 after the end of lactation in sheep and showed that the percentage of apoptotic cells reached a peak at four days of involution and then decreased until 15 days of involution. The same authors reported that the mammary gland completely regressed by 30 to 60 days after weaning.

1.3.3. Impacts of accelerated growth of ewe lambs between weaning and puberty on mammary gland development

1.3.3.1. Milk production

Planes of nutrition influence milk production and the development of the parenchyma and fat pad during the allometric phase (Johnsson and Hart, 1985, Johnsson et al., 1986, Hovey et al., 1999, Hovey and Aimo, 2010, Berryhill et al., 2017). A high plane of nutrition prior to the first breeding at eight months of age was related to a decrease in milk production in ewe lambs (Umberger et al., 1985, McCann et al., 1989). Umberger et al. (1985) found that ewe lambs fed to grow at 200 g/d from early weaning to their first breeding had a lower and less persistent milk yield in their first lactation than ewe lambs fed to grow at 100 g/d. McCann et al. (1989) also reported that ewe lambs that grew more than 200 g/d from weaning to puberty produced less milk than ewe lambs growing at 180 g/d (283 *vs.* 310 g of milk respectively) during their first lactation. Ewe lambs fed *ad libitum* tended to produce less milk during the first lactation than ewe lambs fed to achieve 70% of the average daily gains of the *ad libitum* group with the difference in milk production being significant during the second lactation (Villeneuve et al., 2010b). Sormunen-Cristian and Jauhiainen (2000) reported, however, that increased growth rates (171 g/d *vs.* 196 g/d *vs.* 230 g/d) between weaning and the end of breeding had no effect on milk yield during their first lactation. The difference between these studies may be explained by the number of lambs suckling as ewe lambs were suckling one lamb in Villeneuve et al. (2010b) and ewe lambs in Sormunen-Cristian and Jauhiainen (2000) were suckling twin lambs. The number of times that ewe lambs were milked could also explain these differences (three times in Umberger et al., 1985, once in McCann et al., 1989, 48 times in Sormunen-Cristian and Jauhiainen, 2000, eight times in Villeneuve et al., 2010b).

1.3.3.2. Size and composition of the fat pad

High energy and fat intake have been implicated in an excess of fat accumulation in the mammary fat pad (Johnsson and Hart, 1985, Johnsson et al., 1986, McCann et al., 1989, Peclaris et al., 1997, Hovey et al., 1999, Villeneuve et al., 2010a, Hue-Beauvais et al., 2021). Johnsson and Hart (1985) reported that, at 20 weeks of age, the weight of the mammary fat pad in an *ad libitum* group (fed *ad libitum* between 4 and 20 weeks of age) was twice that measured in a restricted group (fed 120 g/d between 4 and 20 weeks of age; 14.74 g *vs.* 29.99 g respectively). Villeneuve et al. (2010a) reported that ewe lambs fed *ad libitum* from weaning to 19 weeks of age had a heavier fat pad than ewe lambs offered restricted diets (fed to obtain 70% of the average daily gains of the control group using different levels of concentrates). The mammary fat pad produced molecules able to influence cell growth and ductal development such as paracrine growth factors (IGF-1, hepatocyte and fibroblast growth factors), leptin, adiponectin and enzymes able to alter stromal environment (Hovey et al., 1999, Hovey and Aimo, 2010, Palin et al., 2017). Composition and level of dietary fat intake influenced the composition of the fat pad and modified parenchymal growth (McFadden et al., 1990, Hovey et al., 1999, Hue-Beauvais et al., 2021). McFadden et al. (1990) reported that a diet rich in unsaturated fat promoted parenchymal growth in pre-pubertal ewe lambs.

1.3.3.3. Parenchymal development

A high plane of nutrition during the allometric phase reduced parenchymal development in the mammary gland of ewe lambs (Johnsson and Hart, 1985, Johnsson et al., 1986, Peclaris et al., 1997, Hovey and Aimo, 2010, Hue-Beauvais et al., 2021). Parenchymal tissue in a restricted group (fed 120 g/d between 4 and 20 weeks of age), however, tended to occupy a greater mass of the fat pad than in an *ad libitum* group (648 *vs.* 273 g/kg respectively; Johnsson and Hart, 1985). Relative growth analysis showed that the parenchyma of restricted group of ewe lambs increased 3.7 times faster than live weight between four and 20 weeks of age, while in the *ad libitum* group, it increased 2.4 times faster than live weight (Johnsson and Hart, 1985). Villeneuve et al. (2010a) also reported that ewe lambs fed *ad libitum* between weaning and 19 weeks of age tended to have a lighter parenchymal tissue than ewe lambs under restricted diets (fed to obtain 70% of the average daily gains of the control group; 19.3 g *vs.* 27.9 g and 24.4 g, respectively). This reduction in parenchymal development during the allometric phase may explain the impairment of milk production during the first lactation (Umberger et al., 1985).

McFadden et al. (1990) reported that the plane of nutrition (restricted with a growth rate of 120 g/d, a group with a growth of at least 240 g/d and an *ad libitum* group) did not affect the parenchymal development. Indeed, more recently, the impairment in the parenchymal growth was shown to be related to the attainment of puberty at earlier age and so indirectly related to a high plane of nutrition (Meyer et al., 2006a, Berryhill et al., 2017, Van Amburgh et al., 2019). The earlier attainment of puberty shortened the time available for allometric growth to proceed and thus impaired the extent of the ductal network and milk production potential (Hovey and Aimo, 2010, Berryhill et al., 2017, Van Amburgh et al., 2019). The increase of fat deposition and the modifications in the fat pad composition related to a high plane of nutrition have an influence on cell growth and ductal development. In mice, high fat diets resulted in small and fewer alveoli in lactation, low cell differentiation, an enlarged fat pad and an inflammatory process that can lead to impaired milk production and secretion (Hue-Beauvais et al., 2021). The role of high fat diet on adipocytes and their metabolism on the mammary epithelium, however, is not fully understood (Berryhill et al., 2017, Colleluori et al., 2021).

1.3.4. Summary of the impacts of accelerated growth between weaning and puberty of ewe lamb on mammary gland development

A high plane of nutrition (energy and fat) between weaning and puberty in ewe lambs reduced ewe lamb milk production by limiting the parenchymal development and increased the fat deposition in the mammary gland. Recent studies, however, suggest the plane of nutrition would be indirectly related to the impairment of the mammary gland, as a high plane of nutrition increased ewe lamb live weight, which led to an attainment of puberty earlier and ending of the allometric growth of the parenchyma (Meyer et al., 2006a, Van Amburgh et al., 2019). The amount and composition of fat in the diet may, however, have an impact on mammary gland development (Hue-Beauvais et al., 2021). There is a gap of knowledge for the origin and role of fat deposition in the mammary gland, as adipocytes have an endocrine role that also regulates the secretory tissue and ductal development (Hovey et al., 1999, Hovey and Aimo, 2010). Indeed, it is still unknown if having an excess of fat in the mammary gland directly influences the parenchyma or if this has larger positive or negative implications on the mammary gland function.

1.3.5. Measurement of mammary gland and function

The mammary gland and its function can be measured using a large number of methods including *post-mortem* and *in vivo*. The *post-mortem* methods include the measurement of udder weight combined with the determination of udder DNA content, histology and immunoassay (Davis, 2017). The methods performed *in vivo* enable repeated measures on the same animal and include mammary dimensions, imaging methods, DNA content, and gene expression methods (Knight, 2000, Davis, 2017, Narrandes and Xu, 2018). The objective of the mammary investigations in this thesis was to examine the effect of different growth rates between weaning of ewe lambs and their first breeding on their mammary gland development during pregnancy and lactation. To visualise the external and internal mammary structures, mammary dimensions and ultrasound scanning was selected as the most appropriate methods to meet the objective and will be described in this section.

1.3.5.1. Mammary dimensions and morphology

The methods to assess ewe mammary morphology have recently been reviewed by Pourlis (2020). There are two main types of measure of mammary morphology: direct measurement and linear scoring, both types have been used either individually, or combined (Pourlis, 2020). Direct measurements include udder circumference, height, width, teat length, teat angle and teat diameter (Labussière et al., 1981, Milerski et al., 2006, Iniguez et al., 2009, Ayadi et al., 2011, Arcos-Álvarez et al., 2020). Udder volume has been used to examine mammary development profile during pregnancy and lactation in ewes (Davis et al., 1980, Lérias et al., 2014). In dairy cattle, udder volume has been assessed using either the water displacement method or by moulding methods (see review: Davis, 2017). Udder volume can also be calculated using direct measurements as performed by Ayadi et al. (2011) and Arcos-Álvarez et al. (2020) using the following equation:

$$UV = \pi \times R^2 \times UH;\ R = \frac{UC}{2\pi}$$

Where UV = udder volume (cm^3); π = 3.14159; R = radius (cm); UH = udder height (cm); UC = udder circumference (cm).

Linear scores are based on the external appearance of the udder and classified into different typologies. These typologies have been adapted by Gootwine et al. (1980) and Labussière et al. (1981) who created scores for sub-traits of the existing typologies such as teat position, udder shape, teat length and thickness. After multiple adaptations, these scores finally included four traits which describe the general morphology of the mammary gland (de la Fuente et al., 1996, Pourlis, 2020). These four traits were based on the best udder morphology for machine milking in dairy ewes and include udder attachment (also called udder suspension), udder depth, teat position and the degree of separation of the two halves (Casu et al., 2006, Pourlis, 2020). Udder attachment is defined as the ratio between the udder perimeter at its insertion on the abdominal wall and udder height or udder depth score (Casu et al., 2006). Udder depth was defined as the distance between the abdominal wall and the udder cleft with the line joining the hock as a reference (Casu et al., 2006). Teat placement is the distance between the teats, and the degree of separation of the two halves measured the strength of the median ligament (Casu et al., 2006, Pourlis, 2020).

The assessment of mammary dimensions and morphology are useful to assess mammary development through pregnancy and lactation, as repeated measures can be made over time (Lérias et al., 2014, Davis, 2017). In addition, udder morphology has been found to be positively associated with milk yield of dairy ewes (Lérias et al., 2014, Davis, 2017, Arcos-Álvarez et al., 2020, Pourlis, 2020) and therefore, can be used as an indicator of milk yield. Udder dimension measures require the ewe to be standing during measurement, which is relatively easy in dairy ewes as most measures were performed in the milking parlour (Pourlis, 2020), but can be challenging in non-dairy ewes as it would be labour-intensive to restrain a standing ewe. Udder dimensions do not provide any information on changes or the proportions in mammary tissues and cellular development of the mammary gland.

1.3.5.2. Imaging techniques

Imaging techniques have been used to investigate the mammary internal tissues and structures such as computed tomography, X-ray, and magnetic resonance imaging. Magnetic resonance imaging has been used *in vivo* for goats by Fowler et al. (1990) to assess the volume of the mammary parenchyma. These methods generally require the animal to be anesthetised and have challenging logistics, which are not suited to large animals such as cows (Knight, 2000), and therefore, these imaging techniques are not commonly used *in vivo*.

The most widely used imaging technique to examine the mammary internal tissues *in vivo* is ultrasound imaging. There are different ultrasound techniques which provide information on the mammary internal structures (B-mode ultrasound) or on functional parameters of the mammary gland such as the blood flow (Doppler ultrasound) (Barbagianni et al., 2017, Meinecke-Tillmann, 2017).

The use of B-mode ultrasonography on ewe mammary gland has been recently reviewed by Barbagianni et al. (2017). B-mode ultrasound is a non-invasive imaging technique that allows for transcutaneous imaging, which limits animal stress during scanning (Meinecke-Tillmann, 2017). Ultrasound can be used to visualise and assess mammary internal tissues and structures, such as the gland cistern, parenchyma, fat pad and teats (Barbagianni et al., 2017). The ultrasound technique uses a portable machine fitted with a transducer which is applied on the skin (Barbagianni et al., 2017, Meinecke-Tillmann, 2017). The portable aspect of the technique facilitates its on-farm application (Meinecke-Tillmann, 2017). Ultrasonography has been most widely used for dairy breeds to investigate the relationship between mammary internal structures and milk yield of ewes (Nudda et al., 2000, Castillo et al., 2008, Rovai et al., 2008, Makovicky et al., 2017, 2019a,b). A disadvantage of this technique is that it requires a trained and experienced operator to examine and select the images and extract the data using a proprietary software (Molenaar et al., 2020). Depending on the aim of the study, image post-processing is usually required, which can be time consuming and result in delays the assessment of the mammary gland

(Molenaar et al., 2020). In addition, the delineation between mammary tissues can be difficult due to image resolution (Molenaar et al., 2020). Finally, B-mode ultrasonography does not provide information on the cellular activity in the mammary gland, which is related to the lactational performance of the ewe (Boutinaud and Guinard-Flament, 2004).

1.3.6. Indirect measures of ewe milk production

The accurate estimation of milk yield in non-dairy ewes is difficult without disturbing the natural behaviour of animals which could interfere with milk yield and lamb milk intake (Doney et al., 1979, Dove and Freer, 1979, Dove, 1988, van der Linden et al., 2010, McCance, 1959). Lamb milk intake is one component of total milk yield of the ewe but does not necessarily correspond to the total production (Doney et al., 1979, van der Linden et al., 2010). Five methods have been used to estimate either milk yield or lamb milk intake including: lamb suckling, oxytocin method, isotope dilution, udder dimensions and lamb growth. Lamb suckling, isotope dilution and lamb growth are used to estimate lamb milk intake, whereas the oxytocin method and udder dimensions were used to estimate the ewe total milk yield. The associations between udder dimensions and milk yield are inconsistent (Pourlis, 2020), resulting in a less accurate method to estimate ewe milk yield compared to the oxytocin method. The purpose of Chapter 3 was to examine the associations between mammary gland measures and milk yield of ewe lambs, therefore, the oxytocin method was used.

The oxytocin method was developed by McCance (1959) and has been widely used to estimate milk yield of non-dairy ewes (Coombe et al., 1960, Moore, 1967, Doney et al., 1979, Bencini et al., 1992, Cardellino and Benson, 2002, Morgan et al., 2006, 2007, Peterson, 2006, van der Linden et al., 2010, Rosales Nieto et al., 2018a). This method consists of milking ewes with a machine or by hand after an injection of oxytocin. The procedure begins with the separation of ewes and lambs and a first milking to empty the udder. Ewes are milked again after an interval of two to six hours and the quantity of milk at the second milking is measured. Ewes and lambs remain separated until the end of the second milking (McCance, 1959).

The oxytocin method has two main advantages over other methods, firstly it provides an estimate of total milk yield and secondly requires less time and labour compared to the lamb-suckling method (Moore, 1967). The accuracy of this method, however, is dependent on the interval between each milking for the estimation of milk yield (McCance, 1959, Moore, 1967, Doney et al., 1979). A four-hour interval was recommended as the best compromise for the oxytocin method (Doney et al., 1979). The oxytocin method, however, can over-estimate milk yield (McCance, 1959, Moore, 1967, Doney et al., 1979). In addition, milking can result in a greater degree of udder emptying compared to feeding of progeny which then results in an over-stimulation of milk production by the secretory cells (Akers, 2002a). The greater ewe milk yield reported in the literature could also be explained by the effect of oxytocin on milk yield (Doney et al., 1979, Bencini et al., 1992, Bencini, 1995). Oxytocin stimulates milk ejection by inducing the contractions of myoepithelial cells in the mammary gland (Neville et al., 2002), and therefore enables milking of non-dairy animals that would only release the cisternal milk (Lefcourt and Akers, 1983, Bencini, 1995). Doney et al. (1979) suggested that there was a cumulative effect of repeated administration of oxytocin on milk yield. This method also involved a separation of ewes and lambs for several hours which results in behavioural disturbance of the normal lamb suckling behaviour and of the ewe, as handling can temporarily reduce milk yield (Doney et al., 1979). As with the lamb-suckling method, the oxytocin method extrapolates milk yield measured during a period of disturbance of natural behaviour to an undisturbed period of behaviour (Dove and Freer, 1979, Dove, 1988).

1.4. Summary and research objectives

Breeding ewe lambs can increase farm profitability, if well managed, and the number of lambs born per ewe in her lifetime. A minimum live weight of 40 kg at breeding is recommended for breeding Romney-type ewe lambs (Kenyon et al., 2014c). Ewe lambs bred at a heavier live weight can display greater reproductive and lambing performance compared to those bred at a lighter live weight (Corner-Thomas et al., 2014b, 2015a,b, Kenyon et al., 2014c). Increased the growth rate of a ewe lamb between weaning and breeding, however, could impair mammary gland development and its subsequent lactational performance (Umberger et al., 1985, Villeneuve et al., 2010b). This potential impairment of mammary development is not fully understood. Previous studies of ewe lambs investigating the impacts of heavier breeding live weight have limited their investigations to only the first breeding season. There is little information on the potential long-term consequences of a heavier live weights of ewe lambs at their first breeding on their subsequent reproductive performance, mammary development, live weight, and production efficiency.

Therefore, the objectives of this thesis were to:

- Compare and characterise the mammary gland development of ewe lambs who experienced increased growth rate between their weaning as a ewe lamb and their first breeding compared with those with lower growth rates, in their first (Chapter 2) and second pregnancy and lactation (Chapter 4) using ultrasonography.
- Investigate the associations between ewe mammary gland ultrasound measures and the growth rates of their progeny to weaning (Chapter 2 to 4).
- Examine the association between the single-bearing ewe lamb mammary gland ultrasound measures during pregnancy and lactation and their milk production (Chapter 3).
- Investigate the long-term consequences of a heavier ewe lamb live weight at their first breeding on their reproductive and lambing performance (Chapter 5 and 6), and efficiency (Chapter 7) during their first three breeding seasons.
- Examine the relationship between ewe lamb live weight at breeding and ewe mature live weight at 39 months of age (Chapter 7).

Chapter 2

Effect of increased growth rates post-weaning on mammary gland development of ewe lambs

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Haslin E., Corner-Thomas R.A., Kenyon P.R., Molenaar A.J., Morris S.T., Blair H.T. (2021) Mammary Gland Structures Are Not Affected by an Increased Growth Rates of Yearling Ewes Post-Weaning but are Associated with Growth Rates of Single lambs, Animals, 11,3, 884.

2.1. Abstract

This experiment aimed to examine the impacts of an increased growth rate of ewe lambs between three and seven months of age on udder development using ultrasonography and establish whether this method could be used to identify ewe mammary structures that may be indirect indicators of single lamb growth to weaning. Scores to assess udder health (udder and teat palpations, udder depth and symmetry), udder dimensions (height, circumference and volume), depth of gland cistern (GC), parenchyma (PAR) and fat pad (FP) were measured in late pregnancy (P107), early lactation (L29), and at weaning (L100) in 59 single-bearing ewe lambs selected from two treatments. The 'Heavy' group (n = 31) was preferentially fed between weaning and pre-breeding achieving an average live weight of 47.9 ± 0.38 kg at seven months of age. The 'Control' group (n = 28) had an average breeding live weight of 44.9 ± 0.49 kg. Udder dimensions, GC, PAR and FP and single lamb growth rates did not differ ($P > 0.10$) between treatments at any time. Single lamb growth rates to L100 were positively associated ($P < 0.05$) with PAR at P107 and GC at L29. There was no evidence of any negative effects of the liveweight gain treatments on udder development of ewe lambs as measured by ultrasonography. The results suggest that udder ultrasonography has the potential to identify pregnant ewe lambs which would wean heavier single lambs.

2.2. Introduction

An important determinant for achieving puberty and successful breeding of ewe lambs at seven months of age is the attainment of 40-70% of mature weight (see review: Kenyon et al., 2014c). Romney ewe lambs that were 40 to 45 kg at breeding had greater performance than those bred at 35 kg or below, therefore, Kenyon et al. (2014c) recommended a minimum live weight of Romney-type ewe lambs of 40 kg at breeding. Further, heavier live weights at breeding have been shown to improve the reproductive performance of ewe lambs resulting in a greater number of ewe lambs mated during the breeding period, increased fertility rate, litter size and lambing percentage (Kenyon et al., 2014c, Corner-Thomas et al., 2015b). Using this combined knowledge, farmers aim to feed their Romney-type ewe lambs to achieve high growth rates post weaning to ensure they reach live weights greater than 40 kg at breeding. Increased growth rates prior to puberty, however, have been reported to have negative impacts on mammary gland development and milk production in ewe lambs (Johnsson and Hart, 1985, Johnsson et al., 1986, Hovey et al., 1999, Tolman and McKusick, 2001, Hovey and Aimo, 2010, Villeneuve et al., 2010a,b). In addition, McCann et al. (1989) reported that ewe lambs grown at greater rates between weaning and their first breeding at 8 months of age produced less milk (283 *vs.* 310 g in 4 hours) than those with slower growth rates, indicating an impairment of the mammary gland development and/or function. Farmers, therefore, need to balance the desire for heavier live weights at breeding to improve ewe lamb reproductive performance while limiting any practical negative impacts on lactation performance and growth of their progeny to weaning.

Ewe lambs have a period of accelerated growth of parenchymal udder tissue, called allometric phase, between two and five months of age (Anderson, 1975, Johnsson and Hart, 1985, Villeneuve et al., 2010a). During this period, the ductal network of the mammary gland expands extensively into the mammary fad pad (Hovey et al., 1999). The development of the ductal network during this period will determine the future alveolar development, and therefore future milk production (Villeneuve et al., 2010a, Berryhill et al., 2017). A high plane of nutrition prior to puberty has been reported to reduce the development of parenchyma udder tissue (Johnsson and Hart, 1985, Johnsson et al., 1986, Villeneuve et al., 2010a) and increase fat accumulation in the fat pad (Johnsson and Hart, 1985, Johnsson et al., 1986, Hovey et al., 1999), which combined may explain the reported subsequent lower milk production (Umberger et al., 1985, Villeneuve et al., 2010a).

Ewe mammary internal structures can be visualized using ultrasonography (Barbagianni et al., 2017). Specifically, ultrasound has been used to investigate the mammary parenchyma (Petridis et al., 2014, Barbagianni et al., 2015) and the mammary gland cistern (*sinus lactiferous*) (Ruberte et al., 1994, Caja et al., 1999, Castillo et al., 2008, Rovai et al., 2008). In dairy ewes, mammary morphology and milk production were reported as traits of interest in genetic selection, leading to an increase in mammary size and milk yield compared to non-dairy breeds (Carta et al., 2009, Pourlis, 2020). Currently, most studies that have examined mammary structures using ultrasonography utilised dairy breed ewes (Nudda et al., 2000, Castillo et al., 2008, Rovai et al., 2008, Petridis et al., 2014, Barbagianni et al., 2015) with only a small number of studies examining dual-purpose meat and wool breeds (Ruberte et al., 1994, Caja et al., 1999). Ruberte et al. (1994) examined the relationship between mammary ultrasound images and mammary gland anatomy of mature ewes, whereas Caja et al. (1999) focused on measures of cistern size and their correlation with the milk yield of mature ewes. Currently, no studies have used ultrasonography to examine the mammary gland of non-dairy ewe lambs. Over the last 20 years, the technology has improved allowing for more detailed examination of the mammary structures through greater image resolution which allows the assessment of the development of the parenchyma and identification of abnormalities in the parenchyma (Barbagianni et al., 2015, 2017). To the authors' knowledge, this experiment was the first to utilise ultrasound on non-dairy ewe lambs during their first pregnancy and lactation to investigate the relationship between ultrasound measures of ewe lamb udders and the growth of their progeny.

The primary objective of this experiment was to investigate the effects of an increased growth rate between three (weaning) and seven months of age (breeding) on mammary gland structures of single-bearing Romney ewe lambs using ultrasonography. It was hypothesised that ewe lambs with an increased growth rate between weaning and breeding (Heavy) would have a smaller parenchyma and a greater fat pad area than ewe lambs with a lower growth rate (Control). The second objective was to develop an ultrasound technique to identify mammary structures that could be used as indirect indicators of the growth to weaning of the progeny of ewe lambs. It was hypothesised that the ultrasound measurements of the young dam's mammary gland in lactation would be correlated with the early growth of their progeny.

2.3. Materials and Methods

This experiment and all animal handling procedures were approved by the Massey University Animal Ethics Committee (MUAEC-17/16). The experiment was conducted at Massey University's Riverside Farm (latitude: 40° 50' 35 S, longitude: 175° 37' 55 E), 10 km north of Masterton, New Zealand.

2.3.1. Experimental design

Romney ewe lambs (n = 290), born as twins to mature ewes in 2017 were allocated at weaning (3/01/2018; 86 days of age; P-127) to one of two groups using a stratified random sampling procedure to ensure that both treatments had similar (P > 0.10) live weights at P-127. Those allocated to the 'Heavy' (n = 145) group had an average live weight of 28.6 ± 2.4 kg and the 'Control' (n = 145) group 28.7 ± 2.7 kg. The 'Heavy' group was preferentially fed until breeding (10/05/2018; 213 days of age; P0) achieving an average live weight of 47.9 ± 0.36 kg. The 'Control' group had an average live weight of 44.9 ± 0.49 kg at breeding. To achieve these differences in breeding live weight, the two treatments were managed and fed separately from 119 to 94 days prior to breeding (P-119 to P-94) and from P-51 to P0, from P-94 to P-51 both treatments were managed and fed together (Figure 2.1). Both treatments were exposed to vasectomised rams, at a ratio of approximately 1:50. Vasectomised rams were fitted with mating harnesses with crayons for 68 days prior to breeding (145 days of age; P-68; Figure 2.1). Between P-127 and P0, six ewe lambs from the Heavy group and three from the Control group died. Any ewe lamb lighter than 39 kg at the start of breeding (Heavy n = 4; Control n = 7) was not presented for breeding.

At breeding (P0), the Heavy group (n = 135) and the Control group (n = 135) were merged and managed together until weaning (465 days of age; L100). Crayon-harnessed entire Romney rams, at an approximate ratio of 1:40, were introduced for 34 days (P0 – P34). Pregnancy diagnosis was determined by a commercial operator using trans-abdominal ultrasound at P84 (84 days after ram introduction). Single-bearing Romney ewe lambs from each treatment were randomly selected at pregnancy diagnosis (P84; Heavy, n = 31, 52.3 ± 0.85 kg and Control, n = 28, 51.4 ± 0.85 kg; lsmeans ± SEM) from ewe lambs successfully mated during the first 17 days of the breeding period. At P138, ewe lambs from both treatments were randomly assigned to one of four lambing paddocks (average stocking rate 8.02 ewe lambs/ha; Heavy n = 8, 6, 9, 8 and Control n = 9, 5, 9, 5 per lambing paddock, respectively) ensuring ewe lambs from each treatment were in each paddock. All ewe lambs (n = 59) lambed within 15 days (1/10/2018 to 16/10/2018). Lactation was deemed to have begun after the first lamb had been born (1/10/2018; L1). Ewe lambs whose lamb died at birth or during lactation remained with the lactating ewes until weaning. All lambs were weaned at approximately 100 ± 4 days of age (17/01/2018; L100; Heavy = 24 and Control = 24).

2.3.2. Feeding management

The Heavy and the Control ewe lambs were separated from commercial lambs at P-127 and introduced, as a single mob, to lucerne (*Medicago sativa L.*) swards until P-119. Both treatments were grazed on lucerne separately from P-119 to P-94, as a single mob from P-94 to P-51, and then separately from P-51 to P-27 (Control) and from P-51 to P-22 (Heavy; Figure 2.1). Ewe lambs were then grazed on ryegrass

(*Lolium perenne L.*) and white clover (*Trifolium repens L.*) based swards separately from P-27 to P0 (Control) and from P-22 to P0 (Heavy; Figure 2.1).

After the acclimation to lucerne (P-127 to P-119), the Heavy and Control ewe lambs were separated at P-119 to allow the Heavy group to be trained to eat a cereal-based concentrate feed (Figure 2.1). The amount of cereal-based concentrate feed offered to the Heavy group was progressively increased to a maximum of 200 g/head/day. Treatments were merged at P-94 to allow the Control group to be trained to eat the cereal-based concentrate feed, although for a reduced time in order to achieve the target breeding live weights (44 kg; Figure 2.1). Between P-94 and P-51, all ewe lambs were offered 200 g/ewe lamb/day of cereal-based feed. Between P-51 and P0, Control and Heavy ewe lambs were separated, and only Heavy ewe lambs were supplemented to a maximum of 300 g/ewe lamb/day of cereal-based concentrate feed. From P-119 to P-44, the concentrated feed was a cereal-based mix with molasses (Table 2.1), and from P-44 to P0, the concentrated feed was a barley-based mix (Table 2.1). Concentrate feeds are commonly used in this region to ensure ewe lambs reach live weight targets.

From P0 to the weaning of their lambs (L100), both groups were managed as a single cohort and grazed using a rotational grazing system on ryegrass and white clover pasture under commercial New Zealand grazing conditions. Due to low pasture availability in pregnancy (winter), all ewe lambs were also offered lucerne baleage at a rate of approximately 1.0 kg/ewe lamb/day from P34 to P138 (Table 2.1).

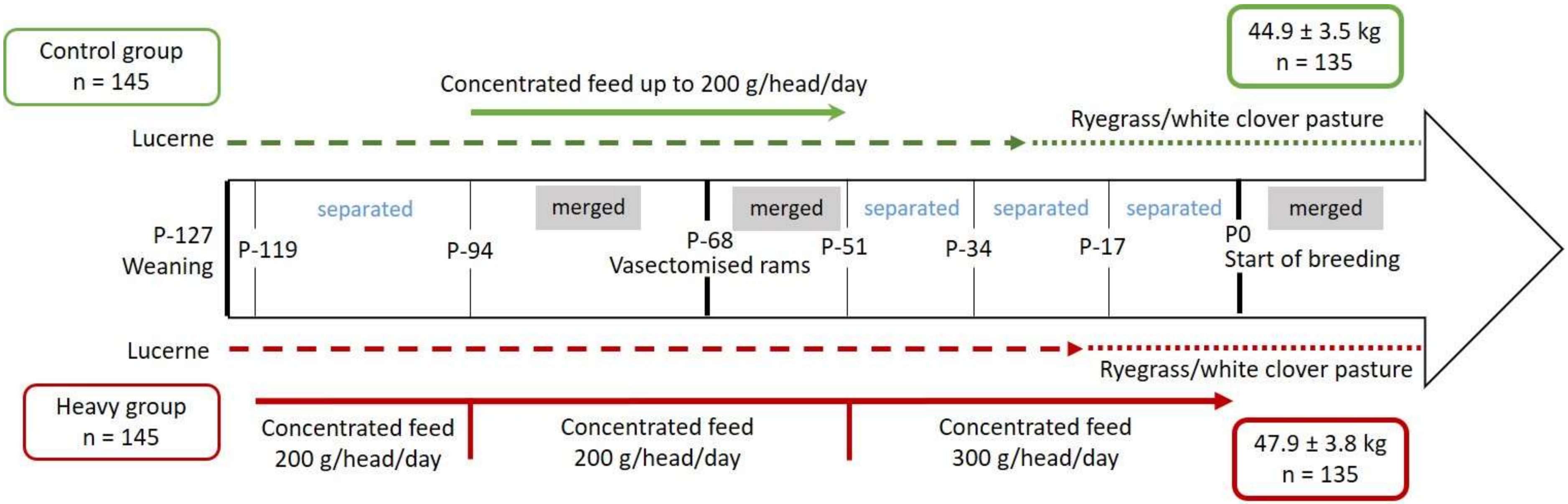

Figure 2.1. Feeding management of the Heavy and Control ewe lambs prior to the start of the breeding period.

2.3.3. Pasture measurements

The pre-grazing cover of pastures was recorded in pregnancy (from P0 to P138; n = 17) and all lambing paddocks fortnightly from P138 to L100 (n = 30). The pre-grazing cover was measured using a plate meter (Ashgrove Pastoral Products, New Zealand, 90 readings per paddock). A standard calibration was used to obtain the herbage mass (herbage mass = [158 x average meter reading] + 200; Hodgson et al., 1999). The pre-grazing pasture mass during pregnancy and lactation was on average 868 ± 39 and 1265 ± 58 kg DM/ha, respectively.

Two samples of lucerne baleage were taken at P50 and P67 to estimate the nutritional composition (Table 2.1). One sample of grain-based mix was taken at P-78 and two samples of barley-based mix at P-44 and P-17 to determine the nutritional values (Table 2.1). These samples were analysed using *in-vitro* methods to determine the nutritional quality; dry matter (DM) and crude protein (CP; Dumas' procedure, AOAC method 968.06 using a Leco total combustion method, LECO corporation, St, Joseph, MI, USA; AOAC, 2019). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were analysed by a Tecator Fibretec System (Robertson and Van Soest, 1981). Metabolizable energy (ME) was calculated using the digestible organic matter in dry matter (Roughan and Holland, 1977).

Table 2.1. Nutritional composition of cereal-based concentrate feed and molasses, barley mix concentrate feed and lucerne baleage (mean ± SEM).

	Cereal-based concentrate + molasses[1]	Barley mix[1]	Lucerne baleage[2]
DM (%)	84.3[3]	85.6 ± 0.8	94.8 ± 0.1
CP (g/100g DM)	16.9	10.5 ± 0.2	13.3 ± 0.05
NDF (g/100g DM)	15.5	17.6 ± 1.3	48.8 ± 0.2
ADF (g/100g DM)	0.5	7.1 ± 0.4	36.1 ± 0.9
ME (MJ/kg DM)	13.2	12.8 ± 0.05	9.6 ± 0.1
SSS (g/100g DM)	53.7	48.5 ± 0.3	-
Lipid (g/100 g DM)	3.3	3.0 ± 0.4	3.6 ± 0.5

DM: Dry Matter; CP: Crude Protein; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; ME: Metabolizable Energy; SSS: Soluble Sugars and Starch; [1] analysed with near infrared reflectance spectroscopy; [2] analysed with wet chemistry methodology; [3] only one sample taken hence no standard error.

2.3.4. Animal measurements

Unfasted live weights of ewe lambs were recorded at P-127, P0, P84, P107, P138, L29 and L100. Body condition score (BCS; Jefferies, 1961, Kenyon et al., 2014b) of ewe lambs were recorded at P-127, P0, P84, P107, L29 and L100. Lambs were tagged within 18 hours of birth, during twice daily lambing rounds, at which time their date of birth, paddock, sex, dam number and birth weights were recorded. Lambs were reweighed at L29 and L100.

2.3.4.1. Udder score and morphology

Ewe udder scoring and morphological trait measurements were performed at P107, L29 and L100 by a single trained operator. The scoring system, adapted from Griffiths et al. (2019b) assessed udder health. The scoring system included palpation of both udder halves and teats and assessed udder symmetry and depth (Table 2.2). Ewe lambs were placed in a sitting position to allow access to the udder for palpations and then in a standing position to assess udder symmetry and udder depth. Morphological traits were measured while ewe lambs were standing.

Morphological measures included udder circumference (UC, cm) above the teats (Sezenler et al., 2016), using a tape (Scrotal Measuring Tape, Shoof international LTD, New Zealand), and the height of each half of the udder (UHR and UHL, cm), using a ruler to measure the distance between the rear udder attachment, measured along the outside edge of the udder, and the udder floor (Ayadi et al., 2011; Figure 2.2). Udder volume (UV, cm^3) was calculated using udder circumference and an average of udder height (UH, cm) according to Ayadi et al. (2011):

$$UV = \pi \times R^2 \times UH; \; R = \frac{UC}{2\pi}$$

Where UV = udder volume (cm^3); π = 3.14159; R = radius (cm); UH = udder height (cm); UC = udder circumference (cm).

Table 2.2 Description of the traits and scores used to assess udder health of ewe lambs in pregnancy and lactation (adapted from Griffiths et al., 2019b).

Score	Description
Udder palpation[a]	
1	Diffuse soft consistency
2	Diffuse firm consistency
3	Soft consistency with nodule(s) – lumps or grainy texture
4	Firm consistency with nodule(s) – lumps or grainy texture
5	Diffuse hard consistency
Teat palpation[a]	
1	Soft consistency
2	Thickened teat orifice
3	Hard consistency
4	Teat orifice obstruction
Udder symmetry[b]	Visible symmetry of the udder halves while the udder is hanging naturally
Symmetrical	Both udder halves were of similar size
Asymmetrical	One udder half was noticeably larger than the other
Udder depth[b]	Distance between the udder cleft and the abdominal wall, taking the line joining the hocks as a reference
1	
2	
3	Udder cleft at hock level
4	
5	Udder cleft at the level of abdominal wall

[a] Ewes examined in a sitting position; [b] Ewes examined in a standing position

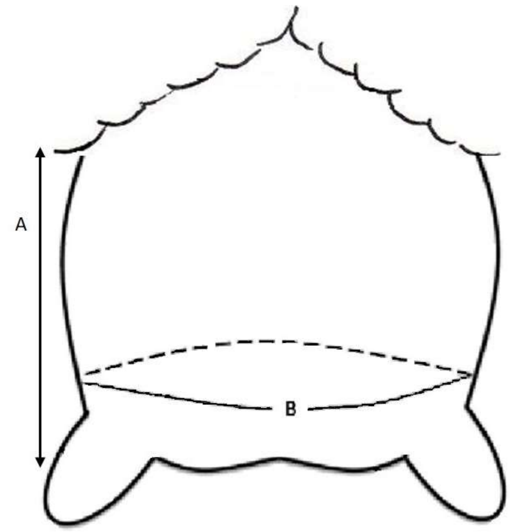

Figure 2.2. Morphological traits of ewe lamb udder. A. Udder height (cm) measured from outside edge of the udder between the ewe leg and the udder floor; B. Udder circumference (cm) taken above the teats (adapted from Sezenler et al. (2016)).

2.3.4.2. Udder ultrasound scanning

Udder ultrasound scans were performed, by a single operator, at P107, L29 and L100. At L29 and L100, ultrasound scans were not conducted for ewe lambs whose lambs had died (Heavy n = 7 and Control n = 4). At L29 and L100, ewe lambs were separated from their lambs four hours prior to the ultrasound scanning to allow the udder to accumulate milk according to Ruberte et al. (1994) and Caja et al. (1999). Ewe lambs were placed in a sitting position (i.e. shearing position, Figure 2.3B & C) to allow easy access to the udder. Ultrasound scans were performed with an ultrasound scanner fitted with a linear transducer with 5.0 – 10.0 MHz imaging frequency (Sonosite M-Turbo Ultrasound with L38xi, Bothell, Washington, USA). Vegetable oil was used as a coupling gel. The transducer was applied on the external base of each teat at a 30° angle from the caudal-cranial axis (Figure 2.3A) with an inclination of approximately 45° in relation to the teat (Albino et al., 2015), as shown in Figure 2.3B. A light and consistent pressure was applied to the udder through the transducer to minimise variations related to pressure on the images. There was some variability in the position of ewe lambs but, the effects of these variations were minimised by indicating to the handler on which position (on right or left leg) the ewe had to be sited for the ultrasound scan (Figure 2.3B & C), and identifying the most representative and consistent mammary structures on the images during the scan prior to capturing images.

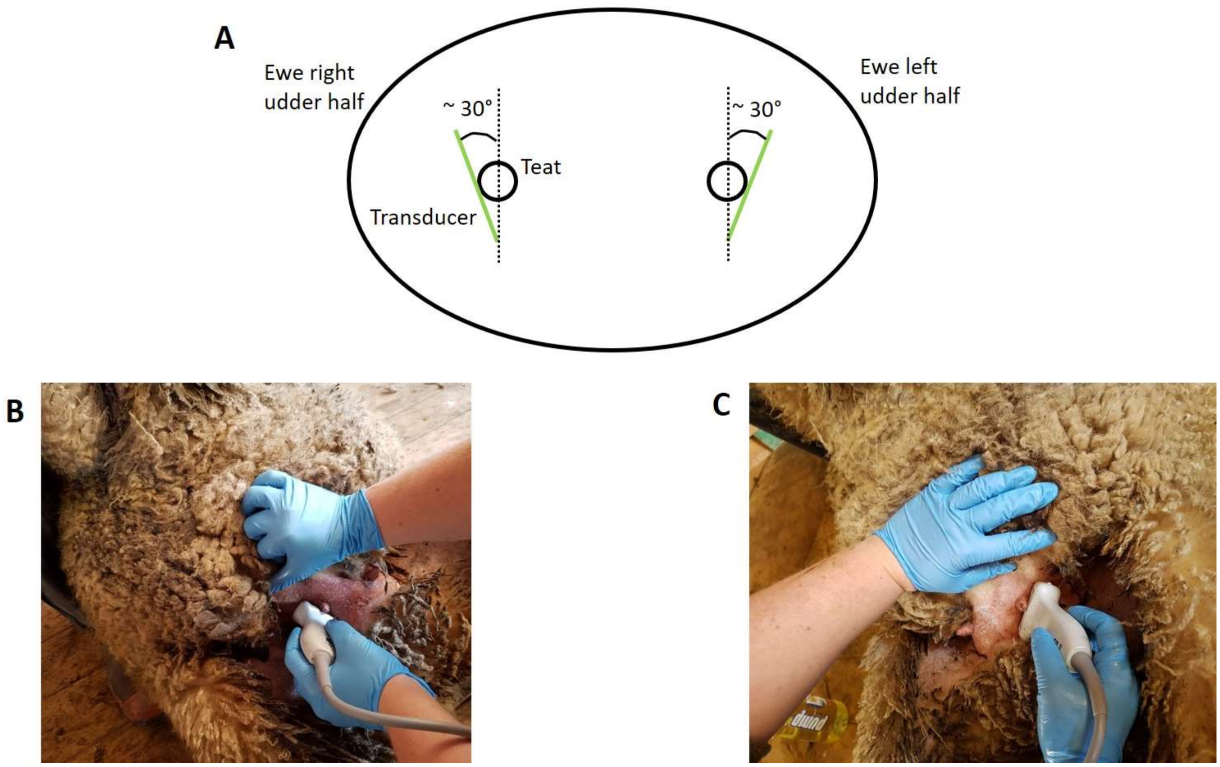

Figure 2.3.(A) Diagram representing the position of the transducer applied on the right and left udder halves when ewe lambs are in a sitting position with solid lines representing the transducer; **(B)** Picture of an ultrasound scan of the right udder half of a ewe lamb in pregnancy (P107); **(C)** Picture of an ultrasound scan of the left udder half of a ewe lamb in pregnancy (P107).

A minimum of three images were captured for each udder half. Images included the gland cistern, mammary parenchyma, putative fat pad and limit between the mammary gland and the abdominal wall. One image of suitable resolution per udder half, where all structures were identifiable and present was selected for image processing (Albino et al., 2017, Molenaar et al., 2020). Udder halves with an udder palpation score of 4 or 5 at a specific time point (pregnancy, early lactation or weaning) were considered "abnormal" (Griffiths et al., 2019b). In order to only observed images of healthy udders, images from "abnormal" udder halves were not included in the image selection (Heavy: 1 ewe with 1 half and Control: 2 ewes with 1 half each).

The processing of the images was undertaken using ImageJ software (Ferreira and Rasband, 2012) as used by Abràmoff et al. (2004). In ImageJ, the scales between pixels and millimetres were calculated based on the number of pixels, the scanning depth (mm) and the probe width (mm; Figure 2.4). The scales of the width and the height of pixels were calculated separately. The number of pixels of the width and the height of the images was estimated using the straight tracer (Furini et al., 2018).

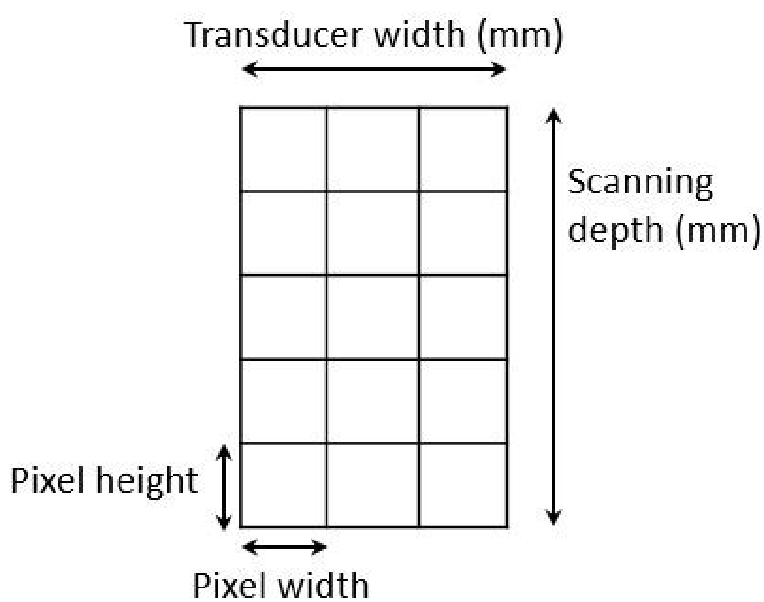

Figure 2.4. Diagram of an ultrasound image and the dimensions needed to calculate the width scale and height scale.

This method relies on the ability of the operator to interpret and identify lines on the images. To standardize the assessment compartment depth, templates were created for each time point and included four representative images from four different ewe lambs with and without the lines drawn for each compartment (Appendix A; Molenaar et al., 2020). The total depth of mammary gland conservative (MTc) was the smallest likely demarcation (abdominal wall) of the mammary gland (Figure 2.5A), whereas the total depth of the mammary gland generous (MTg) was the largest likely demarcation of the mammary gland visible on the image (Figure 2.5A; Molenaar et al., 2020). The MTc, MTg, fat pad (FP), parenchyma (PAR) and gland cistern (GC) depths were estimated at the widest point for each sub compartment, excluding the skin layers, using the straight tracer (Figure 2.5A). These depths were expressed in millimetres.

To assess the development of the parenchyma during pregnancy and lactation, three regions of interest (ROI; Albino et al., 2015) were randomly drawn in the parenchyma area, each square measured 6.7 mm^2 (Figure 2.5B). The brightness of each pixel corresponded to echogenicity and was numerically represented on a scale of 256 levels of grey (Ojala et al., 2002). Echogenicity is defined as the capacity of tissues to interact and reflect the sound waves of the transducer; tissues that reflect a large proportion of the sound waves (e.g. fat tissue) are hyperechoic and have a greater pixel value, whereas tissues that reflect a small proportion of the sound waves (e.g. liquid) are hypoechoic and have a lower pixel value (Strzetelski et al., 2004). This capacity varies with tissues, i.e., liquids have very low echogenicity (Strzetelski et al., 2004) and fat has greater echogenicity but attenuates as the depth increases (Molenaar et al., 2020). The average of each ROI from the same image were averaged and expressed on a 0 to 255 scale.

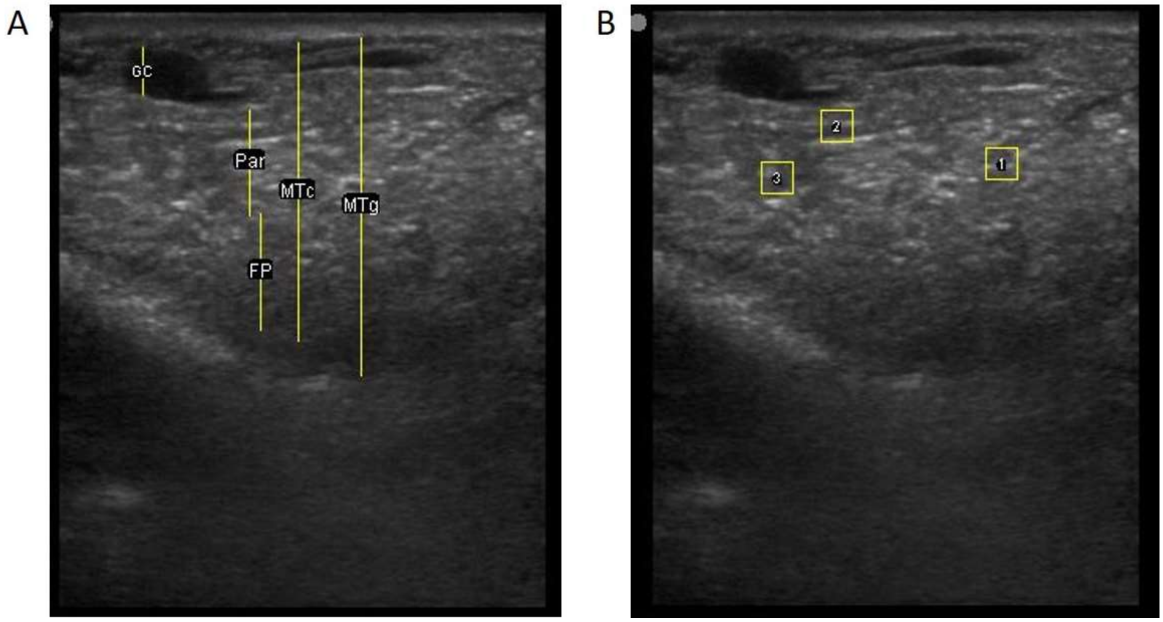

Figure 2.5.(A) Demonstration of delimitations of the mammary total depth conservative (MTc) and generous (MTg), mammary gland cistern (GC), parenchyma (PAR) and the putative fat pad (FP) and **(B)** Demonstration of randomly positioning of regions of interest (1, 2, 3) in the parenchyma.

2.3.5. Statistical analysis

All statistical analyses were conducted with SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Ewes that died (Heavy n = 2) or whose lambs died prior to weaning (Control n = 4 and Heavy n = 5) were excluded from the experiment. The final dataset included 24 ewe lambs and their single lambs in each treatment. A total of 284 images were selected and included in the analyses.

Growth of ewe lambs from weaning (P-127) to breeding (P0) was analysed using a linear mixed model that included treatment (Control *vs.* Heavy) as a fixed effect and age at P-127 as a covariate. Live weight of ewe lambs at P107, L29 and L100 was analysed using a linear mixed model allowing for repeated measures. The model included the fixed effects of treatment, day of measurement (P107, L29 and L100) and their two-way interaction with lambing date included as a covariate. The BCS of ewe lambs was analysed using a generalized linear model allowing for repeated measures with a Poisson distribution and a log transformation. Treatment, day of measurement (P0, P84, P138, L29 and L100) and their two-way interaction were included as fixed effects. Growth of the progeny from birth to L29 and from L29 to L100 was analysed using a linear mixed model allowing for repeated measures, and included treatment and time (birth to L29 and L29 to L100) as fixed effects, date of birth as a covariate and lambing paddock as random effect. Growth of the progeny from birth to L100 was analysed with a linear model including treatment as a fixed effect, date of birth as a covariate and lambing paddock as a random effect.

Udder and teat palpations for each udder half were analysed using generalised linear models allowing for repeated measures, assuming a Poisson distribution and using a log transformation were used to determine whether scores differed between udder halves and treatments. Udder half (right *vs.* left), day of measurement (P107, L29 and L100), treatment and the two-way interactions of day of measurement and treatment, and day of measurement and udder half were fitted as fixed effects, with a Tukey-Kramer adjustment to allow for multiple comparisons. Due to an absence of differences in the palpation scores between treatments ($P > 0.10$), the effects of live weight at P0 on the palpation scores was investigated, therefore, treatment was removed, and the models were re-run with live weight at P0 as a covariate.

Udder depth score was analysed using a generalised linear model allowing for repeated measures, assuming a Poisson distribution and a log transformation.

Treatment, day of measurement and their two-way interaction were fitted in the model, with a Tukey-Kramer adjustment. The model was re-run without treatment as fixed effect and with live weight at P0 as a covariate.

Udder symmetry (yes/no) was analysed with a generalised linear model allowing for repeated measures with a binomial distribution and a logit transformation. The model included treatment and day of measurement as fixed effects and their two-way interaction, with a Tukey-Kramer adjustment. The model was re-run without treatment as fixed effect and with live weight at P0 as a covariate.

Udder circumference (UC) and UV were analysed using general linear mixed models allowing for repeated measures and fitted treatment and day of measurement and their two-way interaction, lambing date lambing date was fitted as a covariate.

Udder height (UH), GC, FP, PAR, MTc, MTg and the ROIs grey scale value of the right and left udder halves were analysed using general linear mixed models allowing for repeated measures. These models included udder half, day of measurement, treatment and two-way interactions between udder halves and day of measurement and treatment and day of measurement as fixed effects, with a Tukey-Kramer adjustment, and lambing date as a covariate and ewe lamb as random effect. Udder half was not significant ($P > 0.10$) in the models constructed for the grey scale values, UH, GC, PAR, MTc and MTg, therefore, an average of the two halves was calculated for each day of measurement and used in further analyses. For FP, udder halves were significantly ($P < 0.05$) different at L100 and therefore the FP measures of the right and left halves at L100, remained separated in the analyses. To determine the effect of individual ewe lamb live weight at P0 on the grey scale values, GC, PAR, FP, MTc and MTg, the linear mixed models were re-run without treatment as a fixed effect and included udder half, day of measurement and their two-way interaction as fixed effects, lambing date and live weight at P0 as a covariate, a two-way interaction between live weight at P0 and day of measurement, and ewe lamb as a random effect.

The residuals were generated using general mixed models to account for potential confounding variables. Ewe live weight, ewe BCS, UV, UH, UC and MTg were adjusted for treatment and lambing date. In the model, PAR, GC and MTc were adjusted for treatment, MTg and lambing date. Lamb growth from birth to L29, from L29 to L100 and from birth to L100 were adjusted for treatment, lambing date, and sex of lamb. Pearson correlations were used to test for linear associations between the residuals of ewe live weight, ewe BCS, UC, UH, UV, GC, PAR, FP, MTc and MTg at each time point (P107, L29, L100) and lamb growth from birth to L29, from L29 to L100 and from birth to L100.

Multiple regression analyses of lamb growth from birth to L29 and from birth to L100 were carried out using general linear models, to examine whether each predictive variable was correlated with lamb growth. Predictive variables correlated with lamb growth with $P \leq 0.20$ were selected and included in the model (Dohoo et al., 2003a). Correlations between selected predictive variables were examined to identify high collinearity (Dohoo et al., 2003b), resulting in the following equations:

Lamb growth from birth to L29 = GC at L29 + MTc at L29 + BCS at P0

Lamb growth from birth to L100 = PAR at P107 + FP at P107 + Ewe LW at L29 + GC at L29 + MTc at L29

Backward manual variable eliminations were used to select the model that best explained the variation in lamb growth from birth to L29 and to L100 by removing predictive variables with $P > 0.10$. Confounding effects were evaluated after each variable removal from the model and were examined by examining changes in predictive variable coefficients. Any non-significant predictive variable causing greater than a 20% change in the model coefficients was considered a confounding variable and included in the model (Dohoo et al., 2003a). No confounding effects were identified in these regressions.

2.4. Results

2.4.1. Growth and live weight

Ewe lambs from the Heavy group had greater growth rates between P-127 and P0 than Control ewe lambs ($P < 0.05$; 147 ± 4.4 *vs.* 133 ± 4.4 g/d, respectively) resulting in a tendency for different live weights at P0 for the Heavy group ($P = 0.09$, 47.5 ± 0.71 *vs.* 45.8 ± 0.71 kg, respectively). Live weight of ewe lambs did not differ ($P < 0.10$) between groups at P107, L29 or L100 (Table 2.3). Ewe lamb BCS did not differ ($P > 0.10$) between treatments at any time point (Table 2.3). Ewe BCS at P138 was greater ($P < 0.05$) than at L29 and L100, which did not differ ($P > 0.10$; Table 2.3). Lamb growth from birth to L29, from L29 to L100 and from birth to L100 did not differ between the treatments ($P > 0.10$; data not shown).

Table 2.3. Effect of time and treatment (Control *vs.* Heavy) on ewe lamb live weight and body condition score (BCS) in pregnancy (P107, P138), early lactation (L29) and at weaning (L100) and lamb live weight in early lactation (L29) and at weaning (L100). Least square means ± SEM

	Pregnancy (P107)	Pregnancy (P138)	Early lactation (L29)	Weaning (L100)
Ewe live weight (kg)				
Control (n = 24)	52.3 ± 0.81^{a}	58.5 ± 0.87^{b}	61.7 ± 1.21^{c}	62.0 ± 1.26^{c}
Heavy (n = 24)	52.4 ± 0.81^{a}	57.8 ± 0.87^{b}	60.8 ± 1.21^{c}	60.2 ± 1.26^{c}
Ewe BCS				
Control (n = 24)		$2.77\ (2.64–2.90)^{b}$	$2.69\ (2.53–2.85)^{a}$	$2.65\ (2.48–2.82)^{a}$
Heavy (n = 24)		$2.69\ (2.58–2.80)^{b}$	$2.46\ (2.30–2.63)^{a}$	$2.42\ (2.24–2.60)^{a}$
Lamb live weight (kg)				
Control (n = 24)			15.0 ± 0.52^{a}	29.9 ± 0.68^{b}
Heavy (n = 24)			15.1 ± 0.52^{a}	29.5 ± 0.68^{b}

^{a,b,c} Means within rows with different superscripts are significantly different ($P < 0.05$).

2.4.2. Udder score and morphology

Udder and teat palpation scores did not differ between the left and the right udder halves at any time point ($P > 0.10$; data not shown). At P107, udder palpation scores in the Control group were greater ($P < 0.05$) than the Heavy group but did not differ at L29 or L100 ($P > 0.10$; Table 2.4). Live weight at P0, irrespective of treatment group, had no effect ($P > 0.10$) on udder palpation scores (data not shown). At L29, udder palpation scores were greater than at either P107 or L100 ($P < 0.05$). Similarly, udder palpation scores at L100 were greater than at P107 ($P < 0.01$; Table 2.4).

Teat palpation scores did not differ ($P > 0.10$) between treatments at any time point (Table 2.4). Ewe lamb live weight at P0, irrespective of treatment, had no impact ($P > 0.10$) on teat palpation, the percentage of ewes with an asymmetric udder or udder depth score (data not shown). Teat palpation scores were greater at L29 than at P107 and L100 ($P < 0.05$) which did not differ ($P > 0.10$; Table 2.4). The percentage of ewe lambs with asymmetric udders did not differ ($P > 0.10$) among treatments nor over time ($P > 0.10$; Table 2.4). Udder depth scores did not differ between treatments ($P > 0.10$) at any time point, but udder depth scores were lower at L29 than at P107 and L100 ($P < 0.01$; Table 2.4).

Udder height (UH) did not differ between the right and the left half of the udder at any time point ($P > 0.10$; data not shown). There was no difference in UH, udder circumference (UC) and udder volume (UV) between treatments at any time point ($P > 0.10$; Table 2.4). Irrespective of treatment, ewe lamb live weight at P0 had no effect ($P > 0.10$) on UH, UC and UV (data not shown). UH, UC and UV changed over time (P < 0.001) with greater values at L29 than at P107 and L100 (P < 0.001; Table 2.4). At L100, UH, UC and UV were greater than at P107 (P < 0.001; Table 2.4).

Table 2.4. Effect of time (P107, L29 and L100) and treatment (Control *vs.* Heavy) on the udder and teat palpations, on the percentage (95% confidence limits) of ewe lambs with asymmetric udders, udder depth score, udder height (UH), circumference (UC) and volume (UV). Least square means ± SEM for palpations, udder depth, udder height, circumference, and volume.

	Pregnancy (P107)	Early lactation (L29)	Weaning (L100)
Control group (n = 24)			
Udder palpation	1.5 ± 0.14**^{a}	1.6 ± 0.13^{c}	1.2 ± 0.07^{b}
Teat palpation	1.0 ± 0^{a}	1.3 ± 0.09^{b}	1.2 ± 0.07^{a}
Asymmetric udders (%)	12.3 (4.24–30.7)	4.2 (0.53–25.4)	12.3 (3.75–33.5)
Udder depth score	5.0 ± 0^{a}	3.8 ± 0.09^{b}	4.0 ± 0^{a}
UH[1] (cm)	1.9 ± 0.09^{a}	10.4 ± 0.21^{c}	8.9 ± 0.26^{b}
UC (cm)	24.0 ± 0.36^{a}	49.5 ± 0.53^{c}	32.5 ± 0.64^{b}
UV (cm^3)	90.7 ± 6.28^{a}	2026.2 ± 55.6^{c}	747.1 ± 47.13^{b}
Heavy group (n = 24)			
Udder palpation	1.1 ± 0.06**^{a}	1.5 ± 0.12^{c}	1.5 ± 0.18^{b}
Teat palpation	1.0 ± 0^{a}	1.3 ± 0.09^{b}	1.2 ± 0.07^{a}
Asymmetric udders (%)	16.7 (6.35–37.3)	16.7 (6.49–36.8)	4.2 (0.59–24.4)
Udder depth score	5.0 ± 0^{a}	3.8 ± 0.08^{b}	4.0 ± 0^{a}
UH[1] (cm)	1.8 ± 0.09^{a}	10.2 ± 0.21^{c}	8.6 ± 0.27^{b}
UC (cm)	24.0 ± 0.35^{a}	48.7 ± 0.53^{c}	33.6 ± 0.64^{b}
UV (cm^3)	85.3 ± 6.14^{a}	1900.6 ± 55.6^{c}	801.8 ± 47.13^{b}

[1] average of height from right and left half; UH: Udder height, UC: Udder circumference, UV: Udder volume; ** Udder palpation differed between treatments (P < 0.01); ^{a,b,c} Within rows, means with different superscripts are significantly different (P < 0.05).

2.4.3. Udder ultrasound measurements

The depth of the gland cistern (GC), parenchyma (PAR), total mammary conservative (MTc), total mammary generous (MTg) and the grey scale values did not differ between udder halves (P > 0.10; data not shown). The depth of the putative fat pad (FP) did not differ at P107 between udder halves (P > 0.10; data not shown), but differed at L100 (P < 0.05) with the left udder half having a deeper FP than the right udder half (19.2 ± 0.91 left *vs.* 15.4 ± 0.99 right). The grey scale values, GC, PAR, FP, MTc and MTg did not differ (P > 0.10) between treatments at any time point (Table 2.5). Ewe lambs in the Heavy group, regardless of day of measurement, tended (P = 0.08) to have larger GC than Control ewe lambs (Table 2.5). Live weight at P0, irrespective of treatment, had no effect (P > 0.10) on GC, FP, MTc, MTg and the grey scale values, but negatively impacted PAR at P107 (P < 0.05; estimate -0.20 mm). The depth of GC, PAR, MTc and MTg were greater (P < 0.001) at L29 than L100 which, in turn, was greater (P < 0.001) than P107, irrespective of treatment (Table 2.5). The depth of FP was greater at L100 than at P107, irrespective of treatment (P < 0.001; Table 2.5).

2.4.4. Correlations between udder measures, ewe live weight, BCS and lamb growth

At P107, UC was positively correlated with UV, UH and PAR (P < 0.05), and GC and PAR were negatively associated with FP (P < 0.01; Table 2.6). Ewe lamb BCS at P138 was positively associated (P < 0.01) with FP and ewe live weight at P107, and negatively associated (P < 0.05) with PAR at P107 (Table 2.6).

At L29, UV was positively correlated with UH and UC (P < 0.05), and PAR was negatively correlated with GC (P < 0.05; Table 2.7). Ewe lamb live weight at L29, irrespective of treatment, was positively associated with ewe BCS (P < 0.01) and UC at L29 (P < 0.05; Table 2.7).

At L100, UV was positively correlated with UH, UC, FP on left udder half (P < 0.05), UH was positively associated with UC and FP on the left udder half (P < 0.05). Ewe live weight was positively associated (P < 0.001) with ewe BCS at L100 (Table 2.8). At L100, FP of the left udder half was negatively correlated with PAR (P < 0.01; Table 2.8).

Table 2.5. Effect of time (P107, L29 and L100) and treatment (Control *vs.* Heavy) on depths of the mammary gland cistern (GC), parenchyma (PAR), fat pad (FP), the total mammary depth conservative and generous (MTc & MTg), and the grey scale value of Region of interest (ROI) in the parenchyma (least square means ± SEM).

	Pregnancy (P107)	Early lactation (L29)	Weaning (L100)
Control group (n = 24)			
GC (mm)	3.57 ± 0.88[a]	16.5 ± 0.88[c]	9.62 ± 0.87[b]
PAR (mm)	10.2 ± 0.41[a]	46.1 ± 1.32[c]	18.9 ± 0.74[b]
FP (mm)	10.3 ± 0.45[a]	-	RS 14.7 ± 1.36[b] & LS 19.7 ± 1.28[b]
MTc (mm)	25.6 ± 0.56[a]	63.1 ± 1.06[c]	44.2 ± 0.98[b]
MTg (mm)	32.2 ± 0.94[a]	65.5 ± 0.93[c]	49.4 ± 0.96[b]
ROI parenchyma (grey scale value)	88.0 ± 2.10	93.6 ± 2.79	88.3 ± 2.23
Heavy group (n = 24)			
GC (mm)	4.37 ± 0.88[a]	18.2 ± 0.87[c]	12.0 ± 0.87[b]
PAR (mm)	8.81 ± 0.41[a]	45.8 ± 1.31[c]	21.6 ± 0.73[b]
FP (mm)	10.4 ± 0.45[a]	-	RS 16.2 ± 1.43[b] & LS 18.7 ± 1.31[b]
MTc (mm)	25.2 ± 0.56[a]	63.5 ± 1.06[c]	47.7 ± 0.94[b]
MTg (mm)	32.3 ± 0.93[a]	67.0 ± 0.93[c]	51.4 ± 0.93[b]
ROI parenchyma (grey scale value)	91.7 ± 2.10	91.5 ± 2.78	90.7 ± 2.22

RS: Right side; LS: Left side; GC: Gland cistern; PAR: Parenchyma; FP: Fat pad; MTc: Total mammary conservative; MTg: Total mammary generous; [a,b,c] Within rows, means with different superscripts are significantly different ($P < 0.05$).

Table 2.6. Correlation coefficients of adjusted residuals of udder volume (UV), circumference (UC), height (UH), gland cistern (GC), parenchyma (PAR) and fat pad (FP) at P107, live weight of ewe lambs (Ewe LW) in pregnancy (P107)[1], body condition score of ewe lambs (Ewe BCS) in late pregnancy (P138), lamb growth from birth to weaning (Birth to L100), birth to early lactation (Birth to L29) and early lactation to weaning (L29 to L100).

	UC	UH	GC	PAR	FP	Ewe LW (P107)	Ewe BCS (P138)	Birth to L100	Birth to L29	L29 to L100
UV	0.738***	0.910***	0.193	0.243	-0.213	-0.016	-0.038	0.041	-0.163	0.175
UC		0.408**	0.251	0.315*	-0.229	-0.062	-0.114	-0.041	-0.056	-0.009
UH			0.109	0.175	-0.168	-0.013	-0.007	0.052	-0.176	0.200
GC				0.040	-0.448**	-0.026	-0.153	-0.110	-0.156	-0.0009
PAR					-0.625***	-0.369**	-0.314*	0.373**	0.091	0.331*
FP						0.257	0.373**	-0.374**	-0.177	-0.246
Ewe LW (P107)							0.413**	0.005	0.126	-0.115
Ewe BCS (P138)								-0.017	0.019	-0.030
Birth to L100									0.480***	0.649***
Birth to L29										-0.351*

*P < 0.05; ** P < 0.01; *** P < 0.001; [1] Data for treatments were pooled, as no differences (P > 0.10) were identified.

Table 2.7. Correlation coefficients of adjusted residuals of udder volume (UV), circumference (UC), height (UH), gland cistern (GC) and parenchyma (PAR) at L29, body condition score and live weight of ewe lambs (Ewe BCS and Ewe LW) in early lactation (L29)[1], lamb growth from birth to weaning (Birth to L100), birth to early lactation (Birth to L29) and early lactation to weaning (L29 to L100).

	UH	UC	GC	PAR	Ewe LW (L29)	Ewe BCS (L29)	Birth to L100	Birth to L29	L29 to L100
UV	0.648***	0.649***	0.006	-0.012	0.254	-0.144	-0.100	-0.010	-0.085
UH		-0.100	0.170	-0.145	0.056	-0.097	-0.052	0.007	-0.043
UC			-0.160	0.128	0.289*	-0.052	-0.003	0.008	-0.009
GC				-0.976***	0.011	-0.017	0.298*	0.284*	0.071
PAR					0.032	0.029	-0.324*	-0.272	-0.105
Ewe LW (L29)						0.500***	0.180	0.113	0.108
Ewe BCS (L29)							0.171	0.183	0.093

*P < 0.05; *** P < 0.001; [1] Data for treatments were pooled, as no differences (P > 0.10) were identified.

Table 2.8. Correlation coefficients of adjusted residuals of udder volume (UV), circumference (UC), height (UH), gland cistern (GC), parenchyma (PAR) and fat pad (FP) from the right and left udder halves at L100, body condition score live weight of ewe lambs (Ewe BCS and Ewe LW) at weaning (L100)[1], lamb growth from birth to weaning (Birth to L100), birth to early lactation (Birth to L29) and early lactation to weaning (L29 to L100).

	UH	UC	GC	PAR	FP Right	FP Left	Ewe LW (L100)	Ewe BCS (L100)	Birth to L100	Birth to L29	L29 to L100
UV	0.771***	0.876***	0.022	-0.181	0.161	0.317*	-0.091	-0.112	0.138	-0.088	0.226
UH		0.394**	0.150	-0.152	0.111	0.309*	-0.206	0.007	0.006	-0.137	0.135
UC			-0.021	-0.121	0.153	0.200	0.0003	-0.101	0.204	-0.058	0.264
GC				-0.291*	-0.226	-0.132	0.009	-0.068	0.111	0.194	-0.045
PAR					-0.210	-0.333*	0.144	-0.062	0.106	-0.105	0.216
FP Right						-0.230	-0.214	-0.067	0.051	0.344*	-0.233
FP Left							-0.151	-0.026	0.122	-0.280	0.379*
Ewe LW (L100)								0.664***	0.040	0.072	-0.017
Ewe BCS (L100)									-0.202	-0.171	-0.068

*P < 0.05; ** P < 0.01; *** P < 0.001; [1] Data for treatments were pooled, as no differences (P > 0.10) were identified.

Lamb growth from birth to L29, irrespective of treatment, was positively associated with GC at L29 ($P < 0.05$; Table 2.6) and FP at L100 on the right half ($P < 0.05$; Table 2.8). Lamb growth from birth to L29 was positively associated with PAR at P107 ($P < 0.05$; Table 2.6) and FP at L100 on the right half ($P < 0.05$; Table 2.8) but negatively associated with FP at P107 ($P < 0.05$; Table 2.6). Lamb growth from birth to L100 was positively associated with PAR at P107 ($P < 0.01$; Table 2.6), GC at L29 ($P < 0.05$; Table 2.7) but negatively associated with PAR at L29 ($P < 0.05$; Table 2.7).

The best model explained 12.2% of the variation in lamb growth from birth to L29 included only the effect of GC at L29 (Lamb growth from birth to L29 = 261.4 (± 35.1) + 4.9 (± 1.9) GC at L29). The difference between a ewe lamb with an average GC at L29 and a GC in the 90th percentile was 6.6 mm (Table 2.9), resulting in a 32.3 g/d difference in lamb growth from birth to L29.

For the period between birth and L100, the best model explained 37.6% of the variation in lamb growth and included the effects of PAR at P107, ewe live weight (LW) at L29 and GC at L29 (Lamb growth from birth to L100 = 37.4 (± 48.5) + 7.2 (± 1.9) PAR at P107 + 2.0 (± 0.65) LW at L29 + 1.2 (± 0.63) GC at L29). The difference between a ewe lamb with an average PAR at P107 and a PAR in the 90th percentile was 2.6 mm (Table 2.9), resulting in a 18.7 g/d difference in lamb growth from birth to L100. The difference between a ewe lamb with an average GC at L29 and a GC in the 90th percentile was 6.6 mm (Table 2.9), resulting in a 7.9 g/d difference in lamb growth from birth to L100.

Table 2.9. Descriptive statistics of the depth of the gland cistern (GC), parenchyma (PAR) and mammary fat pad (FP) in late-pregnancy (P107), early lactation (L29) and weaning (L100), irrespective of treatment.

Descriptor	Minimum	10th percentile	Mean	90th percentile	Maximum
GC (mm)					
P107	1.9	2.4	4.0	7.1	8.3
L29	4.2	8.5	17.2	23.8	30.7
L100	4.3	5.4	10.8	16.2	25.8
PAR (mm)					
P107	5.7	6.8	9.5	12.1	17.2
L29	28.8	37.2	46.0	56.6	60.6
L100	12.3	16.6	20.2	26.3	29.9
FP (mm)					
P107	6.0	8.2	10.4	13.2	15.8
L100-Left	2.3	10.1	19.2	27.1	30.9
L100-Right	4.0	7.5	15.4	24.5	28.7

Left: Left udder half; Right: Right udder half.

2.5. Discussion

2.5.1. Treatment effects

The first objective of this experiment was to investigate the effects of increasing growth rates of ewe lambs between weaning and breeding on mammary gland dimensions and internal structures. It was hypothesised that ewe lambs that were heavier at breeding would have a reduced mammary parenchymal and a greater fat pad area compared with lighter ewes. Despite differences in ewe lamb growth rate between weaning and breeding between treatments, udder scores, mammary morphological and ultrasound measures did not differ, which contrasts with previous studies (Johnsson and Hart, 1985, Umberger et al., 1985, Villeneuve et al., 2010a). The differences in ewe lamb growth rates between treatments from weaning to breeding were small (14 g/d) and the magnitude of growth (less than 150 g/d on average) was lower than achieved in previous studies (i.e., from 173 to 305 g/d; Umberger et al., 1985, Villeneuve et al., 2010a). The small differences in the growth rates of ewe lambs may have impacted the ability of the treatments to alter mammary gland development. There have been differences reported in breeds between studies that may also explain some of differences observed between the present and previous experiments (Hampshire Down, Johnsson and Hart, 1985, Suffolk crossed Dorset, Suffolk, Umberger et al., 1985, Boutsiko, Peclaris et al., 1997, Dorset, Villeneuve et al., 2010a). In this experiment, there was no evidence of any negative impacts on mammary gland development of Romney ewe lambs achieving live weight gains of approximately 147 g/d between their weaning at three months of age and first breeding at seven months of age under New Zealand conditions.

No differences were observed between udder halves for udder height, total depth of the mammary gland conservative and generous, depths of the gland cistern and parenchyma. This finding is consistent with previous studies (Castillo et al., 2008, Petridis et al., 2014, Barbagianni et al., 2015). At weaning, however, the fat pad differed between udder halves. This difference may be explained by lambs showing a preference for one udder half, which may have been over stimulated (Makovicky et al., 2015), particularly among ewes rearing a single lamb. Overstimulation of one udder half may lead to an early partial udder involution of the non-preferred udder half resulting in changes in the mammary tissues (Jena et al., 2018).

Udder and teat palpation scores were greater in early lactation than late pregnancy or weaning, indicating a greater percentage of abnormalities in the mammary gland. This may be due to the intense activity of the production of milk resulting in changes to the consistency of the udder tissue (Boutinaud and Guinard-Flament, 2004, Lérias et al., 2014). Udder depth scores were lower in lactation, indicating that the udder was larger in early lactation than late pregnancy or weaning. This is further supported by the measures of udder height, volume, total depths of the mammary gland over time and previous studies (Lérias et al., 2014, Davis, 2017, Griffiths et al., 2019a,b). Udder measures were greater at weaning than in late pregnancy, which likely indicated that there was some degree of milk production still occurring. These results were consistent with previous findings, which reported that udder volume greatly increases between late pregnancy and the first week of lactation, which is followed by a progressive decrease until total involution between 30 and 60 days after weaning (Tatarczuch et al., 1997, Rovai et al., 2008, Lérias et al., 2014, Davis, 2017). In the current experiment, udder measures in late pregnancy were recorded prior to the period when the major changes would have been expected to occur (approximately 120 to 135 days of pregnancy; Colitti and Farinacci, 2009, Lérias et al., 2014) and therefore may explain the low values observed in late pregnancy. In addition, the ewes in this experiment, were primiparous, and therefore have lower udder volume and smaller parenchyma and gland cistern than multiparous ewes (Fernández et al., 1995, Lérias et al., 2014). The changes of morphological and ultrasound measurements of the mammary gland were consistent with normal mammary gland development during pregnancy and lactation.

2.5.2. Associations between udder measurements and lamb growth

The second objective was to develop an ultrasound technique to identify mammary gland internal structures that could be used as indirect indicators of lamb growth to weaning. Mammary parenchymal depth in late pregnancy measured by ultrasound was positively associated with lamb growth to weaning. Single lambs born to ewe lambs with a large parenchyma in late pregnancy were 1832 g heavier at weaning at 100 days of age than single lambs born to ewe lambs with an average parenchyma in late pregnancy. The mammary parenchyma includes the secretory tissue involved in the production and secretion of milk (Colville, 2007, Akers, 2002b) with the number of secretory cells determining milk production (Capuco et al., 2001, Boutinaud and Guinard-Flament, 2004). Thus, a deeper parenchyma in pregnancy could indicate a greater number of secretory cells and potentially greater milk production. Strzetelski et al. (2004) reported in primiparous dairy heifers that the percentage of secretory tissue in measured by ultrasound was highly positively correlated with milk yield. While the impact of the parenchymal depth in late pregnancy on single lamb growth to weaning was moderate, the results suggested that the ultrasound method could potentially be used as a technique to identify ewe lambs pregnant with a single lamb that would wean heavier lambs.

The gland cistern depth in early lactation, measured by ultrasound, was moderately and positively correlated to lamb growth from birth to early lactation and to weaning. Single lambs born to ewe lambs with a large gland cistern in early lactation were 938 g and 790 g heavier at 29 and 100 days of age respectively, than single lambs born to ewe lambs with an average gland cistern in early lactation. The mammary gland cistern is the cavity into which milk drains between milkings or suckling events (Akers, 2002a). Ewes with larger cisterns have been reported to produce more milk than ewes with smaller cisterns (Caja et al., 1999, Nudda et al., 2000, Castillo et al., 2008, Rovai et al., 2008). It is likely, therefore, that ewe lambs with larger gland cisterns in early lactation would have a greater milk production than those with smaller cisterns. The associations between ultrasound measures and milk production in non-dairy ewe lambs, however, are still unknown. These results further support the use of ultrasound to identify ewe lambs that would wean heavier single lambs. Further research is warranted to confirm these findings and investigate the use of ultrasound to examine the association between mammary gland, milk production and lamb growth in non-dairy ewe lambs.

The late pregnancy ultrasound scan was the easiest to perform, as lactating ewe lambs and their lambs had to be separated and were off feed for four hours pre-scanning, whereas late-pregnant ewe lambs did not require this waiting period. The measurement of the parenchyma in late pregnancy relied on the ability of the operator to identify the demarcation between tissues which can be difficult due to the image resolution and the attenuation of signal as the scanning depth increases

(Molenaar et al., 2020). The measure of the gland cistern was not so reliant on the operator ability, as the gland cistern appears as black on the image (Ayadi et al., 2003, Molenaar et al., 2020). The precise measurement of the parenchyma and gland cistern depth with this ultrasound technique could not be performed at pregnancy diagnosis as the correspondence between millimetres and pixels varies depending on the scanning depth. The ultrasound technique used in this experiment would therefore be challenging to apply on larger flocks of ewe lambs. More research is required to improve the ultrasound technique for its potential application on larger flocks.

2.6. Conclusion

This experiment was the first to use ultrasound to investigate the relationship between udder measurements and the growth of progeny of non-dairy ewe lambs during pregnancy and lactation. The depth of the mammary parenchyma in late pregnancy and the gland cistern in early lactation were indicators of single lamb growth from birth to weaning. Under the conditions of this experiment, there was no evidence of any negative effects of the differing liveweight gain treatments between three and seven months of age on mammary gland development of Romney-type ewe lambs during their first pregnancy and lactation as measured by ultrasonography. The results of the association between lamb growth and mammary ultrasound measures suggest that the ultrasound technique has the potential to identify pregnant ewe lambs which wean heavier single lambs. More research is needed to investigate the use of ultrasound to examine the associations between mammary ultrasound measures, milk production and lamb growth in non-dairy ewes.

Foreword to Chapter 3

The previous chapter (Chapter 2) was the first experiment investigating the mammary gland development of non-dairy ewe lambs using ultrasonography. The depth of the mammary parenchyma in pregnancy and the depth of the mammary gland cistern in early lactation were positive indicators of single lamb growth rates to weaning. These results suggest that these indicators could be potential selection criteria for ewe lambs and that there may be a potential positive relationship between the size of the mammary parenchyma in pregnancy and gland cistern in early lactation and milk production of non-dairy ewe lambs. Chapter 3, therefore, aimed to assess the repeatability of the ultrasound technique used in Chapter 2 and consolidate the previous results and investigate the association between ultrasound measurements and milk yield in non-dairy ewe lambs.

Chapter 3

Association of mammary ultrasound measurements, milk yield of non-dairy ewe lambs, and the growth of their single lambs

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3.1. Abstract

Mammary cistern size has been positively correlated with milk yield (MY) of mature dairy ewes in previous studies, but the association in ewe lambs is unknown. This experiment aimed to examine the associations between mammary ultrasound measures and ewe lamb milk yield at one year of age and determine the accuracy of using maternal mammary ultrasound to predict single lamb growth rates. Single-bearing ewe lambs (n = 45) were randomly selected and 30 were milked once during week three (W3), five (W5), and seven (W7) of lactation. Mammary ultrasound scans were performed at day 110 of pregnancy, W3, W5, W7, and weaning (L69). Single lambs (n = 30) were weighed at birth and at each mammary scanning event. Udder measures explained 26.8%, 21.4%, and 38.4% of the variation in MY at W3, W5 and W7 respectively, and 63.5% and 36.4% of the variation in single lamb growth to W3 and to L69. The ultrasound technique was more accurate in predicting single lamb growth to W3 than MY and may enable the identification of pregnant ewe lambs whose progeny would have greater growth rates. More research is needed to identify accurate indicators of superior milk yield and determine whether ultrasound could be used to select ewe lambs.

3.2. Introduction

Ultrasound scanning is a widely utilised, non-invasive method to examine the mammary gland of ewes and its internal structures (Petridis et al., 2014, Barbagianni et al., 2015, 2017, Makovický et al., 2019a,b). Ultrasonography can also be used for the examination and diagnosis of sheep mammary diseases (Franz et al., 2003, Barbagianni et al., 2015, 2017, Makovicky et al., 2017) and as a technique for animal selection based on mammary structures (Nudda et al., 2000, Castillo et al., 2008, Chapter 2). Currently, most studies have investigated sheep mammary glands of dairy breed ewes, focusing on the relationship between mammary gland cistern (*sinus lactiferous*) size and milk production (Nudda et al., 2000, Rovai et al., 2008, Makovicky et al., 2017, 2019a), and the impacts of management practices such as milking intervals (Castillo et al., 2008) or the drying-off procedure (Petridis et al., 2014). A small number of studies have used ultrasonography to examine the mammary glands of dual-purpose meat and wool breeds (Ruberte et al., 1994, Caja et al., 1999, Chapter 2).

Mammary gland cistern size measured using ultrasound was positively correlated with milk production in mature dairy ewes (Nudda et al., 2000, Rovai et al., 2008, Makovicky et al., 2017, 2019a,b) and in non-dairy mature ewes (Caja et al., 1999). In addition, Barbagianni et al. (2015) reported a negative association between parenchymal grey-scale values on day 145 of pregnancy and the quantities of milk collected after lambing in three- to five-year-old dairy ewes. The authors also reported a negative association between the grey-scale intensity values of the parenchyma three and five days after lambing on ultrasound images and milk quantities collected on the same day. Collectively, these associations suggest it would be possible to use udder ultrasound measurements to select mature ewes with greater potential for milk production. In dairy heifers, the proportion of secretory tissue in the parenchyma measured by ultrasound 15 to seven days prior to calving was highly correlated with milk production over 100 days of lactation. However, the association between ultrasound measurements of mammary gland structures and milk production of dairy and non-dairy ewe lambs during their first lactation is unknown.

Torres-Hernandez and Hohenboken (1980) reported that ewe milk production was positively associated with lamb growth, particularly in early lactation. van der Linden et al. (2010) noted that single lamb growth rates during the first two weeks of lactation were poorly predicted and only moderately predicted by milk yield in week four of lactation. It is possible that ultrasound measurements of the mammary gland structure could be used as an indirect indicator of lamb growth to weaning. Chapter 2 reported the impacts of a heavier live weight of non-dairy ewe lambs at breeding on their mammary gland internal structure size during pregnancy and lactation, and the association between mammary gland structures and progeny growth to weaning. The depth of the mammary gland cistern at day 29 of lactation was moderately positively correlated with single lamb growth from birth to 29 days of age and from birth to weaning (100 days of age). In addition, the depth of the mammary parenchyma at 107 days of pregnancy was positively associated with single lamb growth to weaning. These mammary measurements could be a potential means of selecting non-dairy ewes likely to wean heavier lambs.

The first objective of this experiment was to examine the associations between ultrasound measurements of the mammary gland of non-dairy ewe lambs rearing single lambs and their milk production. It was hypothesised that the depth of the gland cistern would be positively correlated with milk yield of non-dairy ewe lambs. The second objective was to consolidate the results of Chapter 2 and determine if the ultrasound measures was an accurate method to assess ewe lamb milk yield and the growth of their single lambs to weaning.

3.3. Materials and Methods

All animal handling procedures were approved by the Massey University Animal Ethics Committee (MUAEC-19/49). The experiment was conducted at Massey University Riverside Farm (latitude: 40° 50' 35 S, longitude: 175° 37' 55 E), 10 km north of Masterton, and Keeble Farm (latitude: 40° 24' 03 S, longitude: 175° 35' 51 E), 5 km south of Palmerston North, New Zealand.

3.3.1. Experimental design

Romney ewe lambs were bred at seven months of age for two periods of 17 days (P0 to P34). At pregnancy diagnosis (P94; 08/08/2019), 45 single-bearing ewe lambs were randomly selected from those successfully mated in the first 17 days of the breeding period. Only single-bearing ewe lambs were selected as it is more frequent than twin-bearing in ewe lambs (Corner et al., 2013). Ewe lambs were shorn at P102 (16/08/2019) and transported to Keeble Farm at P105 (19/08/2019) for the remainder of the experiment.

From the start of breeding (P0) to the weaning of their progeny (L69), ewe lambs were grazed using a rotational grazing system on ryegrass (*Lolium perenne L.*) and white clover (*Trifolium repens L.*) pasture under New Zealand grazing conditions. Ewe intake was considered to be unrestricted as pre-grazing pasture covers were maintained above 1200 kg DM/ha (Kenyon and Webby, 2017). After moving ewes to Keeble farm, the mean pre-grazing mass offered during pregnancy was 1885 ± 100 kg DM/ha. During lactation, the pre-grazing cover of pastures were recorded fortnightly from P143 (26/09/2019) to weaning and was on average 2347 ± 159 kg DM/ha.

At P143 (26/09/2019), all ewe lambs were moved to their lambing paddock (n = 45; approximately 18.9 ewe lambs/ha). In order to milk all ewe lambs at the same stage of lactation, ewe lambs were divided into three different milking groups based on day of parturition (Table 3.1). Ewe lambs were milked once a week during each of week three (13 to 20 days of lactation; W3), five (27 to 34 days of lactation; W5) and seven (41 to 48 days of lactation; W7) of lactation (Table 3.1). Ewe lambs that did not lamb (n = 2) or whose lamb died at birth or during lactation (n = 12) were excluded from the experiment. Lambs were weaned at approximately 69 days of age (L69; 19/12/2019).

Table 3.1. Description of the milking groups created based on lambing dates in order to milk all ewe lambs in week three (W3), five (W5) and seven (W7) of lactation.

Milking group	n	Lambing dates	Days of lactation at milking		
			W3	W5	W7
1	7	27/09 – 4/10	16	30	44
2	15	5/10 – 11/10	15	29	43
3	21	12/10 – 21/10	18	32	46

3.3.2. Animal measurements

3.3.2.1. Udder scores

Ewe lamb udder scores and morphological measurements were performed at P110 and L69 after ultrasound scanning using the method described in Chapter 2. Briefly, the scoring system included the palpation of ewe both udder halves and teats and assessed udder symmetry and depth (Griffiths et al., 2019b).

Morphological traits were measured as described in Chapter 2 and included udder circumference (UC, cm) and the height of each half of the udder (cm). Udder volume

(UV, cm^3) was calculated using UC and udder height (UH, cm) according to the method of Ayadi et al. (2011):

$$UV = \pi \times R^2 \times UH; \quad R = \frac{UC}{2\pi}$$

Where UV = udder volume (cm^3); π = 3.14159; R = radius (cm); UH = udder height (cm); UC = udder circumference (cm).

3.3.2.2. Udder ultrasound scanning

Udder ultrasound scans were performed by a single operator at 110 days of pregnancy (P110), and at week three (W3), five (W5) and seven (W7) of lactation and at weaning (L69). The ultrasound method was described in detail in Chapter 2. Ultrasound scans were not performed on ewe lambs that did not lamb (n = 2), who had died (n = 1) or whose lambs had died (n = 12). Ultrasound scans were performed with an ultrasound scanner fitted with a linear transducer with an imaging frequency of 5 to 10 MHz (Mindray Digital Ultrasonic Diagnostic Imaging System DP6600 with 75L38EA, ShenZhen, China).

One image of suitable resolution per udder half was selected for image processing. Udder halves which were naturally not producing milk, or that had a palpation score of 4 or 5 and thus considered "abnormal" (Griffiths et al., 2019b; Chapter 2), or identified with mastitis at any time point (W3, W5, W7, L69) were not included in the image selection. Images were discarded for both halves of 2 ewe lambs and one half of 3 ewe lambs.

The drawing templates of the ultrasound images, created for each time point of Chapter 2, were used to standardize the assessment of each compartment depth (Appendix A). Image processing was undertaken using ImageJ software (Ferreira and Rasband, 2012). The total depth of mammary gland conservative (MTc) and generous (MTg), fat pad (FP), parenchyma (PAR) and gland cistern (GC) depth were estimated, in millimetres, at the widest point for each sub-compartment using the straight tracer.

3.3.2.3. Ewe lamb milking

Milking used the "oxytocin method" first described by McCance and Alexander (1959). To enable milk let-down, ewe lambs were given 1 IU of synthetic oxytocin (Oxytocin V, 10 IU/mL, PhoenixPharm, Auckland, New Zealand) intravenously. Ewe lambs were then milked in the morning by machine followed by hand milking to empty the udder. The time of the first milking was recorded. The milking procedure was repeated after a minimum of five hours, when the time and milk weight from each udder half was recorded. Daily milk yield (MY) per udder half and total daily milk yield were calculated using the following formula (van der Linden et al., 2009):

$$Daily\ milk\ yield\ (MY) = \frac{24\ hours}{Time\ between\ milkings} \times Milk\ weight\ at\ 2nd\ milking$$

Lambs were separated from the ewe lambs during the five-hour period, and bottle fed as required and reunited after the second milking.

3.3.2.4. Lamb measurements

Lambs were tagged within 18 hours of birth (during twice daily lambing rounds at approximately 11am and 5pm) at which time their date of birth, sex, dam tag number and birth weights were recorded. Lambs were weighed on each milking day at W3 (approximately 17 days of age), W5 (approximately 31 days of age) and W7 (approximately 44 days of age) between the morning and afternoon milkings and again at weaning (L69).

3.3.3. Statistical analysis

Statistical analyses were conducted using SAS v9.4 (SAS Institute Inc., Cary, NC, USA) and RStudio v1.2. (RStudio Team, PBC, Boston, MA, USA). The final dataset included 30 ewe lambs, their single lambs, and a total of 286 images.

Udder and teat palpations from each udder half (right and left) and udder depth score were analysed using SAS using generalised linear models allowing for repeated measures and assuming Poisson distributions and log transformations (Table 3.1). Udder circumference (UC) and UV per udder, and UH, GC, PAR, FP, MTc, MTg and MY of each udder half were analysed using general linear models allowing for repeated measures (Table 3.2).

The residuals of GC, PAR, FP, MTc, MTg and MY of both udder halves at P110, W3, W5, W7 and L69 were generated using general mixed models using SAS (Table 3.2). Pearson correlations were then used to test for associations between time points (P110, W3, W5, W7 and L69) for the residuals of each ultrasound measure (GC, PAR, FP, MTc and MTg) of each udder half. Pearson correlations were also used to test for linear associations between the residuals of daily MY at W3, W5 and W7 and ultrasound measures (GC, PAR, FP, MTc and MTg) of each udder half at each time point (P110, W3, W5, W7 and L69).

The residuals of the average of UH, UV, UC, GC, PAR, FP, MTc and MTg of both udder halves at P110, W3, W5, W7 and L69, total daily MY at W3, W5 and W7 per udder and lamb growth from birth to W3, birth to W5, birth to W7, W3 to L69, W5 to L69, W7 to L69 and birth to L69 were generated using general mixed models using SAS (Table 3.2). Pearson correlations were then used to test for linear associations between the residuals of lamb growth and the residuals of the average of morphological (UH, UC and UV), ultrasound measures (PAR, FP, GC, MTc and MTg) at each time point and total daily MY at W3, W5 and W7.

Multiple regression analyses of daily MY at W3, W5 and W7 per udder half were undertaken using general linear mixed models with RStudio v1.2. (Packages 'lme4' and 'performance') to enable the calculation of the marginal coefficients of determination (Nakagawa and Schielzeth, 2013). Multiple regression analyses of lamb growth from birth to W3, birth to W5, birth to W7 and birth to L69 were undertaken using general linear models in SAS. Pearson correlations were used to examine whether each predictive variable was individually correlated with daily MY or lamb growth during each period. Predictive variables correlated with daily MY or lamb growth with $P \leq 0.20$ were selected and included in the models (Dohoo et al., 2003a). Correlations between selected predictive variables were examined to identify high collinearity (> 0.80; Dohoo et al., 2003b). In case of a high collinearity between two predictive variables, only one predictive variable was included in the models based on the biological relevance. Two-way interactions between each of the selected variables were individually tested using general linear models. All non-significant ($P > 0.05$) interactions were excluded from the final model. Backward manual variable eliminations were used to select the model that best explained the variation in daily MY and lamb growth by removing predictive variables with $P > 0.10$. Any non-significant ($P > 0.10$) predictive variable causing greater than a 20% change in the model coefficients was considered a confounding variable and included in the models (Dohoo et al., 2003a). Confounding effects were evaluated after each variable was removed from the model by checking the changes in predictive variable coefficients. The random effect of ewe lamb was included in the multiple regression models of daily MY per udder half. The marginal coefficients of determination of the multiple regressions of daily MY per udder half were calculated based on the method of Nakagawa and Schielzeth (2013) and corresponded to the variance explained by the selected predictive variables in the final models.

Table 3.2. Description of the statistical analyses of the udder palpation scores, udder depth scores, udder circumference (UC), udder volume (UV), udder height (UH), depth of the gland cistern (GC), parenchyma (PAR), fat pad (FP), total depth of the mammary gland conservative (MTc) and generous (MTg) and milk yield (MY), and the generation of their residuals.

Dependent variable	Fixed effect	Covariate	Random effect
Palpation scores - udder - teat	Udder half (right vs. left) Time point (P110 and L69) Interaction udder half x time point	Lambing date	
Udder depth score	Time point (P110 and L69)	Lambing date	
UC and UV	Time point (P110 and L69)	Lambing date	
UH, GC, PAR, FP, MTc, MTg and MY	Udder half Time point (P110, W3, W5, W7, L69) Interaction udder half x time point	Lambing date	Ewe lamb
Residuals of GC, PAR, FP and MTc per udder half		MTg Lambing date	
Residuals of UH, MTg and MY per udder half		Lambing date	
Residuals per udder of - UH, UV, UC and MTg - lamb growth - total MY		Lambing date	
Residuals of GC, PAR, FP and MTc per udder		MTg Lambing date	

P110: day 110 of pregnancy; L69: weaning of the lambs; W3: week three of lactation; W5: week five of lactation; W7: week seven of lactation.

3.4. Results

3.4.1. Udder half differences and changes over time

Udder and teat palpations, UH, GC, PAR, FP, MTc and MTg did not differ between udder halves ($P > 0.10$; data not shown). Udder palpation scores did not differ ($P > 0.10$) between P110 and L69 (Table 3.3), however, teat palpation scores, UH, UC and UV had lower values ($P < 0.001$) at P110 than L69 (Table 3.3). Udder depth scores were greater ($P < 0.001$) at P110 than L69.

Table 3.3. Effect of time (day 110 of pregnancy (P110) and weaning of the lambs (L69)) on udder and teat palpations, udder depth score, udder height (UH), circumference (UC), volume (UV). Least square means ± SEM.

	P110	L69
Udder palpation	1.23 ± 0.11	1.57 ± 0.17
Teat palpation	1.0 ± 0^{a}	1.18 ± 0.07^{b}
Udder depth score	4.93 ± 0.05^{b}	3.69 ± 0.06^{a}
UH (cm)	4.90 ± 0.22^{a}	10.3 ± 0.21^{b}
UC (cm)	26.1 ± 0.39^{a}	40.8 ± 0.96^{b}
UV (cm^3)	270.3 ± 13.4^{a}	1393 ± 83.9^{b}

^{a,b} Within rows, means with different superscripts are significantly different ($P < 0.05$).

The depth of GC was smaller ($P < 0.001$) at P110 than at W3, W5, W7 and L69, but was greater ($P < 0.001$) at L69 compared to W3, W5, W7, which did not differ ($P < 0.05$; Figure 3.1). Ewe lambs had a deeper ($P < 0.05$) PAR at W3 and W5 compared to W7, which was greater than L69 which in turn was greater than P110 (Figure 3.1). The depth of FP was lower ($P < 0.05$) at W7 than P110 with all other time points being intermediate ($P > 0.10$; Figure 3.1). The total depth of the mammary gland (MTc and MTg) was lower ($P < 0.01$) at P110 than MTc and MTg at W3, W5, W7 and L69.

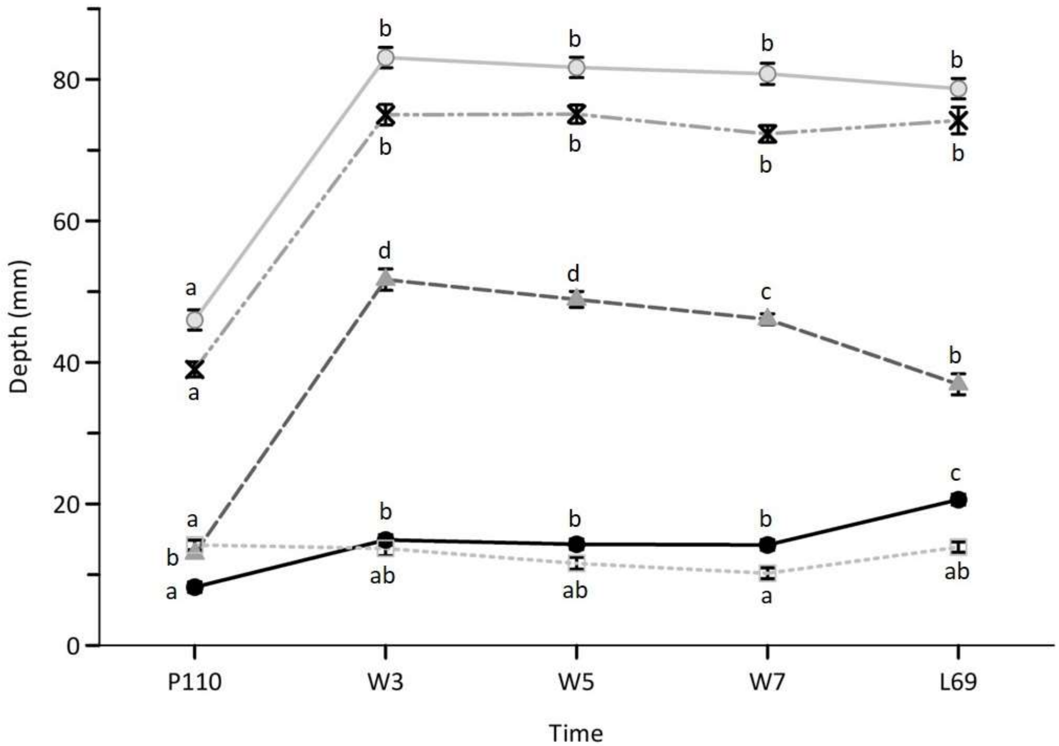

Figure 3.1. Average depths (± SEM) of the mammary gland cistern (GC; black circles – solid line), mammary fat pad (FP; empty squares – dotted line), mammary parenchyma (PAR; grey triangles – dashed line), total depth of the mammary gland conservative (MTc; black crosses – dashed and dotted line) and generous (MTg; grey circles – solid line) in late pregnancy (P110), lactation (W3, W5, W7) and at weaning (L69). Within lines, averages with different letters were significantly different (P < 0.05).

Daily milk yield (MY) of the right udder half was greater than the left udder half at W3 (P < 0.01; Table 3.4). Daily MY of the left udder half (P > 0.10) at W3 and W5 did not differ (P > 0.10) but was lower (P < 0.05) than at W7 (Table 3.4). Daily MY of the right udder half and the total daily MY of the ewe lamb was greater (P < 0.01) at W3 than W5 which in turn was greater (P > 0.10) than W7.

Table 3.4. Effect of time of lactation (week 3 (W3), week 5 (W5) and week 7 (W7)) on daily milk yield of the right and left udder half and total daily milk yield (Total MY). Least square means ± SEM.

Milk yield (g/d)	W3	W5	W7
Left udder half	910 ± 39.4**[b]	876 ± 39.6[b]	723 ± 39.4[a]
Right udder half	1076 ± 38.4**[c]	927 ± 38.9[b]	749 ±38.4[a]
Total MY	1924 ± 65.3[c]	1740 ± 66.1[b]	1421 ± 65.3[a]

**Daily MY of the right udder half differed from MY of the left udder half (P < 0.01); [a,b,c] Within rows, means with different superscripts are significantly different (P < 0.05).

3.4.2. Correlations between ultrasound measures per udder half between time points

The depth of GC per udder half at W3, W5, W7 and L69 were all positively correlated (P < 0.05) but at P110, GC showed no significant (P > 0.10) correlations with values at the other time points (Table 3.5). The depth of MTg at L69 was positively correlated (P < 0.05) with MTg at W3, W5 and W7 but not with MTg at P110 (Table 3.5). The depth of PAR per udder half at P110 was positively correlated (P < 0.05) with PAR at W7 (Table 3.5). No other significant associations (P > 0.10) were found in PAR per udder half between time points (Table 3.5). No significant correlations (P > 0.10) were observed between time points for FP (Table 3.5). At P110, MTc per udder half was positively correlated with MTc at W7 (P < 0.001), but no other significant correlations (P > 0.10) were observed between time points for MTc (Table 3.5).

3.4.3. Associations of daily milk yield and ultrasound measurements per udder half

Daily MY per udder half at W3 was positively associated (P < 0.05) with GC at P110 and W3 (Table 3.6) but negatively associated with FP at P110 (P < 0.001; Table 3.6). Daily MY at W5 was positively correlated (P < 0.05) with MTg at W3 and W5 but was negatively correlated (P < 0.001) with GC at L69 (Table 3.6). Daily MY at W7 per udder half was positively associated with FP at P110 ($P < 0.05$), MTg at W3 and at L69, but was negatively associated ($P < 0.05$) with GC at L69 (Table 3.6). Correlations that were not significant ($P > 0.10$) are presented in Appendix B1.

Table 3.5. Correlation coefficients of residuals of depths of the mammary gland cistern (GC), parenchyma (PAR), fat pad (FP) and total depth of mammary gland conservative (MTc) and generous (MTg) per udder half in late pregnancy (P110), week 3 (W3), week 5 (W5), week 7 (W7) of lactation and at weaning (L69).

	W3	W5	W7	L69
GC				
P110	0.011	-0.058	-0.089	0.102
W3		0.398***	0.257*	0.336*
W5			0.534***	0.504***
W7				0.373**
PAR				
P110	0.196	-0.046	0.302*	-0.068
W3		0.094	-0.017	-0.098
W5			0.085	0.028
W7				0.173
FP				
P110	0.376*	-0.126	0.141	-0.038
W3		0.113	0.193	0.036
W5			-0.204	0.276
W7				0.153
MTc				
P110	0.021	0.004	0.459*	0.063
W3		-0.033	-0.062	-0.218
W5			-0.179	0.200
W7				0.203
MTg				
P110	0.238	-0.031	0.195	0.265
W3		0.193	0.026	0.546***
W5			-0.082	0.297*
W7				0.333*

*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3.6. Correlation coefficients of residuals of daily milk yield (MY) in week 3 (W3), week 5 (W5), week 7 (W7) of lactation, gland cistern (GC) at P110, W3 and L69, fat pad (FP) at P110 and total depth of the mammary gland generous (MTg) at W3, W5 and L69 per udder half.

	MY W3	MY W5	MY W7
GC			
P110	0.314**	0.077	-0.120
W3	0.288*	-0.003	-0.018
L69	-0.030	-0.499***	-0.326*
FP			
P110	-0.409***	-0.053	0.325*
MTg			
W3	0.175	0.327**	0.277*
W5	0.175	0.329**	0.230
L69	0.129	0.178	0.352*

*P < 0.05; ** P < 0.01; *** P < 0.001; P110: day 110 of pregnancy; L69: weaning.

3.4.4. Prediction of daily milk yield per udder half with udder measures

The best regression model for MY at W3 per udder half explained 26.8% of the variation and included the effects of GC, PAR and, FP at P110 and with GC and MTg at W3 (Table 3.7). An average ewe lamb had a gland cistern of 8.24 mm and 14.95 mm whereas a ewe lamb in the 90th percentile had a gland cistern of 10.9 mm and 23.0 mm at P110 and W3 resulting in 48 g/d and 125 g/d difference in milk yield per udder half in week three of lactation, respectively. The best regression model for MY at W5 per udder half explained 21.4% of the variation and included the effects of MTc at W3, PAR and MTc at W5 and the interactions of PAR at W5 with MTc at W3, and MTc at W3 and MTc at W5 (Table 3.7). The best regression model for MY at W7 per udder half explained 38.4% of the variation and included the effects of FP, UH at P110, MTg at W3, MTc at W7 and the interaction of MTg at W3 and UH at P110, and MTc at W7 and UH at P110 (Table 3.7).

3.4.5. Associations between lamb growth and udder measures and milk yield per udder

Lamb growth from birth to week 3 of lactation (W3), birth to W5, birth to W7 and birth to weaning (L69) were positively correlated ($P < 0.05$) with total daily MY at W3, W5 and W7 (Table 3.8). Lamb growth from W3 to L69 and W5 to L69 were positively correlated ($P < 0.05$) with total daily MY at W7 (Table 3.8).

Lamb growth from birth to W3 was negatively correlated ($P < 0.05$) with the average FP of both udder halves at P110 and W3 (Table 3.8). Lamb growth from birth to W5 was negatively correlated ($P < 0.01$) with the average FP of both udder halves at W3 (Table 3.8). Lamb growth from W3 to L69 and birth to L69 were positively correlated ($P < 0.05$) with UC and UV at L69 (Table 3.8). Lamb growth from W7 to L69 was negatively correlated with PAR at W3 ($P < 0.05$; Table 3.8). Non-significant correlations ($P > 0.10$) between udder measurements and lamb growth are presented in Appendix B2.

Table 3.7. Multiple regression coefficients (± SEM) of ultrasound (GC, PAR, FP, MTc and MTg) and morphological measures (UH) in pregnancy (P110), week 3 (W3), 5 (W5) and 7 (W7) of lactation on daily milk yield (MY) in week 3, 5 and 7 of lactation per udder halves (g/d).

Independent variables selected	Daily MY at W3 R^2 = 0.268	Daily MY at W5 R^2 = 0.214	Daily MY at W7 R^2 = 0.384
Intercept	770 ± 302	4966 ± 2358	– 5666 ± 1606
GC (P110)	18.1 ± 9.3[1]	-	-
PAR (P110)	– 9.2 ± 10.2	-	-
FP (P110)	– 13.1 ± 8.2	-	12.0 ± 4.77
UH (P110)	- [3]	-	1146 ± 325
GC (W3)	15.5 ± 7.2	-	-
MTc (W3)	-	– 65.3 ± 31.5	-
MTg (W3)	1.8 ± 3.2	-	41.4 ± 13.1
MTg (W3)*UH (P110) [2]	-	-	– 7.2 ± 2.59
PAR (W5)	-	38.0 ± 26.4	-
MTc (W5)	-	– 80.0 ± 32.0	-
PAR (W5)*MTc (W3)	-	– 0.46 ± 0.36	-
MTc (W3)*MTc (W5)	-	1.18 ± 0.44	-
MTc (W7)	-	-	40.8 ± 16.0
MTc (W7)*UH (P110)	-	-	– 8.0 ± 3.38
Ewe	14238 ± 11175	14118 ± 13208	1112 ± 4182

MY: Milk yield; GC: Mammary gland cistern; PAR: Parenchyma; FP: Fat Pad; UH: Udder height; MTg: Total mammary depth generous; MTc: Total mammary depth conservative; [1] For each 1 mm increase in depth of the mammary gland cistern (GC) in pregnancy (P110), daily milk yield at week 3 of lactation (MY at W3) increased by 18.1 ± 9.3 g/d;); [2] Two-way interaction between MTg at W3 and UH at P110; [3] Independent variable that was not a significant (P > 0.10) predictor of daily milk yield at W3.

3.4.6. Predictions of lamb growth with ultrasound measures

The best regression model for lamb growth from birth to W3 explained 63.5% of the variation and included the effect of the average of GC and FP at P110, FP, PAR and MTc at W3 of both udder halves, and the interaction between PAR and MTc at W3 (Table 3.9). An average ewe lamb had a fat pad of 14.2 mm whereas a ewe lamb in the 90^{th} percentile had a fat pad of 19.0 mm at P110 resulting in 41.7 g/d in difference in single lamb growth from birth to W3. An average ewe lamb had a parenchymal depth of 51.8 mm whereas a ewe lamb in the 90^{th} percentile had a parenchymal depth of 61.8 mm at W3 resulting in 55.4 g/d in difference in single lamb growth from birth to W3. Lamb growth from birth to W5 was not significantly ($P > 0.05$) predicted by ultrasound and morphological measurements per udder (Table 3.9). The best regression model for lamb growth from birth to W7 explained 38.0% of the variation and included the effect of the average of PAR and MTc at W3 of both udder halves, and the interaction between PAR and MTc at W3 (Table 3.9). The best regression model for lamb growth from birth to weaning (L69) explained 36.4% of the variation and included the effect of the average of MTc at W3, FP at W7 and at L69 of both udder halves (Table 3.9).

Table 3.8. Correlation coefficients of residuals of lamb growth from birth to week 3 (Birth to W3), birth to week 5 (Birth to W5), birth to week 7 of lactation (Birth to W7), W3 to weaning (W3 to L69), W5 to weaning (W5 to L69), W7 to weaning (W7 to L69), birth to weaning (Birth to L69), total daily milk yield (MY) in W3, W5 and W7, udder circumference (UC), udder volume (UV) at weaning (L69), the average of both udder halves of parenchyma (PAR) at W3 and fat pad (FP) at P110 and W3.

	Birth to W3	Birth to W5	Birth to W7	W3 to L69	W5 to L69	W7 to L69	Birth to L69
MY							
W3	0.711***	0.631***	0.579***	0.181	0.115	0.033	0.532**
W5	0.500**	0.528**	0.477**	0.168	0.096	0.128	0.466*
W7	0.367*	0.480**	0.628***	0.526**	0.523**	0.296	0.633***
UC							
L69	0.057	0.222	0.349	0.410*	0.269	0.200	0.454*
UV							
L69	0.069	0.190	0.315	0.422*	0.336	0.303	0.455*
PAR							
W3	0.326	0.141	0.103	-0.227	-0.191	-0.378*	-0.037
FP							
P110	-0.370*	-0.299	-0.217	0.059	0.148	0.242	-0.133
W3	-0.592**	-0.534**	-0.369	0.081	0.217	0.397	-0.220

*P < 0.05; ** P < 0.01; *** P < 0.001. P110: day 110 of pregnancy; L69: weaning.

Table 3.9. Multiple regression coefficients (± SEM) of the average of ultrasound (GC, PAR, FP and MTc) measures in pregnancy (P110), week 3 (W3), 5 (W5) and 7 (W7) of lactation on single lamb growth from birth to week 3 (Birth to W3), birth to week 5 (Birth to W5), birth to week 7 of lactation (Birth to W7) and birth to weaning (Birth to L69).

Lamb growth (g/d)	Independent variables selected									
	Intercept	GC (P110)	FP (P110)	FP (W3)	PAR (W3)	PAR*MTc (W3)[2]	MTc (W3)	FP (W7)	FP (L69)	R^2
Birth to W3	– 43.5 ± 1213	– 3.6 ± 6.0[1]	8.11 ± 5.6	– 8.66 ± 2.87	5.72 ± 24	$- 6.7. 10^{-3}$ ± 0.31	1.17 ± 15	- [3]	-	0.635
Birth to W5	NS	-	-	-	-	-	-	-	-	-
Birth to W7	– 1165 ± 524	-	-	-	25.6 ± 10.3	-0.31 ± 0.13	18.1 ± 0.67	-	-	0.380
Birth to L69	- 52.9 ± 101	-	-	-	-	-	4.45 ± 1.40	- 1.14 ± 2.57	2.16 ± 2.11	0.364

GC: Gland cistern; FP: Fat Pad; PAR: Parenchyma; MTc: Total mammary depth conservative; [1] For each 1 mm increase in depth of the gland cistern (GC) in pregnancy (P110), lamb growth decreased by 3.6 ± 6.0 g/d from birth to week 3 of lactation (Birth to W3); [2] Two-way interaction between PAR at W3 and MTc at W3; [3] Independent variable that was not a significant (P > 0.10) predictor of lamb growth from birth to W3.

3.5. Discussion

3.5.1. Prediction of milk production using ultrasound and morphological measures

Milk yield of ewe lambs in this experiment decreased over time, following the normal progress of a lactation (Cardellino and Benson, 2002). In the present experiment, milk yield differed between the right and left udder half at week three of lactation (i.e. near the peak of lactation; Lérias et al., 2014). This difference in production may be explained by a preference of the single lamb for one udder half over the other, which may have resulted in an overstimulation of this udder half (Makovicky et al., 2015). This overstimulation would lead to an increase in milk production to adapt to the demand of the progeny (Lérias et al., 2014). The difference between udder halves, however, did not persist over time.

It was hypothesised that cistern depth of the ewe lamb mammary gland would be positively associated with milk yield. While this was the case, the predictions of milk yield at three, five and seven weeks of lactation from udder ultrasound and morphological measurements were moderate (21, 27 and 38%). The magnitude of this relationship was greater than reported by van der Linden et al. (2010) who found that udder dimensions explained 19% of the variation in milk yield at day 21 and 28 of lactation. The maximum variation explained for milk yield in week seven of lactation using udder measurements was moderate (38%). van der Linden et al. (2010) explained a maximum of 36% of the variation in milk yield at day 35 of lactation using udder dimensions. Arcos-Álvarez et al. (2020) explained 54 to 63% of the variation in milk yield using udder dimensions. In dairy heifers, the proportion of secretory tissue in the parenchyma measured 15 to seven days prior to calving was highly positively correlated (0.80) with milk yield over 100 days of lactation, indicating that this measure was an accurate indicator of milk yield (Strzetelski et al., 2004). In these studies, milk yield was predicted using udder dimensions (van der Linden et al., 2010, Arcos-Álvarez et al., 2020) or ultrasound measurements (Strzetelski et al., 2004). In the current experiment, however, milk yield was predicted using both udder dimensions and internal structures which may explain some of the differences observed. The difference between our data and those of Strzetelski et al. (2004) was likely do to the difference in species and their purpose (dairy heifers *vs.* non-dairy ewe lambs). Milk production is a complex biological process primarily determined by the number of secretory cells and their activity (Capuco et al., 2001, Akers, 2002b, Boutinaud and Guinard-Flament, 2004, Capuco and Akers, 2010). While ultrasound imaging enables the visualisation and assessment of the dimensions of the different tissues in the mammary gland (Molenaar et al., 2013, 2020) and their echo-textural characteristics (Barbagianni et al., 2015, Murawski et al., 2019), it does not provide information on the number and activities of secretory cells. This may explain the moderate prediction of milk yield using ultrasound measurements in the current experiment and is a limitation to the use of ultrasound as a technique to predict milk yield. Further research is needed to identify more accurate indicators of milk yield such as gene expression in ewe mammary gland.

Milk yield in weeks three and five of lactation were only usefully predicted by ultrasound measurements, whereas, in week seven, milk yield was predicted using both ultrasound and morphological measures. Milk yield was better predicted using udder ultrasound measures in late pregnancy and early lactation. Udder measures collected in late pregnancy may enable farmers to identify ewe lambs that may have greater milk yield in lactation earlier and potentially select these ewe lambs.

Mammary gland cistern depth in late pregnancy and early lactation were positively associated with milk yield per udder half in week three of lactation. These findings are consistent with previous studies that reported mature ewes with larger cisterns produced more milk than ewes with smaller cisterns (Caja et al., 1999, Nudda et al., 2000, Rovai et al., 2008). This is perhaps an unsurprising result as the mammary gland cistern is the cavity where milk is stored between suckling events or milkings (Akers, 2002b). Larger cisterns, therefore, enable greater storage capacity for milk until removal (Castillo et al., 2008). The contribution of gland cistern depth in late pregnancy and early lactation to the predicted milk production at week three of lactation, however, was moderate. For example, there were differences of 48 and 125 g/d of milk per udder half during week three of lactation between a ewe lamb with an average cistern depth and those with a larger cistern in late pregnancy and early lactation, respectively. Assuming that milk production is constant during the third week of lactation, these differences would result in an increase of 336 and 875 g of milk per udder half and a total increase of 672 g and 1750 g of milk per ewe lamb during week three of lactation, respectively. Danso et al. (2016) reported that for 1 kg of milk, lamb growth would increase of 130 g between birth and 42 days of age. Hence, using the data of Danso et al. (2016), the difference in milk yield at week three of lactation in the current experiment would lead to an increase of 87 g and 228 g in lamb growth born to ewes with larger gland cistern in late pregnancy and early lactation respectively. Although, the depth of the gland cistern in late pregnancy and early lactation were positively associated with milk yield, the ultrasound method would not be an accurate technique to identify ewe lambs that would have greater milk production.

3.5.2. Prediction of lamb growth rates using ultrasound measures

The prediction of lamb growth to weaning using morphological and ultrasound measurements was moderate (36%), however, lamb growth rates between birth and week three of lactation was better predicted (64%). The percentage of the variation in lamb growth between birth and weaning explained by the model was consistent with that reported in Chapter 2 (37%). During the first three to four weeks of life, lambs are solely dependent on milk to survive (Geenty and Sykes, 1983, Danso et al., 2014). This early reliance of milk likely explains the greater proportion of variation for lamb growth to week three of lactation being explained by udder measurements. In addition, lamb growth was poorly to moderately predicted by milk yield (van der Linden et al., 2010, Danso et al., 2016). The proportion of variation explained for lamb growth to early lactation in the current experiment was greater than that found in Chapter 2 (12%). In the present experiment, lambs were approximately 17 days of age when lactation measurements were recorded, whereas, lambs in Chapter 2 were 29 days of age and, therefore, likely to be less dependent on milk. Regardless of these differences, the models predicted a limited amount of the variation in lamb growth, particularly between birth and weaning. Lamb growth depends on multiple factors, including milk yield and quality (Snowder and Glimp, 1991, Morgan et al., 2007), and the quantity and quality of solid feed available (Danso et al., 2014). It is likely, therefore, that ultrasound and morphological measurements of the udder alone may not explain enough variation in lamb growth to be accurate predictors. Further research is required to identify more accurate indicators of lamb growth.

Lamb growth from birth to week three of lactation was predicted by the ultrasound measures of the fat pad and gland cistern in late pregnancy and of parenchyma and fat pad in early lactation. These findings contrast with those of Chapter 2, where the depth of the gland cistern in early lactation was the only predictor of lamb growth to early lactation. In the current experiment, ultrasound measurements that predicted lamb growth to weaning included measures in week three and seven of lactation and at weaning. This finding also contrasts with the results of Chapter 2 that reported that lamb growth to weaning was predicted by the depth of the parenchyma in late pregnancy and gland cistern depth in early lactation. Mammary gland predictors of lamb growth measured in late pregnancy may enable an early identification of ewe lambs that would have lambs with greater growth rates. More research, however, is needed to determine whether ewe lambs with larger mammary gland cistern or parenchyma would have similar mammary characteristics in subsequent years and, therefore, whether ultrasound could be used to select ewe lambs with superior lactational performance.

The depth of the fat pad in late pregnancy was positively correlated with lamb growth to week three of lactation. The contribution of fat pad in late pregnancy to the model was moderate with lambs born to ewe lambs with a large fat pad in late pregnancy were 626 and 876 g heavier at 15 and 21 days of age respectively, compared to lambs born to ewe lambs with an average fat pad. Lambs born to ewe lambs with large fat pad in late pregnancy would be predicted to be heavier in early lactation when they will be yarded for the first time (approximately 30 days of age) than lambs born to ewe lambs with average fat pad. The fat pad is predominantly composed of adipose and connective tissue (Hovey et al., 1999, Hovey and Aimo, 2010). It has been reported that the amount of adipose tissue in the fat pad dictates the total number of secretory cells (Hoshino, 1978, Hovey et al., 1999), which then determines milk production (Boutinaud and Guinard-Flament, 2004). The fat pad also has local-synthesised IGF-1, which stimulates the growth of mammary parenchyma (Hovey and Aimo, 2010). The fat pad is also involved in lipid storage during pregnancy and supports milk production in lactation with the biosynthesis of lipids (Hovey et al., 1999). Thus, it is perhaps not surprising that a deeper fat pad in late pregnancy was linked with greater lamb growth rates.

The depth of the parenchyma at week three of lactation was moderately and positively associated with lamb growth to week three of lactation. Single lambs born to ewe lambs with a large parenchyma at week three of lactation were 831 and 1163 g heavier at 15 and 21 days of age respectively, than lambs born to ewe lambs with an average parenchymal depth. Lambs born to ewe lambs with large parenchyma depth at week three of lactation would be predicted to be heavier by more than 1 kg in early lactation when they will be yarded for the first time (approximately 30 days of age) than lambs born to ewe lambs with average parenchyma depth. The cells involved in milk production and secretion are located in the mammary parenchyma (Colville, 2007, Lérias et al., 2014) and their number and activity determine the quantity of milk produced (Capuco et al., 2001, Boutinaud and Guinard-Flament, 2004). The development of the parenchyma primarily occurs in late pregnancy (day 110 to 141 of pregnancy; Anderson, 1975, Lérias et al., 2014), thus, a larger parenchyma at week three of lactation may indicate a greater number of secretory cells and possibly greater milk production. The ultrasound method used in the current experiment, therefore, was a potential technique that could be used to determine mammary parenchyma tissue depth as an indicator of early lamb growth.

Among ewe lambs (Chapter 2), the depth of the mammary parenchyma in late pregnancy and the gland cistern in early lactation were indicators of lamb growth to weaning. In this experiment, however, only the total depth of the mammary gland conservative at week three of lactation and depth of the fat pad at week seven of lactation and at weaning were predictors of lamb growth to weaning. The difference in the predictors of lamb growth could be due to differences in the timing of the measurements. Parenchymal tissue development primarily occurs between day 110 and 141 of pregnancy (Nørgaard et al., 2008, Lérias et al., 2014). The ultrasound measurements made in late pregnancy were recorded between 102 and 122 days of pregnancy whereas in Chapter 2, they were recorded between 100 and 115 days of pregnancy. This small difference in timing may explain why the depth of parenchyma in late pregnancy was not a good indicator of lamb growth to weaning in the current experiment. Further research, using a greater number of ewe lambs, is warranted to better understand the indicators of single lamb growth to weaning using ultrasound measurements of the internal structure of ewe lamb mammary gland. This knowledge would determine if ultrasound could be used as an early technique to identify ewe lambs for increased growth of their single lambs.

3.6. Conclusion

Although the mammary gland cistern depths in late pregnancy and third week of lactation were identified as indicators of milk yield in early lactation, the prediction of milk yield using ultrasound measurements was poor. The prediction of single lamb growth from birth to the third week of lactation using udder measurements was high, whereas the prediction of growth to weaning of single lambs was moderate. The size of the mammary fat pad in late pregnancy and the parenchyma in early lactation were indicators of lamb growth to week three of life. This ultrasound method was not an accurate technique to predict milk yield in non-dairy ewe lambs, however, it could potentially provide farmers with a technique for early selection of non-dairy Romney ewe lambs whose progeny would have faster early growth rates. More research is warranted to find more accurate indicators of milk yield and determine whether ultrasound could be used as an accurate technique to identify and select ewe lambs that would have greater single lamb growth rates to weaning.

Foreword to Chapter 4

Chapters 2 and 3 showed contrasting results in the association between mammary ultrasound measures of ewe lambs and the growth rates of their single lambs to weaning. In Chapter 2, the depth of the mammary parenchyma in late pregnancy and gland cistern in early lactation were positively associated with single lamb growth rates to weaning. In Chapter 3, the depth of the fat pad in week seven of lactation and at weaning were negatively and positively associated with lamb growth rates to weaning, respectively. There is, however, no published data on the association between mammary ultrasound measures of older ewes and growth of their progeny to weaning. In addition, Chapter 2 and 3 only investigated single-bearing ewe lambs. One of the objectives of Chapter 4 was, therefore, to investigate the association between mammary internal structures of two-year-old ewes in their second lactation and growth rates to weaning of their single and twin lambs.

Chapter 4

Effect of increased growth rates prior to the first breeding at seven months of age and pregnancy rank on mammary gland of two-year-old ewes

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4.1. Abstract

The current experiment investigated the potential carry-over effects of increased growth rates prior to breeding at seven months of age on mammary gland of two-year-old ewes during their second lactation and examined the association between ewe mammary structures and growth of their progeny. Ewe live weight and mammary ultrasound measures were recorded at 119 days of pregnancy, 29 days of lactation (L29) and at weaning of their progeny (L79) in 64 two-year-old ewes selected from two treatments. The Heavy group (n = 32) was preferentially fed prior to their first breeding at seven months of age achieving an average live weight of 47.9 ± 0.38 kg. The Control group (n = 32) weighed an average of 44.9 ± 0.49 kg at breeding. Lambs (n = 74) were weighed at birth, L29 and L79. Udder ultrasound measures did not differ (P > 0.10) between treatments, indicating no carry-over effects of treatments on mammary gland of two-year-old ewes. The association between ultrasound measures and lamb growth seemed to differ between lamb birth rank. More research is needed to further investigate these associations and determine whether ultrasonography could be used to identify ewes whose progeny would have greater growth rates based on birth ranks.

4.2. Introduction

Higher growth rates between weaning and puberty can have detrimental effects on mammary gland development and milk production in ewe lambs (Johnsson and Hart, 1985, Hovey et al., 1999, Tolman and McKusick, 2001, Villeneuve et al., 2010a, Berryhill et al., 2017). While the exact mechanism is unknown, it is possible these effects are mediated through a reduction in mammary parenchyma and an accumulation of fat in the mammary fat pad (Johnsson and Hart, 1985, Villeneuve et al., 2010a). The allometric phase of mammary gland development in ewe lambs occurs between two and five months of age (Anderson, 1975, Johnsson and Hart, 1985). During this phase, parenchymal development occurs which will determine the future development of alveoli, and subsequently, milk production (Villeneuve et al., 2010a, Berryhill et al., 2017). Increased post-weaning growth rates result in the earlier attainment of puberty in ewe lambs, which has been reported to interrupt the allometric phase (Meyer et al., 2006b, Van Amburgh et al., 2019). Further, Villeneuve et al. (2010b) reported that ewe lambs rearing a single lamb with increased growth rates between weaning and subsequent breeding at seven months of age produced less milk over the subsequent two lactations than those that had slower growth rates. Little is known, however, about potential carry-over effects of increased growth rates of ewe lambs between weaning and their first breeding at seven months of age on the ewe mammary gland and its internal structures.

Mammary gland cistern size during lactation has been positively associated with milk production of both dairy (Nudda et al., 2000, Rovai et al., 2008) and non-dairy ewes (Caja et al., 1999). In Chapter 3, the depth of the mammary gland cistern in late pregnancy and in week three of lactation was positively correlated with milk yield in the third week of lactation of non-dairy ewe lambs rearing single lambs. In Chapter 2, the growth of single lambs to weaning was positively associated with the depth of the parenchymal tissue in late pregnancy and gland cistern size in early lactation. While single lamb growth to weaning was negatively associated with the depth of the mammary fat pad in the seventh week of lactation, it was positively associated with depth of the fat pad at weaning (Chapter 3). Currently, no data have been published on the associations between mammary gland ultrasound measures of older non-dairy ewes and growth of their lambs. In addition, the associations between internal mammary gland structures and twin lamb growth rates to weaning are unknown.

The first objective of the present experiment was to investigate the potential carry-over effects of increased growth rates between weaning and breeding at seven months of age on the mammary gland during the second lactation of two-year-old ewes bearing one or two lambs. The second objective was to investigate the association between mammary internal structures of two-year-old ewes and lamb growth to weaning. It was hypothesised that the mammary gland cistern depth of two-year-old ewes would be positively associated with lamb growth to weaning.

4.3. Materials and Methods

All animal handling procedures were approved by the Massey University Animal Ethics Committee (MUAEC-17/16). The experiment was conducted at Massey University Keeble farm (latitude: 40° 24' 03 S, longitude: 175° 35' 51 E), 5 km south of Palmerston North, New Zealand.

4.3.1. Experimental design

4.3.1.1. Background

The overall experimental design was as previously described in Chapter 2. In short, twin-born Romney ewe lambs (n = 270) were allocated to one of two treatments so that live weight did not differ between treatments at their weaning (28.6 kg ± 0.16; 03/01/2018) at approximately 86 days of age. Post-weaning, the 'Heavy' group (n = 135) was preferentially fed until breeding (10/05/2018), achieving an average live weight of 47.9 ± 0.38 kg and the 'Control' group (n = 135) achieved an average of 44.9 ± 0.49 kg at breeding. Both treatments were managed as one mob and grazed on ryegrass and white clover pasture under commercial New Zealand grazing conditions from breeding as a ewe lamb onwards. Their first set of lambs were weaned at approximately 100 days of lactation (17/01/2019).

4.3.1.2. Present study

Both treatments were rebred as two-year-old ewes at P0 (29/04/2019) to Romney rams for 34 days at a ratio of 1:60. The number of ewes pregnant and the number of foetuses conceived were determined at pregnancy diagnosis using trans-abdominal ultrasound (P93; 25/07/2019). Romney two-year-old ewes from each treatment were selected at pregnancy diagnosis based on two criteria (P93; Heavy, n = 32, 62.5

± 1.13 kg and Control, n = 32, 65.3 ± 1.15 kg; lsmeans ± SEM). Ewes were selected if their udder was ultrasound scanned as a ewe lamb (Chapter 2) and they had weaned a lamb as a ewe lamb (Heavy, n = 23 and Control, n = 23) or if they weaned a lamb as a ewe lamb and were diagnosed pregnant at P93 as a two-year-old ewe (Heavy, n = 9 and Control, n = 9) to enable the inclusion of more single- and twin-bearing ewes. Both treatments included single- (Heavy, n = 15 and Control, n = 14), twin- (Heavy, n = 16 and Control n = 16) and triplet-bearing ewes (Heavy, n = 1 and Control, n = 2). At P134, two-year-old ewes from both treatments were randomly assigned to one of the four lambing paddocks (average stocking rate 13.8 ewes/ha). The lactation period was deemed to have begun after the first lamb had been born (20/09/2019; L1). The lambing period lasted for 22 days (20/09 to 12/10/2019). Ewes whose entire litter died at birth or during lactation were excluded from the reminder of this experiment.

From breeding (P0) to the weaning of their lambs (L79), both groups were managed as a single cohort and grazed together using a rotational grazing system on ryegrass and white clover pasture under commercial New Zealand grazing conditions. The pre-grazing pasture masses during pregnancy and lactation were on average 2209 ± 167 and 1592 ± 63 kg DM/ha, respectively. Ewes were supplemented with approximately 0.5 kg/ewe/day of grass baleage from P0 to P25 (CP 11.5%, NDF 52.9%, ADF 31.4%) and with approximately 200 g/ewe/day of a cereal-based supplement (CP 9.7%, NDF 14.6%, ADF 4.4%) from P0 to P44.

4.3.2. Animal measurements

Live weights of the two-year-old ewes were recorded at P93, P119, P134, 29 days of lactation (L29) and L79. Twice daily lambing observations were conducted between

P134 and L29 during which lambs were tagged and their date of birth, sex, dam number, birth weight and lambing paddock were recorded within 18 hours of birth. Lambs were weighed again at an average of 29 ± 5 (L29) and 79 ± 5 days of age (L79).

4.3.2.1. Udder score and morphology

Udder scores and measures of udder morphology were detailed in Chapter 2 and were performed at P119, L29 and L79. The scoring system included the palpation of both udder halves and teats, and an assessment of udder depth and symmetry (Griffiths et al., 2019b). Morphological measures included udder circumference (UC, cm) and the height of each udder half (cm). Udder volume (UV, cm^3) was calculated using UC and udder height (UH, cm) according to Ayadi et al. (2011):

$$UV = \pi \times R^2 \times UH; R = UC/2\pi;$$

Where UV = udder volume (cm^3); π = 3.14159; R = radius (cm); UH = udder height (cm); UC = udder circumference (cm).

4.3.2.2. Udder ultrasound scanning

The udder ultrasound scanning method was as described in Chapter 2. Ultrasound scans were performed at P119, L29 and L79 by a single operator with an ultrasound scanner fitted with a linear transducer with a 5.0 – 10.0 MHz imaging frequency (Mindray Digital Ultrasonic Diagnostic Imaging System DP6600 with 75L38EA, ShenZhen, China). At L29 and L79, ultrasound scans were not conducted for ewes whose entire litter had died (Heavy n = 3 and Control n = 3).

One image per udder half was selected for image processing which had a suitable resolution per udder half that showed the gland cistern, mammary parenchyma, fat pad and limit between the mammary gland and the abdominal wall (Albino et al., 2017, Molenaar et al., 2020; Chapter 2). Images from "abnormal" udder halves were excluded (Heavy = 4 ewes with 1 half each and 7 ewes with both udder halves at L79 & Control = 2 ewes with 1 half each and 6 ewes with both udder halves at L79).

Image processing was undertaken using the ImageJ software (Ferreira and Rasband, 2012). The total depth of mammary gland conservative (MTc) and generous (MTg), fat pad (FP), parenchyma (PAR) and gland cistern (GC) depths, expressed in millimetres, were estimated at the widest point for each sub compartment. The templates created for each time point in Chapter 2 were used to standardize the assessment of each compartment depth (Appendix A).

4.3.3. Statistical analysis

Statistical analyses were conducted using SAS v9.4 (SAS Institute Inc., Cary, NC, USA) and RStudio v1.2. (RStudio Team, PBC, Boston, MA, USA). Ewes that died (Control n = 2) or whose lambs died prior to weaning (Heavy n = 3 and Control n = 3) and ewes that gave birth to triplet lambs (Heavy n = 1 and Control n = 2) were excluded from the analyses. The final data set included 28 ewes in the Heavy group (12 single- and 16 twin-bearing ewes) and 25 in the Control group (13 single- and 12 twin-bearing ewes), and a total of 269 images.

Growth of ewes in each treatment included in this experiment from weaning to their first breeding at seven months of age and lamb growth from birth to L79 were analysed using a linear mixed model. The model for growth of ewes included treatment (Heavy *vs.* Control) as a fixed effect and ewe date of birth as a covariate. The model for lamb growth from birth to L79 included treatment, birth rank (1 or 2) and sex of the lamb as fixed effects, and lambing date as a covariate.

Ewe live weight at P119, L29 and L79 and lamb growth from birth to L29 and from L29 to L79 were analysed using linear mixed models allowing for repeated measures. The model for ewe live weight included treatment, time (P119, P134, L29 and L79), pregnancy rank (single or twin) and the two-way interaction of treatment with time as fixed effects and lambing date was included as a covariate. The model for lamb growth included treatment, time (birth to L29 and L29 to L79), birth rank and sex of the lamb as fixed effects and the two-way interaction of treatment with time, lambing date as a covariate and ewe as a random effect.

The proportion of "abnormal" udder halves (udder palpation scores of 4 or 5; Griffiths et al., 2019b) and udder symmetry (yes/no) were analysed using a generalised linear model allowing for repeated measures, assuming a binomial distribution and using a logit transformation. Both models included treatment, time point (P119, L29, L79), pregnancy rank and the two-way interaction of treatment with time as fixed effects, and lambing date as a covariate. The model for the proportion of abnormal also included udder half (right *vs.* left) as a fixed effect.

Udder depth score was analysed using a generalised linear model allowing for re-peated measures, assuming a Poisson and using a log transformation. Treatment, time point, pregnancy rank and the two-way interactions of treatment with time, and treatment with pregnancy rank as fixed effects, and lambing date as a covariate.

Udder circumference (UC), UV, UH, GC, FP, PAR, MTc, MTg were analysed using general linear mixed models allowing for repeated measures. The models included treatment, time point, pregnancy rank and the two-way interactions of treatment with time, and treatment with pregnancy rank as fixed effects, and lambing date as a covariate. The models for UH, GC, FP, PAR, MTc and MTg also included udder half and their two-way interaction, and ewe as a random effect.

Ultrasound, morphological measures, ewe live weight and lamb growth for each treatment was pooled as no differences ($P > 0.10$) between treatments were identified. The residuals of the average GC, PAR, FP, MTc, MTg and UH measures of both udder halves, UC, UV, and ewe live weight at P119, L29 and L79 and lamb growth from birth to L29, L29 to L79 and birth to L79 were generated using general mixed models (Chapter 2 and 3). Ewe live weight, UH, MTg, UC and UV were adjusted for treatment and lambing date. In the model, GC, PAR, FP and MTc per udder were adjusted for treatment, MTg and lambing date. Lamb growth was adjusted for treatment, sex of the lamb and lambing date. Pearson correlations were used to test for linear associations between the residuals of lamb growth (birth to L29, L29 to L79, birth to L79) and morphological (UH, UC and UV), ultrasound measures (GC, PAR, FP, MTc and MTg) and ewe live weight at each time (P119, L29, L79).

Multiple regression analyses of lamb growth from birth to L29, L29 to L79 and birth to L79 were undertaken using general linear mixed models with RStudio v1.2. (RStudio Team, PBC, Boston, MA, USA, Packages ‘lme4’ and ‘performance’). Pearson correlations were used to examine whether each predictive variable was individually correlated with lamb growth during each period. Predictive variables that were correlated with lamb growth ($P \leq 0.20$) were included in the models

(Dohoo et al., 2003a). Correlations between selected predictive variables were examined to identify high collinearity (Dohoo et al., 2003b). In the case of a high collinearity between two predictive variables, only one predictive variable was included in the models based on the biological relevance. Two-way interactions between each of the selected variables were individually tested using general linear models, resulting in the following equations:

Lamb growth (birth to L29) = br + UC at P119 + GC at L29 + PAR at L29 + UH at L29

Lamb growth (birth to L79) = br + FP at P119 + GC at P119 + UC at P119 + GC at L29 + PAR at L29 + UH at L29 + UH at L29 x UC at L79 + GC at L79 + MTc at L79 + UH at L79 + UC at L79

Where br = birth rank (single or twin) and UH at L29 x UC at L79 is the interaction between UH at L29 and UC at L79.

Backward manual variable eliminations were used to select the model that best explained the variation in lamb growth by removing predictive variables with $P > 0.10$. Any non-significant ($P > 0.10$) predictive variable causing greater than a 20% change (Dohoo et al., 2003a) in the model coefficients were considered confounding variables and included in the models. Confounding effects were evaluated after each variable was removed from the model by checking the changes in predictive variable model coefficients. Random effects of ewe were included in the models. The marginal coefficients of determination of the multiple regressions were calculated based on the method of Nakagawa and Schielzeth (2013) and corresponded to the variance explained by the selected predictive variables in the final models. To calculate the effects of the depth of mammary internal structures related to lamb growth based on the multiple regression analyses on lamb growth and live weight, the depth of mammary structures in the average and the 90th percentile were used.

4.4. Results

4.4.1. Ewe live weight and lamb growth

Ewes in the Heavy group had greater growth rates between their weaning and first breeding at seven months of age than Control ewes (P < 0.05; 149 ± 4.2 g/d *vs.* 136 ± 4.4 g/d, respectively). At their second breeding, ewe live weight did not differ (P > 0.10) between treatments at any time (Table 4.1). At P119 and P134, twin-bearing ewes were heavier (P < 0.01) than single-bearing ewes (70.0 ± 0.95 kg *vs.* 64.6 ± 1.00 kg at P119 and 73.1 ± 0.94 kg *vs.* 67.6 ± 0.99 kg at P134, respectively), irrespective of treatment. Ewe live weight did not differ (P > 0.10) between single- and twin-bearing ewes at L29 or L79.

Lamb live weight did not differ between treatments (P > 0.10) at birth (Control = 6.96 ± 0.30 *vs.* Heavy = 6.83 ± 0.29), L29 or L79 (Table 4.1). Lamb growth rates from birth to L29, L29 to L79 and from birth to L79 did not differ (P > 0.10) between treatments (data not shown). Single lambs had greater (P < 0.001) growth rates than twin lambs from birth to L29 (singles 289 ± 16 g/d *vs.* twins 219 ± 14 g/d), L29 to L79 (singles 288 ± 12 g/d *vs.* twins 234 ± 11 g/d) and birth to L79 (singles 288 ± 9.5 g/d *vs.* twins 232 ± 8.7 g/d). Female lambs had greater (P < 0.05) growth rates from birth to L79 than male lambs (268 ± 8.0 g/d *vs.* 253 ± 8.0 g/d respectively).

4.4.2. Udder score and morphology

The proportion of abnormal udder palpation scores and ewes with asymmetric udders did not differ (P > 0.10) between treatments or pregnancy ranks (data not shown). There were no abnormal teat palpation scores at any time point (P > 0.10).

Table 4.1. Effect of time (P119, P134, L29 and L79) and treatment (Control *vs.* Heavy) on two-year-old ewe live weight during pregnancy (P119, P134), early lactation (L29) and weaning (L79) and lamb live weight in early lactation (L29) and at weaning (L79). Least square means ± SEM

	Pregnancy (P119)	Pregnancy (P134)	Early lactation (L29)	Weaning (L79)
Ewe live weight (kg)				
Control (n = 25)	68.8 ± 1.00^{b}	71.4 ± 0.99^{c}	67.8 ± 1.28^{b}	63.1 ± 1.06^{a}
Heavy (n = 28)	65.8 ± 0.94^{b}	69.2 ± 0.94^{c}	65.2 ± 1.22^{b}	60.1 ± 1.00^{a}
Lamb live weight (kg)				
Control (n = 25)			13.4 ± 0.42^{a}	27.8 ± 0.78^{b}
Heavy (n = 28)			13.3 ± 0.40^{a}	27.0 ± 0.74^{b}

^{a,b,c} Means within rows with different superscripts are significantly different (P < 0.05).

The proportion of abnormal udder palpation scores was greater (P < 0.05) at L79 than at P119 and L29 (Table 4.2). The proportion of ewes with asymmetric udders was greater (P < 0.05) at L29 and L79 than at P119 (Table 4.2). Udder depth scores did not differ (P > 0.10) between treatments (data not shown) but were greater (P < 0.001) at P119 than L29 which was greater (P < 0.001) than L79 (Table 4.2). At P119 and L29, udder depth scores were greater (P < 0.01) for single-bearing ewes than twin-bearing ewes (mean (95% confidence interval); 4.96 (4.88 – 5.04) *vs.* 4.38 (4.20 – 4.58) at P119 and 4.20 (4.01 – 4.39) *vs.* 3.90 (3.80 – 4.01) at L29 respectively), but did not differ (P > 0.10) at L79.

Udder height (UH), UC and UV did not differ (P > 0.10) between treatments at any time point (data not shown). At P119, UH of the right udder half was greater (P < 0.05) than the left udder half (5.58 ± 0.14 *vs.* 5.29 ± 0.15 respectively), but udder halves did not differ (P > 0.10) at L29 or L79. At L29, UH, UC and UV were greater (P < 0.01) than L79 which in turn was greater (P < 0.01) than at P119 (Table 4.3). At P119, UH, UC and UV were greater (P < 0.01) for twin-bearing ewes than single-bearing (Table 4.3). At L29, UH and UV were greater ($p < 0.05$) for twin-bearing ewes than single-bearing (Table 4.3). At L79, UH, UC and UV did not differ ($P > 0.10$) between birth ranks (Table 4.3).

Table 4.2. Effect of time (P119, L29 and L79) on the proportion (95% confidence intervals) of abnormal udder palpation scores[1] and asymmetric udders (Asymmetry) and least square means (95% confidence intervals) of udder depth score of two-year-old ewes.

	Pregnancy (P119)	Early lactation (L29)	Weaning (L79)
Abnormal udder palpation scores (%)	2.8 (0.84–8.8)[a]	3.7 (1.3–10.3)[a]	29.3 (18.3–43.5)[b]
Asymmetry (%)	3.5 (0.9–13)[a]	14.1 (7.0–26.5)[b]	12.5 (5.9–24.5)[b]
Udder depth score	4.66 (4.56–4.77)[c]	4.05 (3.94–4.15)[b]	3.74 (3.63–3.86)[a]

[1] Abnormal udder palpation included udder palpation score 4 = firm consistency with nodules – lumps or grainy texture, and udder palpation score 5 = diffuse hard consistency; [a,b,c] Means within rows with different superscripts are significantly different ($P < 0.05$).

4.4.3. Udder ultrasound measures

The depth of the gland cistern (GC), parenchyma (PAR), fat pad (FP), total depth of the mammary gland conservative (MTc) and generous (MTg) did not differ ($P > 0.10$) by udder half or treatment at any time point (data not shown). At P119, PAR was greater ($P < 0.01$) for twin-bearing ewes than single-bearing but did not differ ($P > 0.10$) between pregnancy rank at L29 or L79 (Table 4.3). Pregnancy rank had no effect ($P > 0.10$) on GC, MP, MTc and MTg at any time point (Table 4.3). At P119, the GC was smaller ($P < 0.001$) than L29 or L79 (Table 4.3). At L29, PAR, MTc and MTg were greater ($P < 0.05$) than L79 which was in turn greater ($P < 0.05$) than P119 (Table 4.3). At L79, FP was smaller ($P < 0.01$) than P119 (Table 4.3).

Table 4.3. Effect of time (P119, L29 and L79) and pregnancy rank (Single or Twin) on udder height (UH), udder circumference (UC), udder volume (UV), the depths of mammary gland cistern (GC), mammary parenchyma (PAR) and the fat pad (FP), and total depths of mammary gland conservative (MTc) and generous (MTg) of two-year-old ewes. Least square means ± SEM

	Pregnancy (P119)		Early lactation (L29)		Weaning (L79)	
	Single	Twin	Single	Twin	Single	Twin
UH (cm)	4.70 ± 0.19**[a]	6.17 ± 0.19**[a]	10.5 ± 0.25**[c]	11.7 ± 0.24**[c]	10.1 ± 0.29[b]	10.8 ± 0.28[b]
UC (cm)	29.1 ± 0.42*[a]	30.9 ± 0.41*[a]	46.8 ± 0.67[c]	48.5 ± 0.64[c]	39.4 ± 0.62[b]	38.1 ± 0.62[b]
UV (cm^3)	330.5 ± 23.5**[a]	482.9 ± 23.1**[a]	1854 ± 83*[c]	2198 ± 79*[c]	1262 ± 58[b]	1271 ± 58[b]
GC (mm)	8.28 ± 0.54[a]	8.99 ± 0.52[a]	18.1 ± 1.00[b]	16.2 ± 1.01[b]	17.7 ± 1.08[b]	17.8 ± 1.07[b]
PAR (mm)	17.3 ± 0.72**[a]	20.7 ± 0.70**[a]	55.4 ± 1.62[c]	61.8 ± 1.63[c]	31.7 ± 1.85[b]	29.4 ± 1.82[b]
FP (mm)[1]	19.5 ± 0.93[b]	19.2 ± 0.90[b]	-	-	15.9 ± 1.14[a]	18.2 ± 1.13[a]
MTc (mm)	46.0 ± 1.83[a]	49.7 ± 1.76[a]	76.1 ± 1.82[c]	82.0 ± 1.84[c]	67.0 ± 2.20[b]	66.4 ± 2.15[b]
MTg (mm)	52.3 ± 1.04[a]	56.2 ± 0.99[a]	84.5 ± 1.99[c]	90.2 ± 2.01[c]	75.8 ± 2.53[b]	74.7 ± 2.49[b]

[*, **] Means differed between single- and twin-bearing ewes within time point (* $P < 0.05$ and ** $P < 0.01$); [a,b,c] Means within rows (time) with different superscripts are significantly different ($P < 0.05$). [1] The mammary fat pad was not detected on L29 images.

4.4.4. Correlations between lamb growth and udder measures

Lamb growth from birth to L29 was negatively associated ($P < 0.05$) with FP at L79 (Table 4.4). Lamb growth from L29 to L79 was negatively associated ($P < 0.05$) with UV and UH from the left udder half at P119 and positively correlated ($P < 0.05$) with UC, FP and MTg at L79 (Table 4.4). Lamb growth from birth to L79 was negatively associated ($P < 0.05$) with UH of both udder halves and UV at P119 and was positively associated ($P < 0.05$) with UC, UV and MTg at L79 (Table 4.4). Non-significant ($P > 0.10$) correlations are presented in Appendix C.

Table 4.4. Correlation coefficients of residuals of lamb growth from birth to early lactation (Birth to L29), early lactation to weaning (L29 to L79), birth to weaning (Birth to L79) with udder height (UH) in late pregnancy (P119), udder circumference (UC) at weaning (L79), volume (UV) at P119, depth of the mammary fat pad (FP) at L79 and the total depth of the mammary gland generous (MTg) at P119 and L79 of two-year-old ewes.

	Time	Birth to L29	L29 to L79	Birth to L79
UH	P119 RS	-0.138	-0.209	-0.247*
	P119 LS	-0.131	-0.236*	-0.257*
UC	L79	0.155	0.260*	0.328**
UV	P119	-0.119	-0.237*	-0.263*
FP	L79	-0.298*	0.275*	0.074
MTg	P119	0.155	0.169	0.227*
	L79	0.200	0.402**	0.466***

* P < 0.05; ** P < 0.01; *** P < 0.001; RS: Right udder half; LS: Left udder half.

4.4.5. Prediction of lamb growth using udder measures

The best model for lamb growth from birth to early lactation (L29) explained 21.7% of the variation in lamb growth rates and included the effects of birth rank, UH and GC at L29 and the random effect of ewe (Table 4.5). The difference between a ewe with an average GC and a ewe with a GC at L29 in the 90th percentile was 13.3 mm (Table 4.6), resulting in a 26.6 g/d difference in lamb growth from birth to L29.

The best model for lamb growth from birth to weaning (L79) explained 52.9% of the variation in lamb growth rates and included the effects of birth rank, FP and UC at P119, GC and PAR at L29, and GC, MTc, UH and UC at L79, the interaction between UH at L29 and UC at L79, and the random effect of ewe (Table 4.5). The difference between a ewe with an average GC at L29 and a ewe with a GC in the 90th percentile was 13.3 mm (Table 4.6), resulting in a -30.6 g/d difference in lamb growth from birth to L79. The difference between a ewe with an average PAR at L29 and a ewe with a PAR in the 90th percentile was 8.6 mm (Table 4.6), resulting in a -3.10 g/d difference in lamb growth from birth to L79.

Table 4.5. Multiple regression coefficients (± SEM) of the average of ultrasound (FP, GC, PAR, MTc) and morphological (UC, UH) measures in pregnancy (P119), early lactation (L29) and at weaning (L79) and of the random effect of ewe (Ewe) on lamb growth (g/d) from birth to early lactation (Birth to L29) and birth to weaning (Birth to L79).

Independent variables	Birth to L29		Birth to L79	
	Estimate	SEM	Estimate	SEM
Intercept	361	74	– 846	1038
Br1	– 59.2	20	47	15
Br2	59.2	20	– 47	15
FP at P119[1]	-	-	– 0.53	1.8
UC at P119[1]	-	-	2.5	3.4
GC at L29	2.0	1.8	– 2.3	1.7
PAR at L29[1]	-	-	– 0.36	0.9
UH at L29	– 5.7	7.4	– 90.1	100
UH at L29 x UC at L79[1,2]	-	-	– 2.4	2.6
GC at L79[1]	-	-	0.59	1.9
MTc at L79[1]	-	-	1.4	0.7
UH at L79[1]	-	-	– 7.8	7.5
UC at L79[1]	-	-	31	27
Ewe	1.9	44	0.97	0.3

Br: Birth rank; FP: Fat pad; UC: Udder circumference; GC: Gland cistern; PAR: Parenchyma; UH: Udder height; MTc: Total depth of the mammary gland conservative; [1] Dependent variable was not a significant (P > 0.10) predictor of lamb growth from birth to L29; [2] Two-way interaction between UH at L29 and UC at L79.

Table 4.6. Descriptive statistics of the depth of the gland cistern (GC), parenchyma (PAR) and mammary fat pad (FP) in late pregnancy (P119), early lactation (L29) and at weaning (L79), irrespective of treatment.

	Minimum	10th percentile	Mean	90th percentile	Maximum
GC (mm)					
P119	4.85	5.75	8.71	11.8	19.6
L29	8.40	11.5	17.4	23.8	30.7
L79	11.1	11.7	18.3	24.6	33.3
PAR (mm)					
P119	11.1	14.0	19.3	24.2	30.0
L29	44.4	48.5	58.5	67.1	85.7
L79	15.3	20.2	30.5	43.3	56.0
FP (mm)					
P119	7.25	13.3	19.4	24.3	28.2
L79	8.15	11.0	17.5	24.2	29.4

4.5. Discussion

4.5.1. Difference between treatments and pregnancy rank

The dimensions of the mammary internal structures of two-year-old ewes bearing either single or twin lambs and lamb weaning weights did not differ between treatments. These findings are consistent with Chapter 2 that indicated increased growth rates prior to first breeding at seven months of age had no effect on ultrasound measures of the mammary gland during pregnancy and lactation nor subsequent lamb weaning weights. Villeneuve et al. (2010b), however, reported that two-year-old ewes with increased growth rates prior to their first breeding produced less milk in their second lactation and had lighter single lambs at weaning than ewes with slower growth rates. There were greater differences between ewe growth rates (76 to 82 g/d) and greater magnitude in growth (223 to 305 g/d) in the indoor study of Villeneuve et al. (2010b) than in the current experiment (8 g/d difference and 136 to 148 g/d of magnitude), which may explain the difference in findings. The present results suggest that there were no carry-over effects of increased growth rates between weaning and breeding at seven months of age on the morphology and dimensions of the internal structures of the mammary gland of two-year-old ewes. Ultrasound imaging, however, enables only the udder dimensions to be visualized (Molenaar et al., 2020, Chapter 2 and 3) and echo-textural characteristics (Barbagianni et al., 2015, Murawski et al., 2019) of the mammary internal structures to be assessed. Further investigations may be warranted to determine the effect of increasing growth rates prior to the first breeding on the cellular development and function of the ewe mammary gland, particularly if greater live weight differences than seen in this experiment can be achieved.

This is the first experiment that has examined the dimensions of mammary internal structures of twin- and single-bearing ewes using ultrasound. In late pregnancy and early lactation, udder dimensions were greater for twin- than single-bearing ewes. This finding is consistent with previous studies that reported twin-bearing ewes had larger udders than single-bearing ewes (Davis et al., 1980, Kenyon et al., 2011a, Davis, 2017). In addition, in late pregnancy in the current experiment, parenchymal depth was greater for twin- than for single-bearing ewes. The mammary parenchyma includes the milk secretory cells and the ductal network (Akers, 2002a). Hence, a larger parenchyma in late pregnancy could indicate a greater number of secretory cells and the potential for greater milk production. Twin-bearing ewes produce approximately 30 to 50% more milk than single-bearing ewes (Nowak et al., 2008), which would support this suggestion. Interestingly, the depth of the gland cistern did not differ between single- and twin-bearing ewes even though gland cistern size has been shown to be positively correlated with milk production (Caja et al., 1999, Castillo et al., 2008, Rovai et al., 2008). This result could be due a greater suckling frequency of twins than single lambs (Hinch, 1989), thus not requiring a larger gland cistern as milk removal is more frequent. As twin-bearing ewes produce more milk than single-bearing ewes (Nowak et al., 2008), along with having a larger parenchyma, it would be expected that they would also have larger cisterns. The tissues and structure of the mammary gland was shown to change during lactation depending on the number of lambs reared, this is known as mammary plasticity (Boutinaud et al., 2019). The absence of a difference could also be due to the number of lambs reared which may have changed the mammary internal structures.

4.5.2. Predictions of lamb growth using udder ultrasound and morphological measures

The variation in lamb growth rate explained by ewe udder measures was moderate and greater from birth to weaning (53%) than birth to early lactation (22%). The variation in lamb growth rate to weaning explained was greater than that reported in Chapter 2 and 3 in ewe lambs, but still only explained half of the variation in lamb growth to weaning. The differences with the findings of Chapter 2 may be due to the age of the lambs when weaning measures were recorded. Lambs were approximately 100 days of age in Chapter 2 compared with 79 days of age in the current experiment. As lambs grow, their milk intake decreases and intake of solid food increases, therefore, lambs in the current experiment would have been more dependent on milk. The decrease of milk intake and increase of pasture intake in lamb nutrition lead to the start of involution in the mammary gland, which changes the parenchyma and fat pad (Petridis and Fthenakis, 2019). Moreover, the predicted lamb growth rates included twin-lambs, which are known to have slower growth rates than single lambs (Kenyon et al., 2011a). Predictions of lamb growth using ultrasound measures, therefore, may differ between single and twin lambs and may explain the differences with Chapter 2 and 3 which included only single lambs. The differences observed could also be due to the age of the ewes. In Chapter 2 and 3, ultrasound scans were performed on ewe lambs. Ewes have been reported to produce less milk during their first than second lactation (Morgan et al., 2006, Villeneuve et al., 2010b), which may also result in differences as ewes age (Knight et al., 1995, Cardellino and Benson, 2002). Lamb growth is affected by multiple factors, including milk yield and composition and solid feed quantity and quality (Danso et al., 2014). Although mammary ultrasound measures were positively associated with milk yield in ewe lambs (Chapter 3) and mature ewes (Caja et al., 1999, Rovai et al., 2008), these measures do not provide information on milk composition or the lamb's herbage intake, which may explain why only half of the variation in lamb growth could be explained.

In the current experiment, it was hypothesised that the gland cistern depth of two-year-old ewes would be positively associated with lamb growth to weaning. The depth of the gland cistern in early lactation was positively associated with predicted lamb growth to early lactation but was negatively associated with lamb growth to weaning. The difference in lamb growth to early lactation between ewes with average and large gland cisterns was moderate (less than 900 g in early lactation), which was lower than that reported in Chapter 2. The negative association between the gland cistern in early lactation and lamb growth to weaning, in the present experiment, indicates that ewes with larger cisterns had lambs with slower growth rates to weaning than ewes with smaller cistern, resulting in a difference of 2.4 kg in lamb weaning weight (79 days of lactation). This finding contrasts with that reported in Chapter 2 for ewe lambs, that the gland cistern was positively associated with lamb growth to weaning. This negative association could be due to the inclusion of both single and twin lambs in the regression model. The mammary gland cistern is the cavity in which milk is stored between milkings or suckling events (Akers, 2002b). Gland cistern size has been positively correlated with milk yield, indicating that ewes with larger cistern had a greater milk yield than ewes with smaller cistern (Caja et al., 1999, Castillo et al., 2008, Rovai et al., 2008). It is also known that twin-bearing ewes produce more milk than single-bearing ewes (Nowak et al., 2008) and that twin lambs have slower growth rates than single lambs (Torres-Hernandez and Hohenboken, 1980, Kenyon et al., 2011a). Hence, it would be expected that twin-bearing ewes would have larger cisterns and lambs with slower growth rates than single-bearing ewes. Similarly, the depth of the parenchyma in early lactation was negatively associated with predicted lamb growth to weaning resulting in a difference in lamb weaning weight of less than 300 g. Further investigations are needed to determine the associations between single- and twin-lamb growth rates to weaning and the depth of the ewe mammary gland cisterns. This knowledge would determine if ultrasound could be used as a technique to identify ewes for increased lamb growth rates based on birth ranks.

4.6. Conclusion

No carry-over effects of increased growth rates prior to breeding at seven months of age were observed on morphology and the dimensions of internal structures of the mammary gland of two-year-old ewes during their second lactation. This experiment was the first to compare mammary glands of twin- and single-bearing ewes using ultrasound. The depth of the gland cistern in early lactation was positively associated with lamb growth rates to early lactation but, was negatively associated with growth rates to weaning. Further investigations are warranted to investigate the associations between ultrasound measures of the mammary gland and growth rates of lambs depending on their birth rank and to determine whether ultrasonography could be used as a technique for farmers to identify and select ewes that would have lambs with greater growth rates based on their birth ranks.

Foreword to Chapters 5 and 6

In Chapters 2 and 4, subsets of ewes from each treatment were selected to develop a mammary ultrasound method to investigate the mammary gland development of ewes with an increased growth rates between weaning and their first breeding. These experiments showed no evidence of an impairment of the mammary gland of two-year-old ewes, growing at approximately 150 g/d between weaning and their first breeding, to the end of their second lactation. To determine the long-term impacts of breeding heavier ewe lambs on their performance and live weight, reproductive performance and subsequent live weight of all ewe lambs present in each treatment were recorded and examined for their first (Chapter 5), second and third (Chapter 6) breeding seasons.

Chapter 5

Impact of heavier live weight of ewe lambs at breeding on their reproductive performance and performance of their progeny to weaning during their first breeding season

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5.1. Abstract

Ewe lamb live weight at breeding is positively associated with reproductive performance and has led to the recommendation of a minimum breeding live weight of 40 kg. This experiment examined the effects of heavier breeding live weight of ewe lambs on their live weight, reproductive performance, and progeny performance to weaning. Ewe lambs (n = 270) were allocated to one of two treatments. The Heavy group (n = 135) was preferentially fed until breeding, achieving an average live weight of 47.9 ± 0.36 kg, while the Control group (n = 135) had an average of 44.9 ± 0.49 kg. Both groups were exposed to crayon-harnessed vasectomised rams for 68 days before breeding, and then were bred to entire rams for 34 days. Crayon marks, fertility, litter size, lambing percentage, ewe live weights, survival and live weights of their progeny were recorded. The Heavy group had a greater odds of being mated in the first 17 days of the breeding period ($P < 0.05$), mated in the entire breeding period ($P < 0.01$) and a greater litter size ($P < 0.01$) than the Control group. There was a 28% increase in fertility and a 59% increase in lambing percentage in the Heavy group compared to the Control group. Progeny survival and live weights did not differ ($P > 0.05$) by treatment. There were curvilinear relationships between ewe lamb breeding live weight and the probability of being mated and fertility. Heavier breeding live weight improved reproductive performance of ewe lambs and emphasised the importance of an adequate breeding live weight for reproductive success in ewe lambs. They also suggest that ewe lambs should be bred at a live weight of 50 to 55 kg to maximise reproductive performance without reducing ewe live weight or the progeny performance.

5.2. Introduction

If appropriately managed, breeding ewe lambs at seven to eight months of age can have multiple advantages for farmers, such a greater number of lambs born per year, increased ewe lifetime performance and farm profitability, (Young et al., 2010, Kenyon et al., 2014c). Breeding ewe lambs can also allow for more efficient use of spring pastures, through increased feed demand during lactation (McCall and Hight, 1981, Kenyon et al., 2011b, 2014c). In New Zealand, only 40% of ewe lambs are bred each year, which is primarily due to the perception that there may be negative effects on two-year-old reproductive performance, their low and varied reproductive performance as a ewe lamb and the difficulty in achieving target pre-breeding live weights (Kenyon et al., 2004b, 2014c, Beef + Lamb New Zealand, 2018). It is generally accepted that for successful breeding, ewe lambs should have reached 60% of their expected mature live weight by the start of the breeding period (Kenyon et al., 2014a). The current recommended minimum live weight for Romney-based ewe lambs, therefore, is 40 kg at breeding (Kenyon et al., 2014c).

The live weight of ewe lambs at breeding is positively related to their breeding performance (Kenyon et al., 2014c), ovulation rate (Paganoni et al., 2014a), fertility rate (ewe lamb pregnant per ewe lamb presented for breeding; Corner-Thomas et al., 2015b) and reproductive rate (foetuses per ewe lamb presented for breeding; Corner-Thomas et al., 2015b). It has been reported, however, that there is a plateau in the effect of live weight at breeding on reproductive performance of ewe lambs (Rosales Nieto et al., 2013a,b, Corner-Thomas et al., 2015b). The point at which this plateauing occurs, however, has not been well defined. Further, the association between ewe lamb live weight at breeding and the birthweight of progeny has been inconsistent between studies, but in general the relationships were positive (Morris et al., 2005, Kenyon et al., 2006, Corner-Thomas et al., 2014b). Given these positive relationships and no formal indication of a maximum live weight, some New Zealand farmers have increased their minimum pre-breeding live weight target for ewe lambs in excess of 40 kg, as a means to improve their reproductive performance. It is unknown, however, if breeding ewe lambs at a heavier live weight may have negative impacts on ewe lamb performance, subsequent live weight or performance of their lambs to weaning. This experiment, therefore, investigated the impact of a heavier live weight at breeding on the reproductive performance of ewe lambs in their first breeding season and their progeny to weaning. It was hypothesised that ewe lambs bred at heavier live weights would have improved reproductive performance compared to lighter ewe lambs at breeding.

5.3. Material and methods

The experiment and all animal handling procedures were approved by the Massey University Animal Ethics Committee (MUAEC-17/16). The experiment was conducted at Massey University Riverside Farm (latitude: 40° 50’ 35 S, longitude: 175° 37’ 55 E), 10 km north of Masterton, New Zealand.

5.3.1. Experimental design

5.3.1.1. Background

The experimental design has been previously described in Chapter 2. Briefly, at weaning, at approximately 86 days of age (127 days prior to breeding; d86), 270 twin-born Romney ewe lambs were allocated to one of the two treatments (Heavy *vs.* Control), using a stratified randomly sampling procedure to ensure that the average live weight of the treatments did not differ (28.6 kg ± 0.16). The ‘Heavy’ group (n = 135) was then preferentially fed until their first breeding (10/05/2018; d213) achieving an average live weight of 47.9 ± 0.38 kg. The ‘Control’ group achieved an average live weight of 44.9 ± 0.49 kg at d213.

5.3.1.2. Present experiment

Both treatments were exposed to vasectomised rams, at a ratio of approximately 1:50, which were fitted with mating harnesses with crayons for 68 days prior to breeding (145 days of age; d145). Immediately after removal of vasectomised rams, crayon-harnessed entire Romney rams, at an approximate ratio of 1:40, were introduced for 34 days (d213 – d247). At breeding (d213), the Heavy (n = 135) and the Control group (n = 135) were merged and managed together until weaning (465 days of age; d465). Pregnancy diagnosis was determined by a commercial operator using trans-abdominal ultrasound at d301 (88 days after the ram introduction). Ewe lambs were shorn by commercial operators at d337. In late pregnancy (d351), pregnant ewe lambs (Heavy n = 101; Control n = 66) were assigned to one of eight lambing paddocks (average stocking rate 7.74 ewe lambs/ha) based on their pregnancy rank: single- (Heavy n = 11, 7, 12, 10, 5, 9, 6, 9 and Control n = 9, 6, 9, 5, 6, 7, 7, 7 in each lambing paddocks respectively) or twin-bearing (Heavy n = 6, 8, 7, 5, 3, 0, 3, 0 and Control n = 0, 4, 0, 4, 2, 0, 0, 0), and period of conception (first period or second 17-day period of breeding). Ewe lambs that were identified as non-pregnant (Heavy n = 34; Control n = 69) were separated from the group at d351 until d465. From eight days before the expected start of lambing until all ewe lambs had lambed, ewe lambs were checked twice a day to identify newly lambed ewes and record lamb birth data. At d394, lambs were ear-marked and their tail was docked. At d465, lambs were weaned and vaccinated. Pregnant ewes who subsequently lost their lambs at birth or during lactation remained with the lactating ewes until d465.

5.3.2. Pasture measurements

The pre-grazing cover of pastures was recorded during pregnancy (from d213 to d351; n = 17) and fortnightly for all lambing paddocks from d351 to d465 (n = 34). The pre-grazing cover was firstly assessed using a plate meter (Ashgrove Pastoral Products, New Zealand, 90 readings per paddock) and herbage mass estimated using a standard calibration (herbage mass = [158 x average meter reading] + 200; Hodgson et al., 1999).

Two samples of lucerne baleage were taken at d263 and d280 to estimate the nutritional composition as described and presented in Chapter 2. Pasture grab samples (Frame, 1993; n = 51) were collected in pregnancy (n = 17) and fortnightly in all lambing paddocks from d351 to d465 (n = 34) to estimate the nutritional composition of pasture offered (Table 5.1). Samples were frozen at -20°C until analysis. Percentages of CP, ADF, NDF, lipid and ME (MJ/kg) of pasture samples were estimated with near infrared reflectance spectroscopy using a Bruker MPA NIR spectrometer (Bruker, Ettigen, Germany).

Table 5.1. Nutritional composition[1] of ryegrass/white clover pasture in pregnancy (d213 to d351) and in lactation (d351 to d465). Means ± SEM

	Pregnancy (d213 to d351)	Lactation (d351 to d465)
CP (g/100g DM)	32.3 ± 1.17	21.4 ± 0.79
NDF (g/100g DM)	37.6 ± 1.17	37.7 ± 0.99
ADF (g/100g DM)	17.4 ± 0.58	20.4 ± 0.57
ME (MJ/kg DM)	12.2 ± 0.14	12.0 ± 0.14
SSS (g/100g DM)	6.31 ± 0.64	12.5 ± 0.50
Lipid (g/100 g DM)	4.24 ± 0.13	3.40 ± 0.12

DM: Dry Matter; CP: Crude Protein; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; ME: Metabolizable Energy; SSS: Soluble Sugars and Starch; [1] analysed with near infrared reflectance spectroscopy.

5.3.3. Animal measurements

In this experiment, ewe lamb sex and that of their co-twin were recorded within 18 hours of birth in 2017, during twice daily lambing rounds (Pettigrew et al., 2018). Ewe lambs were weighed at weaning (d86), d113, d127 and then every 17 days from the introduction of vasectomised rams (d145) until the end of the breeding period (d247). Ewe lambs were weighed approximately monthly from d247 to late pregnancy (d351) and again in early lactation (d394) and at weaning (d465). Ewe lambs were weighed unfasted which took a similar amount of time on each weighing day. Ewe lambs were weighed using a purpose-built sheep weighing crate (Tru-Test group, model XR5000). At d337, ewe lambs were shorn, and their fleece weighed.

Body condition scores (BCS; range 1-5 and 0.5 units; Jefferies, 1961, Russel et al., 1969) were also recorded at d213, d247, d301, d351, d394 and d465.

Every 17 days after the introduction of the vasectomised rams, the colour of the crayon attached to the mating harness was changed and the rump crayon marks on ewe lambs were recorded on four occasions (d162, d179, d196, d213). Crayon marks from vasectomised rams were used as an indicator of oestrous behaviour. Marks on the rump of the ewe lambs were recorded using a three-point scoring system: a score of 1 – a fine narrow mark, 2 – a medium mark and 3 – a large area on the rump covered (Rosales Nieto et al., 2015). Only scores 2 or 3 were accepted as indicating that mating had occurred, while a score of 1 was considered to have been the result of accidental contact.

During the breeding period (d213 to d247), the crayon colour on mating harnesses of entire rams was changed after 17 days. After completion of the 34-day breeding period, rams were removed. Ewe lambs were then identified to mating categories: mated only in the first 17-day period (first crayon colour only on their rump), second 17-day period (second crayon colour only), both 17-day periods (both crayon colours) or not mated at all (no crayon colour). At pregnancy diagnosis (d301), pregnancy and the number of foetuses (0, 1 or 2) were determined.

Lambs were tagged within 18 hours of birth, their birth rank, date of birth, sex and dam were identified, and birthweights were recorded. Dam maternal behaviour score (MBS; 1-5 score with 1 = ewe flees and does not return during tagging and 5 = ewe touches recorder during tagging; O'Connor et al., 1985) was recorded. These observations were made by one of two-trained operators. The number of people present during the observation of MBS was also recorded. Lambs were weighed in early lactation (d394) and at weaning (d465). At d465, one-year-old ewes were identified to a ewe lamb status: either 1. weaned at least one lamb or 2. failed to wean a lamb(s) when ewe lambs aborted or whose lamb(s) died before weaning or 3. ewe lambs not diagnosed as pregnant.

5.3.4. Statistical analysis

All statistical analyses were conducted using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). During the lambing period, a one-year-old ewe died in the Heavy group and two died in the Control group. These ewes were excluded from the analyses of lambing and lamb performance. All non-significant ($P > 0.10$) interactions were removed from final models.

The analysis of ewe lamb live weight aimed to compare the treatments from d86 to d465. Live weights of ewe lambs at d86, d213, d301, d351, d394 and d465 were analysed with a linear mixed model allowing for repeated measures (Table 5.2). The analysis of BCS of ewe lambs aimed to compare the treatments from d213 to d465. BCS at d213, d301, d351, d394 and d465 was analysed with a generalised linear model with repeated measures and Poisson distribution and log transformation (Table 5.2). BCS at d465 was analysed using a generalised linear model assuming a Poisson distribution and a log transformation (Table 5.2).

Ewe lambs marked by vasectomised rams with a mark score 2 or 3 between d145 and d213 (hereafter termed mated) were considered to have expressed oestrous behaviour. The proportion of ewe lambs that expressed oestrous behaviour was analysed with a generalised linear model with a binomial distribution and a logit transformation (Table 5.2). A linear model was used to analyse live weight at d213 of ewe lambs based on the presence of a crayon mark (Table 5.2).

The proportion of ewe lambs marked by an entire ram (i.e. mated) during the overall breeding period, first 17-day period only, second 17-day period only and mated in both periods was analysed with generalised linear model with binomial distributions and logit transformations (Table 5.2). Due to the low number of animals with BCS 2.0 and 4.0, the BCS of ewe lambs at breeding was divided in three categories: ≤ 2.5 (n = 144), 3.0 (n = 86) and ≥ 3.5 (n = 40). A logistic regression model was constructed for the proportion and the 95% confidence intervals of ewe lambs mated during breeding with treatment, live weight at d213 and their two-way interaction as fixed effects. A linear model was used to determine the differences in live weight at d213 depending on breeding performance (mated *vs.* non-mated; Table 5.2).

Fertility rate (ewe lambs pregnant/ewe lambs presented for breeding) and the proportion of ewe lambs identified as single- or twin-bearing at d301 were analysed with generalised linear models with binomial distributions and logit transformations (Table 5.2). A logistic regression model was constructed for fertility rate and the 95% confidence intervals with treatment and live weight at d213 as fixed effects. Litter size at d301 was analysed with a generalised linear model with a Poisson distribution and a log transformation (Table 5.2). Two linear models were used to determine the differences in live weight at d213 depending on fertility (pregnant *vs.* non-pregnant) or the number of foetuses identified at d301 (0 *vs.* 1 *vs.* 2; Table 5.2).

The proportion of ewe lambs assisted during lambing, ewe lambs that weaned a lamb, failed to wean a lamb or that were not pregnant at d465 (ewe lamb status) were analysed using generalised linear models with binomial distributions and logit transformations (Table 5.2).

The MBS and the lambing percentage (lambs weaned/ewe presented for breeding) were analysed using generalised linear models with Poisson distributions and log transformations (Table 5.2).

Survival rate of lambs to d465 (weaning) was analysed with a generalised linear model with a binomial distribution and a logit transformation (Table 5.2). Live weights of lambs at birth, d394 and d465 were analysed with a linear mixed model allowing for repeated measures (Table 5.2). Lamb growth from birth to weaning (d465), birth to early lactation (d394) and d394 to d465 were analysed using linear models (Table 5.2).

Table 5.2. Description of the statistical analyses of ewe lamb live weight, body condition score (BCS), the proportion of ewe lambs marked by vasectomised rams, mated in the first, second or both 17-day periods, or not mated, identified as pregnant, single- or twin-bearing at pregnancy diagnosis (d301), litter size, the proportion of ewe lambs assisted during lambing, the proportion of ewes that either weaned a lamb, failed to wean a lamb or were not pregnant as a ewe lamb, maternal behaviour score, lambing percentage (lambs weaned per ewe presented at breeding), lamb survival to weaning, lamb live weights and lamb growth.

Dependent variable	Fixed effects	Covariates	Random effects
Ewe lamb live weights	Treatment (Heavy *vs.* Control); Day of measurement (d213, d301, d351, d394, d465); Number of foetuses (0, 1, 2) Two-way interaction treatment and day of measurement.		
BCS of ewe lambs	Treatment; Day of measurement (d213, d301, d351, d394, d465); Number of foetuses; Two-way interaction treatment and day of measurement.		
BCS at weaning	Treatment; Ewe lamb status; Two-way interaction		
Proportion of ewe lambs marked by vasectomised rams	Treatment; Sex of the co-twin (male, female); Two-way interaction		
Live weight at d213	Either crayon marks by vasectomised rams (yes, no), or Mated *vs.* non-mated, or Pregnant *vs.* non-pregnant, or Number of foetuses Treatment; Sex of the co-twin; Two-way interactions		
Proportion of ewe lambs mated during - 34 days of breeding - first 17 days only - second 17 days only - both 17-day periods	Treatment; Sex of the co-twin; BCS at breeding (≤ 2.5, 3.0, ≥ 3.5); Two-way interactions	Live weight at d213 Liveweight change from d86 to d213	
Proportion of ewe lambs - pregnant at d301 - single-bearing at d301 - twin-bearing at d301	Treatment; Sex of the co-twin; BCS at breeding (≤ 2.5, 3.0, ≥ 3.5); Two-way interactions	Live weight at d213 Liveweight change from d86 to d213	

Table 5.2. continued

Dependent variable	Fixed effects	Covariates	Random effects
Litter size at d301	Treatment; Sex of the co-twin; BCS at breeding (≤ 2.5, 3.0, ≥ 3.5); Two-way interactions	Live weight at d213 Liveweight change from d86 to d213	
Proportions of one-year old ewes assisted during lambing	Treatment; Lamb birth rank; Two-way interaction	Live weight at d213 Liveweight change from d86 to d213	
Ewe lamb status (weaned a lamb or failed to wean a lamb or not pregnant)	Treatment and re-run with number of foetuses	Live weight at d213 Liveweight change from d301 to d351	
Maternal behaviour score	Treatment; Lamb birth rank (single, twin); Lambing paddock; Number of people present; Operator Two-way interactions		
Lambing percentage	Treatment; Sex of the co-twin	Live weight at d213	
Lamb live weights	Treatment; Lamb birth rank; Sex of lambs; Day of measurement (birth, d394, d465); Two-way interactions	Date of birth	Dam Lambing paddocks
Lamb survival to d465	Treatment; Lamb birth rank; Sex of lambs; Date of birth; Lambing paddock Interaction treatment x sex of lambs Interaction treatment x birth rank	Lamb birthweight	
Lamb growth - birth to d394 - d394 to d465 - birth to d465	Treatment; Lamb birth rank; Lamb rearing rank Two-way interactions	Date of birth	Dam

d86: weaning of ewe lambs; d213: start of breeding; d351: late pregnancy; d394: early lactation; d465: weaning of the progeny.

5.4. Results

Pre-grazing ryegrass and white clover pasture masses during the breeding period, pregnancy, and lactation were on average 1129 ± 98, 868 ± 39 and 1265 ± 58 kg DM/ha, respectively.

5.4.1. Ewe live weight

Heavy and Control groups had similar live weights ($P > 0.10$) at d86 (28.7 ± 0.23 *vs.* 29.1 ± 0.26 kg, respectively), however, the Heavy group were heavier ($P < 0.001$) from the start of breeding (d213) to d351 than the Control group (Figure 5.1). Ewe lamb live weight did not differ ($P > 0.10$) between Heavy and Control groups at d394 and d465 (Figure 5.1). At d465, irrespective of treatment, one-year-old ewes that failed to wean a lamb were heavier ($P < 0.001$) than one-year old ewes that weaned a lamb or that were not pregnant (70.1 ± 1.2 *vs.* 61.7 ± 0.5 and 62.1 ± 0.6 kg, respectively). Live weight at d465 did not differ ($P > 0.10$) between one-year-old ewes that weaned a lamb and those that were not pregnant.

5.4.2. Ewe body condition score

Ewe lambs from the Heavy group had greater ($P < 0.05$) BCS at d213 (2.97 ± 0.04 *vs.* 2.51 ± 0.04, respectively) and d301 (2.82 ± 0.04 *vs.* 2.63 ± 0.04, respectively) than Control ewe lambs. At d351, d394 and d465, BCS did not differ ($P > 0.10$) between treatments (average 2.81 ± 0.03 at d351, 2.75 ± 0.03 at d394 and 2.75 ± 0.03 at d465). At d465, irrespective of treatment, BCS did not differ ($P > 0.10$) between non-pregnant ewes and ewes that weaned a lamb and ewes that failed to wean a lamb (data not shown).

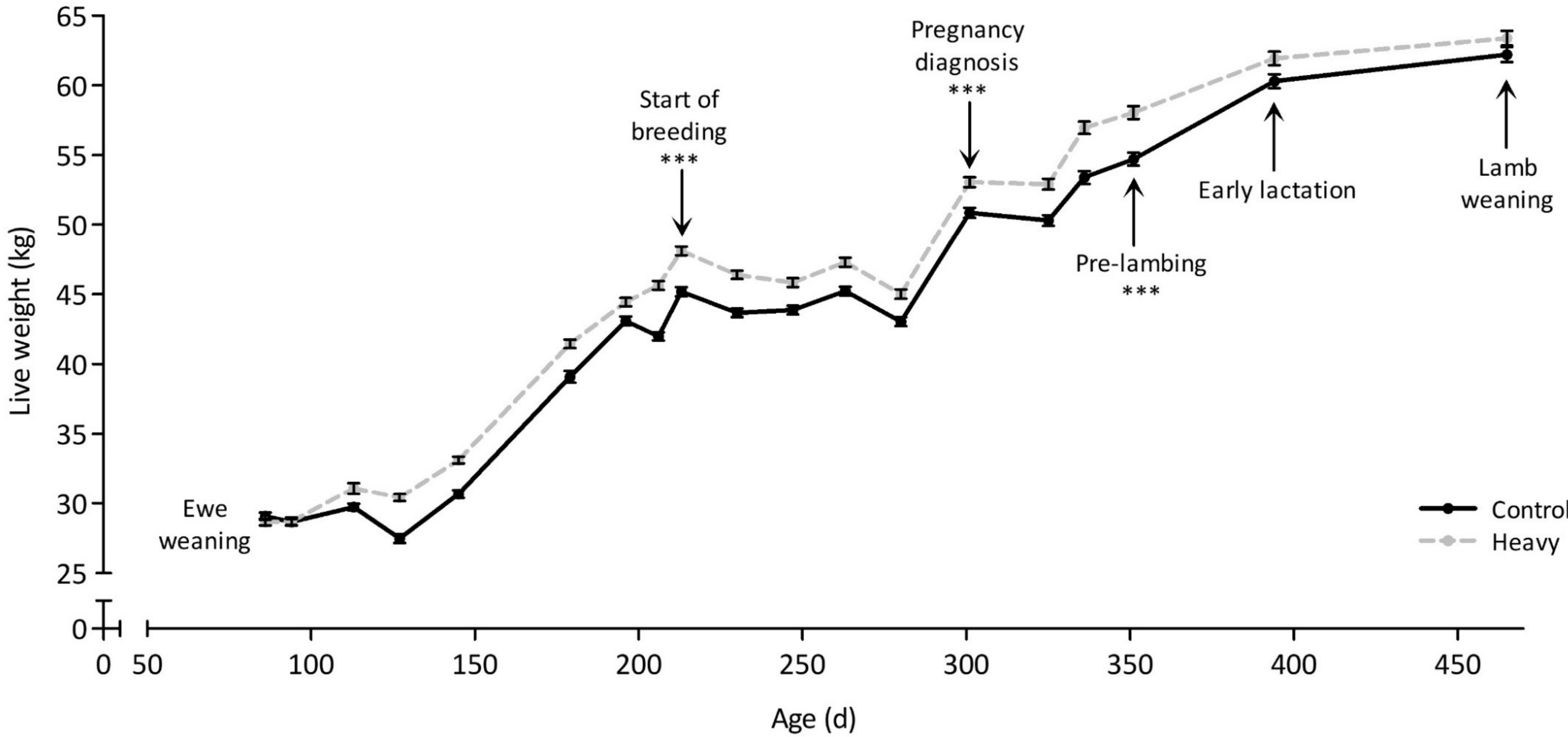

Figure 5.1. Live weight (± SEM) of all ewe lambs in the Heavy (n = 135; dotted line) and the Control (n = 135; solid line) groups from when they weaned as a lamb (d84) to when they weaned their lamb (d465). *** for P < 0.001.

5.4.3. Crayon marks prior to breeding

From d145 to d213, 1.5% of the Heavy group and 3.0% of the Control group had a vasectomised ram harness mark score of 2 or 3 (Table 5.3). Treatment and the sex of the co-twin (data not shown) had no effect ($P > 0.10$) on the proportion of ewe lambs marked by vasectomised rams from d145 to d213.

Marked ewe lambs with a ewe co-twin were heavier than marked ewe lambs with a ram co-twin and non-marked ewe lambs, regardless of the sex of their co-twin (mean ± s.e.m.; 48.7 ± 0.62 *vs.* 45.8 ± 0.78, 46.5 ± 0.35 and 45.7 ± 0.34 kg for marked with ewe co-twin, marked with ram co-twin, non-marked ewe with a ram co-twin and non-marked ewe with a female co-twin, respectively). No other combination between marked ewe lambs and the sex of the co-twin differed in live weight.

5.4.4. Breeding pattern

A greater percentage of ewe lambs in the Heavy group were mated during the 34-day breeding period than Control ewe lambs ($P < 0.05$; Table 5.3). The inclusion of live weight at d213 as a covariate for the proportion of ewe lambs mated during the entire breeding period resulted in the difference between treatments becoming a tendency ($P < 0.10$). BCS at breeding and the sex of the co-twin had no effect ($P > 0.10$) on the proportion of ewe lambs that were mated during the entire breeding period. The probability of being mated during the entire breeding period increased in the Heavy group by 3% for each additional kilogram of live weight up to 45 kg, and then by 2% from 46 to 50 kg with little apparent benefit above 50 kg (Figure 5.2A). In the Control group, the probability of being mated increased by 4% up to 45 kg, 3% from 46 to 50 kg and 2% from 51 to 55 kg with little apparent benefit above 55 kg (Figure 5.2A). Ewe lambs mated during the entire breeding period, irrespective of treatment, were heavier at d213 than ewe lambs that were not mated (46.8 ± 0.25 *vs.* 45.1 ± 0.42 kg; $P < 0.001$). Ewe lambs that were mated and had an ewe co-twin were heavier than ewe lambs that were not mated and ewe lambs that were mated and had a ram co-twin ($P < 0.05$; data not shown).

A greater percentage of Heavy ewe lambs were mated only in the first 17-period compared to Control ewe lambs ($P < 0.05$; Table 5.3). The percentage of ewe lambs that were mated in the first 17-day period did not differ ($P > 0.10$) by BCS at breeding or sex of the co-twin. The addition of live weight at d213 as covariate to the model resulted in treatment effect becoming non-significant ($P > 0.10$). There was no effect ($P > 0.10$) of group or sex of the co-twin (data not shown) on the proportion of ewe lambs identified as being mated in the second 17-day period only or mated in both 17-day periods ($P > 0.10$; Table 5.3). The proportion of ewe lambs identified as mated in the second 17-day period only, did not differ ($P > 0.10$) by group and BCS, however, ewe lambs with a BCS 3.0 were more likely to be identified as mated in both 17-day periods than ewe lambs with BCS ≤ 2.5 ($P < 0.05$). There were no differences between BCS ≤ 2.5 and ≥ 3.5 or between BCS 3.0 and ≥ 3.5.

Ewe lamb live weight showed an interaction ($P < 0.05$) of the sex of the co-twin with mating categories. Ewe lambs, only mated in the first 17-day period only and had ewe co-twin, were heavier than those only mated in the first 17-day period with a ram co-twin (47.8 ± 0.39 *vs.* 46.0 ± 0.40 kg, respectively; $P < 0.05$). Ewe lambs only mated in the first 17-day period with an ewe co-twin were heavier than those that were not mated, irrespective of the sex of co-twin (47.8 ± 0.39 *vs.* 44.8 ± 0.60 kg for ewe co-twin and 45.5 ± 0.59 kg for ram co-twin; $P < 0.05$). Ewe lambs mated in both 17-day periods with a ewe co-twin were heavier than those that were not mated with a ewe co-twin (48.9 ± 1.2 *vs.* 44.8 ± 0.60 kg; $P < 0.05$). No other combination of mating category and the sex of the co-twin differed ($P > 0.10$) in live weight at d213 (data not shown).

5.4.5. Pregnancy status and rank

Ewe lambs in the Heavy group were more likely to be diagnosed as pregnant than the Control group (Table 5.3). The probability of becoming pregnant increased in the Heavy group by 4% for each additional kilogram of live weight up to 45 kg, by 3% from 45 to 50 kg, and by 2% from 50 to 52 kg with little apparent benefit above 52 kg (Figure 5.2B). In the Control group, the probability of becoming pregnant increased by 3% up to 41 kg, 4% from 41 to 50 kg, 3% from 50 to 54 kg and 2% from 54 to 58 kg (Figure 5.2B). The sex of the co-twin had no effect ($P > 0.10$) on the proportion of ewe lambs identified as pregnant (data no shown). Ewe lambs with a BCS at breeding ≥ 3.5 were more likely ($P < 0.05$) to be diagnosed as pregnant than ewe lambs with a BCS ≤ 2.5 (78.0% (58.7 – 89.9) *vs.* 58.2% (48.8 – 66.9), respectively). Ewe lambs with a BCS 3.0 did not differ ($P > 0.10$) from ewe lambs with BCS at breeding ≥ 3.5 and ≤ 2.5 for being pregnant. When adjusted for live weight at d213, the treatment effect became a tendency ($P < 0.10$). Similarly, the addition of liveweight change from d86 to d213 as a covariate in the model resulted in treatment effect becoming non-significant ($P > 0.10$). Ewe lambs diagnosed as pregnant at d301 were heavier ($P < 0.05$) at d213 than non-pregnant ewe lambs, regardless of treatment (47.2 ± 0.27 *vs.* 45.15 ± 0.35 kg, respectively).

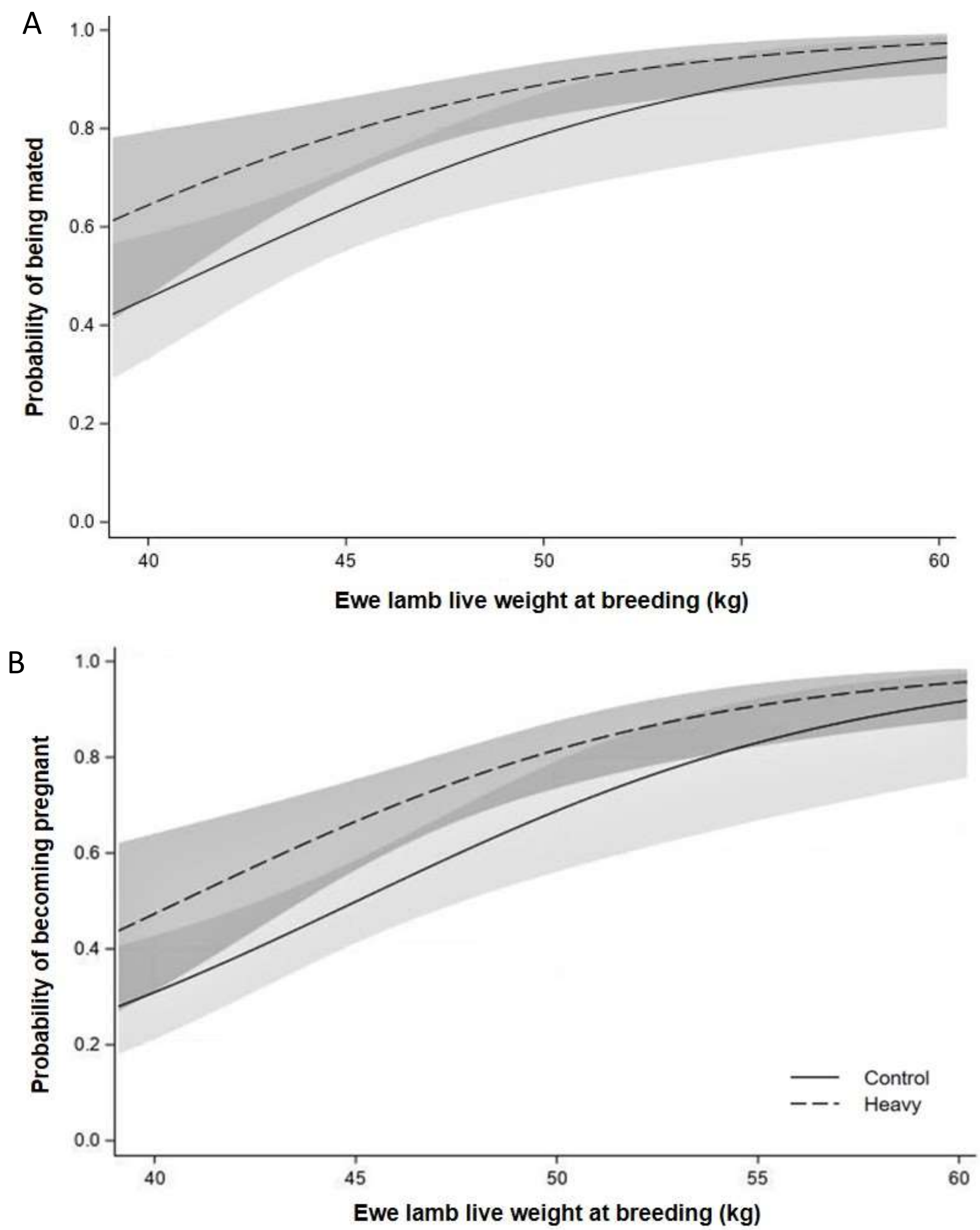

Figure 5.2 (A) The probability of being mated during the breeding period and **(B)** The probability of ewe lambs becoming pregnant in Heavy (n = 135; dotted line) and Control (n = 135; solid line) groups in relation to their live weight at breeding (logit predictions and 95% confidence intervals shown).

Table 5.3. Effect of treatment (Control *vs.* Heavy) on the percentage (95% confidence limits) of ewe lambs identified as marked before breeding, in each mating category (first, second, both 17-day periods or not mated), pregnancy rank at d301 (non-pregnant, single- or twin-bearing), litter size (foetuses/ewe lamb presented for breeding), lambing percentage (lamb weaned/ewe lamb presented for breeding) and on the ewe lamb status at weaning (d465; weaned a lamb, failed to wean a lamb or non-pregnant)[2].

	n	Control	n	Heavy	*P value*
Vasectomised ram crayon mark score (%)					
Marked	4	3.0 (1.1 – 7.6)	2	1.5 (0.4 – 5.7)	NS
Non-marked	131	97.0 (92.4 – 98.9)	133	98.5 (94.3 – 99.6)	NS
Mating category (%)					
First 17-day period only	67	54.8 (43.8 – 65.3)^{a}	93	69.9 (61.3 – 77.3)^{b}	0.020
Second 17-day period only	7	3.5 (1.3 – 9.0)	11	7.5 (3.9 – 13.7)	NS
Both periods	10	9.5 (4.7 – 18.3)	10	6.2 (3.2 – 11.8)	NS
Non-mated	51	30.6 (21.0 – 42.0)^{a}	21	14.9 (9.6 – 22.3)^{b}	0.004
Pregnancy rank at d301 (%)					
Non-pregnant	69	39.6 (29.0 – 51.3)^{a}	34	22.9 (16.2 – 31.4)^{b}	0.006
Single-bearing	56	45.8 (35.5 – 56.5)	69	50.5 (41.9 – 59.1)	NS
Twin-bearing	10	9.1 (4.6 – 17.0)^{a}	32	24.5 (17.8 – 32.7)^{b}	0.004
Litter size at d301	66	0.65 (0.50 – 0.84)^{a}	101	0.99 (0.84 – 1.18)^{b}	0.008
Lambing percentage (%)	60	44.4 (34.5 – 57.2)^{a}	95	70.4 (57.5 – 86)^{b}	0.005
Ewe lamb status[1] (%)					
Weaned a lamb	56	42.1 (34.0 – 50.6)^{a}	83	61.9 (53.5 – 69.8)^{b}	0.001
Failed to wean a lamb	8	5.3 (2.5 – 10.6)^{a}	17	11.9 (7.4 – 18.6)^{b}	0.049
Non-pregnant	69	52.6 (44.2 – 61.0)^{b}	34	26.1 (19.4 – 34.2)^{a}	< 0.001

NS: Non-significant; ^{a,b} Within rows, means with different superscripts are significantly different ($P < 0.05$); [1] Dead ewes (2 Control and 1 Heavy) were not included in the ewe lamb status at d465.

Ewe lambs in the Heavy group were more likely to be diagnosed as twin bearing than Control ewe lambs ($P < 0.05$; Table 5.3). The proportion of ewe lambs carrying twins did not differ by BCS at d213 and sex of the co-twin (data not shown). The inclusion of live weight at d213 as a covariate was not significant ($P > 0.10$), whereas the addition of liveweight change from d86 to d213 resulted in treatment effect on the probability of carrying twins becoming a tendency ($P = 0.081$).

5.4.6. Litter size

Ewe lambs in the Heavy group had greater litter size at d301 than Control group ewe lambs (Table 5.3). On average, for each additional kilogram of live weight at d213, irrespective of treatment, there was a 6.3% increase in litter size ($P < 0.01$). Ewe lambs with a BCS at d213 ≥ 3.5 tended ($P = 0.056$) to have greater litter sizes than those with BCS ≤ 2.5. There were no differences ($P > 0.10$) in litter size between BCS ≤ 2.5 and 3.0 or between BCS 3.0 and ≥ 3.5 at d213 (data not shown). When adjusted for live weight at d213, the treatment effect on litter size became a tendency ($P < 0.10$). The addition of liveweight change from d86 to d213 as a covariate resulted in treatment effect becoming non-significant ($P > 0.10$). Ewe lambs that were identified as twin bearing were heavier at d213, irrespective of treatment, than single-bearing and non-pregnant ewe lambs (48.5 ± 0.54 *vs.* 46.7 ± 0.31 and 45.1 ± 0.35 kg respectively; $P < 0.001$). Non-pregnant ewe lambs were lighter at d213 ($P < 0.01$), irrespective of treatment, compared with single- and twin bearing ewe lambs.

5.4.7. Lambing performance of one-year-old ewes

Treatment had no effect ($P > 0.10$) on the probability of a one-year-old ewe being assisted during lambing. One-year-old ewes carrying singles, irrespective of treatment, however, were more likely to be assisted (P = 0.001; data not shown) than those carrying twins. The addition of lamb birthweight as a covariate resulted in birth rank effect becoming non-significant (P > 0.10). Maternal behaviour score did not differ (P > 0.10) by either treatment or birth rank (data not shown).

One-year-old ewes in the Heavy group weaned more lambs (P < 0.01) than Control ewes (Table 5.3). The inclusion of live weight at d213 as a covariate resulted in treatment effect becoming non-significant (P > 0.10). The lambing percentage did not differ by sex of the co-twin (P > 0.10; data not shown).

One-year-old ewes in the Heavy group were more likely (P < 0.01) to wean a lamb than those in the Control group (Table 5.3). The addition of the number of foetuses in the model resulted in the treatment effect becoming non-significant (*P* > 0.10). When adjusted for live weight at d213, the treatment effect became non-significant (*P* > 0.10). A greater percentage (P < 0.05) of one-year-old ewes in the Heavy group failed to wean a lamb compared with one-year-old ewes in the Control group (Table 5.3). The inclusion of the number of foetuses in the model resulted in the treatment effect becoming non-significant (*P* > 0.10). When adjusted for live weight change between d301 and d351, treatment effect became non-significant (P > 0.10).

5.4.8. Lamb performance

Treatment had no effect (P > 0.10) on lamb birth and weaning weights (Table 5.4). Males were heavier than females at birth (4.5 ± 0.09 *vs.* 4.2 ± 0.09 kg, respectively; P < 0.01). In addition, lambs born as singles, irrespective of treatment, were heavier than twins (3.8 ± 0.12 *vs.* 4.9 ± 0.08 kg, respectively). Lambs reared as singles were heavier at d465 than lambs reared as twins (27.7 ± 0.47 *vs.* 24.3 ± 0.78 g/d; P < 0.01).

Single-born lambs, irrespective of treatment, were more likely to survive to weaning than twin-born lambs (mean percentage (95% confidence limits); 88.3% (81.0 – 93.1) *vs.* 62.7% (47.8 – 75.5); P < 0.001). The addition of lamb birthweight as covariate to the model resulted in the birth rank becoming non-significant (P > 0.10). Sex of the lamb (data not shown), lambing paddock (data not shown), date of birth (data not shown) and dam treatment had no effect on the probability of lambs surviving to weaning (Control 83.1% (70.2 – 91.1) *vs.* Heavy 78.8 (68.7 – 86.2); P > 0.10). Lambs that survived to d465 were heavier at birth (P < 0.05) than lambs that did not, regardless of treatment (4.46 ± 0.08 *vs.* 3.98 ± 0.12 kg, respectively).

Treatment and the sex of the lamb had no effect (P > 0.10) on lamb growth from birth to d394 (data not shown), d394 to d465 (data not shown) or birth to d465 (Table 5.4). Lambs reared as a single had greater growth rates, irrespective of treatment, than lambs reared as twins from birth to d394 (368 ± 19 *vs.* 276 ± 33 g/d, respectively; P < 0.05), d394 to d465 (198 ± 5.2 *vs.* 187 ± 8.6 g/d; P < 0.05) and birth to d465 (240 ± 4.7 *vs.* 206 ± 7.9 g/d; P < 0.01).

Table 5.4. Effect of treatment (Control *vs.* Heavy) on lamb live weight at birth and weaning, growth from birth to weaning and survival rate. Least square means ± SEM for birth and weaning weight of lambs and lamb growth from birth to weaning, and percentage (95% confidence limits) of lambs that survived to weaning.

	n	Control	n	Heavy	*P value*
Lamb birth weight (kg)	73	4.35 ± 0.11	130	4.34 ± 0.08	NS
Single	55	4.87 ± 0.11	68	4.91 ± 0.09	
Twin	18	3.95 ± 0.25	62	3.75 ± 0.13	NS
Lamb weaning weight (kg)	60	26.2 ± 0.52	95	25.8 ± 0.42	NS
Reared as Single	52	28.0 ± 0.63	71	27.4 ± 0.50	
Reared as Twins	8	23.5 ± 1.36	24	24.4 ± 0.88	NS
Lamb growth to weaning (g/d)	60	225.9 ± 5.2	95	220.5 ± 4.2	NS
Reared as Single	52	245.1 ± 6.3	71	236.5 ± 5.0	
Reared as Twins	8	195.7 ± 13.5	24	207.4 ± 8.8	NS
Survival rate[1] (%)	60	83.1 (70.2 – 91.1)	95	78.8 (68.7 – 86.2)	NS
Single	49	91.6 (80.7 – 96.6)	57	85.5 (74.5 – 92.2)	
Twin	11	67.0 (39.1 – 86.5)	38	69.1 (53.3 – 81.3)	NS

NS: Non-significant; [1] Number of lambs at weaning/number of lambs born.

5.5. Discussion

5.5.1. Ewe lamb live weight and BCS

Ewe lambs from the Heavy group were approximately 3 kg heavier than Control ewe lambs by breeding, thus demonstrating the success of the two feeding treatments. Despite both treatments being managed together after breeding, the live weight difference between treatments persisted until their last live weight measurement in late pregnancy. This finding was consistent with the results of Thompson et al. (2019) who reported that a difference of 2.2 kg at breeding was maintained until pre-lambing. The absence of a liveweight difference during lactation could be due to the Control group showing compensatory growth as a result of having a lower maintenance nutritional requirement (Nicol and Brookes, 2017). It could also be due to the Heavy group having a greater percentage of twin-bearing ewe lambs, which have greater requirements in late pregnancy and lactation (Kenyon et al., 2008a), which may have negatively affected their growth and live weight. Ewe BCS differed between treatments at d213 (breeding) and d301 (pregnancy diagnosis) with Heavy having greater BCS than Control, but no longer differed thereafter. These differences in BCS, however, were relatively small and corresponded to the liveweight differences observed, as one BCS unit in ewe lambs has been reported to be equal to approximately 7.0 kg of live weight (Kenyon et al., 2014b).

5.5.2. Ewe lamb reproductive performance

It was hypothesised that heavier ewe lambs at breeding would have improved reproductive performance compared to lighter ewe lambs. A greater percentage of ewe lambs in the Heavy group was mated, diagnosed as pregnant and twin-bearing than the Control ewe lambs. These percentages, combined, resulted in a greater litter size, lambing percentage and a greater percentage of ewe lambs that weaned a lamb in the Heavy compared with the Control group, which supported the hypothesis.

In the current experiment, prior to the breeding period, there was a low percentage of ewe lambs that were marked by the vasectomised rams in both treatments with no difference found between treatments. A low percentage (< 4%) of ewe lambs being marked prior to breeding with similar live weight profiles was reported in previous studies (Kenyon et al., 2005, Corner et al., 2013). This may have been due to the potential negative effects of the phytoestrogen, coumestan, which can be found in lucerne and can inhibit the expression of oestrous (Kelly et al., 1976). It is generally recommended ewes are removed from lucerne for approximately three weeks prior to breeding to reduce the potential negative effects (McGaveston, 2012). In this experiment, ewe lambs were removed from lucerne swards 27 and 22 days before breeding for the Control and Heavy group, respectively. The low percentage of ewe lambs marked by vasectomised rams, observed in the present experiment, suggest that few ewe lambs had attained puberty prior to breeding. More than 50% of ewe lambs in both treatments, however, were mated in the first 17 days of breeding, indicating either they had reached puberty before mating or that the timing of breeding matched the onset of puberty. These low percentages may be explained by the first oestrus being silent or of weak intensity, and for a shorter duration among ewe lambs compared to adult ewes (Dýrmundsson, 1981, Kenyon et al., 2014c), and therefore oestrous may not have been detected by the vasectomised rams.

Ewe lambs from the Heavy group were more likely than the Control group to be mated during the 34-day breeding period. The inclusion of breeding live weight in the model changed the treatment effect to a tendency, indicating that the greater live weight of the Heavy compared to the Control group explained much of the observed treatment effect. These results are consistent with Kenyon et al. (2004a, 2009, 2014c), who reported a positive association with ewe lamb breeding live weight and percentage of ewes mated during the breeding period. In the current experiment, ewe lambs from the Heavy group were more likely to be mated in the first 17-day period of breeding only. This finding is consistent with previous studies Kenyon et al. (2004a, 2005), who reported a positive relationship between the live weight of ewe lambs at breeding and the proportion mated by entire rams in the first 17 days of breeding. It is known that heavier ewes attain puberty at an earlier age than lighter ewes (Valasi et al., 2012, Edwards and Juengel, 2017). Farmers should therefore aim to breed heavier ewe lambs to increase the number of ewe lambs mated in the breeding period and thus increase their probability of becoming pregnant.

In this experiment, the probability of a ewe lamb being mated by the entire ram during the 34-day breeding period and fertility plateaued at approximately 50 kg and 52 kg for ewe lambs in the Heavy group and at 55 kg and 58 kg for the Control group, respectively. This plateauing effect has been previously reported in ewe lambs for fertility (Rosales Nieto et al., 2013a, Corner-Thomas et al., 2015b) and reproductive rates (Corner-Thomas et al., 2014b, 2015b). Corner-Thomas et al. (2015b), with Highlander composite, Romney, Coopworth and Romney × Finnish and Coopworth × Finnish Landrace crosses breeds, reported that fertility and reproductive rate plateaued for ewe lambs live weights greater than 47.5 kg. The reason for the difference in live weight at which performance plateaued between studies is unknown but may be due to breed or liveweight range used. Corner-Thomas et al. (2015b) reported a range of breeding live weights of 32.5 kg to > 52.5 kg, whereas the range of breeding live weight in this experiment was 39.1 kg to 60.2 kg. Combined these data suggest there is a plateau in Romney-type ewe lambs in terms of reproduction between 48 and 55 kg. Farmers should, therefore, breed their ewe lambs between 50 and 55 kg to maximise the proportion of ewes bred and their fertility. More research is needed to determine why the plateau occurs and why they differed between treatments.

Ewe lambs in the Heavy group were more likely to be identified as pregnant than ewe lambs in the Control group. A positive correlation was found between live weight at breeding and fertility (ewes pregnant per ewe bred) as has previously been reported (Ferguson et al., 2011, Paganoni et al., 2014a, Corner-Thomas et al., 2015b, Thompson et al., 2019). This positive correlation may have been due to an early attainment of puberty that led to a greater proportion of ewe lambs being mated during the 34-day breeding season resulting in an increased probability of becoming pregnant. In addition to increase the proportion of ewe lambs mated during the breeding period, aiming for heavier ewe lamb live weight at breeding increase the proportion of ewe lambs pregnant.

Ewe lambs in the Heavy group gave birth to a greater litter size than Control ewe lambs and a greater percentage of ewes were diagnosed as twin-bearing in the Heavy group compared to the Control group. This finding is in agreement with previous studies that also reported a positive relationship between ewe lamb live weight at breeding and the percentage of twin-bearing ewes (Drew et al., 1973, Bichard et al., 1974, Kenyon et al., 2005, Thompson et al., 2019) and litter size (Paganoni et al., 2014a, Corner-Thomas et al., 2015b). The magnitude of the increase in litter size was similar to that reported among Merino ewe lambs (4.5 - 4.8%; Rosales Nieto et al., 2013a,b). These increases in litter size and proportion of ewe lambs identified with twins could be related to an earlier puberty attainment that led to ewe lambs being mated during their second or third oestrous. It has been reported that ewes in their second and third oestrous have a greater number of viable embryos than those in their first oestrous (Hare and Bryant, 1985, Kenyon et al., 2014c). A greater percentage of one-year-old ewes in the Heavy group lost their lambs either at birth or during lactation compared to the Control group. This difference between treatments was likely due to the greater percentage of twin litters born to ewe lambs in the Heavy group. This was further supported when the number of foetuses was added to the model, the treatment effect was no longer significant. Twin lambs born to ewe lambs generally have lower survival rates than singles which is driven by lower birthweights, a greater rate of dystocia and starvation exposure (Sawalha et al., 2007, Young et al., 2010, Pettigrew et al., 2018). Given twin born lambs born to ewe lambs have display lower survival and weaning weights (Pettigrew et al., 2020), and ewe lambs that have twins are generally lighter at weaning (Schreurs et al., 2010b) than those that had singles, this is something that farmers need to consider when setting breeding live weight target.

A greater percentage of one-year-old ewes in the Heavy group weaned a lamb than in the Control group. This difference between treatments was likely due to the greater number of ewe lambs that were pregnant in the Heavy group combined with the greater number of twin-bearing ewe lambs. Further, when the number of foetuses was added as a covariate to the model, the treatment effect was no longer significant. These results are in agreement with Griffiths et al. (2016) who reported that multiple-bearing ewe lambs were more likely to rear a lamb to mid-lactation than single-bearing ewe lambs. In the current experiment 169% more ewe lambs in the Heavy group were twin-bearing than in the Control group.

Heavy ewes weaned 58% more lambs (per ewe presented for breeding) than the Control group, matching previous research that reported a positive relationship between ewe lamb live weight at breeding and lambing percentage (Kenyon et al., 2004c, 2014c). The Heavy group weaned 0.7 lambs per ewe presented for breeding and the Control group weaned 0.4 lambs. Previously, the lambing percentage of ewe lambs in New Zealand was reported to be approximately 60% (Kenyon et al., 2004c). The difference of lambing percentage between treatments was driven by the greater proportion of ewes identified as pregnant and a greater twining rate in the Heavy compared to the Control group, combined with similar survival rate of lambs from birth to weaning between treatments.

5.5.3. Lamb performance

Lamb live weights at birth, weaning and lamb growth rate from birth to weaning did not differ between treatments. The absence of difference in birthweight supports the previous findings (Mulvaney et al., 2010, Thompson et al., 2019), but also contrasts with studies that reported a small positive effect of breeding live weight on lamb live weight at birth of less than 100 g per 1 kg of live weight (Kenyon et al., 2006, Schreurs et al., 2010b, Corner-Thomas et al., 2014a). Combined, these data suggest that the impact of live weight at breeding of ewe lambs was relatively small.

Farmers should, therefore, recognise that increased lamb birthweight will not be one of the benefits of heavier ewe lamb live weight at breeding.

Previous studies have reported positive associations between live weight at breeding and lamb growth (Kenyon et al., 2006) and between ewe lamb live weight at breeding and lamb weaning weight (Mulvaney et al., 2010, Schreurs et al., 2010b, Kenyon et al., 2014c). Mulvaney et al. (2010) reported an increase of 284 g weaning live weight per additional kilogram of ewe lamb live weight at breeding between two treatments with 6 kg-difference ('Light' had an average 36.2 kg and 'Heavy' had an average of 42.6 kg at breeding). In the present experiment, treatments differed by 3 kg at breeding and thus may have limited the identification of difference in lamb weaning live weight. In addition, in the previous studies, lambs were weaned either earlier (71 and 81 days old; Mulvaney et al., 2010, Schreurs et al., 2010b) or later at 109 days of age (Kenyon et al., 2006) than the present experiment. Similarly, ewe lamb live weights at breeding were lower (Kenyon et al., 2006, Mulvaney et al., 2010, Morris et al., 2005) or similar (Corner-Thomas et al., 2014a) to the Control group and lower than the Heavy group in this experiment. More research is needed to investigate whether there is a plateau at which lamb weaning weight is not affected by ewe lamb live weight at breeding. Lamb growth and weaning weight, in this experiment, were not affected by heavier live weight of ewe lambs at breeding. Hence, there appears to be little benefit for farmers in terms of lamb growth and weaning weight from increasing breeding live weight of ewe lambs.

Treatment did not affect survival of progeny to weaning. Birthweight was one of the main factors that affected lamb survival (Everett-Hincks and Dodds, 2008, Schreurs et al., 2010a, Mulvaney et al., 2012, Dwyer et al., 2015), thus the absence of a difference in lamb birthweight likely explains why no survival effects were identified in this experiment and why twin-born lambs had a lower survival rates than single-born lambs. Farmers therefore need to consider whether increasing the ewe lamb live weight at breeding is likely to result in more ewe lambs carrying twins and a lower survival rate of those lambs from birth to weaning.

5.6. Conclusion

Ewe lambs bred at an average of 47.9 kg (Heavy) had greater breeding performance, fertility, litter size and lambing percentage and had similar live weights at the weaning of their progeny than ewe lambs bred at an average of 44.7 kg (Control). The difference in breeding live weight of ewe lambs, however, did not impact their progeny's live weight, survival or growth to weaning. Curvilinear relationships between ewe lamb live weight at breeding and ewe lamb breeding performance and fertility were identified. These data suggest that the optimal ewe lamb live weight at breeding would be between 50 and 55 kg, but may vary based on breed. More research is needed to determine the key factors for optimal ewe lamb live weight at breeding. These results suggest that farmers should bred their ewe lambs between 50 and 55 kg to maximise fertility and lambing percentage without reducing ewe live weight nor the performance of their progeny to weaning.

Chapter 6

Breeding heavier ewe lambs did not impact their subsequent two and three-year-old ewe live weight and reproductive performance

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6.1. Abstract

The current experiment investigated the effects of growing ewe lambs to a heavier live weight at breeding on their subsequent reproductive performance and live weight at two and three years of age, and the growth of their progeny to weaning. Two groups of ewe lambs were bred at seven months of age at an average pre-breeding live weight of either 47.9 ± 0.36 kg (Heavy; n = 135) or 44.9 ± 0.49 kg (Control; n = 135) (Chapter 2, 4 and 5). Breeding performance, fertility, litter size, lambing percentage, ewe wither height, live weight, progeny survival, and live weight were recorded at two and three years of age. None of the variables measured differed between the Heavy and Control ewes at either two or three years of age ($P > 0.10$). This suggests that farmers can breed their Romney-type ewe lambs at an average live weight of 48 kg without any negative impacts on reproductive performance at two or three years of age nor on their progeny's live weight or growth to weaning. Although breeding heavier ewe lambs improved reproductive performance during their first year, further investigations are needed to assess their overall lamb production and efficiency over multiple breeding seasons.

6.2. Introduction

Ewe lambs can be bred at seven months of age and potentially improve farm income through an increase of the number of lambs born on farm per year and increased ewe lifetime production (Kenyon et al., 2014c). Ewe lambs need to reach approximately 60% of their mature live weight in order to attain puberty and be successfully bred (Kenyon et al., 2014a,c). Currently, the recommended minimum pre-breeding live weight target for Romney-based ewe lambs is 40 kg (Kenyon et al., 2014c). Heavier live weights at breeding have been shown to result in a greater percentage of ewe lambs mated (Kenyon et al., 2005), increased litter size and fertility rate (Corner-Thomas et al., 2015b), and a greater lambing percentage (Kenyon et al., 2014c) compared to lighter ewe lambs. In 2018, a study was designed (Chapter 5) to investigate the impact of a heavier live weight at breeding at seven months of age on subsequent reproductive performance of the ewes and on the growth and survival to weaning of their progeny. Two groups of ewe lambs were bred at seven months of age at an average pre-breeding live weight of either 47.9 ± 0.36 kg (Heavy group) or 44.9 ± 0.49 kg (Control group). The results of that experiment (Chapter 5) showed that a greater percentage of ewe lambs in the Heavy group were mated during the breeding period, and had a greater fertility, litter size and lambing percentage than Control ewe lambs. The live weight difference at breeding, however, had no effect on the live weight, growth, or survival of their progeny (Chapter 5).

At breeding at 18 months of age, ewes first bred as a ewe lamb can have lower live weights than those that were not bred (McMillan and McDonald, 1983, Kenyon et al., 2008b, Keady and Hanrahan, 2021). Further, some studies have shown at weaning at two years of age, there is a reduction in live weight of ewes first bred as a ewe lamb compared to ewes that were not (Baker et al., 1978, Kenyon et al., 2008b). Kenyon et al. (2011b) and Thomson et al. (2020) both reported that ewes that had lambed as a ewe lamb were lighter at breeding at 30 months of age than ewes that had not lambed, but differences were no longer apparent at weaning at three years of age. Fertility, litter size and lambing percentage of two-year-old ewes, first bred as a ewe lamb, have been reported to be either lower (Baker et al., 1978, Kenyon et al., 2008b), similar (McMillan and McDonald, 1983, Kenyon et al., 2008b) or improved (Moore et al., 1983, Afolayan et al., 2008) compared to two-year-old ewes not bred as a ewe lamb. The growth, weaning weight and survival to weaning of lambs born to two-year-old ewes that were first bred as a ewe lamb, have been reported to be lower (Afolayan et al., 2008, Morel et al., 2010), similar (Cannon and Bath, 1969) or improved (McCall and Hight, 1981, McMillan and McDonald, 1983) compared to the progeny born to ewes not bred as a ewe lamb. At three years of age, the litter size of ewes that had lambed as a ewe lamb was either similar (Baker et al., 1978, 1981, Ponzoni et al., 1979) or greater (Morel et al., 2010, Kenyon et al., 2011b)to ewes that had not lambed as a ewe lamb. These inconsistent results may be due to the reduction in live weight at breeding at two years of age, however, more research is needed to better understand these inconsistencies.

Thomas and Berger (2009) investigated the impacts of prepubertal growth rate of dairy ewe lambs on their subsequent reproduction and milk production. Ewe lambs were offered either *ad libitum* or restricted feeding between being weaned at 50 days of age and five months of age, which resulted in a 1.8 kg difference in live weight at their first breeding. No differences between treatments in fertility, number of lambs born per ewe lambing and milk production to four years of age were reported (Thomas and Berger, 2009). Currently, no studies have investigated the effects of a heavy breeding live weight of ewe lambs at two and three years of age on the performance of their progeny to weaning. In the 2018 study (Chapter 5), ewe lambs in the Heavy group were 3-kg heavier than the Control ewe lambs at breeding and throughout pregnancy, however, there was no difference in live weight at the weaning of their progeny at 16 months of age.

The current experiment examined the effects of a heavier ewe lamb live weight at breeding at seven months of age on their reproductive performance, body size and live weight during their second and third breeding seasons at two and three years of age and on the performance of their progeny to weaning. Further, it enabled the examination of the impacts of weaning of a lamb as a ewe lamb on subsequent performance. It was hypothesised firstly, that ewes bred as a ewe lamb at a heavier live weight would have similar reproductive performance to those bred at a lighter live weight, and secondly that ewes that weaned a lamb as a ewe lamb would be lighter during their second and third breeding season compared to ewes that failed to wean a lamb.

6.3. Material and methods

The experiment was conducted at Massey University's Riverside farm (latitude: 40° 50' 35 S, longitude: 175° 37' 55 E), 10 km north of Masterton, and Keeble farm (latitude: 40° 24' 03 S, longitude: 175° 35' 51 E), 5 km south of Palmerston North, New Zealand. The experiment and all animal handling procedures were approved by the Massey University Animal Ethics Committee (MUAEC-17/16).

6.3.1. Experimental design

6.3.1.1. Background

The overall experimental design has been previously described in Chapters 2 and 5. Briefly, at weaning, 270 twin-born Romney ewe lambs were allocated to one of two treatments so that live weight did not differ between treatments (28.6 kg ± 0.16; 03/01/2018) at approximately 86 days of age (d86). Post-weaning, the 'Heavy' group (n = 135) was preferentially fed until breeding (10/05/2018; d213), achieving an average live weight of 47.9 ± 0.38 kg and the 'Control' group achieved an average of 44.9 ± 0.49 kg at d213. Both treatments were managed as a single cohort and grazed on ryegrass/white clover pasture under commercial New Zealand grazing conditions from d213 until the weaning of their first lambs (d465).

6.3.1.2. Present experiment

The present experiment reports the performance of ewes from both treatments between the weaning of their first lambs at one year of age (d465, 17/01/2019) until the weaning of their third set of lambs at 39 months of age (d1159, 11/12/2020).

Two years of age

At d465, live weight of ewes in the Heavy (n = 131) and Control group (n = 130) did not differ (61.9 ± 0.89 kg and 61.5 ± 0.96 kg respectively; Chapter 5). Prior to d479, ewes were managed at Riverside farm after which they were transported to Keeble farm in January 2019. At d567 (29/04/2019), crayon-harnessed entire Romney rams, at an approximate ratio of 1:60, were introduced for 34 days (d567 to d601), with crayons changed after 17 days (d584). Ewes were shorn by commercial operators at d638. Pregnancy diagnosis was determined at d660 using trans-abdominal ultrasound (31/07/2019). All ewes were drenched with anti-anthelmintics at d589 and d683.

In late pregnancy (d701), pregnant ewes from the Heavy (n = 119) and the Control groups (n = 124) were allocated to one of 11 lambing paddocks (average stocking rate 13.8 ewes/ha) based on their pregnancy rank: single (total n = 40; n = 5, 4, 3, 5, 4, 2, 2, 3, 7, 2, 3; total n = 40 Heavy and 5, 12, 10, 7, 4, 2, 2, 7, 3, 2, 3; total n = 57 Control per lambing paddock), twin (n = 2, 15, 11, 12, 4, 3, 2, 4, 17, 3, 2; total n = 75 Heavy and 2, 17, 10, 13, 4, 3, 2, 3, 7, 2, 2; total n = 65 Control respectively) or triplet (n = 3, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1; total n = 4 Heavy and n = 2, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0; total n = 2 Control) and the period of conception (first or second 17-day period). Ewes from both treatments were present in each lambing paddock. Non-pregnant ewes (n = 8 Heavy; n = 4 Control) were separated from the pregnant ewes at d701 and grazed separately until weaning (d800). Pregnant ewes were vaccinated against clostridial diseases at d689, approximately 15 days prior to the start of lambing. During the lambing period (eight days before the expected start of lambing until all ewes had lambed), ewes were checked twice daily to identify newly-lambed ewes.

In early lactation (d746), when lambs were on average 24 days of age, they were ear-marked and their tail was docked. At d800, when lambs were on average 78 days of age, lambs were weaned and vaccinated against clostridial diseases and scabby mouth. Between d567 and d701, six ewes died or were euthanised due to welfare issues (3 Control and 3 Heavy) and between d701 and d800, five ewes died or were euthanised (4 Control and 1 Heavy).

At d810 ewes were shorn by a commercial operator. Ewes whose lamb died at birth or during lactation remained with the lactating ewes until d800. All ewes were drenched with anti-anthelmintics at d834 and d901. Between d800 and d913, three ewes died or were euthanised (2 Control and 1 Heavy).

Three years of age

At d913 (9/04/2020), ewes (Heavy, n = 125 and Control n = 122) were bred to crayon harnessed Suffolk rams at an approximate ratio of 1:50 for 34 days (d913 to d947). Ewe were shorn by a commercial operator at d962 and pregnancy diagnosis was determined at d995 (30/06/2020) by trans-abdominal ultrasound. At d1050 (24/08/2020), ewes (n = 245) were allocated to 12 lambing paddocks based on their treatments: Heavy (n = 11, 17, 16, 11, 7, 10, 5, 8, 5, 8, 12, 15 per lambing paddock, respectively) or Control (n = 9, 16, 14, 13, 8, 16, 5, 4, 2, 9, 15, 9, respectively) and on the period of conception (first or second 17-day period). Ewes from both treatments were present in each lambing paddock. Pregnant ewes were vaccinated against clostridial diseases at d1037, approximately 15 days prior to the start of lambing. Non-pregnant ewes (n = 3 Heavy; n = 2 Control) were grazed with lactating ewes until weaning (d1159). During the lambing period, ewes were checked twice daily to identify newly-lambed ewes. In early lactation (d1095), at an average of 27 days of age, lambs were ear-marked and their tail was docked. At d1159, at an average of 91 days of age, lambs were weaned and vaccinated against clostridial diseases and scabby mouth. Ewes whose lamb died at birth or during lactation remained with the lactating ewes until d1159. Between d913 and d1050, two Control ewes died or were euthanised and between d1050 and d1159, one ewe in the Heavy group was euthanised.

6.3.2. Feeding management

The Heavy and Control ewes were grazed together on ryegrass/white clover pasture under commercial grazing conditions from the weaning of their first lambs (d465) until the end of the current experiment (d1159). The pre-grazing pasture masses were recorded at pre-breeding (n = 1) and from d567 to d701 (n = 15). Pre-grazing pasture masses were recorded fortnightly between d701 and d800 (n = 41), d913 and d1050 (n = 14) and d1050 to d1159 (n = 40). The pre-grazing pasture sward height was firstly assessed using a rising plate meter (Ashgrove Pastoral Products, New Zealand, 80 readings minimum per paddock) and then using a standard calibration the herbage mass was estimated (herbage mass = [158 x average meter reading] + 200; Hodgson et al., 1999). At two years of age, the pre-grazing pasture masses during pregnancy (d567 to d701), lactation (d701 to d800) were on average 2209 ± 167 kg DM/ha, 1592 ± 63 kg DM/ha, respectively. At three years of age, the pre-grazing pasture masses in pregnancy (d913 to d1050) and lactation (d1050 to d1159) were on average, 1814 ± 144 kg DM/ha and 1433 ± 586 kg DM/ha, respectively.

Due to dry summer conditions (Beef + Lamb New Zealand, 2019b), ewes were supplemented with one bale of grass baleage per day (approximately 0.9

kg/ewe/day) from d504 to d524 and half a bale from d524 to d592 (approximately 0.5 kg/ewe/day; Table 6.1). Ewes were also supplemented with approximately 200 g/ewe/day of a cereal-based supplement from d537 to d611 (Table 6.1).

One sample of grass baleage was taken at d547 and one sample of cereal-based supplement was taken at d554 to determine their nutritional composition (Table 6.1). Samples were analysed using *in-vitro* methods to determine the nutritional quality; dry matter (DM) and crude protein (CP; Dumas' procedure, AOAC method 968.06 using a Leco total combustion method, LECO corporation, St, Joseph, MI, USA; AOAC, 2019). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were analysed by a Tecator Fibretec System (Robertson and Van Soest, 1981). Metabolizable energy (ME) was estimated using the digestible organic matter in dry matter (Roughan and Holland, 1977).

Grass grab samples (Frame, 1993; n = 97) were collected to estimate the nutritional composition of the pasture offered (Table 6.1). Grab samples were collected at pre-breeding (n = 1), during pregnancy (d567 to d701; n = 15), fortnightly between d701 and d800 (n = 41), pregnancy at three years of age (d913 to d1050; n = 14) and from d1050 to d1159 (n = 14). Grab samples were frozen at -20°C until analysis. The percentage of CP, ADF, NDF, lipid and ME (MJ/kg) on DM basis was estimated with near infrared reflectance spectroscopy (NIR) using a Bruker MPA NIR spectrometer (Bruker, Ettigen, Germany).

Table 6.1. Nutritional composition of ryegrass/white clover pasture in 2019 (d567 to d800) and 2020 (d906 to d1159), cereal-based supplement and grass baleage (mean ± SEM).

	Ryegrass/white clover pasture (d567 to d800)[1]	Ryegrass/white clover pasture (d906 to d1159)[1]	Cereal-based supplement[2, 3]	Grass baleage[2, 3]
DM (%)	-	-	86.2	91.7
CP (g/100g DM)	21.0 ± 0.53	22.3 ± 0.72	9.7	11.5
NDF (g/100g DM)	43.2 ± 0.69	42.0 ± 0.89	14.6	52.9
ADF (g/100g DM)	21.3 ± 0.44	21.5 ± 0.57	4.4	31.4
ME (MJ/kg DM)	11.3 ± 0.11	11.3 ± 0.14	-	-
SSS (g/100g DM)	3.67 ± 0.06	3.68 ± 0.06	-	-
Lipid (g/100 g DM)	12.0 ± 0.26	14.3 ± 0.52	-	-

DM: Dry Matter; CP: Crude Protein; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; ME: Metabolizable Energy; SSS: Soluble Sugars and Starch; [1] analysed with near infrared reflectance spectroscopy; [2] analysed using wet chemistry methodology; [3] only one sample was taken hence no standard error.

6.3.3. Animal measurements

At d465, ewe lambs were retrospectively identified to one of three ewe lamb status categories: 'weaned a lamb' if the ewe lamb had weaned at least one lamb, 'failed to wean a lamb' if the ewe lamb was diagnosed as pregnant as a ewe lamb but failed to wean a lamb or 'not pregnant' if the ewe lamb was diagnosed as not pregnant.

Ewes were weighed unfasted at the start and end of each breeding period (d567 and d601, and d906 and d947), at pregnancy diagnosis (d660 and d995), in late pregnancy (d701 and d1050), early lactation (d746 and d1095) and at weaning (d800 and d1159). At d638, ewes were shorn and their fleeces were weighed. The ewe wither height (Cockrem and Rae, 1959) was recorded at d535, d567, d660, d701, d800, d995 and d1159. The wither height was used as a measure of skeletal development and body size of the ewe (Gregory, 1933, Holmes, 1973). Body condition score (BCS; range 1-5 and 0.5 units; Jefferies, 1961, Russel et al., 1969) was recorded at d567, d601, d660, d746, d800, d995, d1050, d1095 and d1159.

After completion of each 34-day breeding period, ewes were identified to one of four mating categories: mated in the first period only (first crayon colour only on their rump), mated in the second period only (second crayon colour only), mated in both 17-day periods (both crayon colours) or not mated at all (no crayon colour). At pregnancy diagnosis (d660 and d995), fertility (pregnant or not pregnant) and the number of foetuses (0, 1, 2 or 3) was determined.

During each lambing period, lambs were tagged within 18 hours of birth, their birth rank, date of birth, sex, dam, and birthweight were recorded. Maternal behaviour score (MBS; 1-5 score with 1 = ewe flees and does not return during tagging and 5 = ewe touches recorder during tagging; O'Connor et al., 1985) was recorded. These observations were made by one of two-trained operators. The number of people present during the MBS observation was also recorded. Lambs were weighed in early lactation (d746 and d1095) and at weaning (d800 and d1159).

Ewe status at d800 and d1159 was identified as: 'weaned a lamb' if a ewe had weaned at least one lamb, 'failed to wean a lamb' if a ewe was diagnosed pregnant but failed to wean a lamb or 'not pregnant' if a ewe was diagnosed as not pregnant.

6.3.4. Statistical analysis

All statistical analyses were conducted using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). At two years of age, triplet lambs were excluded from the lamb performance analyses (i.e. lamb survival, lamb live weight and lamb growth) due to the low number of lambs (n = 6 Control & n = 12 Heavy). Data from each year were analysed separately.

Ewe live weights and wither heights at two and three years of age and lamb live weights were analysed using linear mixed models allowing for repeated measures (Table 6.2). Body condition scores at two and three years of age were analysed using generalised linear models for repeated measures with a Poisson distribution and log transformation (Table 6.2). Fleece weight at d638 and lamb growth to weaning were analysed using linear mixed models (Table 6.2).

The proportion of ewes that were marked by a ram (i.e. mated) in the first period only, second period only, both 17-day periods and not mated at all, identified with 0, 1, 2 or 3 foetuses at d660 and at d995, not pregnant, failed to wean a lamb and weaned at least one lamb at d800 and at d1159 and lamb survival to weaning were analysed with generalised linear model with a binomial distribution and logit transformation (Table 6.2). Models in which treatment was significant ($P < 0.05$) were re-run with live weight at d567 or live weight at ewe lamb breeding (d213) fitted as a covariate.

Litter size at d660 and d995, ewe MBS and lambing percentage (lambs weaned/ewe presented for breeding) were analysed using generalised linear models with a Poisson distribution and log transformation (Table 6.2).

Table 6.2. Description of the statistical analyses of ewe live weight, body condition score (BCS), wither height, fleece weight, the proportion of ewes mated in the first, second or both 17-day periods, or not mated, identified as not pregnant, single-, twin or triplet-bearing at pregnancy diagnosis (d660 and d995), litter size, the proportion of ewes that were not pregnant or failed to wean a lamb or weaned a lamb at weaning (d800 and d1159), maternal behaviour score, lambing percentage (lambs weaned per ewe presented for breeding), lamb survival to weaning, lamb live weights and lamb growth to weaning.

Dependent variable	Fixed effect	Covariate	Random effect
Ewe live weights and BCS - d567 to d800 - d906 to d1159	Treatment (Control *vs.* Heavy); Day of measure (d567, d660, d701, d746, d800 and d906, d995, d1050, d1095, d1159); Ewe lamb status (wean a lamb, failed to wean a lamb, not pregnant); Number of foetuses (0, 1, 2, 3) Interaction treatment x day of measure Interaction ewe lamb status x day of measure		
Ewe wither height	Treatment; Ewe lamb status; Day of measure (d567, d660, d701, d746, d800 and d995, d1050, d1095, d1159); Number of foetuses Interaction treatment x day of measure Interaction ewe lamb status x day of measure		
Fleece weight at d638	Treatment; Ewe lamb status		
Proportion of ewes mated - in first 17-day period - in second 17-day period - in both periods - not mated	Treatment; Ewe lamb status		
Proportion of ewes at d660 and d995 - not pregnant - single-bearing - twin-bearing - triplet-bearing	Treatment; Ewe lamb status; Ewe status at d800	Live weight at d567	
Litter size	Treatment; Ewe lamb status;		
Proportion of ewes at d800 and d1159 that - were not pregnant - failed to wean a lamb - weaned a lamb	Treatment; Ewe lamb status Ewe status at d800 only included in the model for d1159.		
Maternal behaviour score	Treatment; Ewe lamb status; Observer; Number of people present; Lambing paddock	Date of birth	

Table 6.2. continued

Dependent variable	Fixed effect	Covariate	Random effect
Lambing percentage	Treatment; Ewe lamb status		
Lamb survival	Treatment; Ewe lamb status; Birth rank; Sex of the lamb	Date of birth	
Lamb live weights	Treatment; Ewe lamb status; Sex of the lamb; Birth rank; Day of measure (birth, d746, d800 and birth, d1050, d1159) Interaction treatment x day of measure Interaction ewe lamb status x day of measure	Date of birth	Ewe
Lamb growth to weaning	Treatment; Ewe lamb status; Sex of the lamb; Birth rank	Age at weaning	Ewe

d567: two-year-old ewe breeding; d638: shearing; d701: late pregnancy at two years of age; d746: early lactation; d906: three-year-old ewes breeding; d1050: late pregnancy at three years of age; d1095: early lactation.

6.4. Results

6.4.1. Ewe live weight, BCS, fleece weight and wither height

Ewe live weight, BCS, wither height (Table 6.3) and fleece weight at d638 (4.33 ± 0.05 kg Control *vs.* 4.42 ± 0.04 kg Heavy) did not differ ($P > 0.10$) between treatments at any time point between d567 and d1159.

Ewes that had weaned a lamb as a ewe lamb, irrespective of treatment, were lighter ($P < 0.05$) between d567 and d701 than the two other ewe groups (i.e. ewes that were not pregnant and ewe that failed to wean a lamb as a ewe lamb; Figure 6.1). The live weight of ewes that failed to wean a lamb did not differ ($P > 0.10$) at any time point from those that were not pregnant as a ewe lamb (Figure 6.1). From d746 to d1159, ewe live weights did not differ ($P > 0.10$) by ewe lamb status (i.e. weaned a lamb, failed to wean a lamb and not pregnant; Figure 6.1).

Ewes that weaned a lamb as a ewe lamb had a lower BCS at d567 ($P < 0.05$) than those that were non pregnant or failed to wean a lamb (2.74 ± 0.07 *vs.* 3.18 ± 0.08 and 3.38 ± 0.17 BCS, respectively), irrespective of their treatment. At d660, ewes that weaned a lamb had lower BCS than ewes that were not pregnant as a ewe lamb ($P < 0.05$; 3.15 ± 0.08 *vs.* 3.42 ± 0.09, respectively). BCS did not differ ($P > 0.10$) by ewe lamb status between d701 and d1159.

Ewes that were not pregnant as a ewe lamb had a heavier ($P < 0.001$) fleece than those that weaned a lamb or failed to wean a lamb as a ewe lamb (4.61 ± 0.04 kg *vs.* 4.15 ± 0.04 kg and 4.36 ± 0.09 kg, respectively). The wither height of ewes did not differ ($P > 0.10$) at any time point between ewe lamb status or the number of foetuses conceived at two- and three years of age (data not shown).

Table 6.3. Effect of treatment (Control *vs.* Heavy) on live weight, body condition score (BCS) and wither height of ewes from breeding at two-years of age (d567) to the weaning of their third litter (d1159). Least square means ± SEM

	Live weight (kg)		BCS		Height (cm)	
	Control	Heavy	Control	Heavy	Control	Heavy
2 years of age[2]						
Breeding (d567)	68.2 ± 0.88	68.7 ± 0.86	3.00 ± 0.09	3.14 ± 0.09	63.6 ± 0.32	63.7 ± 0.31
Pregnancy diagnosis (d660)	68.3 ± 0.86	68.2 ± 0.85	3.26 ± 0.09	3.34 ± 0.09	59.1 ± 0.29	59.8 ± 0.28
Late-pregnancy (d701)	75.9 ± 0.88	76.7 ± 0.86	3.22 ± 0.10	3.23 ± 0.09	62.2 ± 0.30	62.6 ± 0.29
Early lactation (d746)	71.0 ± 1.07	71.1 ± 1.04	2.74 ± 0.08	2.73 ± 0.07	-	-
Weaning (d800)	70.7 ± 1.02	70.7 ± 0.95	2.65 ± 0.08	2.64 ± 0.08	62.8 ± 0.33	63.1 ± 0.31
3 years of age[1,2]						
Breeding (d906)	66.9 ± 0.95	67.0 ± 0.88	-	-	-	-
Pregnancy diagnosis (d995)	75.2 ± 0.94	74.7 ± 0.88	3.25 ± 0.08	3.25 ± 0.09	60.7 ± 0.40	60.8 ± 0.38
Late-pregnancy (d1050)	93.5 ± 1.03	93.1 ± 0.96	3.60 ± 0.08	3.64 ± 0.08	-	-
Early lactation (d1095)	75.6 ± 1.01	76.0 ± 0.94	2.91 ± 0.08	2.91 ± 0.08	-	-
Weaning (d1159)	73.0 ± 1.15	73.2 ± 1.08	2.88 ± 0.08	2.94 ± 0.09	63.9 ± 0.42	64.6 ± 0.39

[1] Due to Covid-19, BCS and height were not recorded at d906; [2] The wither height was not recorded at d746, d1050 and d1095.

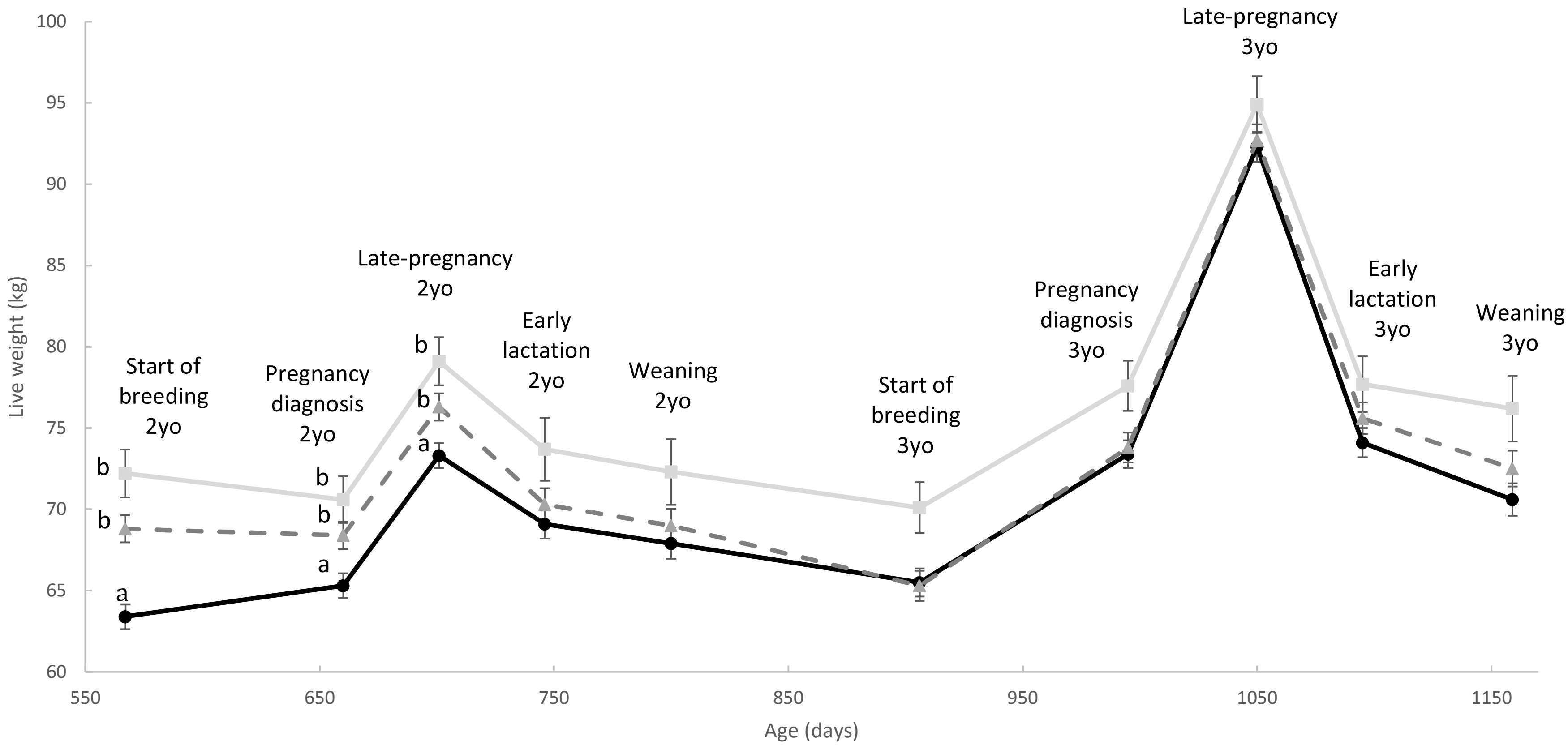

Figure 6.1. Live weight (± SEM) of ewes at two (2yo) and three years of age (3yo) of ewe lamb that failed to wean a lamb as a ewe lamb (n = 24; square – grey solid line), were not pregnant (n = 103; triangles – dotted line) or weaned a lamb as a ewe lamb (n = 134; circles – black solid line) from the start of breeding at two-years of age (d567) to the weaning of their third litter (d1159). Means with differing letters (a, b) are significantly different ($P < 0.05$).

6.4.2. Breeding pattern

At two years of age, treatment had no effect (P > 0.10) on the proportion of ewes that were not mated during the 34-day breeding period or those mated only in the second 17-day period (Table 6.4). Ewes in the Control group, however, were more likely to be mated in the first 17 days of the period than the Heavy group (P < 0.01; Table 6.4). More ewes from the Heavy group were mated in both 17-day periods (i.e. returned to service) than the Control group (P < 0.01; Table 6.4). The treatment effects on the proportion of ewes mated only in the first 17-day period and on the proportion of ewes mated in both periods remained significant even after the addition of breeding live weight at d213 or d567 as a covariate.

At three years of age, all ewes were mated during the 34-day breeding period. Treatment had no effect (P > 0.10) on the proportion of ewes mated only in the first, second or in both 17-day periods of the breeding period of three-year-old ewes (Tables 6.4). Ewe lamb status had no effect (P > 0.10) on the proportion of ewes in each mating category at either two or three years of age (Table 6.5).

6.4.3. Pregnancy rank and litter size

Neither treatment or ewe lamb status had an effect (P > 0.10) on the proportion of ewes identified as not pregnant, twin- and triplet-bearing at d660 or d995 and a litter size at two and three years of age (Table 6.4 & 6.5). At two years of age, ewes in the Control group tended to be more likely (P = 0.06) to be identified as single-bearing than the Heavy group (Table 6.4). When adjusted for live weight at d567, the tendency was no longer apparent (P > 0.10). At three years of age, the proportion of ewes identified as single-bearing at d995 did not differ between treatments (P > 0.10; Table 6.4).

Ewe lamb status had no effect (P > 0.10) on the proportion of ewes identified as single-bearing at two years of age (Table 6.5). At three years of age, however, ewes that were not pregnant as a ewe lamb were more likely (P < 0.05) to be identified as single-bearing compared to those that weaned a lamb. Three-year-old ewes that weaned a lamb as a ewe lamb were more likely (P < 0.01) to be identified as twin-bearing than ewes that were not pregnant (Table 6.5). Ewe status at d800 had no effect (P > 0.10) on the proportion of ewes identified as not pregnant, single-, twin- and triplet-bearing nor on litter size at d995.

6.4.4. Lambing performance of ewes

Treatment had no effect (P > 0.10) on the ewe MBS at two and three years of age (percentage (95% confidence limits); two years of age Heavy 3.18 (2.7–3.7) *vs.* Control 3.03 (2.6–3.6) and at three years of age Heavy 3.48 (2.6–4.6) *vs.* Control 3.26 (2.5–4.3)). At two years of age, ewes that were not pregnant as a ewe lamb had lower (P < 0.05) scores than ewes that weaned a lamb and ewes that failed to wean a lamb (2.53 ± 0.20 *vs.* 3.31 ± 0.24 and 3.56 ± 0.49, respectively). At three years of age, ewe lamb status had no effect (P > 0.10) on the MBS (data not shown).

At two and three years of age, treatment and ewe lamb status had no effect (P > 0.10) on the proportion of ewes that weaned a lamb, failed to wean a lamb or were not pregnant at d800 and d1159, respectively (data not shown), and on lambing percentage (lambs weaned/ewes presented for breeding; Tables 6.4 & 6.5). Ewes that weaned a lamb at d800 were more likely (P < 0.01) to wean a lamb at d1159 than ewes that failed to wean a lamb or were not pregnant at d800 (percentage (95% confidence limits); 92.9% (87.5–96.1) *vs.* 71.1% (45.6–87.9) and 66.4% (36.4–87.2), respectively). Ewe status at d800 had no effect (P > 0.10) on the proportions of ewes that failed to wean a lamb and were not pregnant at d800 and d1159 (data not shown).

6.4.5. Lamb performance

Survival to weaning of the progeny of two and three-year-old ewes did not differ (P > 0.10) between treatments (Table 6.6). At two-years of age, ewe lamb status had no effect (P > 0.10) on lamb survival, however, at three years of age, lambs born to ewes that were not pregnant as a ewe lamb were more likely (P < 0.01) to survive compare to those born to ewes that failed to wean a lamb (87% (79.1-92.2) *vs.* 62.5% (43.8-78.1), respectively). There was no difference (P > 0.10) in lamb survival between lambs born to a ewe that had weaned a lamb, was not pregnant or failed to wean a lamb as a ewe lamb (data not shown). At two and three years of age, female lambs tended (P = 0.09 and P = 0.08) to have a greater survival than male lambs, irrespective of ewe treatment (at two years of age 90.2% (84.7-93.9) *vs.* 84.4% (77.5-89.5) and at three years of age 81.5% (72.4-88.1) *vs.* 73.0% (62.2-81.5)).

The live weight of lambs born to two- and three-year-old ewes did not differ (P > 0.10) between treatments (Table 6.6) or ewe lamb status (data not shown) at any time. At two years of age, single lambs were heavier (P < 0.05) than twin lambs at all time points, irrespective of treatment (7.97 ± 0.15 *vs.* 6.58 ± 0.14 kg at birth; 14.3 ± 0.32 *vs.* 12.1 ± 0.28 kg at d746; 29.1 ± 0.43 *vs.* 24.8 ± 0.37 kg at d800, respectively). Similarly, at three years of age, single lambs were heavier (P < 0.001) than twin lambs which were heavier (P < 0.001) than triplet lambs at all time points (7.2 ± 0.11

Table 6.4. Effect of treatment (Control *vs.* Heavy) on the percentage (95% confidence limits) of ewes identified as mated in the different mating categories, pregnancy ranks and litter size at pregnancy diagnosis (d660 and d995) and lambing percentage at two and three years of age.

	n	Control	n	Heavy	*P value*
2 years of age					
Mating categories (%)					
First 17-day period	108	82.5 (73.4–89.0)[a]	87	67.4[b] (57.2–76.1)[b]	0.006
Second 17-day period	5	4.0 (1.4–10.8)	6	4.3 (1.6–10.7)	NS
Both 17-day periods	6	5.6 (2.4–12.2)[a]	26	19.6[b] (12.7–29.1)[b]	0.001
Non-mated	10	6.0 (2.5–13.9)	8	6.2 (2.7–13.7)	NS
Pregnancy rank at d660 (%)					
Non-pregnant	4	4.1 (1.5–10.7)	8	7.6 (3.8–14.8)	NS
Single-bearing	57	41.0 (30.8–52.2)	40	29.7 (21.2–39.8)	0.06
Twin-bearing	66	52.2 (41.5–62.6)	75	59.4 (49.2–68.8)	NS
Triplet-bearing[1]	2	-	4	-	NS
Litter size at d660[2]	129	1.48 (1.24–1.76)	127	1.56 (1.32–1.83)	NS
Lambing percentage[3] (%)	124	115.0 (94.5–139.8)	126	127.5 (106.4–152.8)	NS
3 years of age					
Mating categories					NS
First 17-day period	113	92.1 (83.8–96.3)	117	92.8 (85.6–96.5)	NS
Second 17-day period[4]	2	-	0	-	-
Both 17-day periods	7	4.8 (1.8–12.2)	8	6.4 (2.8–14.1)	NS
Pregnancy rank at d995 (%)					
Non-pregnant	2	1.68 (0.42–6.47)	3	2.42 (0.78–7.23)	NS
Single-bearing	33	25.5 (17.1–36.3)	27	24.5 (16.8–34.3)	NS
Twin-bearing	83	69.9 (58.9–78.9)	88	66.2 (55.9–75.2)	NS
Triplet-bearing	3	3.1 (0.96–9.6)	7	5.7 (2.5–12.5)	NS
Litter size at d995[2]	121	1.76 (1.49–2.07)	125	1.75 (1.50–2.04)	NS
Lambing percentage[3] (%)	119	132.8 (109–161)	123	135.8 (113–163)	NS

[a,b] Means between columns with differing superscripts are different ($P < 0.05$). NS: Non-significant ($P > 0.10$); [1] The proportion of ewes bearing triplets were very low (10^{-5}) and therefore were not display in this table; [2] Foetuses at pregnancy diagnosis/ewes presented for breeding; [3] Lambs weaned/ewes presented for breeding; [4] The proportion of ewes mated in the second 17-day period of breeding in the Heavy group was 0, therefore, no model was run.

Table 6.5. Effect of ewe lamb status (weaned a lamb *vs.* failed to wean a lamb *vs.* non-pregnant) on the percentage (95% confidence limits) of ewes identified as mated in the different mating categories, pregnancy ranks and litter size at pregnancy diagnosis (d660 and d995) and lambing percentage at two and three years of age.

		n	Weaned a lamb	n	Failed to wean a lamb	n	Non-pregnant
2 years of age							
Mating categories (%)	First 17-day period	98	77.0 (68.8–83.6)	17	72.0 (50.5–86.6)	80	78.0 (68.5–85.2)
	Second 17-day period	7	5.3 (2.5–10.8)	1	4.5 (0.6–26.2)	3	2.9 (0.9–8.9)
	Both 17-day periods	20	11.8 (7.1–19.2)	5	16.8 (6.5–37.1)	7	6.0 (2.7–12.9)
	Non-mated	6	4.6 (2.1–9.9)	1	4.5 (0.6–26.2)	11	10.7 (5.9–18.7)
Pregnancy rank at d660 (%)	Non-pregnant	5	3.4 (1.4–8.4)	3	11.9 (3.6–32.6)	4	4.1 (1.6–10.5)
	Single-bearing	48	37.6 (29.6–46.3)	7	28.9 (13.6–51.1)	42	39.6 (30.4–49.6)
	Twin-bearing	74	55.8 (47.1–64.2)	14	57.8 (36.8–76.4)	53	53.7 (43.8–63.3)
	Triplet-bearing[2]	4	2.9 (0.9–7.6)	0	-	2	2.1 (0.5–7.9)
Litter size at d660		131	1.58 (1.38–1.81)	24	1.44 (1.02–2.04)	101	1.54 (1.31–1.80)
Lambing percentage[1] (%)		127	126.8 (109–148)	23	102.3 (68.9–151.7)	100	136.9 (115–163)
3 years of age							
Mating categories (%)	First 17-day period	119	94.4 (88.6–97.3)	20	90.3 (68.1–97.6)	91	92.1 (84.7–96.1)
	Second 17-day period[2]	1	-	1	-	0	-
	Both 17-day periods	6	4.6 (2.1–10.1)	1	4.4 (0.6–26.2)	8	8.3 (4.2–15.8)
Pregnancy rank at d995 (%)	Non-pregnant[2]	1	0.79 (0.11–5.4)	0	-	4	4.1 (1.54–10.4)
	Single-bearing	18	14.3 (9.2–21.7)[a]	6	28.7 (13.4–51.2)[ab]	36	35.6 (25.6–45.8)[b]
	Twin-bearing	101	80.4 (72.6–86.6)[a]	14	62.8 (40.9–80.5)[ab]	56	57.5 (47.3–667.1)[b]
	Triplet-bearing	6	4.3 (1.8–9.8)	2	8.2 (1.9–29.2)	2	3.2 (1.0–9.9)
Litter size at d995		126	1.89 (1.66–2.15)	22	1.82 (1.33–2.48)	98	1.57 (1.34–1.84)
Lambing percentage[1] (%)		126	152.4 (132–176)	20	113.7 (76–170)	96	139.7 (118–166)

[a,b] Means between columns with differing superscripts are different ($P < 0.05$); [1] Lambs weaned/ewes presented for breeding; [2] The proportion of ewes bearing triplets, mated in the second 17-day period and non-pregnant were 0, therefore the model was not run.

vs. 6.0 ± 0.07 *vs.* 4.8 ± 0.19 kg at birth; 17.1 ± 0.35 *vs.* 14.1 ± 0.20 *vs.* 11.2 ± 0.57 kg at d1095; and 32.6 ± 0.62 *vs.* 27.6 ± 0.36 *vs.* 23.8 ± 0.99 at d1159, respectively). The growth to weaning of lambs born to two- and three-year-old ewes did not differ (P > 0.10) between treatments (Table 6.6) or ewe lamb status (data not shown).

Table 6.6. Effect of treatment (Control *vs.* Heavy) on lamb birth and weaning (d800 and d1159) weights, growth from birth to weaning (d800 and d1159) and survival rate. Least square means ± SEM for birth and weaning weights of lambs and lamb growth from birth to weaning, and percentage (95% confidence limits) of lambs that survived to weaning (d800 and d1159).

	n	Control	n	Heavy	*P value*
2 years of age					
Lamb birth weight (kg)	180	7.32 ± 0.15	186	7.23 ± 0.15	NS
Lamb weight at d800 (kg)	153	27.0 ± 0.42	160	26.3 ± 0.43	NS
Lamb growth birth to d800 (g/d)	153	251.4 ± 3.5	160	248.7 ± 3.6	NS
Survival rate[1] (%)	153	87.0 (77.6–92.9)	160	87.6 (78.5–93.2)	NS
3 years of age					
Lamb birth weight (kg)	206	6.01 ± 0.10	223	6.04 ± 0.09	NS
Lamb weight at d1159 (kg)	171	28.4 ± 0.46	185	27.9 ± 0.41	NS
Lamb growth from birth to d1159 (g/d)	171	253.7 ± 6.9	185	245.0 ± 6.2	NS
Survival rate[1] (%)	171	75.1 (64.4–83.4)	185	80.4 (71.8–86.9)	NS

NS: Non-significant (P > 0.10); [1] Lambs that survived to weaning (d800 and d1159) / lambs born.

6.5. Discussion

6.5.1. Treatment effects

Ewe live weight, BCS and wither height did not differ between the Heavy and Control group from breeding to weaning their lambs at either two or three years of age. Despite a significant difference in live weight at their first breeding and pregnancy as a ewe lamb (Chapter 5), ewes from the two treatments had similar body sizes (live weight and height) at two and three years of age. Previous studies have reported that when live weights differed at weaning of the first litter, it also differed at breeding at two years of age (Dýrmundsson, 1973, Baker et al., 1978, McMillan and McDonald, 1983). The two treatments in the current experiment differed by 3-kg at their first breeding, although this difference was no longer present at the weaning of their first set of lambs (Chapter 5). This absence of a difference at weaning may explain why ewe live weight did not differ at breeding at two years of age and subsequently. These results suggest that heavier live weight at ewe lamb breeding did not impact the growth and development of the ewe nor the mature size up to 39 months of age.

It was hypothesised that ewes in the Heavy and Control group would have similar reproductive performance during their second and third breeding seasons. Fertility, litter size, maternal behaviour score, lambing percentage, and ewe status at weaning did not differ between treatments at two or three years of age which supports this hypothesis. In addition, lamb performance did not differ between treatments. Previous studies have reported a positive relationship between ewe breeding live weight and reproductive performance (Brown et al., 2005, 2015, Scaramuzzi et al., 2006) and with the growth of their progeny (Brown et al., 2005, Schreurs et al., 2012). The similar live weight and BCS of ewes observed throughout the present experiment, likely explain the absence of a difference in reproductive performance and performance of their progeny to weaning between treatments.

The results from the current experiment suggest that a heavier ewe lamb live weight at breeding did not affect subsequent live weight, BCS, reproductive performance of ewes at two and three years of age nor the performance of their progeny to weaning. Farmers can, therefore, breed their ewe lambs at an average live weight of 48 kg without any negative impacts on the performance to three years of age. Given that heavier ewe lamb breeding live weights improved reproductive performance (Chapter 5), further investigation is needed to determine the effect of the efficiency of ewes bred as a ewe lamb at a heavier live weight over the three breeding seasons.

6.5.2. Impact of ewe lamb status on performance at two and three years of age.

It was hypothesised that ewes that weaned a lamb as a ewe lamb would be lighter at their second breeding compared with ewes that failed to wean a lamb and those that were not pregnant. Two-year-old ewes that weaned a lamb as a ewe lamb were lighter from breeding to pre-lambing and had a lower BCS at breeding than those that failed to wean a lamb or did not conceive as a ewe lamb. These differences were consistent with previous studies of Keane (1974), Kenyon et al. (2008b) and Thomson et al. (2020). Differences in live weight were likely due to the effects of the previous lactation on the two-year-old ewe. Milk production has a high energy requirement which is met from both nutrition and body reserves (Akers, 2002a) which can lead to a loss of live weight and BCS. The live weight difference observed during pregnancy between ewes that weaned a lamb or were not pregnant as a ewe lamb was presumably due to the carry-over effects of a lighter live weight at breeding. During the period between lactation at two years of age and weaning at three years of age, the differences in live weight and BCS were no longer observed. These results support previous findings that the impacts of lambing as a ewe lamb on live weight do not persist after the second breeding season (Cannon and Bath, 1969, Keane, 1974, Baker et al., 1978, 1981). Kenyon et al. (2011b) and Thomson et al. (2020), however, reported a live weight difference persisted until pre-lambing at three years of age between ewes that had lambed compared with those that were not pregnant. Kenyon et al. (2008b) reported a greater difference in live weight at the second breeding than in the current experiment which may explain the persistence of live weight difference in their trial. Combined, the results suggest the reduced live weight and BCS at breeding and during pregnancy at two years of age of ewes that weaned a lamb as a ewe lamb did not persist in lactation at two years of age or at three years of age and did not negatively affect their mature live weight.

At two and three years of age, breeding performance, fertility, litter size and lambing percentage did not differ between ewe lamb status. These results support previous studies that reported no differences in breeding performance (Kenyon et al., 2008b) and fertility (Baker et al., 1981, Kenyon et al., 2008b, 2011b) between ewes that lambed as a ewe lamb and those that did not. Other studies, however, reported improved ewe fertility at two years of age (McCall and Hight, 1981, Moore et al., 1983, Fogarty et al., 2007), litter size (Afolayan et al., 2008, Morel et al., 2010) and the number of lamb weaned between two and four years of age (Baker et al., 1981, McCall and Hight, 1981, McMillan and McDonald, 1983, Fogarty et al., 2007) for ewes that lambed as a ewe lamb compare to those that had not.

Lamb live weights and growth rate to weaning did not differ between ewe lamb status at two or three years of age. These results support previous studies that lambing as a ewe lamb had no effect on the birthweight, weaning weight and growth rates of lambs born to two- and three-year-old ewes (Keane, 1974, McMillan and McDonald, 1983, Kenyon et al., 2008b). These results, however, contrast with Kenyon et al. (2011b) who reported that three-year-old ewes that had lambed as a ewe lamb gave birth to lighter lambs than ewes that had not lambed, although this difference was no longer present at weaning. The results suggest that ewe lamb status does not impact growth or live weight of the progeny to weaning during the second and third breeding seasons of the ewe. At three years of age, however, lambs born to ewes that were not pregnant as a ewe lamb were more likely to survive to weaning than lambs born to those that failed to wean a lamb, which had a high mortality rate (37.5%). These results indicate that ewes that failed to wean a lamb as a ewe lamb would potentially negatively impact lamb survival.

The difference in the performance of ewes and their progeny at two and three years of age with previous studies could be due to the experimental design. Firstly, different classifications of ewe lambs were used in the current experiment. In previous studies, ewe lambs were identified as "mated and lambed" or "mated but not lambed" (Baker et al., 1981, McMillan and McDonald, 1983, Moore et al., 1983, Kenyon et al., 2008b, 2011b). In this experiment, however, ewe lambs were identified as either "weaned a lamb", "not pregnant" or "failed to wean a lamb" which included ewes that were diagnosed as pregnant but either aborted their lamb or whose entire litter died at birth or during lactation, which was similar to Fogarty et al. (2007). The classification used in the current experiment accounted for the potential physiological consequences of a pregnancy and a lactation on the performance of ewes for those that weaned a lamb. Lactation can lead to physiological changes such as a loss of body condition and live weight (Akers, 2002a, Cranston et al., 2017). Physiological changes related to lactation could potentially impact subsequent ewe performance. Secondly, these differences could be due to the breed used. Baker et al. (1981), McMillan and McDonald (1983), Kenyon et al. (2008b) and (2011b) used Romney or crossbred Romney and Border Leicester, whereas Moore et al. (1983) used Romney, Coopworth and Perendale, Fogarty et al. (2007) used crossbred Merino and Border Leicester, East Friesian, Finnsheep, Coopworth, White Suffolk, Corriedale and Booroola Leicester. Breeds differ in their reproductive performance, for example Romney have an average lambing percentage of 110 to 150%, Merino 75 to 110% and East Friesian 160 to 330% (Cranston et al., 2017). These differences could lead to different physiological responses between breeds to the same treatment or event. Finally, in some studies ewes that lambed as a ewe lamb were preferentially fed which could have limited the identification of potential negative impacts of lambing as a ewe lamb (Keane, 1974, Moore et al., 1983, Thomson et al., 2020). The absence of a difference in reproductive performance at two-years of age despite a live weight difference at breeding is, however, unclear and requires more investigation.

6.6. Conclusion

Breeding ewe lambs at a heavier live weight had no effect on ewe live weight, BCS and reproductive performance during their second and third breeding seasons or on the performance of their progeny to weaning. Live weight and BCS of ewes that weaned a lamb as a ewe lamb were reduced during their second breeding and pregnancy, but these impacts did not persist after their second lambing. Successfully weaning of a lamb as a ewe lamb did not impact subsequent reproductive performance of ewes at two and three years of age or live weights of their progeny. These results suggest that farmers can breed their Romney-type ewe lambs at an average live weight of 48 kg without any negative impact on reproductive performance of two and three-year-old ewes or live weight of their progeny to weaning. Breeding heavier ewe lambs was, however, reported to improve reproductive performance during the first breeding season. Further investigations would be needed to assess the overall production and efficiency of ewes bred at a heavier live weight as a ewe lamb over multiple breeding seasons.

Foreword to Chapter 7

During their first breeding season (Chapter 5), ewe lambs from the Heavy group had greater reproductive performance and were heavier during pregnancy than ewe lambs from the Control group. During their second and third breeding seasons (Chapter 6), however, neither reproductive performance nor ewe live weight differed between treatments. In addition, treatments had no effect on performance of the progeny to weaning over the three breeding seasons (Chapters 5 and 6). In Chapter 7, data from Chapters 5 and 6 were combined to determine the effect of breeding heavier ewe lambs on efficiency and overall lamb production of ewes over their first three breeding seasons.

Chapter 7

Effect of breeding heavier ewe lambs on lamb production and efficiency over their first three breeding seasons and their mature weight

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7.1. Abstract

The purpose of this experiment was to examine the effect of breeding heavier ewe lambs on lamb production and their efficiency over their first three breeding seasons. Two groups of ewe lambs were bred at seven months of age at an average pre-breeding live weight of either 47.9 ± 0.36 kg (Heavy; n = 135) or 44.9 ± 0.49 kg (Control; n = 135). Ewe live weight, number of lambs born and weaned, and lamb live weight were recorded, and efficiency was calculated for each ewe. Although the total lamb production did not differ between treatments, when data were pooled, heavier ewe lambs at breeding had a greater number and weight of lambs at weaning over the three-year period. Breeding heavier ewe lambs had no effect on efficiency over the three-year period. There was, however, a positive relationship between ewe lamb breeding live weight and their mature weight. These results suggest that, although, breeding heavier ewe lambs had a positive effect on total lamb production, it had no effect on efficiency over the first three breeding seasons. Before final recommendations can be made, lifetime performance and longevity to five years of age of heavier ewe lambs at breeding is required.

7.2. Introduction

Breeding ewe lambs at seven months of age can increase farm productivity, the number of lambs born per ewe in her lifetime and enable early selection of replacement ewes (Kenyon et al., 2014c). A significant driver of the reproductive performance of ewe lambs is ensuring they are of suitable live weight at breeding, with the positive relationship between live weight at breeding and reproductive performance being well documented (Mulvaney et al., 2010, Corner-Thomas et al., 2014b, 2015b, Kenyon et al., 2014c). Kenyon et al. (2014c) recommended that a minimum live weight target should be 40 kg at breeding for Romney-type ewe lambs in New Zealand. To date, studies have focused on the effects of breeding a ewe lamb on lifetime performance and efficiency compared to those not bred until 17-19 months of age (Baker et al., 1981, Morel et al., 2010, Kenyon et al., 2011b, Thomson et al., 2020), rather than the influence of their ewe lamb breeding live weight per se.

Thomas and Berger (2009) investigated the impacts of greater prepubertal growth rates of dairy ewe lambs on their subsequent reproductive performance. They reported no effect of greater growth rates (245 g/d *vs.* 327 g/d) on fertility or number of lambs born to four years of age (Thomas and Berger, 2009). In Chapter 5, the Heavy and Control Romney ewe lambs showed a three kg difference in live weight at breeding (47.9 ± 0.36 *vs.* 44.9 ± 0.49 kg, respectively). It was observed that the Heavy group had greater reproductive performance than the Control group at first breeding season (Chapter 5), however, during their second and third breeding seasons, reproductive performance did not differ (Chapter 6). The long-term consequences of breeding Romney ewe lambs at heavier live weight on reproductive efficiency over multiple breeding seasons is unknown.

The efficiency of a ewe is related to her ability to wean a large number of heavy lambs relative to her live weight (Coop and Hayman, 1962, Baker et al., 1973). Sheep efficiency can be defined as the ratio of total weaning weight of her litters and her live weight at breeding. While, feed efficiency can be defined as the ability of a ewe to convert feed into live weight of lambs weaned (Sise et al., 2009), it can be estimated by dividing the total kg of lambs weaned by the estimated ewe feed intake over a period of time. A major component of efficiency, therefore, is the ewe mature live weight, as a heavier ewe will have greater maintenance requirements compared to a lighter ewe (Sise et al., 2009, Nicol and Brookes, 2017). Based on similar lamb production, a lighter ewe would, therefore, be more efficient than a heavier ewe (Baker et al., 1973, Holmes, 1973). Thus, it is of interest to determine if ewe lamb breeding live weight and later live weight affect lifetime efficiency.

The current experiment had three objectives. Firstly, to examine the effect of a heavier live weight at the first breeding at seven months of age, on productive performance and the efficiency of ewes over their first three breeding seasons. Secondly, to determine the effect of successfully weaning a lamb as a ewe lamb on ewe performance and efficiency over their first three breeding seasons. Thirdly, to examine the impact of breeding heavier ewe lambs on feed requirements and mature live weight at 39 months of age.

7.3. Materials and methods

The experiment was conducted at Massey University 's Riverside farm (latitude: 40° 50' 35 S, longitude: 175° 37' 55 E), 10 km north of Masterton, and at Keeble farm (latitude: 40° 24' 03 S, longitude: 175° 35' 51 E), 5 km south of Palmerston North, New Zealand. The experiment and all animal handling procedures were approved by the Massey University Animal Ethics Committee (MUAEC-17/16). Data from Chapter 5 and 6 were used to undertake additional analysis and the related experimental design has been previously described in detail in Chapters 2, 5 and 6.

7.3.1. Animal measurements

Unfasted live weights of ewes were recorded at d86 (weaning), d94, d113, d127, d145, d179, d196, d206, d213 (ewe lamb breeding in 2018), d230, d247, d263, d280, d301 (pregnancy diagnosis 2018), d325, d336, d351 (pre-lambing 2018), d394, d465 (weaning 2018), d504, d535, d567 (breeding of two-year-old ewes in 2019), d584, d604, d660 (pregnancy diagnosis 2019), d683, d701 (pre-lambing 2019), d746, d800 (weaning 2019), d906 (breeding of three-year-old ewes in 2020), d947, d995 (pregnancy diagnosis 2020), d1050 (pre-lambing 2020), d1095 and d1159 (weaning 2020) (see Figure 7.1).

At pregnancy diagnosis (d301, d660 and d995), ewes were identified as pregnant or not pregnant and the number of foetuses were recorded. Each year, the number of lambs born, and the number of lambs weaned for each ewe were recorded. Ewe deaths were recorded throughout the three years. During the current experiment, ewes were only removed from the experiment for welfare reasons.

During lambing periods, within 18h of birth, lambs were identified to their mother, and their date of birth, sex, and birth rank were recorded. Live weights of lambs born in each year were recorded within 18h of birth, at d394, d746 and d1095 and at weaning (d465, d800 and d1159).

7.3.2. Data management

7.3.2.1. Ewe lamb status

For data analysis, at weaning in 2018 (d465; i.e. after their first lambing), ewes were retrospectively identified to one of three ewe lamb status categories: 'weaned a lamb' when ewes weaned at least one lamb as a ewe lamb, 'failed to wean a lamb' when ewe lambs were identified as pregnant and aborted or whose lamb died before d465 or 'not pregnant'.

7.3.2.2. Number and live weight of lambs

The total number of lambs born, and the total number of lambs weaned over three years (2018, 2019 and 2020) was calculated for each individual ewe. The total birthweight of lambs over the three years was calculated by summing the birthweights of lambs born to each individual ewe from each year. Similarly, the total progeny live weight in early lactation (approximately 25 days of age) and at weaning over the three years was calculated for each ewe by summing the yearly live weights of lambs. Ewes that gave birth to multiple lambs of which one or more lambs died, had the total lamb live weight calculated based on the live weights of the surviving lambs. Ewes that did not conceive in a given year were given a zero for the number of lambs born, weaned and lamb birth and weaning weights for that year. Ewes that had no surviving lambs in a given year were given a zero for the number of lambs weaned and lamb weaning weight for that year. Ewes that died were removed from the data set from that point onwards.

7.3.2.3. Efficiency

Production efficiency was calculated for each year over the three-year period using the yearly litter weaning weight divided by ewe live weight at breeding of each year (2018, 2019 and 2020) as per Pettigrew et al. (2019).

Ewe live weights during pregnancy were adjusted using the Gompertz equation (Freer et al., 2007) to calculate conceptus-free live weight based on lambing dates and litter birthweights. A transformational regression was used to fit a polynomial spline curve to the adjusted live weights of each individual ewe as undertaken by Pettigrew et al. (2019). Spline knots were placed at birth, weaning of the ewe (d86) and thereafter every breeding (d213, d567 and d906), pre-lambing (d351, d701 and d1050) and weaning (d465, d800 and d1159). Order two, three and four polynomials were fitted to ewe adjusted live weights. The best polynomial spline model was selected using the Akaike information criterion (AIC), coefficient of determination (r^2), coefficient of correlation (r), mean square prediction error (MSPE), root of the mean square prediction error (RMSPE), and relative prediction error (RPE) (O'Neill et al., 2013).

The MSPE is an indicator for the error of predicted values relative to the observed values (Halas et al., 2004), is expressed in kg^2 and was calculated as follows:

$$MSPE = \frac{1}{n}\sum(Actual_i - Predicted_i)^2$$

Where n is the number of experimental observations; *Actual* and *Predicted* are the actual and predicted live weights; $i = 1, 2, \ldots, n$ (Halas et al., 2004).

The RMSPE is the accuracy of prediction, is expressed in kg and was calculated as follows (O'Neill et al., 2013):

$$RMSPE = \sqrt{MSPE}$$

The RPE was calculated as follows (O'Neill et al., 2013):

$$RPE = \frac{\text{RMSPE}}{Actual_m} \times 100$$

Where $Actual_m$ is the mean actual live weight. The lower the MSPE, RMSPE and RPE, the more accurate are the predictions in the model.

Based on the above criteria, the third order polynomial spline was selected as the most appropriate model to predict ewe adjusted live weights, as the criteria of order 3 and 4 were very similar, with order 4 adding little benefit (Table 7.1). A daily live weight prediction was generated for each ewe from their weaning as a lamb (d86), until their death or the end of the experiment (d1159), as undertaken by Pettigrew et al. (2019).

Table 7.1. Prediction accuracy of second, third and fourth order polynomial spline models for the prediction of ewe adjusted live weight over three years.

Model	n	AIC[a]	r^2	r	MSPE[a] (kg^2)	RMSPE[a] (kg)	RPE[a] (%)
Order 2	9118	17,502	0.980	0.990	6.81	2.61	5.06
Order 3	9118	15,925	0.983	0.991	5.73	2.39	4.64
Order 4	9118	15,763	0.983	0.992	5.63	2.37	4.59

n: number of experimental observations; AIC: Akaike information criterion; r^2: coefficient of determination; r: coefficient of correlation; MSPE: mean square prediction error; RMSPE: root of the mean square prediction error and RPE: relative prediction error; [a] The lower the MSPE, RMSPE and RPE are, the more accurate the predictions are.

As per the study of Pettigrew et al. (2019), the predicted daily live weights from the spline models between the start of the treatments (d86) to the weaning of their third litter (d1159) were used to determine the daily energy requirements for each ewe for their maintenance and live weight change (Nicol and Brookes, 2017). Litter birth dates and weights were used to determine daily energy requirements for pregnancy (Freer et al., 2007, Nicol and Brookes, 2017). Ewes that died between breeding and pregnancy diagnosis were considered as not pregnant and therefore no pregnancy requirement was calculated until death. Ewes which were identified as pregnant with one or more foetuses and that died between pregnancy diagnosis and lambing, were given an average date of birth and lamb birthweight for their treatment for that year. Ewes that were identified as pregnant at pregnancy diagnosis, but that did not lamb were assumed pregnant and to have aborted just before the expected lambing date and were given an average lambing date and lamb birthweight for their treatment for that year. Ewe lactation energy requirements were calculated with the energy required to produce milk, which was based on ewe milk yield and days of lactation (Freer et al., 2007) and the proportion of their litter's energy requirement provided by pasture, which depends on lamb energy requirement for maintenance and growth to weaning (Freer et al., 2007, Nicol and Brookes, 2017). Ewe milk yield was modelled from Peart et al. (1975) based on week of lactation, and number of lambs reared, as undertaken by Pettigrew et al. (2019). Lamb energy requirements met by milk or pasture were calculated based on birth date and weight, weaning date and weight, and average daily gain between birth and weaning (Freer et al., 2007, Nicol and Brookes, 2017). Lamb growth was assumed to be linear between birth and weaning (Pettigrew et al., 2019). Lambs that died before weaning (d465, d800 and d1159) or that did not have live weights at weaning were assumed to have died at birth, therefore, no lamb or ewe lactation energy requirements were calculated for these lambs. Equations for the estimation of ewe daily energy requirements are presented in Appendix D.

The estimated total energy requirement between d86 and d1159 was calculated for each ewe, and based on this, a total predicted pasture intake (kg DM) was also estimated. The total predicted pasture intake was based on the total estimated energy requirement for each ewe and the average energy content of pastures (i.e. ryegrass/white clover) over a year in the Central North Island of New Zealand (10.3 ± 0.51 MJME/kg DM; Litherland et al., 2002). A feed efficiency value for each individual ewe between d86 and d1159 (total progeny weaning weight/total predicted pasture intake) was calculated based on the total progeny weaning weight and the total predicted pasture intake over the three breeding seasons.

7.3.2.4. Survival

All ewes alive at the end of the experiment (d1159) were censored at that date for the experiment survival analysis. In this experiment, ewes were only culled for welfare reasons, therefore a hypothetical culling policy was imposed retrospectively on ewes to simulate commercial farming conditions, as undertaken by Pettigrew et al. (2019). In the hypothetical culling policy, in 2018, ewe lambs that were identified as non-pregnant at pregnancy diagnosis (d301) were culled, as suggested by Kenyon (2012) as a means of increasing flock productivity. Ewe lambs whose lambs died before weaning (d465) were not culled. In the hypothetical culling policy, at two and three years of age, ewes were culled if they did not conceive at pregnancy diagnosis (d660 and d995) or if all their lambs died by day 25 of lactation (d746 and d1095) or by weaning (d800 and d1159) which is normal farm practice in New Zealand.

7.3.2.5. Feed requirements required to wean an extra lamb

Due to an absence of a significant ($P > 0.10$) difference between ewe lamb breeding treatments (i.e. Control *vs.* Heavy), the total number of lambs weaned over three breeding seasons and live weight at ewe lamb breeding (d213) were pooled. To determine the relationship of individual live weights at d213 and the total number of lambs weaned over the three breeding seasons, a generalised linear model was used. The model assumed a Poisson distribution and used a log transformation and included ewe lamb live weight at d213 as a covariate. Ewe lamb live weight at d213 that corresponded to the weaning of three or four lambs over three breeding seasons were estimated with the generalised linear model, and were 44.3 and 59.0 kg, respectively.

To estimate the feed required to wean an additional lamb over three breeding seasons, simulations of two scenarios were run. In the first scenario (Scenario 1), ewes were weaned at d86 at an average live weight of 28.6 kg (actual average weaning weight of the Control and Heavy groups) and were bred at d213 at 44.3 kg, which was the predicted ewe live weight at d213 required to successfully wean three lambs over three breeding seasons in this data set, from the above generalised linear model. In the second scenario (Scenario 2), ewes were also weaned at d86 at an average live weight of 28.6 kg, but were bred at d213 at an average 59.0 kg, which corresponded to the estimated ewe breeding live weight to successfully wean four lambs over three breeding seasons in this data set, from the above generalised linear model.

Daily live weights of a ewe in each scenario, were calculated from d86 to d213 based on the average weaning weight (28.6 kg), and the corresponding ewe lamb live weight at d213 required to wean either three or four lambs over the three breeding seasons as estimated from the generalised linear model (44.3 kg and 59.0 kg, respectively). Daily energy requirements of ewes in each scenario were then estimated between d86 and d213 using maintenance and live weight gain energy requirements adapted from Nicol and Brookes (2017). The total energy requirements between d86 and d213 for ewes in both scenarios were calculated. To determine the total estimated pasture intake required to meet the total energy requirements of ewes in each scenario, the total energy requirements between d86 and d213 were divided by the yearly average energy content per kg DM of pastures in the Central North Island of New Zealand (10.3 ± 0.51 MJME/ kg DM; Litherland et al., 2002). The daily estimated pasture intake was calculated based on the total estimated pasture intake required to meet the total energy requirements of ewes in each scenario between d86 and d213. The total live weight of lambs weaned by ewes in each scenario over their first three years of production was estimated using a linear regression model between ewe live weight at d213 and total progeny weaning weight, as described in the Statistical analyses section.

Starting from the estimated breeding live weights to wean four lambs (i.e. 59.0 kg), simulations were run using different ewe lamb growth rates between d86 and d213 (150 g/d, 200 g/d and 250g/d) to determine ewe lamb live weight at weaning (d86), the total estimated energy required, total pasture intake and daily pasture intake between d86 and d213. The total and daily pasture intake calculations were based on the yearly average energy content per kg DM of pastures (10.3 ± 0.51 MJME/ kg DM; Litherland et al., 2002).

7.3.3. Statistical analyses

All statistical analyses were conducted using SAS v9.4 (SAS institute Inc., Cary, NC, USA). Ewe live weights have been previously reported in Chapter 5 for live weight in 2018 as ewe lambs and in Chapter 6 for live weight in 2019 and 2020 as two- and three-year-old ewes.

The yearly number of lambs born, and number of lambs weaned per ewe presented for breeding were analysed with generalized linear models assuming Poisson distributions and using a log transformation. The models included treatment (Control *vs.* Heavy), ewe lamb status (weaned a lamb *vs.* failed to wean a lamb *vs.* not pregnant) and year as fixed effects, and ewe as a random effect.

The yearly litter birth weight, litter weight in early lactation (approximately 25 days of age), litter weaning weight per ewe presented for breeding, and yearly production efficiency were analysed with linear mixed models. Treatment, ewe lamb status and year were included as fixed effects and ewe was included as a random effect.

The total number of parities, lambs born, and lambs weaned over the three years were analysed using generalized linear models assuming Poisson distributions and using a log transformation. Treatment and ewe lamb status were included as fixed effects. The total progeny weaning weight over three breeding seasons, and the estimated feed efficiency between d86 and d1159 were analysed with linear models including treatment and ewe lamb status as fixed effects.

Ewe survival analysis was carried out using the age at which ewes died or were removed prior to the end of this experiment (d1159). In addition, a hypothetical culling policy was applied to ewes, retrospectively. Survival analyses of the trial ewes, and the hypothetical culling policy were compared between treatments and ewe lamb statuses.

Due to an absence of significant ($P > 0.10$) differences between treatments, live weight at ewe lamb breeding (d213) and their three-year-old live weight at weaning (d1159) were pooled. A linear regression model was constructed to examine the relationship between the live weight of ewe lambs at d213 and their mature weight at d1159, irrespective of treatment. Ewe lamb status and pregnancy rank (single- or twin-bearing) as a ewe lamb at d301 was also included in the model.

A linear regression model was constructed to examine the relationship between ewe live weight at d213 and the total progeny weaning weight over their first three years of production, irrespective of treatment.

7.4. Results

7.4.1. Ewe live weight

Differences in ewe lamb live weight between the Heavy and the Control group in 2018 were reported in detail in Chapter 5 and the liveweight differences at two and three years of age (2019 and 2020) in Chapter 6 (Figure 7.1). Briefly, ewes in the Heavy group were heavier (P < 0.001) than those in the Control group from d213 to d351 after which time they did not differ (P > 0.10; Chapter 5 and 6; Figure 7.1).

Liveweight differences between ewe lamb status (i.e. weaned a lamb *vs.* conceived but failed to wean a lamb *vs.* not pregnant) at two and three years of age were reported in detail in Chapter 6. Briefly, ewes that weaned a lamb as a ewe lamb were lighter (P < 0.05) than ewes that conceived but failed to wean a lamb or were not pregnant from d567 to d701, but did not differ (P > 0.10) after that (Chapter 6). The live weight of ewes that failed to wean a lamb and ewes that were not pregnant as a ewe lamb did not differ (P > 0.10) at any time point (Chapter 6).

7.4.2. Lamb production

The yearly number of lambs born and weaned per ewe across the three years did not differ (P > 0.10) between treatments (Table 7.2). Based on ewe lamb status, ewes that successfully weaned a lamb as a ewe lamb gave birth to a greater (P < 0.001) number of lambs per year across the three years than those not pregnant as a ewe lamb. The number of lambs born per year to ewes that failed to wean a lamb at d465 (i.e. weaning after first lambing) was intermediate and did not differ (P > 0.10) from either ewes that weaned a lamb or that were not pregnant as a ewe lamb (Table 7.2). Ewes that weaned a lamb as a ewe lamb weaned a greater (P < 0.001) number lambs

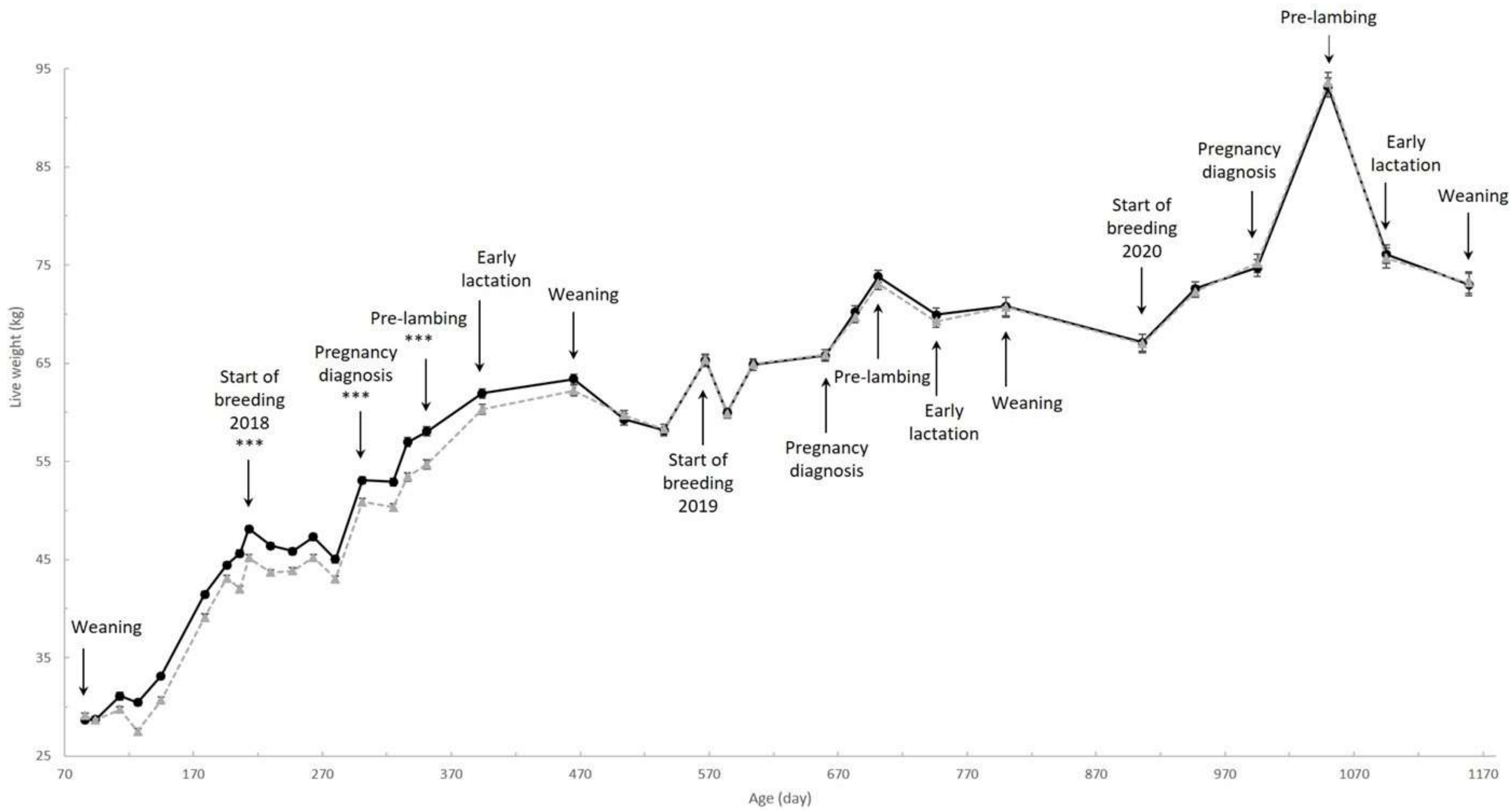

Figure 7.1. Live weight (± SEM) of all ewes in the Heavy (black solid line) and the Control (grey dotted line) groups from their weaning (d86) to the weaning of their third set of lambs at three years of age (d1159). *** indicating treatment differences at $P < 0.001$.

per year than ewes that were not pregnant or failed to wean a lamb as a ewe lamb, which did not differ ($P > 0.10$; Table 7.2).

The yearly litter weight at birth, early lactation and weaning per ewe presented for breeding over the three-year period did not differ ($P > 0.10$) between the Heavy and Control group (Table 7.3). Ewes that weaned a lamb as a ewe lamb had greater ($P < 0.001$) yearly litter birth weights across the three years than ewes that failed to wean a lamb, which had a greater ($P < 0.001$) litter birthweight than ewes that were not pregnant (Table 7.3). Ewes that weaned a lamb as a ewe lamb had greater ($P < 0.01$) yearly litter weights in early lactation and at weaning over the three-year period than ewes that failed to wean a lamb and were not pregnant as a ewe lamb, which did not differ ($P > 0.10$; Table 7.3).

The total number of parities, lambs born, lambs weaned and total progeny weaning weight over the three-year period did not differ ($P > 0.10$) between treatments (Table 7.4). Ewes that were not pregnant as a ewe lamb had a lower ($P < 0.01$) total number of parities and number of lambs born over three years than both ewes that weaned a lamb or that failed to wean a lamb as a ewe lamb, which did not differ ($P > 0.10$). Ewes that weaned a lamb as a ewe lamb weaned more lambs ($P < 0.05$) over three years than ewes that were not pregnant or that failed to wean a lamb as a ewe lamb, which did not differ ($P > 0.10$). The total progeny weaning weight over three years was greater ($P < 0.05$) for ewes that weaned a lamb as a ewe lamb than ewes that were not pregnant, which was greater ($P < 0.05$) than ewes that failed to wean a lamb.

Table 7.2. The effect of treatment (Control *vs.* Heavy) and ewe lamb status at d465[1] (weaned a lamb, failed to wean a lamb[2], non-pregnant) on least square means (95% confidence intervals) of the yearly number of lambs born (NLB) and yearly number of lambs weaned (NLW) per ewe presented for breeding over the first three years of production.

	n	NLB/ewe	NLW/ewe
Treatments			
Control	135	1.17 (1.04 – 1.30)	0.84 (0.74 – 0.96)
Heavy	135	1.25 (1.13 – 1.39)	0.90 (0.79 – 1.02)
P Value		0.265	0.386
Ewe lamb status at d465[1]			
Weaned a lamb	139	1.44 (1.32 – 1.56)^{b}	1.21 (1.10 – 1.33)^{b}
Failed to wean a lamb[2]	25	1.26 (1.03 – 1.55)^{ab}	0.65 (0.49 – 0.86)^{a}
Non-pregnant	103	0.98 (0.87 – 1.10)^{a}	0.84 (0.74 – 0.95)^{a}
P Value		< 0.001	< 0.001

^{a,b,c} Means between rows with differing superscripts are different ($P < 0.05$); [1] Ewes were retrospectively allocated to the categories at the weaning of their first lambs (d465); [2] Were identified as pregnant at d301 but did not wean a lamb as a ewe lamb.

Table 7.3. The effect of treatment (Control *vs.* Heavy) and ewe lamb status at d465[1] (weaned a lamb, failed to wean a lamb, non-pregnant) on least square means (± SEM) of the yearly litter weight (kg) at birth, early lactation and weaning per ewe over the three-year period, and the yearly production efficiency (yearly litter weaning weight/ewe breeding live weight) across their first three years of production.

	n	Litter weight at birth (kg)	Litter weight in early lactation (kg)	Litter weight at weaning (kg)	Yearly production efficiency
Treatments					
Control	135	7.97 ± 0.19	13.4 ± 0.50	26.5 ± 0.99	0.420 ± 0.017
Heavy	135	8.11 ± 0.18	13.9 ± 0.47	26.9 ± 0.92	0.422 ± 0.015
P Value		0.539	0.334	0.718	0.897
Ewe lamb status at d465[1]					
Weaned a lamb	139	9.28 ± 0.16^{c}	17.9 ± 0.41^{b}	35.3 ± 0.80^{b}	0.609 ± 0.014^{c}
Failed to wean a lamb[2]	25	8.08 ± 0.37^{b}	10.6 ± 0.96^{a}	20.0 ± 1.91^{a}	0.278 ± 0.032^{a}
Non-pregnant	103	6.76 ± 0.18^{a}	12.4 ± 0.47^{a}	24.7 ± 0.93^{a}	0.376 ± 0.016^{b}
P Value		< 0.001	< 0.001	< 0.001	< 0.001

^{a,b,c} Means between rows with differing superscripts are different ($P < 0.05$); [1] Ewes were retrospectively allocated to the categories at the weaning of their first lambs (d465); [2] Was identified as pregnant at d301 but did not wean a lamb as a ewe lamb.

7.4.3. Efficiency

The yearly production efficiency (litter weaning weight/ewe breeding live weight per year) did not differ (P > 0.10) between treatments (Table 7.3). Ewes that weaned a lamb as a ewe lamb had a greater (P < 0.01) yearly production efficiency than ewes that were not pregnant, which was greater (P < 0.01) than ewes that failed to wean a lamb as a ewe lamb (Table 7.3).

The total predicted pasture intake and estimated feed efficiency over the first three years did not differ (P > 0.10) between treatments (Table 7.4). Ewes that weaned a lamb as a ewe lamb had a greater (P < 0.01) total predicted pasture intake over the first three years than both ewes that were not pregnant and those that conceived but failed to wean a lamb as a ewe lamb (Table 7.4). Ewes that weaned a lamb as a ewe lamb had greater (P < 0.001) feed efficiency between d86 and d1159 than those that were not pregnant, which in turn had greater efficiency (P < 0.001) than ewes that conceived but failed to wean a lamb (Table 7.4).

7.4.4. Ewe survival to three years of age (d1159)

The actual survival of ewes to d1159 did not differ (P > 0.10) between ewe lamb treatments and ewe lamb status (Figure 7.2A & B). The predicted survival of ewes from the Heavy group, based on a hypothetical culling policy, was greater than the predicted survival of ewes from the Control group (P < 0.001; Figure 7.3A). The predicted survival to d1159 of ewes that weaned a lamb as a ewe lamb was greater (P < 0.001) than the predicted survival of ewes that failed to wean a lamb as a ewe lamb (Figure 7.3B).

Table 7.4. The effect of treatment (Control *vs.* Heavy) and ewe lamb status at d465[1] (weaned a lamb, failed to wean a lamb[2], non-pregnant) on least square means (95% confidence intervals) of total number of parities, lambs born and weaned over the first three years of production, and least square means (± SEM) of total progeny weaning weight (kg) over the three-year period, total predicted pasture intake (kg DM) between weaning of the ewe (d86) and the weaning of the third litter (d1159), and estimated feed efficiency (total progeny weaning weight/total predicted pasture intake; kg/kg DM) over the first three years of production.

	n	Total number of parities	Total number of lambs born	Total number of lambs weaned	Total progeny weaning weight (kg)	Total predicted pasture intake (kg DM)	Feed efficiency (kg/kg DM)
Treatments							
Control	135	2.40 (2.11 – 2.74)	3.54 (3.18 – 3.94)	2.60 (2.29 – 2.97)	72.3 ± 3.19	1484.9 ± 35.5	4.72 ± 0.17
Heavy	135	2.40 (2.12 – 2.71)	3.85 (3.48 – 4.24)	2.79 (2.47 – 3.15)	76.4 ± 3.02	1501.7 ± 33.5	4.85 ± 0.16
P Value		0.973	0.202	0.336	0.302	0.698	0.517
Ewe lamb status at d465[1]							
Weaned a lamb	139	2.81 (2.55 – 3.11)[b]	4.36 (4.03 – 4.73)[b]	3.69 (3.38 – 4.02)[b]	98.2 ± 2.61[c]	1590 ± 29.0[b]	6.15 ± 0.14[c]
Failed to wean a lamb[2]	25	2.68 (2.11 – 3.41)[b]	3.82 (3.13 – 4.67)[b]	2.01 (1.53 – 2.65)[a]	52.9 ± 6.13[a]	1437 ± 68.1[ab]	3.43 ± 0.32[a]
Non-pregnant	103	1.83 (1.59 – 2.12)[a]	3.01 (2.69 – 3.37)[a]	2.64 (2.34 – 2.98)[a]	72.0 ± 3.07[b]	1453 ± 34.1[a]	4.77 ± 0.16[b]
P Value		< 0.001	< 0.001	< 0.001	< 0.001	0.004	< 0.001

[a,b,c] Means between rows with differing superscripts are different (P < 0.05); [1] Ewes were retrospectively allocated to the categories at the weaning of their first lambs (d465); [2] Was identified as pregnant at d301 but did not wean a lamb as a ewe lamb.

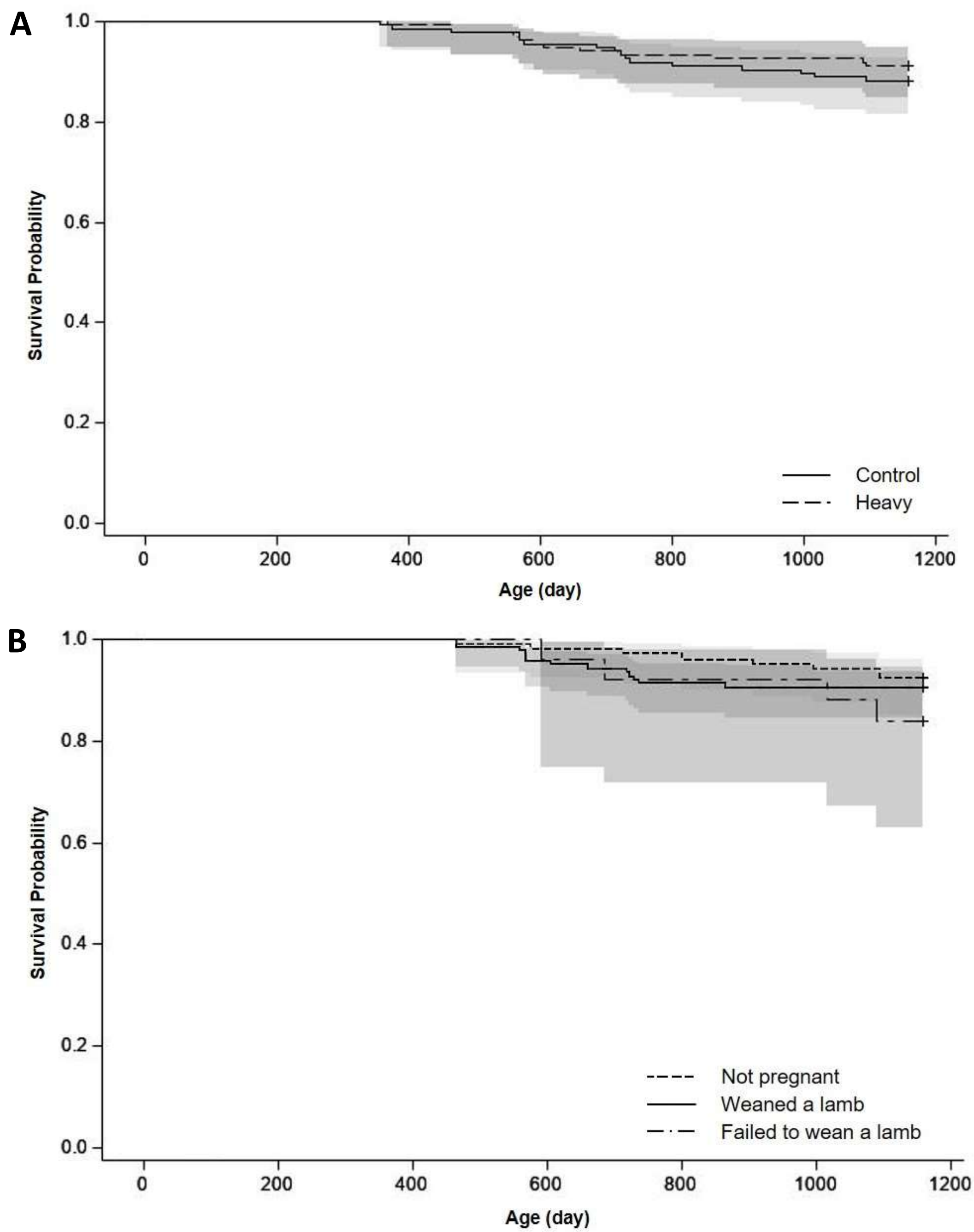

Figure 7.2. Actual survival curves and 95% confidence interval (grey area) of the ewes based on **(A)** treatment (Control or Heavy) and **(B)** ewe lamb status (not pregnant or weaned a lamb or failed to wean a lamb) for the three years of the experiment until 1159 days of age. Ewes were only culled for welfare reasons.

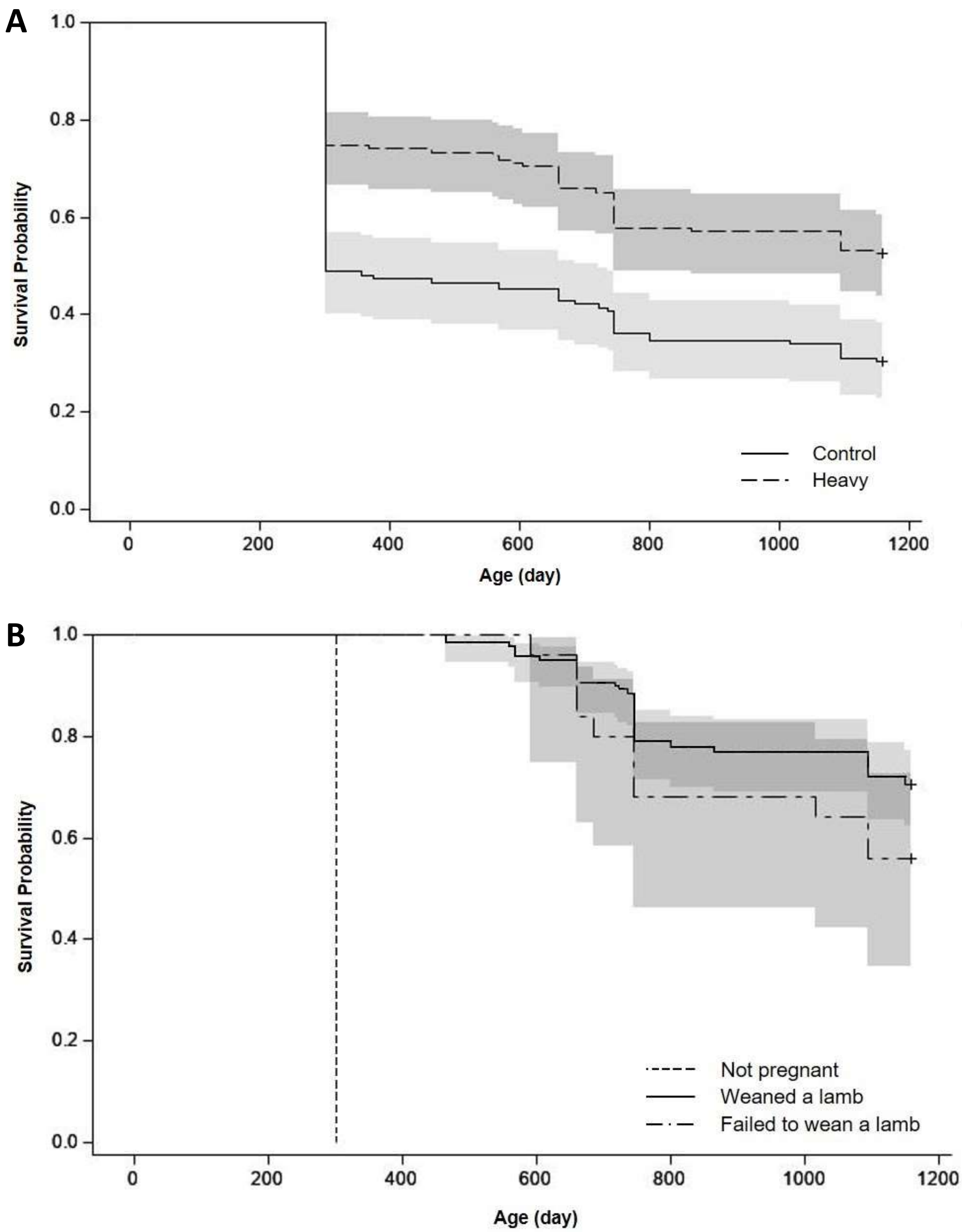

Figure 7.3. Predicted survival curves and 95% confidence interval (grey area) of ewes based on **(A)** treatments (Control or Heavy) and **(B)** ewe lamb status (not pregnant or weaned a lamb or failed to wean a lamb) for the three-year period of the experiment at 1159 days of age with the hypothetical culling policy: ewe lambs that were not pregnant were culled at pregnancy diagnosis (d301) and ewe lambs whose lambs died were not culled, and at two and three years of age, non-pregnant ewes at pregnancy diagnosis (d660 and d995) and ewes whose lambs died before weaning (d800 and d1159) were culled.

7.4.5. Relationship between ewe lamb breeding live weight and ewe mature weight

As there was no difference (P > 0.10) in live weight at d1159 between treatments, data of the two treatments were pooled. Ewe live weight at weaning at three years of age (d1159) was positively associated (P < 0.01) with ewe live weight at their first breeding (d213) (r^2 = 0.113; ewe live weight at d1159 = 45.5 (± 5.23) + 0.62 (± 0.11) ewe live weight at d213; Figure 7.4). For each 1 kg increase in breeding live weight of ewe lambs (d213), mature live weight at 39 months of age (d1159) increased by 0.62 ± 0.112 kg (Figure 7.4).

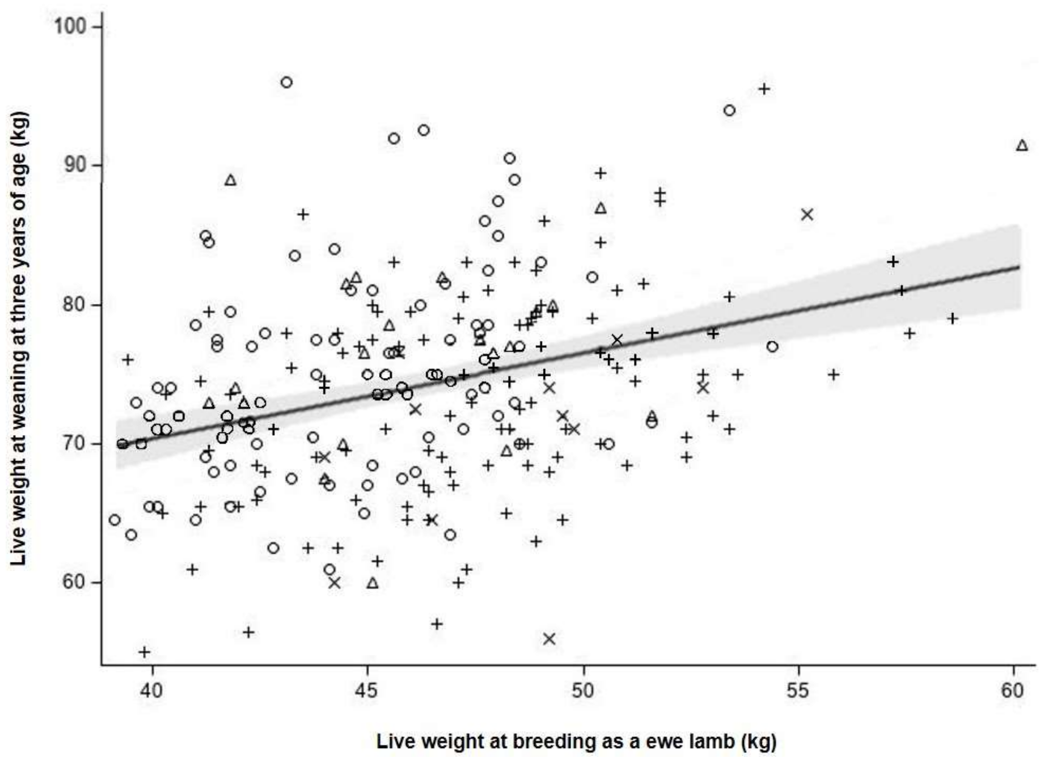

Figure 7.4. Ewe live weight at weaning at 39 months of age (d1159) in relation to their live weight at their first breeding at seven months of age (d213) displaying their status (failed to wean a lamb (triangles) or not pregnant (circles)) and pregnancy rank (single-bearing (plus) or twin-bearing (crosses)) as a ewe lamb. Predictions and 95% confidence intervals shown.

7.4.6. Feed requirement to wean an additional lamb

No difference ($P > 0.10$) in the total number of lambs weaned over the three-year period was found between treatments, therefore, data from the two treatments were pooled. Ewe lamb live weight at d213 was positively associated ($P < 0.05$) with total number of lambs weaned and the total progeny weaning weight over the three-year period (Figure 7.5 & 7.6). In order to wean an additional lamb across three years, ewe lambs would need to be 15-kg heavier at breeding (44.3 kg *vs.* 59.0 kg; Figure 7.5). Based on Figure 7.6, a ewe lamb weighing 44.3 kg or 59.0 kg at her first breeding would be expected to wean 79.2 kg or 104.9 kg of lambs over the first three breeding seasons, respectively. In order to be 15-kg heavier at d213, a ewe would need to eat an additional 95.5 kg DM, which corresponds to an additional 0.75 kg DM per day from d86 to d213 (Table 7.5). The estimations of the weaning weight and daily pasture intake to achieve 59.0 kg at breeding with growth rates of 150, 200 and 250 g/d between weaning (d86) and breeding as a ewe lamb (d213) are presented in Table 7.6.

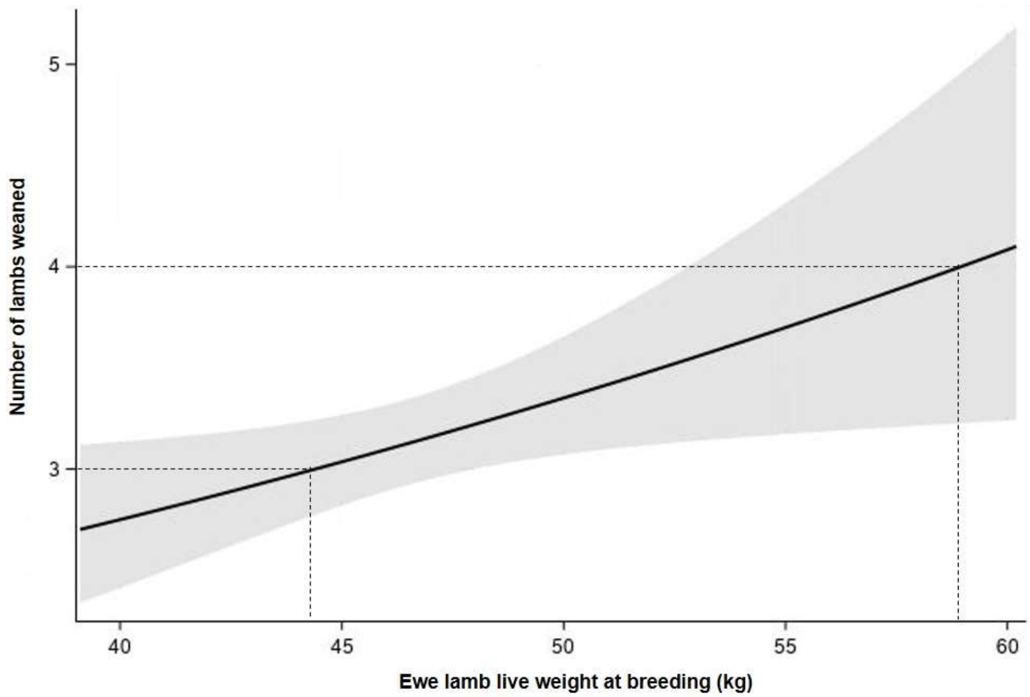

Figure 7.5. Total number of lambs weaned over the three-year period in relation to ewe lamb live weight at breeding (d213) (log predictions (solid line) and 95% confidence intervals shown (grey area)), and determination of ewe lamb live weight at breeding for three or four lambs weaned over the three-year period (dotted lines).

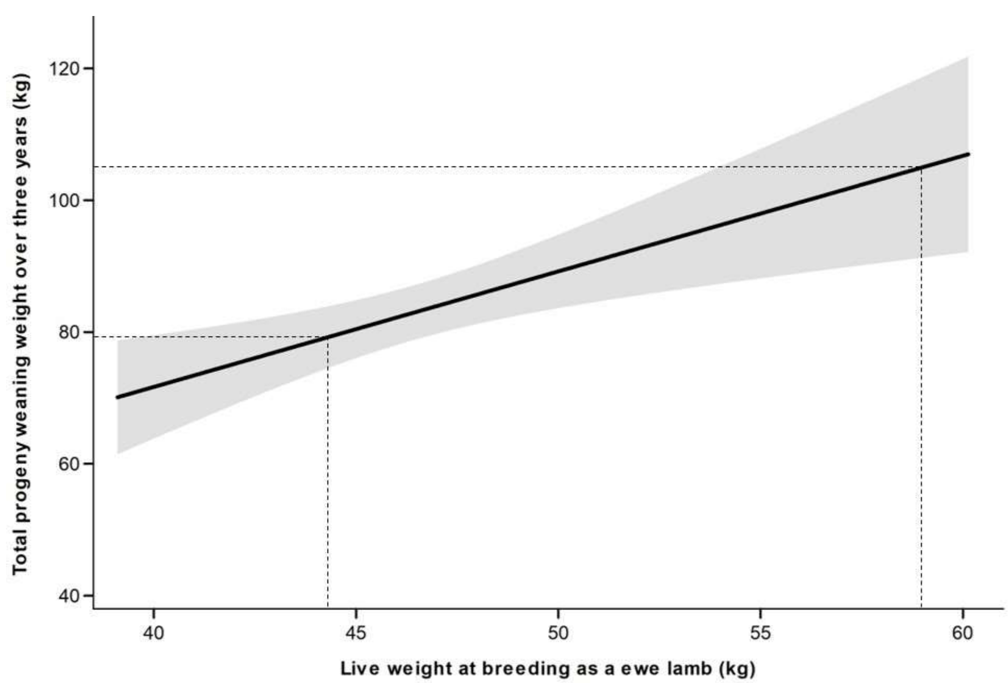

Figure 7.6. Total progeny weaning weight (kg) over the first three years of production in relation to ewe lamb live weight at breeding (d213). Predictions and 95% confidence intervals shown ($r^2 = 0.044$), and determination of the total progeny weaning weight for a ewe lamb weighing either 44.3 kg or 59.0 kg at her first breeding (dotted lines).

Table 7.5. Total estimated ewe energy requirement[1], pasture intake[2] and daily pasture intake of ewes between their weaning (d86) and first breeding at seven months of age (d213) for a ewe in Scenario 1 (ewe live weight at d86 = 28.6 kg and at d213 = 44.3 kg) and Scenario 2 (ewe live weight at d86 = 28.6 kg and at d213 = 59.0 kg).

	Scenario 1 3 lambs weaned over the three-year period	Scenario 2 4 lambs weaned over the three-year period
Live weight at d86 (kg)	28.6	28.6
Live weight at d213 (kg)	44.3	59.0
Average daily gain (g/d)	123.6	239.4
Total estimated energy requirement[1] (MJME)	1520.6	2504.1
Total estimated pasture intake[2] (kg DM)	147.6	243.1
Estimated daily pasture intake (kg DM/day)	1.16	1.91

[1] Estimations were based on the maintenance energy requirements and growth energy requirements (Nicol and Brookes, 2017); [2] Total estimated energy requirement (MJME) divided by the average energy content per kg DM of pastures in the Central North Island of New Zealand (10.3 ± 0.51 MJME/kg DM; Litherland et al., 2002).

Table 7.6. Estimations of the weaning weight, total energy requirement[1], total pasture intake[2] and daily pasture intake of a ewe weighing 59.0 kg at breeding at seven months of age and growing at either 150 g/d, 200 g/d or 250 g/d between weaning (d86) and breeding (d213).

	Average daily gain		
	150 g/d	200 g/d	250 g/d
Live weight at d213 (kg)	59.0	59.0	59.0
Estimated weaning weight at d86 (kg)	39.95	33.6	27.25
Total estimated energy requirement[1] (MJME)	2031.8	2309.0	2553.1
Total estimated pasture intake[2] (kg DM)	197.3	224.2	247.9
Estimated daily pasture intake (kg DM/day)	1.55	1.77	1.95

[1] Estimations were based on the maintenance and growth energy requirements (Nicol and Brookes, 2017); [2] Total estimated energy requirement (MJME) divided by the average energy content per kg DM of pastures in the Central North Island of New Zealand (10.3 ± 0.51 MJME/kg DM; Litherland et al., 2002).

7.5. Discussion

7.5.1. Effect of heavier ewe lamb live weight on lamb production and efficiency

The first objective of the current experiment was to examine the effects of a heavier breeding live weight, as a ewe lamb, on ewe performance and efficiency over their first three breeding seasons. Treatment had no effect on lamb production over the three years, however, when data were pooled, a heavier live weight at breeding was positively associated with the total lambs produced. The addition of ewe lamb status in the statistical models created a confounding effect with treatment and obscured any effect of the treatment. In Chapter 5, the Heavy group had greater fertility than the Control group, therefore, there was a greater proportion of ewes from the Heavy group that 'weaned a lamb as a ewe lamb' and a greater proportion of ewes from the Control group that were 'not pregnant'. The large range of ewe lamb breeding live weight within each treatment (Heavy 41.0 to 60.2 kg and Control 39.1 to 57.6 kg) may have limited the identification of an effect of a heavier live weight and explained the differences in results between treatment and pooled data. These results suggest that there is a positive effect of heavier live weight at ewe lamb breeding on the total number of lambs produced over the first three breeding seasons, which was due to improved ewe lamb reproductive performance.

Yearly production efficiency (litter weaning weight/ewe breeding live weight) and feed efficiency (total progeny weaning weight/estimated pasture intake) did not differ between treatments over the three-year period. The Control group was lighter at ewe lamb breeding and weaned less lambs than the Heavy group (Chapter 5). The 3-kg difference in ewe lamb breeding live weight between treatments, combined with the greater lamb production of ewe lambs in the Heavy group, resulted in production efficiency being similar between treatments. At two and three years of age, lamb production and ewe breeding live weight did not differ between treatments (Chapter 6). Over the first three breeding seasons, the total progeny weight weaned and estimated pasture intake did not differ between treatments, therefore, there was no difference in the feed efficiency.

Yearly production efficiency in the current experiment was similar to that reported by Pettigrew et al. (2021) of ewes born as either a single or twin to a ewe lamb during their first two productive years (0.39 kg lamb weaned/kg at ewe breeding per year). Yearly values, however, were lower than those reported by Pettigrew et al. (2019) for ewes born as twins to mature ewes over eight years of production (0.70 kg lamb weaned/kg at ewe breeding). The differences between these studies might be explained by the eight-year period considered by in Pettigrew et al. (2019), the age at first breeding (seven or 18 months of age), and dam age (ewe lambs or mature ewes) which can affect body size (Pettigrew et al., 2021).

The actual survival rate of the ewes did not differ between treatments over the three-year period. When a hypothetical culling policy was applied, however, the Heavy group showed greater predicted survival to 39 months of age than the Control group. The hypothetical culling policy consisted of the culling of non-pregnant ewes at pregnancy diagnosis and two- and three-year-old ewes whose lambs died before weaning, which is a policy commonly employed on commercial New Zealand farms. The lower fertility of the Control group compared to the Heavy group as ewe lambs, which was mainly driven by live weight at ewe lamb breeding (Chapter 5), explained this difference in ewe predicted survival.

A heavier live weight at ewe lamb breeding had a positive effect on total lamb production, which was driven by the greater reproductive performance of heavier ewe lambs during their first breeding season (Chapter 5). There was, however, no effect of ewe live weight on production efficiency and feed efficiency over their first three breeding seasons. Predicted ewe survival within the flock to 39 months of age was improved for ewe lambs bred at a heavier live weight. Breeding heavier ewe lambs may also impact ewe lifetime production and efficiency and, therefore, needs to be further examined before final recommendations can made to farmers. Ewe performance should be followed to at least five or six years of age, when culling for age traditionally occurs in New Zealand.

7.5.2. Impact of successfully weaning a lamb as a ewe lamb on subsequent production and efficiency

Ewes that weaned a lamb as a ewe lamb had greater total lamb and yearly production and feed efficiency over their first three breeding seasons than the two other groups (i.e. failed to wean a lamb and not pregnant). This is consistent with Baker et al. (1981) who reported that ewes that lambed as a ewe lamb had a greater weaning rate and lamb weaning weight than those that did not, up to four years of age. Kenyon et al. (2011b), however, reported that lambing as a ewe lamb had no effect on lamb weaning weight at three years of age. In the current experiment, ewes that weaned a lamb as a ewe lamb were lighter at two-year-old breeding than the two other groups (Chapter 6), however, they produced heavier litters than the other two groups, which explained the greater production efficiency of ewes that had weaned a lamb. Ewes that weaned a lamb as a ewe lamb had the greatest total progeny weaning weight and had similar pasture intake to ewes that failed to wean a lamb, which explained their greater feed efficiency. Ewe survival did not differ between ewe lamb status, however, if a hypothetical culling policy had been applied, ewes that weaned a lamb were more likely to survive to 39 months of age than ewes that failed to wean a lamb.

Ewes that failed to wean a lamb as a ewe lamb had the lowest production and feed efficiency over the three-year period. These results are consistent with Amer et al. (2009) who reported that ewes whose entire litter died prior to weaning had lower lamb and litter weaning weight during the following lambing compared to ewes that successfully weaned a lamb. Griffiths et al. (2018) also reported that the risk of losing the entire litter at two years of age was greater for ewes whose entire litter had died during their first lactation compared to ewe lambs that successfully weaned a lamb. Fogarty et al. (2007), however, reported that lamb production of ewes that did not lamb as a ewe lamb, and ewes that lambed and lost their lambs, did not differ over the first three breeding periods. In that study, ewes that were identified as pregnant, but did not lamb were not included in the analyses. In the current experiment, ewes that aborted were included in the analysis and likely explained the difference in production. While not statistically different, ewes that failed to wean a lamb had lower yearly litter weaning weight and were three-kilograms heavier (Chapter 6) than ewes that had not been pregnant as a ewe lamb. Collectively, these differences explain why yearly production efficiency was lower in ewes that failed to wean a lamb than the two other ewe groups. Similarly, ewes that failed to wean a lamb had the lowest feed efficiency and total progeny weaning weight but had similar total pasture intake compared the two other ewe groups. In addition, ewes that failed to wean a lamb were less likely to survive to 39 months of age than ewes that weaned a lamb as a ewe lamb.

In summary, ewes that weaned a lamb as a ewe lamb were more efficient on a per kg of lamb weaned per kg of dry matter eaten basis than ewes that were not pregnant which in turn were more efficient than ewes that failed to wean a lamb. The results suggested that farmers should cull ewe lambs whose entire litter die prior to weaning in order to improve flock performance and efficiency. A limitation of this experiment, however, was the low number of ewe lambs that failed to wean a lamb (< 30), therefore, these results should be interpreted with caution. Further investigations are needed using a larger number of ewes to determine the consequences of failing to wean a lamb as a ewe lamb.

7.5.3. Requirements to wean an additional lamb over the first three breeding seasons

The estimated live weight at ewe lamb breeding required to wean four lambs over the first three breeding seasons was 59.0 kg. This estimated live weight was high as the mature weight of Romney ewes ranges from 55 to 70 kg (Cranston et al., 2017). The low performance of the Control group (lighter animals) during the first breeding season (44% of lambs weaned per ewe lamb bred; Chapter 5) and the high proportion of single-bearing ewes across the entire population in the second breeding season (40%; Chapter 6) may explain the high estimated live weight required to wean four lambs in three productive years. In Chapter 5, a live weight range of 50-52 kg at breeding was identified as the optimal live weight to maximise breeding performance and fertility in ewe lambs. Further studies are required before the identification of optimal ewe lamb breeding live weight to maximise their lamb production over their first three breeding seasons is fully understood.

To achieve the estimated 59.0 kg at ewe lamb breeding, ewe lambs weaned at 28.6 kg would need to grow 240 g/d between weaning and their first breeding with a daily pasture intake of 1.91 kg DM/d. This daily pasture intake is high for a 28.6-kg ewe lamb as it corresponds to 6.6% of ewe lamb weaning weight and therefore is unlikely to be achieved. The maximum daily DM intake of a non-pregnant ewe has been estimated to be 3.5% of the ewe live weight (Cranston et al., 2017). Daily pasture intake depends on the average energy content of the pasture (Nicol and Brookes, 2017), which was 10.3 MJME/kg DM for ryegrass/white clover pasture in this experiment. If grazed on a herbage of higher quality (MJME/kg DM), the estimated daily pasture intake to achieve 59.0 kg would be reduced which would be more biologically feasible. Herbages such as chicory (*Cichorium intybus L.*), plantain (*Plantago lanceolata L.*), herb-clover mixes, lucerne (*Medicago sativa L.*) are known to have higher quality over the summer/autumn period that a grassed based sward (Lindsay et al., 2007, Ekanayake et al., 2019) and therefore could be an option.

Simulations were run to identify the weaning weight required if subsequent growth rates were 150, 200 or 250 g/d in order to achieve the 59.0 kg target at ewe lamb breeding. At growth rates of 150 and 200 g/d, ewe lambs would need to be heavier at weaning (40 and 33.6 kg, respectively) than that achieved in the current experiment. Alternative forages such as herb-clover mix and lucerne increased lamb growth rates to weaning and lamb weaning weight (Kenyon et al., 2010a, Hutton et al., 2011, Corner-Thomas et al., 2020). In the present experiment, due to management requirements, ewe lambs were born and weaned later than the usual management practice resulting in 127 days between weaning and their first breeding instead of a more traditional 150 days (Cranston et al., 2017). These additional 23 days would allow for lower live weight of ewe lambs at weaning (36.5 and 29.0 kg respectively) to achieve 59.0 kg at breeding as a ewe lamb.

There was a positive relationship between ewe lamb live weight at breeding and their live weight at 39 months of age, indicating that there was a carry-over effect of greater breeding live weights. Ewe mature live weight is one of the primary components of ewe efficiency (Holmes, 1973, Conington et al., 2004, Sise et al., 2009). A heavier mature ewe would, therefore, result in greater maintenance energy requirements and overall feed demand (Brown et al., 2015, Nicol and Brookes, 2017). To be as efficient, a heavier mature ewe would need to produce either more or heavier lambs to compensate for the additional feed needed to meet her energy requirements (Holmes, 1973, Dickerson, 1978, Brown et al., 2015). A heavier live weight at breeding is known to increase ewe reproductive performance (Ferguson et al., 2011, Brown et al., 2015), therefore, there is value in following the ewes in this experiment until they are culled for age at five years of age to examine whether the efficiency of heavier ewes is affected.

7.6. Conclusion

Breeding ewe lambs at heavier weights had a positive effect on the total lamb production to weaning at 39 months of age, however, there was no effect on production and feed efficiency over the first three breeding seasons. Ewes that successfully weaned a lamb as a ewe lamb had the greatest performance and efficiency, whereas ewes that failed to wean a lamb had the lowest performance and efficiency. There was a positive relationship between ewe lamb breeding live weight and their live weight at 39 months of age. These results suggest that farmers should aim to breed their ewe lambs at heavier live weights to improve their overall performance. Before final recommendations can be made, performance of this ewe group to five years of age is required.

Chapter 8

General discussion and conclusion

8.1. Introduction

Ewe lamb breeding is a management tool that farmers can use to improve ewe lifetime performance and farm profitability (Kenyon et al., 2014c, Farrell et al., 2021). A key component to the success of breeding ewe lambs is that they attain 50 to 70% of their expected mature live weight prior to breeding (Dýrmundsson, 1973, Kenyon et al., 2014c). The current recommendation for breeding Romney ewe lambs is a minimum pre-breeding live weight of 40 kg (Kenyon et al., 2014c). It has been well established that heavier ewe lambs at breeding display greater reproductive performance than their lighter counterparts (Mulvaney et al., 2010, Corner-Thomas et al., 2014b, 2015b, Kenyon et al., 2014c). To improve the reproductive performance of ewe lambs, therefore, farmers could aim to grow them to live weights above 40 kg at breeding. Increased growth rates of ewe lambs post-weaning can, however, have detrimental effects on their mammary gland development and subsequent lactational performance (Johnsson and Hart, 1985, Umberger et al., 1985, Villeneuve et al., 2010a,b). Previous research has focused on the effects of heavier ewe lamb breeding live weights on their first breeding season performance only, therefore, little is known about the potential long-term impacts on subsequent reproductive performance, mammary gland development, live weight, and efficiency.

The primary aims of this thesis were to:

- Determine the effect of an increased growth rates between weaning and first breeding at seven months of age on mammary gland development of ewe lambs in their first and second pregnancy and lactation using ultrasonography.

- Investigate the associations between ewe mammary ultrasound measurements and the growth rate of progeny to weaning.
- Examine the association between the single-bearing ewe lamb mammary ultrasound measurements and milk production.
- Investigate the long-term consequences of heavier ewe lamb live weights at first breeding on reproductive and lambing performance, and efficiency during the first three breeding seasons.
- Examine the relationship between ewe lamb live weight at breeding and ewe mature live weight at 39 months of age.

8.2. Summary of the main findings

Chapter 2 – Effect of increased growth rates post-weaning on mammary gland development of ewe lambs

The focus of Chapter 2 was to investigate the effects of an increased growth rate between weaning and the first breeding on mammary development of single-bearing ewe lambs during their first pregnancy and lactation. Mammary gland morphology and the depth of mammary internal structures, as measured with ultrasound, and growth of their progeny did not differ between ewe lamb treatments. The absence of a difference in mammary gland development, may be explained by the relatively small difference in growth rates between treatments (133 *vs.* 147 g/d) and/or low growth rates of ewe lambs in both treatments. Interestingly, the depth of the mammary gland cistern in lactation and the mammary parenchyma in late pregnancy was positively related with ewe lamb progeny growth rate to weaning. This may suggest that the ultrasound method has the potential to be used to identify ewe lambs that would wean heavier single lambs.

Chapter 3 – Association among mammary ultrasound measures, milk yield of non-dairy ewe lambs, and the growth of their single lambs

Based on the findings of Chapter 2, a further study was designed with a second cohort of single-bearing ewe lambs to determine whether mammary ultrasound measures could be used as an indirect indicator of milk yield and subsequent lamb growth. The depth of the gland cistern in pregnancy and lactation were both positively correlated with milk yield in the third week of lactation. The prediction of milk yield using ultrasound, however, was poor, indicating that it was not suitable as a technique to accurately predict milk yield of ewe lambs. Ewe lamb mammary ultrasound measures and morphology explained more than half of the variation in single lamb growth to the third week of lactation. Both the depth of the fat pad in late pregnancy and parenchyma in third week of lactation were positive indicators of lamb growth to the third week of lactation. This indicates that ultrasound could potentially enable the identification of ewe lambs that would have lambs with faster early lactation growth rates. The reason for the differences in this experiment compared to Chapter 2 is unknown, thus further research is needed.

Chapter 4 – Effect of increased growth rates prior to the first breeding at seven months of age and pregnancy rank on mammary gland of two-year-old ewes

Villeneuve et al. (2010b) reported that there were carry-over effects of increased growth rates prior to puberty on lactational performance of ewes until their second lactation. Hence, this Chapter was designed to investigate the potential carry-over effects on a subset of ewes from each treatment. The association between ultrasound measures and single- and twin-lamb growth rates was also examined. No carry-over effects of increased growth rates between weaning and breeding at seven months of age on morphology and measures of the mammary internal structures were observed at two years of age. These results were likely due to the small difference in ewe growth rates of each ewe subset from each treatment (136 *vs.* 149 g/d) between weaning and their first breeding. At the growth rates observed in this experiment, farmers do not need to be concerned about carry-over effects of growth rates prior to breeding on ewe mammary gland. In addition, the gland cistern in early lactation was positively associated with lamb growth to early lactation but was negatively associated with lamb growth to weaning. This negative association could suggest there are differing associations between mammary ultrasound measures and lamb growth based on birth rank, and therefore, requires further investigation.

Chapter 5 – Impacts of heavier ewe lambs at breeding on reproductive performance and performance of their progeny to weaning during their first breeding season

The focus of this Chapter was to investigate the effects of a heavier live weight of Romney ewe lambs at breeding (44.9 kg *vs.* 47.9 kg) on their live weight, reproductive performance, and performance of their progeny to weaning during the first breeding season. Heavier ewe lambs at breeding remained heavier than Control ewe lambs throughout pregnancy, but not in lactation. Heavier ewe lambs at breeding had greater breeding performance, fertility, litter size and weaned more lambs than their Control counterparts. Lamb survival and live weights, however, did not differ. Analyses of the pooled liveweight data indicated a curvilinear relationship between ewe lamb breeding live weight and the probability of being mated, and fertility rate. Combined, data from both treatments indicate that heavier ewe lamb at breeding had better reproductive performance during their first breeding season than lighter ewes. To maximise fertility rate, ewe lambs should be bred at 50-52 kg.

Chapter 6 – Breeding heavier ewe lambs did not impact their subsequent two- and three-year-old ewe live weight and reproductive performance

The effect of a heavier ewe lamb at breeding on two- and three-year-old ewe performance was investigated. Ewe reproductive performance, BCS, wither height and mature live weight did not differ between treatments. Further, successfully weaning a lamb as a ewe lamb did not impact subsequent reproductive performance or live weight of their progeny. Live weight and BCS of ewes that weaned a lamb as a ewe lamb were reduced during their second breeding and pregnancy, but these differences did not persist after their second lambing. Combined, these data indicate that to three years of age a difference in ewe lamb breeding live weight did not have any long-term effects on ewe live weight, reproductive performance and performance of their progeny to weaning.

Chapter 7 – Effect of breeding heavier ewe lambs on lamb production and efficiency over their first three breeding seasons and their mature weight

This chapter examined the effects of breeding heavier ewe lambs on ewe production and feed efficiency over their first three breeding seasons and ewe mature live weight at 39 months of age. Although the total lamb production and ewe efficiency did not differ between treatments, when data were pooled, heavier ewe lambs at breeding had more lambs that were heavier at weaning over their first three breeding seasons than lighter ewes. In addition, ewes that weaned a lamb as a ewe lamb had the greatest production and efficiency, whereas ewes that failed to wean a lamb as a ewe lamb had the lowest production and efficiency. There was a positive relationship between ewe lamb breeding live weight and their live weight at 39 months of age. These results suggest that breeding ewe lambs at heavier weights improved total lamb performance but had no effect on ewe efficiency. More research is needed to consolidate these results and examine the effect of breeding heavier ewe lambs on their lifetime production and efficiency.

8.3. Practical implications and recommendations

Based on the findings of this series of experiments and literature relevant to ewe lamb breeding, the following guidelines are suggested for farmers to breed their ewe lambs:

- Farmers should aim for a minimum live weight of 40 kg to breed Romney ewe lambs (Kenyon et al., 2014c).
- To maximise Romney ewe lamb breeding performance and fertility and to increase their lamb production over their first three breeding seasons, farmers should aim for an average live weight of approximately 50-52 kg at breeding.
- Average ewe lamb growth rates of 150 g/d between weaning and their first breeding did not negatively affect ewe mammary gland development over the first two breeding seasons. Farmers should, therefore, aim to achieve similar ewe lamb growth rates prior to their first breeding to avoid potential negative impacts on mammary gland. This will likely require providing a high-quality herbage and allowances well above maintenance requirements.
- Breeding heavier ewe lambs did not affect ewe production efficiency (total kg of lambs weaned/ewe breeding live weight) or feed efficiency (total kg of lambs weaned/kg DM) over their first three breeding seasons.
- Farmers should consider culling ewe lambs whose entire litter died during lactation at weaning in order to improve the performance and efficiency of their flock.

- Preliminary results suggest that the ultrasound technique has the potential to be used to identify ewe lambs that would wean heavier lambs and, therefore, could be used by the sheep breeding industry to select these ewe lambs, providing further research is undertaken.

8.4. Limitations of the study

8.4.1. One breed investigated

In all experimental Chapters of this thesis, Romney-type ewe lambs were used to examine the consequences of breeding heavier ewe lambs. Caution would therefore be needed when transferring the results of this thesis to other breeds, in particular dairy breeds. Romney and Romney-composites are currently one of the most common breeds of sheep in New Zealand (Cranston et al., 2017), therefore the results of our study would be of interest for most of New Zealand farmers. Investigating the consequences of a heavier ewe lamb live weight at breeding would be of interest and provide information on other breeds and general management of ewe lambs breeding.

8.4.2. Ewe position for udder ultrasound scanning changed the shape of the mammary gland

The ultrasound technique used in this thesis (Chapters 2 to 4) required non-dairy ewes to be restrained in a sitting position in order to allow for easy access to the mammary gland. Restraining ewes in this way was time and labour intensive. The sitting position resulted in changes to the shape of the mammary gland and internal structures, in particular the mammary gland cistern which is a cavity (Akers, 2002b). The intention of this study was not to assess the size of the mammary structures, although the change of shape could have impacted the association between mammary structures and lamb growth and milk yield. All animals, however, were examined this way across both treatments, thus any measurement bias occurred for both treatments. To avoid this potential measurement bias in the future, a system needs to be developed to enable the ultrasound scan to be conducted while non-dairy ewes are standing, as is achieved in dairy ewe studies (Petridis et al., 2014, Barbagianni et al., 2015). The mammary gland and its structures would retain their natural shape and any association between the mammary gland and milk yield or lamb growth rate would be more accurately reflected. A system with an up-lifted crate could be used (see Section 8.5. Future Research).

8.4.3. Udder ultrasound scanning environment

The ultrasound machine used in Chapter 2 was changed for practical reasons for Chapter 3 and 4 (Sonosite M-Turbo Ultrasound to Mindray Digital Ultrasonic Diagnostic Imaging System DP6600). This resulted in differences in image resolution and dynamic range between Chapters. The dynamic range is the parameter the most frequently used for image quality in ultrasound studies and is defined as the ratio of the largest and smallest signal levels (Lee et al., 2015). Within each chapter, all ewes were scanned with the same ultrasound machine. These differences in image quality across the chapters impacted the assessment by the operator of the tissues present in the mammary gland (parenchyma and fat pad) particularly of images with a lower image quality (Chapters 3 and 4). This could potentially explain the inconsistent results found among single-bearing ewe lambs in Chapters 2 and 3. Changes in image quality, particularly in the dynamic range, however, did not impact the assessment of easily identifiable structures such as the gland cistern and the limits between the mammary gland and the abdominal wall. To improve ultrasound studies, an ultrasound machine with high image quality and high dynamic range should be used to enable a more accurate assessment of mammary tissues.

In Chapters 2 and 4, ewes from both treatments were scanned on a raised shearing platform, but in Chapter 3 in lactation, ewes were scanned on the floor of the milking shed at the same level as the ultrasound operator. This difference in environment influenced the way ewes were scanned and potentially the ultrasound images generated, increasing the measurement bias in the results. Within Chapter 2 and 4, however, the ultrasound scanning environment was similar and consistent in both treatments, and therefore would not have impacted the results within experiments.

8.4.4. Number of ewe lambs in the milking experiment

The ultrasound experiment examining the relationship between ewe lamb milk production and mammary ultrasound measures (Chapter 3) was performed on a limited number of animals (n = 30). Overall, 33.3% of the selected ewe lambs (n = 15) were excluded after lambing due to abortion (4.4%), death (2.2%) or death of their lamb (26.7%). These exclusions resulted in a reduction of the statistical power of the analyses, which may have impacted on the predictions of milk yield and single lamb growth rates. A power analysis, conducted using the results of the correlation between the gland cistern and milk yield of ewe lambs at week three of lactation ($r = 0.288$; $P < 0.05$) of Chapter 3 and using a statistical power of 0.80 (i.e. the probability of correctly rejecting the null hypothesis), calculated that 92 ewe lambs would be required to be milked and scanned. Hence, to improve this experiment, a greater number of non-dairy ewe lambs should be used for milking and ultrasound scanning.

8.4.5. Liveweight difference at ewe lamb breeding

The difference of three kilograms in ewe lamb live weight at breeding was achieved by differential feeding of ewe lambs from a similar starting live weight at weaning of 28.6 kg. The target was to achieve average live weights of 53.0 kg for the Heavy group and 43.0 kg for the Control group, which was a greater difference than was actually achieved (Heavy 47.9 kg and Control 44.9 kg; Chapter 2, 4 and 5 to 7). This relatively small difference in live weight could have limited the ability of the study to detect positive or negative consequences on ewe reproductive performance, live weight, mammary gland development and the performance of progeny during the first, second and third breeding seasons. To achieve a greater live weight difference at breeding, the two treatments could have been fed separately between weaning and the first breeding to provide more flexibility to adjust the feed allowances. The use of alternative forages such as herb-clover mixes could also have increased live weight at breeding in a prospective study (see Section 8.5. Future research). A greater difference could also be achieved in retrospectively allocating ewe lambs to one of the two treatments based on their live weight at breeding in a retrospective study (see Section 8.5. Future research). The three-kilogram difference in average live weight at breeding between treatments did, however, result in differences in reproductive performance during the first breeding season.

The range of ewe lamb live weights at breeding was large within each treatment group, with a range of 41.0 to 60.2 kg in the Heavy group and 39.1 to 57.6 kg in the Control group. These large ranges, which resulted in a high degree of overlap, may have also limited the identification of the effects of a heavier live weight at breeding on ewe and lamb performance. A greater absolute live weight difference between groups, and less overlap, would likely have provided a greater opportunity to identify potential positive or negative impacts of heavier live weight at ewe lamb breeding on ewe performance. More compact live weight ranges could be achieved by either conducting a retrospective or a prospective study controlling for the liveweight difference at breeding and compact liveweight range (see Section 8.5. Future research).

8.4.6. Live weight and condition prior to the second breeding

During the second year of experiment (2019; Chapter 4 and 6), spore counts for *Pithomyces chartarum* which causes facial eczema (FE) were high, resulting in ewes being administered with rumen zinc boluses. The boluses (n = 2) were administered at a 6-weekly interval prior to breeding at 18 months of age. Prior to breeding, some ewes lost live weight and condition, which was later diagnosed as being due to zinc toxicity by post-mortem necropsy on four ewes and suspected on three ewes. This loss of live weight and condition was particularly noted among ewes that were already light or in low condition. As a result, breeding at 18 months of age was delayed by a month to allow ewes to be supplementary fed both before breeding and until the end of the foetal implantation period (approximately 21 days after mating; Cranston et al., 2017). Ewes from both treatments were affected by this situation, therefore, the comparison of the treatments remained valid. The performance of the ewes at two years of age could have been influenced, as ewes that lost live weight and condition prior to breeding would be disadvantaged compared to those that did not. Currently, there are three main strategies to protect ewes from FE by either introducing the genes for FE tolerance in the genetic of the ewes or treating ewes with zinc using a drench or a ruminal bolus to reduce the incidence and severity of FE or spraying a fungicide on pastures (Grace, 2010, Beef + Lamb New Zealand, 2019a). Ewes in the current study were not tolerant to FE and therefore needed to be treated with zinc every year. Using ruminal boluses protects ewes for four to six weeks and is less labour-intensive compared to drenching which needs to performed weekly or fortnightly and (Beef + Lamb New Zealand, 2019a). Hence, treating ewes with zinc ruminal bolus seems to be the best practice during the FE season.

8.4.7. Ewe lambs bred at 50 kg and above

In Chapter 7, data from the two treatments were pooled as one population to determine the relationship between ewe lamb breeding live weight and ewe mature weight, and to estimate the feed required to breed heavier ewe lambs. In the whole population considered in Chapter 7, the number of ewe lambs above 50 kg at breeding was limited (12.6%) and above 55 kg was very low (3.0%) compared to the number of ewes bred between 40 and 45 kg (42.6%). This distribution of live weights limited the identification of the effects of heavier (50 kg and above) live weight at ewe lamb breeding on performance and efficiency. Having a greater number of ewes with the heavier live weights at ewe lamb breeding would have allowed the investigation of heavier live weight. In order to grow more ewe lambs to a heavier live weight (above 50 kg), ewe lambs from the Heavy group could have been fed separately from the Control group from their weaning to their first breeding. This separation would have allowed an increase in the quantity of concentrate feed distributed to the Heavy group earlier than what was achieve in the current study.

8.5. Future research

8.5.1. Effect of birth and rearing rank on mammary gland structures

The associations between ewe lamb mammary ultrasound measures and single lamb growth rates were inconsistent across experiments (Chapters 2-3). In addition, these associations appeared to differ based on lamb birth rank among two-year-old ewes in their second lactation (Chapter 4). The effect of birth rank on the association between ultrasound measures and lamb growth, however, was not investigated due to the small number of ewes in each birth rank category in Chapter 4. The mammary gland is known to have significant cellular plasticity (Boutinaud et al., 2019), allowing it to adapt to the number of lambs suckling. To date, the relationship between the number of lambs reared and mammary ultrasound measures have not been investigated in ewes. It would, therefore, be of interest to investigate the effect of lamb birth and rearing rank on the association between ewe mammary ultrasound measures of ewes and the growth of their progeny. It would be recommended to use a larger number of ewes in each category of birth and rearing rank, at least 118 ewes, based on a power analysis using the depths of gland cistern in lactation in single- and twin-bearing ewes (18.1 ± 1.0 and 16.2 ± 1.01 respectively; Chapter 4) and using a statistical power of 0.80. This type of investigation would establish whether measurements during the first pregnancy and lactation would enable this to be used as an early selection technique.

8.5.2. Persistence of mammary structure size over subsequent years

The mammary parenchyma in late pregnancy and the depth of the gland cistern in early lactation have been identified as positive indicators of single lamb growth to weaning for lambs born to ewe lambs (Chapter 2). If easily identified, these structures could potentially enable farmers to identify ewe lambs that would be more likely to wean heavier lambs. It is not known, however, whether those indicators are persistent in subsequent lactations and whether a ewe identified with large gland cisterns would have large gland cisterns in subsequent lactations. An investigation of the repeatability of the results and the persistence of the gland cistern and parenchyma depth across years and lactations is required. To determine this, an experiment should be conducted using the same animal positions and quality of ultrasound machine, which were limitations in Chapter 3 and 4 respectively.

8.5.3. Improvement of the ultrasound scanning technique

If ultrasonography was to be used as a selection technique in the future, it will need to be improved for a larger scale application. The following changes would need to be made:

- *limit ewe handling to reduce labour*. In this study, ewes were placed in a sitting position for scanning, however, scanning the ewes while standing could provide information on the natural shape of the mammary structures. A system similar to that used for pregnancy scanning, whereby ewes are restrained standing in a up-lifted crate could be considered. This system would reduce the labour required but could result in an increase in the time required to scan each ewe as they would be difficult to immobilise.
- *reduce the waiting period for ultrasound scanning in lactation*. The method used in Chapters 2 to 4 during lactation required waiting for a period of approximately four hours. This waiting period allowed the udder to accumulate milk to correctly visualise, assess and compare mammary

structures with similar gland filling. This waiting period limits the applicability of using ultrasound scanning in a commercial farming system. Ultrasound scanning ewes without waiting period would likely create a bias in the comparison of udder measures that differ in filling. Further investigation would be needed to determine alternative methods of scanning ewes with similar udder filling and the potential impact of various length of separation from lambs.

- *reduce the time required for image processing*. The ideal situation would be to measure mammary structures at the time of scanning and directly provide information about the ewe. The precise measurement of mammary structures, however, varies depending on the scanning depth. These variations mean, for example, that a gland cistern that looks large on the screen does not necessarily have a large measurement. The measures at the time of scanning would therefore be challenging. Using the previously recorded data on ewe lambs, it may be possible to determine categories with depth ranges for each mammary structure in which a ewe lamb could be classified as small, medium or large, based on the different time points and scanning depths. Using this type of classification system, measurements could be made at the time of scanning but would considerably increase the total time of scanning. Prior to this study, a significant number of ewe lambs would need to be ultrasound scanned to determine the range of depths, and the relationship with lamb growth traits. Further the range of measures at the time of scanning would likely require an experienced operator.

8.5.4. Increase of the liveweight difference at ewe lamb breeding

In this thesis, the two treatments (Heavy and Control) only differed by three kilograms at ewe lamb breeding, which likely limited the identification of negative consequences of a heavier live weight (Chapter 5 to 7). It would, therefore, be of interest to investigate the effects of a heavier live weight with a greater difference in live weight at ewe lamb breeding over their lifetime. Two different methods could be used:

- *a retrospective cohort study* where ewe lambs would be retrospectively allocated to a "Heavy" or a "Control" group based on their live weight at breeding at seven months of age to compare their lifetime performance and efficiency. This approach would reduce the live weight range at breeding within each group and increase the live weight difference between groups. As ewe lambs would retrospectively be assigned to a group, it would be possible to ensure that the live weight ranges of each group did not overlap. It would also be possible to only select a proportion of the heaviest and the lightest ewe lambs to increase the live weight difference and reduce the live weight range at ewe lamb breeding. A sufficient number of ewe lambs, however, would need to be included in each group to maintain a statistical power of 0.80. The ewe lambs would not be selected randomly which may result in different genetic composition between groups. This approach, however, would potentially include some confounding effects (i.e. different birth ranks, weaning weight, feeding management, etc.) which could be accounted for, if known, in the analysis but can reduce statistical power. Such an approach may potentially exclude explanatory variables. Further, in a retrospective approach, the two groups would not necessarily need to be treated differently (i.e. different feeding management) to reach the different ewe lamb breeding live weight, hence, the reason why some ewe lambs grow faster would remain completely unknown. These limitations would likely impact the results and conclusions of the study and any findings found would need to acknowledge these limitations.

- *a prospective study*, similar to that undertaken in this thesis, where ewe lambs would be allocated to the different feeding treatments at weaning to lead to differing live weight at breeding. To increase the difference in live weight, the two treatments could be fed separately from weaning to breeding to enable for more flexibility in feed adjustments. In the present study, ewe lambs were offered concentrate feeds to avoid the drought in summer 2018. It could be beneficial to investigate the use different alternative forage crops such as herb-clover mix (including chicory, plantain, red and white clover) to increase live weight at ewe lamb breeding. Herb-clover mixes can increase live weight at weaning of lambs (Kenyon et al., 2010a, Hutton et al., 2011) and live weight of their dams at weaning compared to ryegrass/white clover pastures (Corner-Thomas et al., 2020). Using alternative forage crops to increase live weight of ewe lambs at breeding would match with the low input farming system in New Zealand. Two options could be considered either offering different herbage allowances of herb-clover mix between treatments or comparing two feed types (i.e. herb-clover mix *vs.* ryegrass and concentrate feed) from weaning to breeding. This approach would result in a greater live weight range at breeding than in the retrospective approach as ewe lambs would be grazing in pasture resulting in a challenging assessment and adjustment of individual intakes. To reduce the live weight range, the selection of ewe lambs could be based on a narrow range of weaning weights. Further, with this approach, there would be uncertainty in achieving a greater difference in ewe lamb breeding live weight than was achieved in the current study. A greater number of ewe lambs could be selected at the start of the feeding treatments in order to allow some ewes in each treatment to be removed to increase the live weight difference between treatments at breeding, while maintaining a sufficient number of ewe lambs in each group to have a statistical power of 0.80. The number of confounding effects and missing explanatory variables could also be reduced with this approach compared to a retrospective approach.

8.5.5. Lifetime performance of heavier ewe lambs at breeding

Reproductive performance and live weight of ewes bred at a heavier live weight as a ewe lamb did not differ between Heavy and Control ewe lambs during their second and third breeding seasons (Chapter 6). It would be beneficial to continue the investigations on the effects of a heavier live weight at ewe lamb breeding over their lifetime, as potential consequences on efficiency or longevity could be identified (Baker et al., 1981, Kenyon et al., 2011b, Thomson et al., 2020). Using lifetime data, to at least five years of age, would add to the information available in Chapters 5 to 7 about breeding heavier ewe lambs and would benefit farmers in their management of ewe lamb breeding.

8.5.6. Impact of not rearing a lamb as a ewe lamb on subsequent ewe reproductive performance

Over the three years of the experiments in this thesis, ewes that were identified pregnant but failed to wean a lamb as a ewe lamb had the lowest efficiency and total weight of lamb weaned compared to ewes that weaned a lamb or did not conceive as a ewe lamb. These results were consistent with the results of Amer et al. (2009) who reported that mature ewes whose entire litter died during the previous lambing had less lambs born, lambs with lower survival and lighter lambs at weaning at their next lambing than ewes that reared a lamb. The number of ewe lambs that failed to wean a lamb in this thesis, however, was low (< 30), hence, these results should be interpreted with caution and require further investigations. The examination of reproductive performance of a larger group of ewe lambs that failed to wean a lamb would be important for the sheep industry and provide information on the management and culling of ewe lambs.

8.5.7. Economic analysis of the effect of a heavier live weight at ewe lamb breeding

An economic analysis of breeding heavier ewe lambs is required to assess the profitability and the potential benefits of this practice. Breeding ewe lambs has been shown to increase farm profitability in both New Zealand (Farrell et al., 2020, 2021) and Australia (Tocker et al., 2020, Young and Thompson, 2021). In addition, the reproductive performance of ewe lambs can have a positive effect on farm profitability (Farrell et al., 2020, 2021). These studies, however, have not investigated the potential effects of a heavier breeding live weight on ewe efficiency or lifetime performance of ewes bred as a ewe lamb, on farm profitability. Accounting for the cost of growing ewe lambs to heavier live weights at breeding and the income from their lifetime lamb production needs to be addressed. Multiple scenarios could be compared with different live weights of ewe lambs at breeding based on existing reproductive data. An economic analysis would benefit farmers in their decision regarding management of ewe lambs.

8.6. Conclusion

Increased growth rates of 150 g/d prior to the first breeding did not negatively impact ewe lamb mammary gland development to their second weaning. Breeding heavier ewe lambs improved the overall reproductive performance of ewe lambs and increased the total lamb production over three breeding seasons. Heavier ewe lamb live weights, however, did not negatively affect ewe efficiency to 39 months of age but may increase ewe live weight at 39 months of age. Collectively, the results of this work suggest that farmers should aim to breed their Romney ewe lambs at a heavier live weight of approximately 50-52 kg to maximise ewe lamb reproductive performance and, if well managed, increase ewe performance over the first three breeding seasons. Before final recommendations can be made, impacts on lifetime performance and longevity of heavier ewe lambs at breeding need to be examined. The ultrasound technique used in this thesis enabled the visualisation and measurement of internal structures of the mammary gland. Evidence of positive associations between mammary ultrasound measures and growth rates of the progeny to weaning were found. These associations indicate that mammary ultrasound scanning has the potential to be used to select ewes which would wean heavier lambs, although additional research is required.

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Appendices

Appendix A. Drawing templates of mammary ultrasound images in late pregnancy, early lactation and weaning in ewe lambs

Table A1. Drawing template of mammary ultrasound images in late pregnancy (107 days of pregnancy; P107) in four different ewe lambs

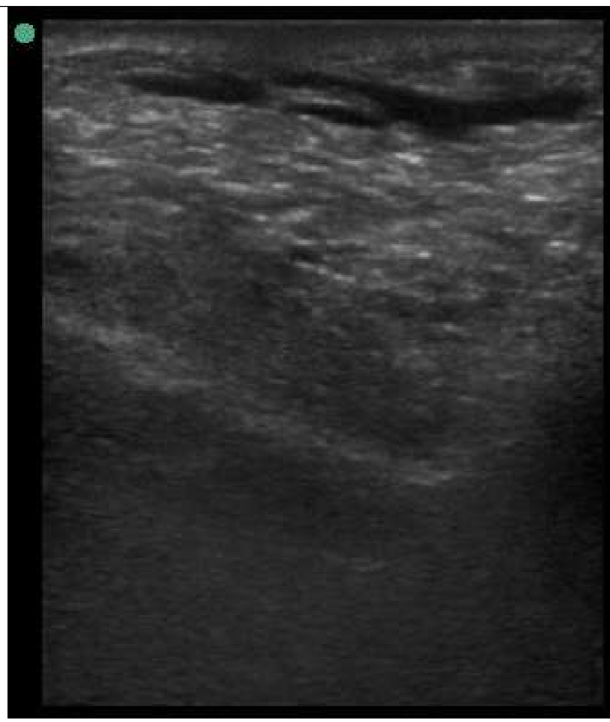
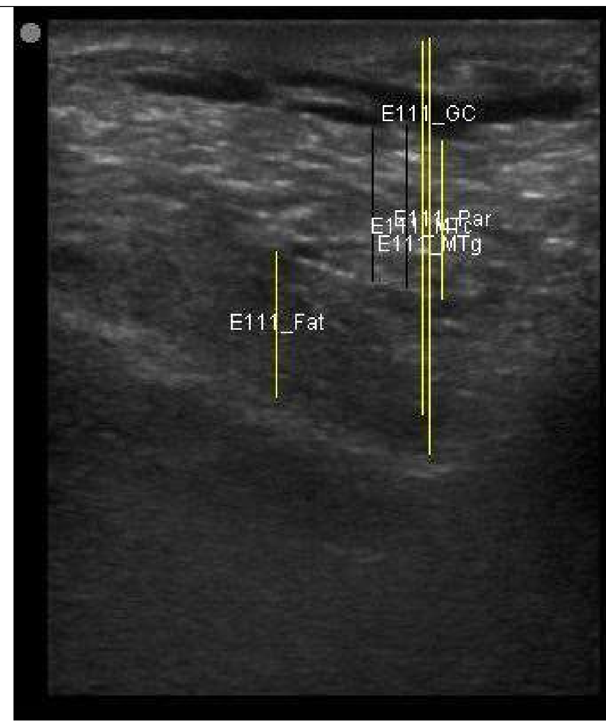
Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
113 days of pregnancy Scanning depth 4.7 cm			MT generous: 28.9 mm MT conservative: 25.9 mm Gland cistern: 3.3 mm Parenchyma: 10.9 mm Fat Pad: 10.0 mm

Table A1 continued

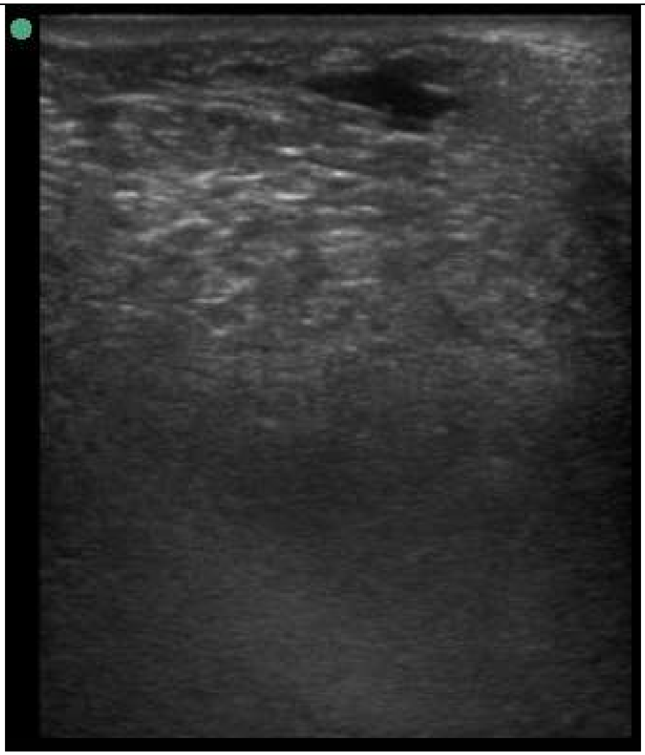
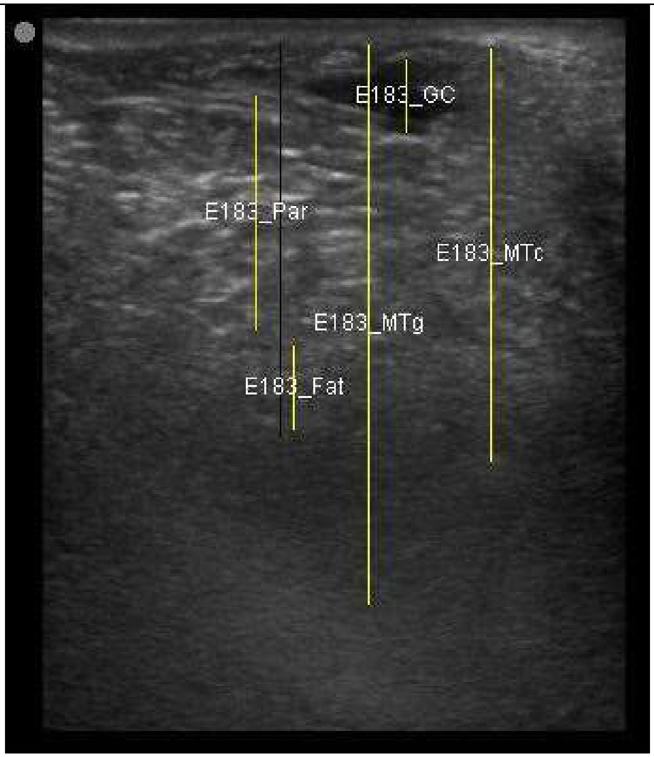
Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
109 days of pregnancy Scanning depth 4.7 cm			MT generous: 36.8 mm MT conservative: 27.2 mm Gland cistern: 4.7 mm Parenchyma: 15.4 mm Fat Pad: 5.5 mm

Table A1 continued

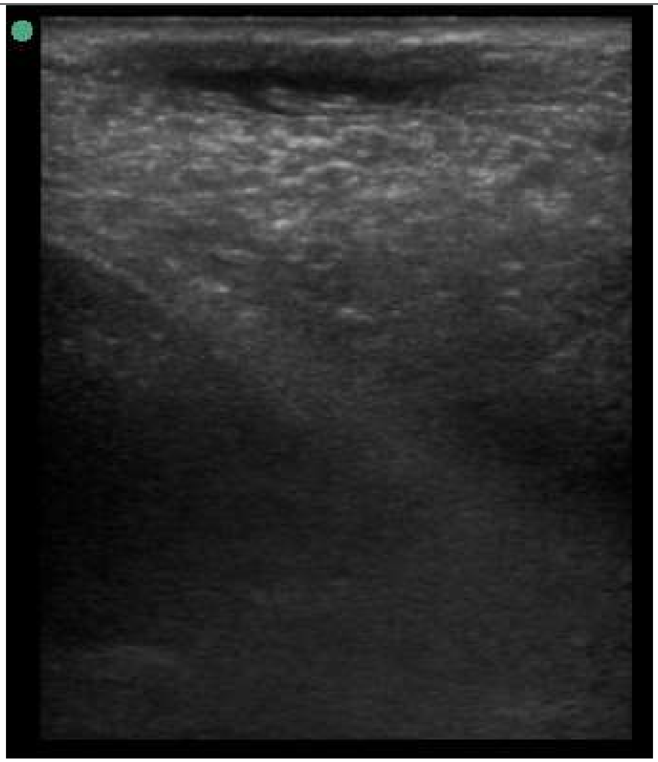
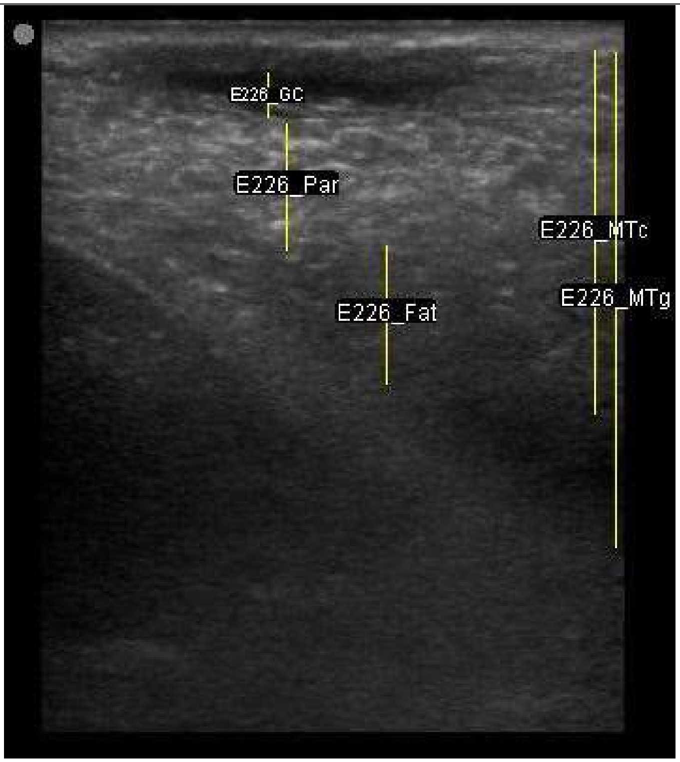
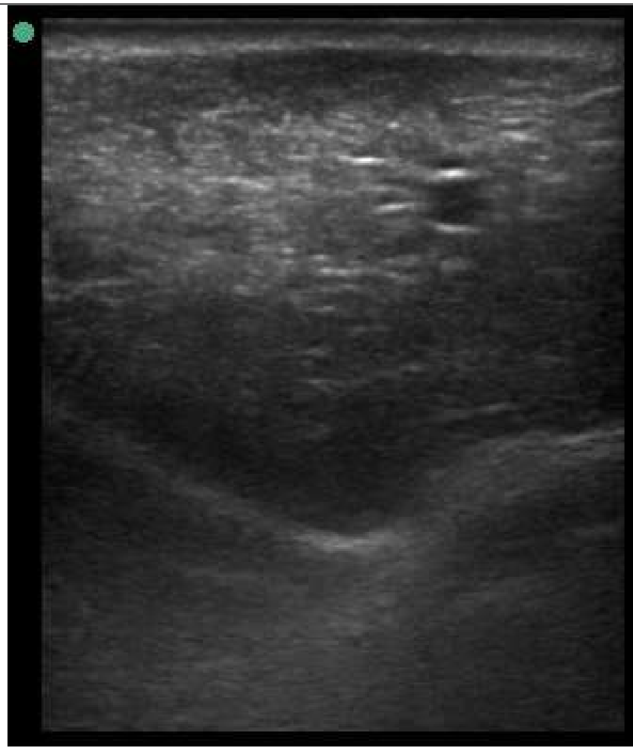
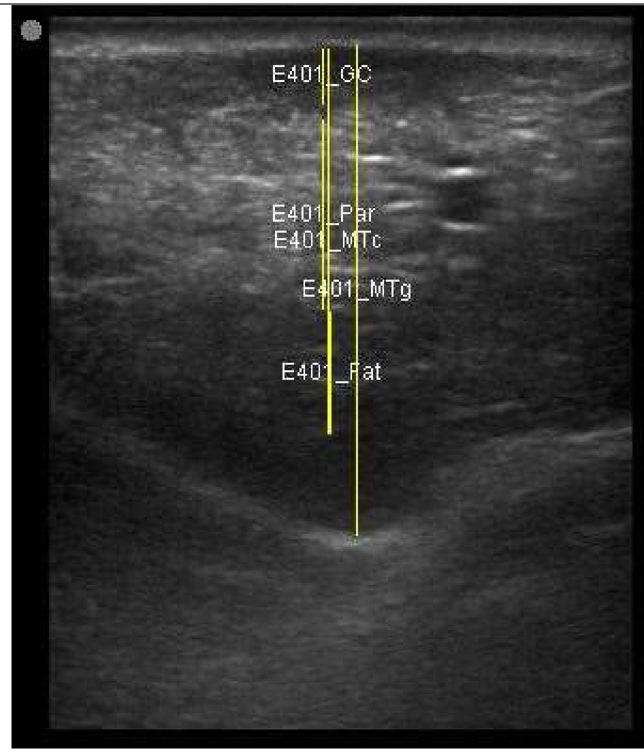
Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
107 days of pregnancy Scanning depth 4.7 cm			MT generous: 31.7 mm MT conservative: 22.3 mm Gland cistern: 4.5 mm Parenchyma: 8.3 mm Fat Pad: 7.8 mm

Table A1 continued

Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
107 days of pregnancy Scanning depth 4.7 cm			MT generous: 32.2 mm MT conservative: 25.3 mm Gland cistern: 3.6 mm Parenchyma: 12.4 mm Fat Pad: 7.9 mm

MT: Total depth of mammary gland

Table A2. Drawing template of mammary ultrasound images in early lactation (29 days of lactation; L29) in four different ewe lambs

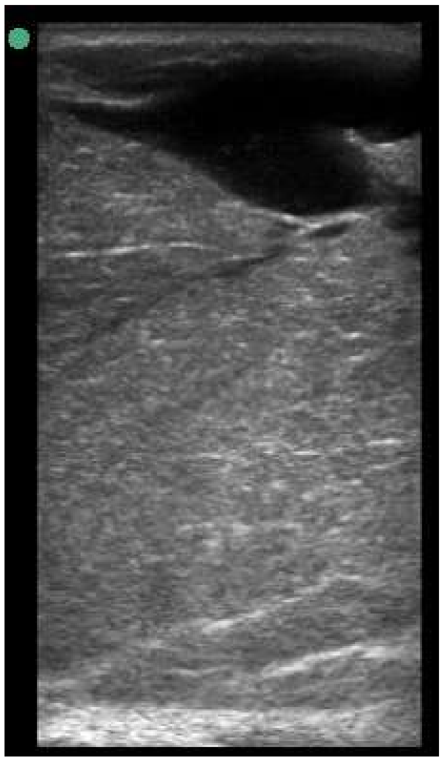
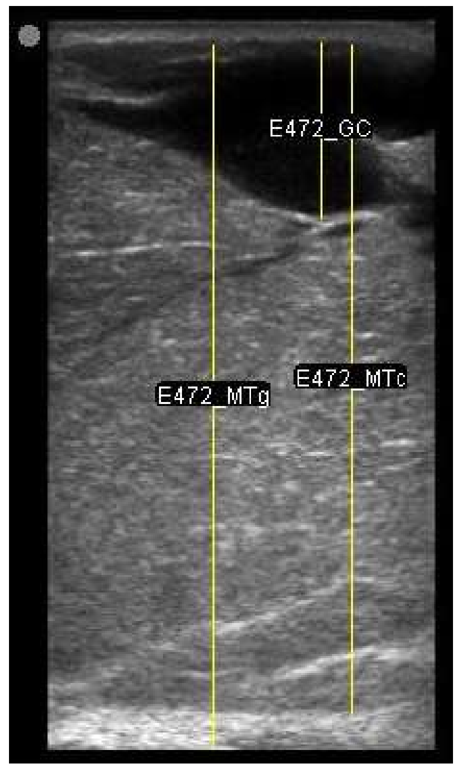
Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
32 days of lactation Scanning depth 7.3 cm			MT generous: 70.6 mm MT conservative: 67.2 mm Gland cistern: 17.6 mm Parenchyma: 49.6 mm

Table A2 continued

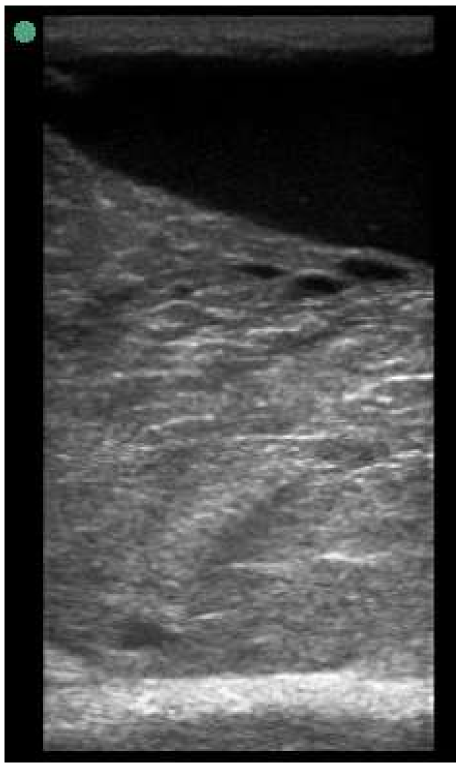
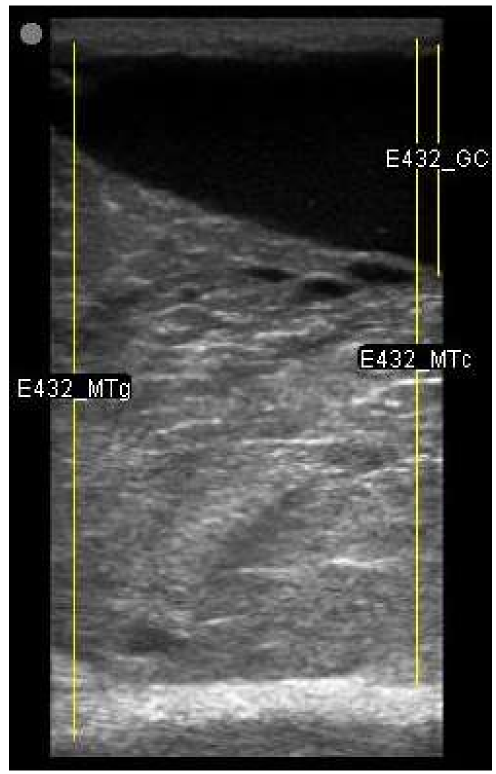
Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
26 days of lactation Scanning depth 7.3 cm			MT generous: 68.9 mm MT conservative: 63.9 mm Gland cistern: 22.8 mm Parenchyma: 41.1 mm

Table A2 continued

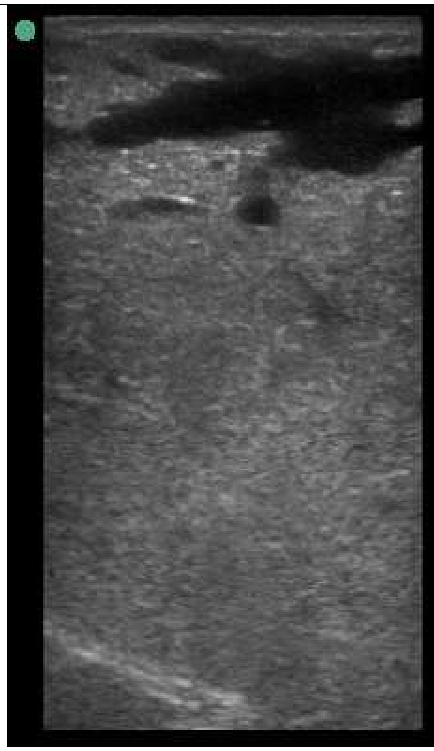
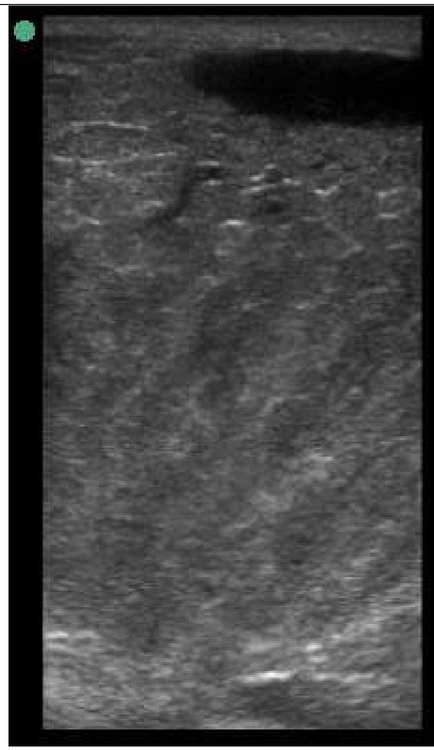
Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
34 days of lactation Scanning depth 7.3 cm			MT generous: 70.8 mm MT conservative: 67.4 mm Gland cistern: 14.5 mm Parenchyma: 52.9 mm

Table A2 continued

Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
26 days of lactation Scanning depth 7.3 cm			MT generous: 68.5 mm MT conservative: 65.0 mm Gland cistern: 8.1 mm Parenchyma: 56.9 mm

MT: Total depth of mammary gland

Table A3. Drawing template of mammary ultrasound images at weaning (100 days of lactation; L100) in four different yearling ewes

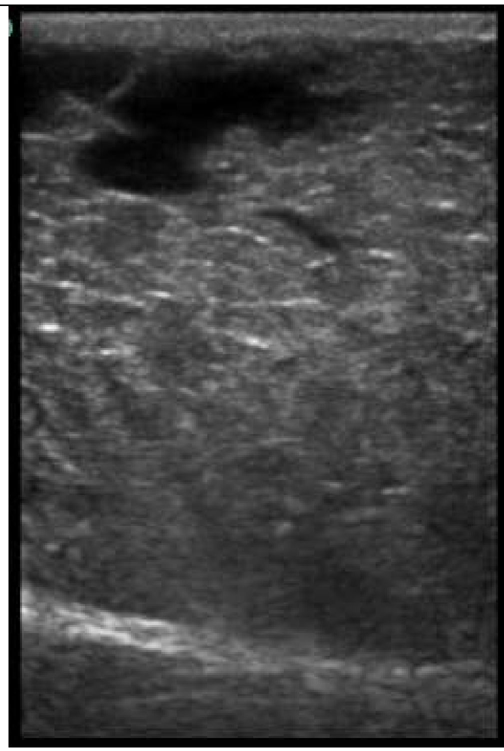
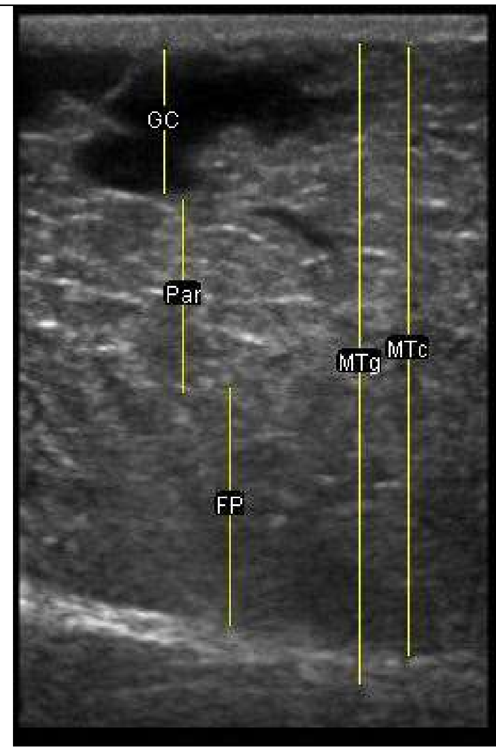
Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
104 days of lactation Scanning depth 5.9 cm			MT generous: 53 mm MT conservative: 50.4 mm Gland cistern: 11.9 mm Parenchyma: 15.9 mm Fat Pad: 19.7 mm

Table A3 continued

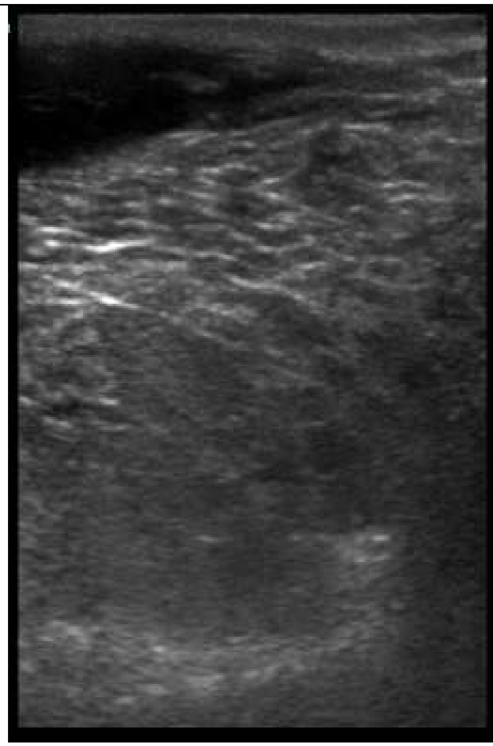
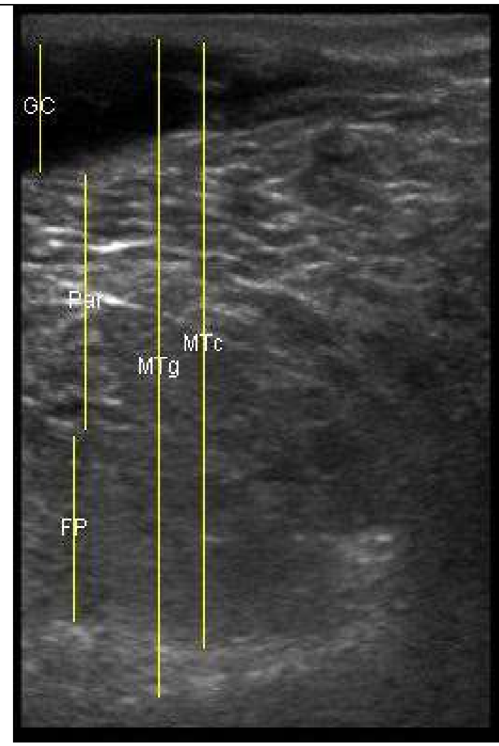
Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
106 days of lactation Scanning depth 5.9 cm			MT generous: 54.4 mm MT conservative: 50.1 mm Gland cistern: 10.5 mm Parenchyma: 21.1 mm Fat Pad: 15.3 mm

Table A3 continued

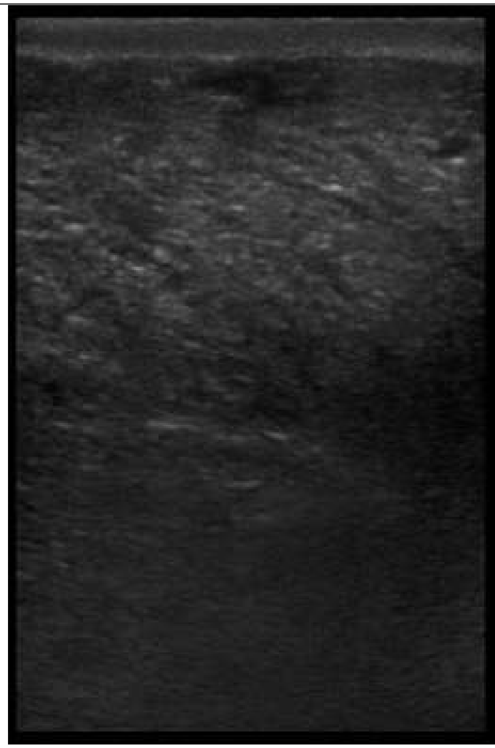
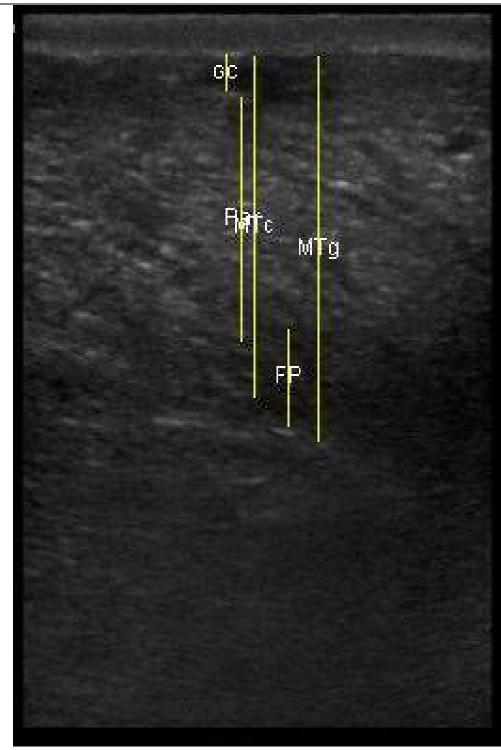
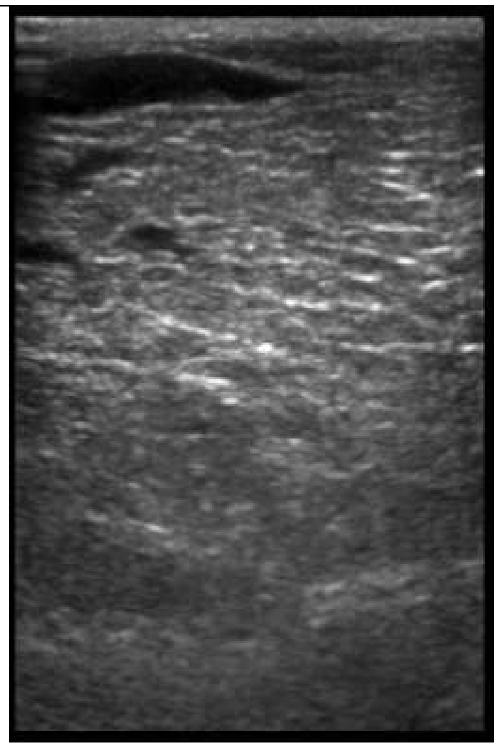
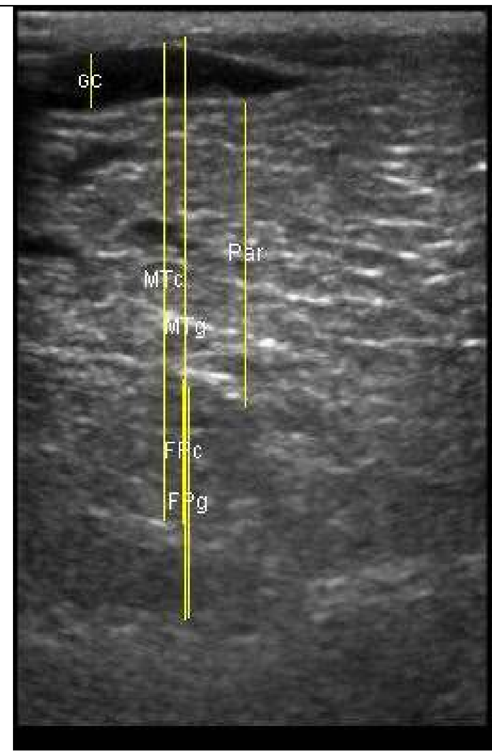
Time point & scanning depth	Raw image	Measurement of different udder structures	Depth (mm)
100 days of lactation Scanning depth 5.9 cm			MT generous: 31.8 mm MT conservative: 28.2 mm Gland cistern: 3 mm Parenchyma: 20.1mm Fat Pad: 8 mm

Table A3 continued

<table>
<tr><th>Time point & scanning depth</th><th>Raw image</th><th>Measurement of different udder structures</th><th>Depth (mm)</th></tr>
<tr><td>105 days of lactation

Scanning depth 5.9 cm</td><td></td><td>
</td><td>MT generous: 48.2 mm

MT conservative: 39.5 mm

Gland cistern: 4.4 mm

Parenchyma: 25.1 mm

Fat conservative: 11.9 mm

Fat generous: 19 mm</td></tr>
</table>

MT: Total depth of mammary gland

Appendix B. Correlations between udder measurements, milk yield and lamb growth

Table B.1. Correlation coefficients of residuals of daily milk yield (MY) in week 3 (W3), week 5 (W5), week 7 (W7) of lactation, udder height (UH) in pregnancy (P110) and at weaning (L69), mammary gland cistern (GC), parenchyma (PAR), fat pad (FP), total depth of the mammary gland conservative (MTc) and total depth of the mammary gland generous (MTg) at P110, W3, W5, W7, L69 per udder half.

	MY W3	MY W5	MY W7
UH			
P110	0.013	-0.186	-0.256
L69	-0.006	0.116	0.143
GC			
W5	-0.008	-0.141	-0.065
W7	-0.014	-0.080	0.040
PAR			
P110	-0.172	-0.016	-0.167
W3	0.014	-0.036	-0.048
W5	0.028	0.220	0.011
W7	-0.016	0.113	-0.030
L69	0.026	0.121	0.185
FP			
W3	-0.112	-0.047	0.297
W5	0.057	-0.128	-0.199
W7	-0.046	0.073	0.031
L69	-0.0007	0.273	0.028
MTc			
P110	-0.170	-0.133	0.037
W3	-0.055	-0.129	-0.131
W5	0.052	-0.047	-0.101
W7	-0.129	-0.029	-0.008
L69	0.022	0.104	0.147
MTg			
P110	0.119	-0.107	0.120
W7	0.209	0.002	0.219

P110: day 110 of pregnancy; L69: weaning.

Table B.2. Correlation coefficients of residuals of lamb growth from birth to week 3 of lactation (Birth to W3), birth to week 5 of lactation (Birth to W5), birth to week 7 of lactation (Birth to W7), L3 to weaning (W3 to L69), W5 to weaning (W5 to L69), W7 to weaning (W7 to L69), birth to weaning (Birth to L69), the average of both udder half of udder height (UH) in pregnancy (P110) and at weaning (L69), udder circumference and volume at P110, the average of both udder half of gland cistern (GC), mammary parenchyma (PAR) and fat pad at P110, W3, W5, W7, L69.

	Birth to W3	Birth to W5	Birth to W7	W3 to L69	W5 to L69	W7 to L69	Birth to L69
UH							
P110	-0.043	-0.280	-0.269	-0.138	0.017	0.117	-0.167
L69	0.031	0.015	0.040	0.134	0.139	0.222	0.152
UC							
P110	-0.172	-0.171	-0.053	0.088	0.130	0.048	-0.021
UV							
P110	-0.085	-0.234	-0.162	-0.020	0.092	0.100	-0.080
GC							
P110	0.290	0.134	0.082	-0.007	0.018	0.091	0.147
W3	-0.085	0.093	0.090	0.168	0.063	0.113	0.117
W5	-0.126	0.067	0.126	0.253	0.158	0.062	0.126
W7	0.031	0.126	0.133	0.040	-0.076	-0.154	0.051
L69	-0.057	-0.045	-0.043	-0.004	-0.053	-0.086	-0.042
PAR							
P110	-0.008	-0.127	-0.160	-0.209	-0.133	-0.120	-0.175
W5	0.098	-0.009	-0.028	-0.031	0.041	0.163	0.049
W7	-0.012	-0.167	-0.282	-0.302	-0.248	-0.079	-0.259
L69	-0.081	-0.077	-0.061	-0.068	-0.071	-0.107	-0.087
FP							
W5	0.012	-0.090	-0.119	-0.120	-0.132	-0.118	-0.117
W7	0.090	0.171	0.228	0.265	0.259	0.262	0.291
L69	0.126	0.257	0.275	0.267	0.228	0.235	0.287

P110: day 110 of pregnancy; L69: weaning.

Appendix C. Associations among lamb growth and udder measurements and two-year-old ewe live weight.

Table C.1. Correlation coefficients of residuals of lamb growth from birth to early lactation (Birth to L29), early lactation to weaning (L29 to L79), birth to weaning (Birth to L79) and udder height (UH) with udder height (UH), circumference (UC), volume (UV), live weight (Ewe LW), depth of the mammary gland cistern (GC), parenchyma (PAR) and the total depth of the mammary gland conservative (MTc) and generous (MTg) of two-year-old ewes in late-pregnancy (P119), early lactation (L29) and at weaning (L79).

	Birth to L29	L29 to L79	Birth to L79
UH			
L29	-0.031	-0.011	-0.023
L79	-0.122	-0.062	-0.123
UC			
P119	-0.119	-0.118	-0.168
L29	0.200	-0.107	0.021
UV			
L29	0.094	-0.093	-0.023
L79	0.008	0.112	0.110
Ewe LW			
P119	-0.115	-0.023	-0.073
L29	0.220	0.046	0.159
L79	-0.093	-0.041	-0.072
GC			
P119	-0.028	-0.155	-0.152
L29	0.106	0.090	0.112
L79	0.171	-0.089	0.012
PAR			
P119	0.038	-0.001	0.030
L29	-0.103	-0.103	-0.147
L79	-0.027	-0.139	-0.124
FP			
P119	0.032	0.038	0.058
MTc			
P119	0.210	-0.084	0.039
L29	0.011	0.029	0.025
L79	-0.231	0.215	0.087
MTg			
L29	0.193	0.101	0.196

Appendix D. Ewe energy requirement equations

Ewe maintenance *(Nicol and Brookes, 2017)*

$$ME\_b = Species \times Sex \times 0.28 \times EXP\ (-0.03 \times Age\ in\ years) \times (Predicted\ live\ weight\text{^}0.75)\ /\ km$$

where: Species = 1.0 for sheep

Sex = 1.0 for females

km = M/D × 0.02 + 0.5

where M/D = Energy concentration of the feed (MJME/ kg DM) = 10.3 MJME/kg DM in this experiment (Litherland et al., 2002)

Ewe liveweight change *(adapted from Nicol and Brookes, 2017)*

$$ME\_g = 1.1 \times LWC \times NE\_g\ /\ k\_g$$

where: LWC = liveweight change (kg/d)

$NE\_g = 0.92 \times ((6.7 + (((920 \times LWC)/(4 \times SRW^{0.75})) - 1)) + (20.3 - (((920 \times LWC)/(4 \times SRW^{0.75})) - 1)) / (1 + EXP\ (-6 \times ((predicted\ live\ weight/SRW) - 0.4))))$

SRW = mature body size = 65 kg for Romney

k_g = (M/D × 0.042) + 0.006

k_g (lactating) = 0.95 × ((M/D × 0.02) + 0.4)

Ewe pregnancy requirement *(Nicol and Brookes, 2017)*

$$ME\_p = (Total\ birth\ weight)/4 \times EXP(7.64 - 11.46 \times (EXP(-0.00643 \times day\ of\ pregnancy))) \times 0.0737 \times EXP(-0.00643 \times day\ of\ pregnancy))/k\_p$$

where: k_p = 0.133

Lactation requirements *(Freer et al., 2007, Nicol and Brookes, 2017)*

Ewe metabolizable energy (ME) required to produce milk (Ewe lactation)

$$Ewe\ lactation = (((0.328 \times 8.0) + (0.0025 \times day\ of\ lactation) + 2.203) \times daily\ milk\ yield)/\ k\_l$$

where: daily milk yield was predicted from Peart et al. (1975) based on days of lactation and number of lambs reared

k_l = 0.95 × ((M/D × 0.02) + 0.4)

Lamb net energy (NE) requirement for maintenance for each lamb (LmNEm)

$$LmNEm = Species \times Sex \times 0.28 \times EXP\ (-0.03 \times age\ in\ years) \times (predicted\ live\ weight^{0.75})$$

where: Species = 1.0 for sheep

Sex = 1.075 for lambs

Lamb NE requirement for growth for each lamb (LmNEg)

$$LmNEg = 0.92 \times ((6.7 + (((920 \times LWC)/(4 \times (SRW \times 1.2)\hat{}0.75\ )) - 1)) + (20.3 - (((920 \times LWC)/(4 \times (SRW \times 1.2)\hat{}0.75)) - 1))\ /\ (1 + EXP\ (-6 \times ((predicted\ live\ weight/(SRW \times 1.2)) - 0.4)))))) \times 1.1 \times LWC$$

where: LWC = liveweight change (kg/d)

SRW = 65 kg

Proportion of the lamb requirement met by DE in milk (%MilkDE)

$$\%MilkDE = (0.26 - (0.015 \times day\ of\ \ lactation\ /\ 7))\ /\ 0.23$$

If day of lactation = 0, %MilkDE = 0

If %MilkDE > 1, %MilkDE = 1

Proportion of the lamb NE requirement for maintenance and growth met by milk (%MilkNE)

$$\%MilkNE = \%MilkDE \times ((LmNEm/(0.9 \times 0.82)) + (LmNEg/(0.7 \times 0.82))))\ /\ (((LmNEm/(0.9 \times 0.9)) + (LmNEg/(0.7 \times 0.9))) + (\%MilkDE \times (((LmNEm/(0.9 \times 0.82)) + (LmNEg/(0.7 \times 0.82))) - ((LmNEm/(0.9 \times 0.9)) + (LmNEg/(0.7 \times 0.9))))))$$

Proportion of the lamb ME requirement for maintenance and growth met by pasture (LmMEp)

$$LmMEp = ((LmNEm/k\_m) + (LmNEg/k\_gain)) \times (1 - \%MilkNE)$$

where: k_m = (M/D × 0.02) + 0.5

k_gain = 0.7

Total ewe daily requirement

Total daily energy requirement = Ewe maintenance + Ewe liveweight change + Ewe pregnancy + Ewe lactation + LmMEp

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E. Haslin, R.A. Corner-Thomas, P.R. Kenyon, S.T. Morris, H.T. Blair, 2021, Effects of Increased Growth Rates Prior to the First Breeding as Ewe Lambs and Pregnancy Rank on Mammary Glands of Two-Year-Old Ewes, Ruminants, 1, 72-86.

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