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Molecular genetic analysis of IGF1 in Romney sheep and its role in growth

A Dissertation
submitted in partial fulfilment
of the requirements for the Degree of
Bachelor of Agricultural Science (Honours)
at
Lincoln University
by
Olivia Margaret Ellis

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science (Honours)

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In an effort to improve livestock breeding, recent focus has been on using marker-assisted selection (MAS) to increase the accuracy of the choices made in selecting breeding stock. MAS may increase the annual rate of genetic gain in livestock by as much as 15-30%. Traits that determine the economic value of livestock are of primary concern in livestock breeding. Insulin-like Growth Factor 1 (IGF-1: also known as somatomedin C) affects young animal growth and a range of other anabolic processes in adults from a number of species, but little is known about its role in sheep. Variation in the IGF-1 gene (\textit{IGF1}) has also been reported in other animal species, but once again little is known about ovine \textit{IGF1} variation. Using a polymerase chain reaction – single strand conformational polymorphism (PCR-SSCP) approach, 50 New Zealand (NZ) Romney rams were investigated to ascertain whether variation existed in two regions (an exon 2 fragment and an exon 3 fragment) of ovine \textit{IGF1}. Two PCR-SSCP banding-patterns were discovered for each region, with one or a combination of two banding patterns detected for each sheep. For each region, these patterns were named \textit{A} and \textit{B}, and upon sequencing a unique DNA sequence was identified. 150 lambs (obtained from the NZ Romney Progeny Test; 2007-present), that were the progeny of a single ram that produced lambs over two seasons (2007 and 2009) were investigated for the association analysis. Phenotypic data for growth and carcass traits were available for these lambs and statistical analyses (Minitab® v17) were performed using stepwise regression to assess the effect of the presence or absence of the \textit{IGF1} variants on the various lamb phenotypes. In these analyses the presence of the exon 2 \textit{IGF1} \textit{A} variant was associated (\textit{P} = 0.049) with increased birth weight in the 2007 lambs, although this effect did not persist in the 2009 lambs, or when the data from both years was combined. For the 2009 lambs, the presence of exon 2 \textit{A} was associated (\textit{P} = 0.017) with an increased growth rate from birth to tailing and this effect persisted (\textit{P} = 0.035) when the 2007 and 2009 lamb data was combined. Trends (0.05 < \textit{P} < 0.2) were observed between the presence of exon 2 \textit{A} and increased tailing weight and growth from birth to weaning in the 2009 lambs and these trends persisted when the data from the 2007 and 2009 lambs was combined. No effects were observed for
the exon 3 genotypes in 2007, but in 2009, there was a trend for AA to be associated with increased tailing weight and weaning weight, with the tailing weight effect persisting when both years’ data was combined.

Overall these results suggest that variation in *IGF1* could be of value as a genetic marker to complement genetic evaluations for early growth in sheep.

**Keywords:** *IGF1*, sheep, growth trait, variation, production, growth promoting effect, Romney, genetic selection
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Chapter 1

Introduction

In the livestock species that are farmed for meat production, increased productivity is achieved by selection for increased number of offspring, increased growth rates and increased muscling, such that larger numbers of offspring reach market weights more rapidly and with a greater meat yield (Dwyer & Bunger, 2012). In New Zealand, the sheep industry places a strong emphasis on selection for rapid lamb growth and high meat yield to obtain premium prices, and to free up land more rapidly for other stock classes. This can also reduce labour inputs and animal health costs (Golding et al., 2008; Judson et al., 2009).

Lamb growth rate is an important driver of farm profit. Faster growing lambs achieve target weights earlier and can be weaned for slaughter earlier to take advantage of meat schedule premiums (Mathias-Davis et al., 2013). Faster growing lambs also have a higher gross efficiency of conversion (kg lamb meat/unit feed intake) than slower growing lambs of the same weight (Elmes, 2013).

Birth weight, growth rate prior to weaning and weaning weight are three growth traits that have an important role in lamb meat production. Therefore, breeding for optimal birth weight and increased weight gains, is a major factor in sheep breeding programmes. Growth rate is related to muscle protein accretion, this being the difference between the rates of muscle protein synthesis and degradation. The higher the protein accretion at a set rate of degradation, the higher the growth rate of the animal (Therkildsen & Oksbjerg, 2009).

Insulin-like growth factor 1 (IGF-1) is an endocrine growth factor produced largely by the liver. It mediates some of the metabolic effects of growth hormone and it is therefore associated with lean tissue accretion (Gluckman et al., 1991). The IGF-1 gene (IGF1) is therefore considered to be a candidate gene for predicting growth and meat quality traits in animal genetic improvement schemes. It is accordingly the focus of the research in this dissertation.
Chapter 2

Literature Review

In an effort to improve livestock breeding, recent focus has been on using marker-assisted selection (MAS) to increase the accuracy of the choices made in selecting breeding stock. MAS may increase the annual rate of genetic gain in livestock by 15-30% and without increasing the risk involved in breeding schemes, such as increasing variation in response to livestock selection (Ge et al., 2001 Irvin & Simmen, 2001).

Traits that determine the economic value of livestock are of primary concern in breeding. In this context, Insulin-like Growth Factor 1 (IGF-1: also known as somatomedin C) is known for its role in young animal growth and also for its contribution to a range of anabolic processes in adults and mature animals. IGF-1 was identified in 1957 by Salmon & Daughaday and designated “sulphation factor” because of its ability to stimulate sulphate incorporation into rat cartilage (Laron, 2001). It is now known to be part of the growth hormone (GH) axis and thus involved in animal growth. GH is understood to stimulate the generation of IGF-1 in the liver and regulate the paracrine production of IGF-1 in a number of other tissues (Laron, 2001).

Many components of the GH axis have been demonstrated to affect animal growth, including GH itself, the GH receptor and IGF-1; while other non-axis proteins are also involved including leptin (LEP), pituitary-specific transcription factor-1 (POU1F1), myostatin (MSTN) and the products of the bone morphogenetic protein (BMP) genes. These hormones and proteins are necessary for among other things bone formation, in-utero growth, pre-weaning growth, body condition changes and muscle growth (Supakorn, 2008).

The objective of this study is to use PCR-SSCP analysis to find if variation exists in the ovine IGF-1 gene (IGF1) and then ascertain whether that variation affects lamb growth.

2.1 Sheep Production

2.1.1 The importance of growth on sheep production systems

The New Zealand meat industry earned $7.5 billion in export revenue in the year ended March 2016 (MIA, 2016) and this sector has always been a principle driver of New Zealand’s economy. Producing fast growing lambs with lean carcasses is of economic importance for the industry, and while animal growth and meat yield are essential to farmers and meat processors, market signals suggest meat quality traits are becoming progressively more important to consumers too.
Animal growth is affected by both environmental and genetic factors. Genetic selection has the potential to improve growth traits and contribute to the profitability and sustainability of the sheep industry. Sheep have been subject to strong artificial selection for numerous production traits and of these, growth is a fundamental determinant of sheep production efficiency. Compromised growth rates lead to an increased number of days to slaughter and accordingly an increased potential for lamb losses, adverse effects of parasitism, increased endemic disease rates on farm and the inefficient use of feed. Thus, as the number of days to slaughter increases, the production efficiency decreases.

Growth occurs when energy and protein availability exceeds maintenance requirement. The lower the growth rates, the longer it takes to achieve finishing weights. This results in accumulating maintenance energy costs and reduced production energy efficiency, and therefore feed use efficiency increases as growth rates increase.

Growth rates and growth efficiencies of farm animals vary widely both within and between breeds (Mears, 1995). The biological processes responsible for expressing the genetic potential for growth of meat producing animals are not fully understood. Animals that grow faster appear to utilise nutrients more efficiently and to partition metabolites into muscle and adipose tissue differently than slower growing animals.

Most traits with economic interest in animal production show continuous variation. However, their underlying genetic nature is usually very complex. Growth involves complex mechanisms that are influenced by genetic, nutritional and environmental factors. Lamb gender is one of the most important factors affecting growth performance and gain (Abdullah et al., 2010). It has an important influence on early post-natal growth along with other environmental influences. Ram lambs are characterised by having higher birth weights and growth rates than ewe lambs (Peeters et al., 1996). When slaughtered at the same age, carcasses from ewe lambs are lighter and fatter than those from wethers and rams (Bennet et al., 1991). Depending on the feed availability, ram and cryptorchid lambs are very similar in growth rates, and approximately 10-20% better than ewes and wethers (wethers are 8 to 12% faster growing than ewes). They are also more likely to lay down lean muscle than fat (Kerr, 2000).

2.1.2 Growth in Romney Sheep

The NZ Romney sheep breed was chosen for this study as it is the most common breed of sheep in New Zealand. Developed in New Zealand from the Romney Marsh, a breed that evolved in wet marshlands in Southern England, the Romney Marsh developed the strong constitution necessary to
survive in a harsh environment. These qualities have been passed on through the generations and have been instrumental in the breed’s success.

The Romney Marsh was first introduced into New Zealand in 1853, and by the beginning of the twentieth century the breed had shown a remarkable growth in popularity (Meadows, 1997). In 1956, in recognition of the changes it had undergone from its original ancestors, the name of the breed changed to NZ Romney. Since then, the Romney has been used to produce the Perendale (a Romney x Cheviot cross) and the Coopworth (a Romney x Border Leicester cross) and today the NZ Romney, Perendale and Coopworth are the most common breeds in NZ for mating to terminal sires to produce lambs for meat production.

The NZ Romney is a dual purpose breed with well-recognised performance for meat and wool production. Both males and females have a medium-large, well-formed and heavy body. The NZ Romney is well suited to high rainfall areas and is widespread throughout the country in almost every type of farming environment. They create large, prime lambs that produce carcasses ranging from 15-22kg in weight by the end of summer. Because of the popularity of the NZ Romney breed, an understanding of factors that affect its potential for growth could be of benefit to the New Zealand sheep industry.

2.2 Insulin-like Growth Factor 1 (IGF-1)

Many aspects of nutrient utilisation and partitioning in ruminant animals are regulated by the endocrine system, with GH, IGF-1 and insulin having primary roles (Etherton & Kensinger, 1984). The IGF-1 gene (IGF1) is considered to be a candidate gene for predicting growth and meat quality traits in animal genetic improvement schemes (Machado et al., 2003). The gene is located on chromosome 3 in sheep and is near a quantitative trait locus (QTL) for growth rate and production.

IGF-1 has been identified as an important growth hormone in many animals as it is associated with lean tissue accretion. It shares considerable structural homology with insulin and exerts insulin-like effects on food intake and glucose metabolism. IGF-1 mediates the anabolic and linear growth promoting effect of pituitary GH, but also has a GH-independent growth stimulating effect, which in respect to cartilage cell growth, may be optimised through a synergistic action with GH (Laron, 2001) (Figure 1).
Figure 1: The Growth Hormone Axis. CNS, Central Nervous System; GH, Growth Hormone; GHBP, GH Binding Protein; GH-S, GH Secretagogues; IGF-1; IGFBPs, IGF Binding Proteins; +, stimulation; -, inhibition (Laron, 2001).

The primary action of IGF-1 is mediated through binding to its specific receptor, the IGF-1 receptor (IGF-1R). This receptor is present on many cell types and in numerous tissues. Binding to IGF-1R initiates intracellular signalling and IGF-1 is one of the most powerful natural activators of the AKT-signalling pathway. This pathway promotes growth and proliferation, but it is also a powerful inhibitor of programmed cell death. IGF-1 is secreted by many tissues and the secretory site seems to determine its actions (Laron, 2001). Most IGF-1 is however secreted by the liver and it is then transported to other tissues, where it acts as an endocrine hormone (Merimee & Laron, 1996).

The predicted amino acid sequence of ovine IGF-1 differs from the human, bovine and porcine IGF-1 by a single amino acid substitution at position 66, (alanine is substituted for proline), and it differs from rat and mouse IGF-1 at amino acid positions 4 and 5, respectively. The ovine IGF-1 amino-terminal peptide is one amino acid longer than other mammalian IGFs, due to the presence of an extra glutamine residue that is present at the putative boundary of exon 1 and exon 2 (Wong et al., 1989). Overall, there is very little difference between ovine IGF-1, human and other animal IGF-1s, so it is assumed the protein has important and conserved metabolic functions and therefore that nucleotide changes in IGF-1 are rarely tolerated.

The regulation of circulating IGF-1 levels is controlled by both genetic and metabolic factors, nutritional status and disease-related physiological conditions (Franco et al., 2014). It has been revealed that circulating IGF-1 levels correlate with age, are maximal during puberty and progressively reduce in the course of adult life. Age-dependent decreases in IGF-1 levels are probably a factor in quite a diverse range of illnesses (Franco et al., 2014). The heritability estimates for
circulating human IGF-1 level variation are consistently statistically significant and range from 40% to 60% depending on the population studied (Pantsulaia et al., 2005).

Blood concentrations of IGF-1 and its associated specific binding proteins (Binoux, 1995) are under nutritional and growth hormone control, such that IGF-1 levels are high when nutrition is optimal and drop sharply when nutrition is inadequate (Wylie, 1995). In effect, IGF-1 constitutes a putative mechanistic link between the genetic potential for growth and the metabolic response to nutrition (Wylie et al., 1997). Nutrition has many interacting effects upon the IGF system, including direct effects of certain nutrients upon hepatic IGF expression, and indirect effects via insulin and via changes in hepatic GH receptors (Ketelslegers et al., 1995).

2.2.1 Growth Promoting Effects of IGF-1

IGF-1 is an important determinant of foetal growth (Varela-Nieto & Chowen, 2005) and in humans and other mammals a positive phenotypic correlation exists between foetal IGF-1 concentration at term and birth mass (Breier et al., 1988; Gluckman et al., 1983). In mice, genetically divergent IGF-1 lines differ, such that high IGF-1 expression line mice have heavier foetal weights (Kroonsberg et al., 1989) and display heavier liveweight and differential organ growth (Siddiqui et al., 1992) in comparison to their low expression line counterparts.

In sheep, low birth weights are associated with lower lamb survival rates to weaning (Dalton et al., 1980). Therefore, identification and manipulation of any factor that improves foetal growth has the potential to result in higher lamb survival (Kenyon et al., 2010). Accordingly, the findings in humans and mice would suggest that higher IGF-1 concentrations would have a positive effect on foetal growth and birth weight in sheep.

Mears (1995) found plasma IGF-1 concentrations at several stages of growth were positively correlated with both the past and the future growth rates of the lambs. Fast growing lambs generally had higher plasma IGF-1 concentrations than their slower growth counterparts. The positive correlation between plasma IGF-1 concentrations and growth rates is the most consistent relationship reported for a hormone and ruminant growth rate, thus plasma IGF-1 concentrations at an early age may be useful indicators of growth rate potential and help in selection of fast growing animals.

2.2.2 IGF-1 Deficiency

IGF-1 deficiency causes under-development and weakness in the muscular system of humans (Brat et al., 1997). It impairs and weakens hair (Lurie et al., 2001) and nail growth.
IGF-1 deficiency during childhood causes dwarfism. In one report from the United Kingdom, a child with a deletion of exons 4 and 5 of IGF1 and had severe growth retardation (Woods et al., 1996). In 1966 and 1968 a new type of dwarfism was described, that was characterised by high serum GH values and further research revealed that these patients could not generate IGF-1 (Laron et al., 1968; Laron et al., 1966).

Laron (2001) found that newborns with Laron Syndrome (IGF-1 deficiency) were slightly shorter at birth (42-47cm) than healthy babies (49-52cm), therefore IGF-1 potentially influences linear intrauterine growth. Children with hereditary IGF-1 deficiencies have reduced size at birth and throughout childhood, and skeletal maturation is retarded, as is organ growth (Laron, 1984). Abnormalities include having a small brain (Laron, 2001), a small heart (cardiomicria), acromicria (small chin due to underdevelopment of the facial bones) and small hands and small feet (Laron et al., 1968; Laron et al., 1966).

Impaired growth and skeletal development in the absence of IGF-1 has also been reported in mice (Laron, 2001). Knockout of the IGF-1 gene, or the IGF-1 receptor gene, reduces the size of mice by 40% -45% (Accili et al., 1999 Park, & Rother, 1999). Absence of the mouse IGF-1 receptor gene can also be lethal due to respiratory failure caused by reduced development of the diaphragm and intercostal muscles.

2.3 Variation in ovine IGF1

Blood IGF-1 concentrations in sheep (and cattle and pigs) are sex and age dependent (Mears, 1995; Roberts et al., 1990). There is very little data on primary breed influences. A divergence in IGF-1 concentrations was found between lines of lambs selected for high and low weaning weights and for high and low estimated carcass lean content (Cameron, 1992). However, the usefulness of measuring IGF-1 concentrations in young sheep as a predictor of future growth or carcass quality characteristics is not well understood and appears to be conflicted.

Wylie et al. (1997) reported that typically higher serum IGF-1 concentrations in single lambs were consistent with a higher nutrient intake from their mothers. There was however a fall in lamb serum IGF-1 concentrations between weeks 8 and 20, which is consistent with the progressive reduction in milk supply to the lamb as lactation proceeded. In contrast, Roberts et al. (1990) found no significant change in serum IGF-1 until about 7 and 9 months in male and female lambs respectively, this being coincident with pubertal changes in both genders.

Breier et al. (1986) and Bass et al. (1991) demonstrated nutritional control of the hepatic growth hormone/ IGF-1 axis in steers and lambs respectively. Under optimal or supra-optimal nutrition, circulating IGF-1 concentrations are maximal, permitting high rates of peripheral tissue protein
anabolism whilst, under nutritional inadequacy, and particularly during fasting or starvation (Wylie, 1995), circulating concentrations of IGF-1 are reduced, leading to probable reductions in peripheral tissue anabolism.

Wylie et al. (1997) also found that serum IGF-1 levels correlated with eye-muscle area, and were higher in naturally leaner rams and wethers than in ewes. This is consistent with the view that IGF-1 is implicated in lean tissue development. Plasma IGF-1 concentrations in intact ram lambs were greater (P<0.05) by 12 weeks of age than ewe lambs (Mears, 1995), and by the time the ram lambs were 19 weeks old, their plasma IGF-1 concentrations were three times those of ewe lambs. Increases in circulating sex steroids at puberty are thought to cause marked increases in plasma IGF-1 concentrations, with testosterone having a much greater impact than oestrogen (Roberts et al., 1990). IGF-1 was also found to be higher in Texel-sired lambs, which is consistent with the lower carcass fat content and higher carcass lean content of the Texel breed. This suggests potential for IGF-1 as a marker of lean deposition in general, and of eye-muscle area in particular.

Medrano and Bradford (1991) described IGF-1 as an ‘unlikely’ selection aid for growth rate in sheep but their interpretation may have been compromised by the high variability observed in IGF-1 levels between animals. In contrast, Mears (1995) determined that IGF-1 levels early in lamb life (7 weeks) were sufficiently well correlated with liveweight gain over weeks 4 to 20 (r = 0.584; P = 0.001) to be a useful indicator of growth potential. This is in agreement with the findings of Roberts et al. (1990), whereby plasma IGF-1 is positively correlated with liveweight between 3 and 13 months of age in New Zealand Romney lambs.

### 2.4 Conclusion

IGF-1 was first discovered as a skeletal growth factor that is mainly produced by the liver and acts on mediating the effects of the pituitary gland on whole body somatic growth. It is an important hormone for growth, mediating the anabolic and linear growth promoting effect of pituitary GH. It has a GH-independent growth stimulating effect, which with respect to cartilage cells is possibly optimised by synergistic interaction with GH (Laron, 2001). IGF-1 plays an important role in growth, lactation, reproduction, foetal development and other physiological processes.

Variation in IGF-1 levels correlate with both eye-muscle area and liveweight gain. Therefore, plasma IGF-1 concentrations at an early age may be useful indicators of growth rate potential and help in selection of fast growing meat producing animals and other useful production traits. Human IGF-1 concentrations have moderate to high heritabilities of 0.4 to 0.6 and cattle GH concentrations are highly heritable (Lovendahl et al., 1994). If the heritability of ovine IGF-1 levels were similarly
inherited, selection for rapid growth based on plasma IGF-1 concentrations would be beneficial in improving the growth rate of livestock.

A few studies have been carried out on IGF1 and its relationship with growth and development traits of animals, but very little has been studied with respect to variation in IGF1 in Romney Sheep. The studies that have been carried out are somewhat contradictory. There is therefore a need to undertake further quantitative molecular genetic analysis to study the relationship between IGF1 and growth traits in sheep.
Chapter 3
Materials and Methods

3.1 Sheep investigated and DNA purification

Fifty New Zealand (NZ) Romney rams were investigated to ascertain whether variation exists in the ovine \( IGF1 \) gene. For the association analysis 150 lambs that were the progeny of a single ram that produced lambs over two seasons were investigated. Phenotypic data for growth and carcass traits was available for these lambs from the NZ Romney Progeny Test (2007-present).

All lambs were ear-tagged with a unique identification number within 12 hours of birth, and birth date, birth weight, tailing weight, birth rank (i.e. whether they were a single, twin or triplet), rearing rank, gender and dam number were recorded. All of the ewes and lambs were brought together at tailing (lambs were aged between 2–6 weeks old) and remained together until weaning. At weaning each lamb was weighed and their pre-weaning growth rate was calculated as the difference between weaning weight and birth weight divided by age in days (expressed as grams/day).

At weaning, lambs were separated according to gender. Only male lambs were used in carcass trait analyses, as female lambs were retained as flock replacements. At weaning, those lambs weighing 36 kg and over were drafted and sent to the Alliance Group meat processing plant at Pukeuri, NZ. Two subsequent drafts occurred at four weekly intervals. At the second draft, male lambs were drafted at weights over 30 kg and draft three consisted of all the remaining male lambs regardless of weight. Draft age and weight were recorded for each male lamb.

Hot carcass weights (H-W) were measured directly on the processing chain. H-W is the weight in kilograms of the carcass components minus the pelt, head and gut. Video imaging analysis (VIASCAN\textsuperscript{TM} Sastek), developed by Meat and Livestock Australia and described in Hopkins \textit{et al.} (2004) was used to estimate the following carcass traits: lean meat yield (expressed as a percentage of H-W) in the leg (leg yield), loin (loin yield) and shoulder (shoulder yield) expressed as a percentage of H-W), total yield (the sum of the leg, loin and shoulder yields for any given carcass), the proportion leg yield, the proportion loin yield and the proportion shoulder yield. The proportion yield of leg, loin or shoulder is the yield of the specific area divided by the total yield expressed as a percentage.

Samples of blood from these sheep were collected directly onto FTA cards (Whatman BioScience, Middlesex, UK) and DNA for analysis was purified from 1.2 mm punches from the cards, using a two-step procedure described by Zhou \textit{et al.} (2006).
3.2 PCR primers and PCR amplification

PCR primers (Exon2UP 5'-CTGCTCAGAGTCACATC-3', Exon2DOWN 5'-GCTGAAACACTAGGCTCG-3' and Exon3UP 5'-GACTGCTGGAGATATACTGG-3', Exon3DOWN5'-CTGGTGCTCTCCCTCTG-3') were designed based on ovine *IGF1* sequences X51357 and X69473, to amplify an exon 2 fragment and an exon 3 fragment of *IGF1*. The primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA).

PCR amplification was undertaken using the purified genomic DNA on one punch of the FTA paper, 0.25 μM of each primer, 150 μM of each dNTP (Bioline, London, UK), 2.5 mM of Mg²⁺, 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany) and 1× reaction buffer supplied in a 15-μL reaction.

The thermal profile for amplification consisted of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 62°C and 30 s at 72°C, with a final extension of 5 min at 72°C. This was done in S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA).

Amplicons were visualised by electrophoresis in 1% agarose (Quantum Scientific, Queensland, Australia) gels, using 1 x TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na2EDTA) containing 200 ng/mL of ethidium bromide.

3.3 Polymorphism screening and sequencing of allelic variants

PCR amplicons were subject to SSCP analysis. A 0.7-μL aliquot of each amplicon was mixed with 7 μL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol) and after denaturation at 95°C for 5 min, the samples were cooled rapidly on wet ice and loaded on 16 cm × 18 cm, 14% acrylamide: bisacrylamide (37.5:1) (Bio-Rad) gels.

Electrophoresis was performed using Protean II xi cells (Bio-Rad), at 200 V for 20 h at 25-30°C in 0.5 × TBE buffer. The gels were silver-stained by the method of Byun et al. (2009).

3.4 Statistical Analysis

Statistical analyses were performed through Minitab® 17 using stepwise regression to assess the effect of the presence or absence of the *IGF-1* variants on lamb growth rates and weights. The effects of the presence of each variant on growth were assessed using a series of ‘single variant’ general linear model (GLMs) in which each variant was analysed separately. The presence of a variant will be coded as either 1 (present) or 0 (absent) for the various analyses.
In all regression models there were five categorical predictors: sex, birth rank, rearing rank, year (if 2007 and 2009 were used in the same model) and the variant (Ex 2 A, Ex 2 B, Ex 3 A, and Ex 3 B). The GLMs will be used to assess whether the presence or absence of a particular variant is associated with birth weight, tailing weight, weaning weight, growth rate from birth to tailing, growth rate from birth to weaning and growth rate from tailing to weaning.
Chapter 4
Results

4.1 Variation in ovine *IGF1*

There were two PCR-SSCP banding patterns discovered for the ovine *IGF1* in both regions amplified, with one or a combination of two banding patterns detected for each sheep (Figure 2 and Figure 3). These banding patterns represented different nucleotide sequences.

![Figure 2: PCR-SSCP of exon 2 of ovine *IGF1*. Two banding patterns signify two variants (A and B) and come in either heterozygous or homozygous forms](image)

![Figure 3: PCR-SSCP of exon 3 of ovine *IGF1*. Two banding patterns signify two variants (A and B) and come in either heterozygous (Abdullah et al.) or homozygous forms (Lovendahl et al.)](image)
Three genotypes were detected among the progeny for exon 2 and two genotypes were detected among the progeny for exon 3. The sire chosen had a genotype of AB for exon 2 and AA for exon 3, therefore a progeny genotype of BB in exon 3 is not possible (Figure 3).

Table 1 shows the genotype frequencies for the IGF1 sequences found in the Romney sheep studied.

Table 1: IGF1 exon 2 and 3 frequencies in NZ Romney lambs in 2007, 2009 and 2007/09 combined

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 2</td>
<td>2007</td>
<td>24</td>
<td>43</td>
<td>14</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29.6%</td>
<td>53.1%</td>
<td>17.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>12</td>
<td>41</td>
<td>16</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.4%</td>
<td>59.4%</td>
<td>23.2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>36</td>
<td>84</td>
<td>30</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24.0%</td>
<td>56.0%</td>
<td>20.0%</td>
<td></td>
</tr>
<tr>
<td>Exon 3</td>
<td>2007</td>
<td>77</td>
<td>4</td>
<td>0</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95.1%</td>
<td>3.9%</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>56</td>
<td>13</td>
<td>0</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>81.2%</td>
<td>18.8%</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>133</td>
<td>17</td>
<td>0</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>88.7%</td>
<td>11.3%</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4: Sequence variation in a selected region of intron 1, exon 2 and intron 2 of ovine IGF1.

Bars represent nucleotides identical to the nucleotides of A and B sequence. Lower case and upper case letters refers to intron 1 and exon 2, respectively. The arrow shows the first nucleotide of exon 2 of ovine IGF-1, based on the ovine sequence (GenBank accession number X51357) with a total of 451 base pairs (bp). Also shown in Figure A 1: IGF-1 Exon 2 Transcript Sequence in Appendix A.

Figure 5: Sequence variation in a selected region of intron 2, exon 3 and intron 3 of the ovine IGF1.

Bars represent nucleotides identical to the nucleotides of A and B sequence. Lower case and upper case letters refers to intron 2 and exon 3, respectively. The arrow shows the first nucleotide of exon 3 of ovine IGF1, based on the ovine sequence (GenBank accession number X69473) with a total of 389 base pairs. Also shown in Figure A 2: IGF-1 Exon 3 Transcript Sequence in Appendix A.
Cloning of PCR amplicons representative of the unique SSCP patterns followed by DNA sequencing, revealed two different DNA sequences for Exon 2 and Exon 3 (Figure 4 and Figure 5, respectively). Both of these sequences shared high homology to the reported human sequence (GenBank accession number X51357 and X69473). The IGF-1 sequences were named A and B.

Two novel sequences (A and B) of IGF-1 Exon 2 were identified in a fragment of 451bp, which encodes part of intron 1, exon 2 and intron 2 of this gene. The A and B sequence was differentiated by variation in intron 1 and the B sequence was identified by a synonymous substitution located in exon 2 (Appendix A; Figure A 1).

Two novel sequences (A and B) of IGF-1 Exon 3 were also identified in a fragment of 389bp, which encodes part of intron 2, exon 3 and intron 3. The A and B sequences were differentiated by two variations in intron 2 (Appendix A; Figure A 2).
4.2 Associations between variation in *IGF1* and growth traits

In the analysis of all of progeny (150), the exon 2 *IGF-1* A sequence was associated with variation in growth traits, both within (Table 2 and Table 3) and across years (Table 4).

Table 2: Association of *IGF1* exon 2 variants with various growth traits (mean ± SE) within the 2007 Progeny

<table>
<thead>
<tr>
<th>Trait</th>
<th>Variant</th>
<th>n</th>
<th>Absent</th>
<th>Present</th>
<th>Absent</th>
<th>Present</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Wt. (kg) A</td>
<td>14</td>
<td>67</td>
<td>4.65 ± 0.26</td>
<td>5.21 ± 0.13</td>
<td>0.049</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth Wt. (kg) B</td>
<td>24</td>
<td>57</td>
<td>5.22 ± 0.29</td>
<td>5.17 ± 0.19</td>
<td>0.996</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tailing Wt. (kg) A</td>
<td>14</td>
<td>67</td>
<td>15.13 ± 1.55</td>
<td>16.46 ± 0.57</td>
<td>0.614</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tailing Wt. (kg) B</td>
<td>24</td>
<td>57</td>
<td>16.83 ± 0.75</td>
<td>16.09 ± 0.57</td>
<td>0.326</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning Wt. (kg) A</td>
<td>14</td>
<td>67</td>
<td>28.38 ± 2.16</td>
<td>29.41 ± 0.79</td>
<td>0.405</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning Wt. (kg) B</td>
<td>24</td>
<td>57</td>
<td>29.41 ± 1.05</td>
<td>29.23 ± 0.81</td>
<td>0.862</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GR Birth to Tailing (g/day) A</td>
<td>14</td>
<td>67</td>
<td>0.261 ± 0.02</td>
<td>0.271 ± 0.01</td>
<td>0.543</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GR Birth to Tailing (g/day) B</td>
<td>24</td>
<td>57</td>
<td>0.272 ± 0.01</td>
<td>0.268 ± 0.01</td>
<td>0.784</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GR Birth to Weaning (g/day) A</td>
<td>14</td>
<td>67</td>
<td>0.264 ± 0.01</td>
<td>0.275 ± 0.01</td>
<td>0.371</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GR Birth to Weaning (g/day) B</td>
<td>24</td>
<td>57</td>
<td>0.272 ± 0.01</td>
<td>0.275 ± 0.01</td>
<td>0.744</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GR Tailing to Weaning (g/day) A</td>
<td>14</td>
<td>67</td>
<td>0.268 ± 0.02</td>
<td>0.278 ± 0.01</td>
<td>0.521</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GR Tailing to Weaning (g/day) B</td>
<td>24</td>
<td>57</td>
<td>0.268 ± 0.01</td>
<td>0.280 ± 0.01</td>
<td>0.326</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Predicted means, standard errors and P values from GLMs. P < 0.05 are in bold, whereas 0.05 ≤ P < 0.2 in ‘single variant’ models are in italics.

The presence of A is associated (P<0.05) with a higher mean birth weight (5.21 ± 0.13).
Table 3: Association of IGF1 exon 2 variants with various growth traits (mean ± SE) within the 2009 Progeny\textsuperscript{1}

<table>
<thead>
<tr>
<th>Trait</th>
<th>Variant</th>
<th>n</th>
<th>Single-variant model</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Birth Wt. (kg)</td>
<td>A</td>
<td>16</td>
<td>53</td>
<td>6.06 ± 0.24</td>
<td>5.82 ± 0.14</td>
<td>0.337</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12</td>
<td>57</td>
<td>6.04 ± 0.26</td>
<td>5.83 ± 0.14</td>
<td>0.453</td>
</tr>
<tr>
<td>Tailing Wt. (kg)</td>
<td>A</td>
<td>16</td>
<td>53</td>
<td>14.74 ± 0.69</td>
<td>16.02 ± 0.40</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12</td>
<td>57</td>
<td>16.84 ± 0.77</td>
<td>15.50 ± 0.40</td>
<td>0.109</td>
</tr>
<tr>
<td>Weaning Wt. (kg)</td>
<td>A</td>
<td>16</td>
<td>53</td>
<td>37.62 ± 1.37</td>
<td>39.38 ± 0.90</td>
<td>0.178</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12</td>
<td>57</td>
<td>40.19 ± 1.44</td>
<td>38.75 ± 0.93</td>
<td>0.324</td>
</tr>
<tr>
<td>GR Birth to Tailing (g/day)</td>
<td>A</td>
<td>16</td>
<td>53</td>
<td>0.302 ± 0.01</td>
<td>0.333 ± 0.01</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12</td>
<td>57</td>
<td>0.329 ± 0.01</td>
<td>0.326 ± 0.01</td>
<td>0.848</td>
</tr>
<tr>
<td>GR Birth to Weaning (g/day)</td>
<td>A</td>
<td>16</td>
<td>53</td>
<td>0.331 ± 0.01</td>
<td>0.350 ± 0.01</td>
<td>0.107</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12</td>
<td>57</td>
<td>0.350 ± 0.01</td>
<td>0.345 ± 0.01</td>
<td>0.709</td>
</tr>
<tr>
<td>GR Tailing to Weaning (g/day)</td>
<td>A</td>
<td>16</td>
<td>53</td>
<td>0.341 ± 0.02</td>
<td>0.355 ± 0.01</td>
<td>0.321</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12</td>
<td>57</td>
<td>0.356 ± 0.02</td>
<td>0.351 ± 0.01</td>
<td>0.764</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Predicted means, standard errors and P values from GLMs. P < 0.05 are in \textbf{bold}, whereas 0.05 \leq P \leq 0.2 in ‘single variant’ models are in \textit{italics}.

The presence of A is associated (P < 0.05) with a higher mean growth rate from birth to tailing (0.333 ± 0.01). The presence of A shows a trend for being associated (0.05 \leq P < 0.2) with a higher mean tailing weight (16.02 ± 0.40), weaning weight (39.38 ± 0.90) and growth rate from birth to weaning (0.350 ± 0.01). The presence of B shows strong trends (0.05 \leq P < 0.2) with a higher mean tailing weight (15.50 ± 0.40).
Table 4: Association of \textit{IGF1} exon 2 variants with various growth traits (mean ± SE) within the 2007 and 2009 Progeny combined\(^1\)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Variant</th>
<th>n</th>
<th>Absent</th>
<th>Present</th>
<th>Absent</th>
<th>Present</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Wt. (kg)</td>
<td>A</td>
<td>29</td>
<td>120</td>
<td>5.00 ± 0.19</td>
<td>5.12 ± 0.12</td>
<td>0.527</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>35</td>
<td>114</td>
<td>5.23 ± 0.17</td>
<td>5.04 ± 0.12</td>
<td>0.281</td>
<td></td>
</tr>
<tr>
<td>Tailing Wt. (kg)</td>
<td>A</td>
<td>29</td>
<td>120</td>
<td>15.25 ± 0.62</td>
<td>16.39 ± 0.37</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>35</td>
<td>114</td>
<td>16.99 ± 0.54</td>
<td>15.95 ± 0.39</td>
<td>0.057</td>
<td></td>
</tr>
<tr>
<td>Weaning Wt. (kg)</td>
<td>A</td>
<td>29</td>
<td>120</td>
<td>28.63 ± 1.02</td>
<td>30.19 ± 0.65</td>
<td>0.087</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>35</td>
<td>114</td>
<td>30.53 ± 0.87</td>
<td>29.75 ± 0.70</td>
<td>0.368</td>
<td></td>
</tr>
<tr>
<td>GR Birth to Tailing (g/day)</td>
<td>A</td>
<td>29</td>
<td>120</td>
<td>0.252 ± 0.01</td>
<td>0.273 ± 0.01</td>
<td>\textbf{0.035}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>35</td>
<td>114</td>
<td>0.273 ± 0.01</td>
<td>0.269 ± 0.01</td>
<td>0.720</td>
<td></td>
</tr>
<tr>
<td>GR Birth to Weaning (g/day)</td>
<td>A</td>
<td>29</td>
<td>120</td>
<td>0.266 ± 0.01</td>
<td>0.281 ± 0.01</td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>35</td>
<td>114</td>
<td>0.280 ± 0.01</td>
<td>0.279 ± 0.01</td>
<td>0.979</td>
<td></td>
</tr>
<tr>
<td>GR Tailing to Weaning (g/day)</td>
<td>A</td>
<td>29</td>
<td>120</td>
<td>0.269 ± 0.01</td>
<td>0.280 ± 0.01</td>
<td>0.249</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>35</td>
<td>114</td>
<td>0.276 ± 0.01</td>
<td>0.280 ± 0.01</td>
<td>0.616</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Predicted means, standard errors and P values from GLMs. P < 0.05 are in \textbf{bold}, whereas 0.05 ≤ P < 0.2 in ‘single variant’ models are in \textit{italics}.

The presence of A is associated (P<0.05) with a higher mean growth rate from birth to tailing (0.273 ± 0.01). The presence of A shows strong trends (0.05 ≤ P < 0.2) with a higher mean tailing weight (16.39 ± 0.37), weaning weight (30.19 ± 0.65) and growth rate from birth to weaning (0.281 ± 0.01). The presence of B shows strong trends (0.05 ≤ P < 0.2) with a higher mean tailing weight (15.95 ± 0.39).
Table 5: Association of *IGF1* exon 3 variants with various growth traits (mean ± SE) within the 2007 Progeny

<table>
<thead>
<tr>
<th>Trait</th>
<th>n</th>
<th>Single-variant model</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
<td>AA</td>
<td>AB</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>Birth Wt. (kg)</td>
<td>77</td>
<td>4</td>
<td>5.07 ± 0.12</td>
<td>6.07 ± 0.50</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>Tailing Wt. (kg)</td>
<td>77</td>
<td>4</td>
<td>16.30 ± 0.53</td>
<td>17.75 ± 1.74</td>
<td>0.385</td>
<td></td>
</tr>
<tr>
<td>Weaning Wt. (kg)</td>
<td>77</td>
<td>4</td>
<td>29.28 ± 0.74</td>
<td>30.09 ± 2.43</td>
<td>0.730</td>
<td></td>
</tr>
<tr>
<td>GR Birth to Tailing (g/day)</td>
<td>77</td>
<td>4</td>
<td>0.269 ± 0.01</td>
<td>0.272 ± 0.03</td>
<td>0.927</td>
<td></td>
</tr>
<tr>
<td>GR Birth to Weaning (g/day)</td>
<td>77</td>
<td>4</td>
<td>0.274 ± 0.01</td>
<td>0.270 ± 0.02</td>
<td>0.873</td>
<td></td>
</tr>
<tr>
<td>GR Tailing to Weaning (g/day)</td>
<td>77</td>
<td>4</td>
<td>0.276 ± 0.01</td>
<td>0.267 ± 0.03</td>
<td>0.719</td>
<td></td>
</tr>
</tbody>
</table>

1Predicted means, standard errors and P values from GLMs. P < 0.05 are in **bold**, whereas 0.05 ≤ P < 0.2 in ‘single variant’ models are in *italics.*

The presence of *AB* shows strong trends (0.05 ≤ P < 0.2) with a higher mean birth weight (6.07 ± 0.50).

Table 6: Association of *IGF1* exon 3 variants with various growth traits (mean ± SE) within the 2009 Progeny

<table>
<thead>
<tr>
<th>Trait</th>
<th>n</th>
<th>Single-variant model</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
<td>AA</td>
<td>AB</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>Birth Wt. (kg)</td>
<td>56</td>
<td>13</td>
<td>5.92 ± 0.14</td>
<td>5.70 ± 0.25</td>
<td>0.422</td>
<td></td>
</tr>
<tr>
<td>Tailing Wt. (kg)</td>
<td>56</td>
<td>13</td>
<td>16.29 ± 0.52</td>
<td>15.22 ± 0.83</td>
<td>0.189</td>
<td></td>
</tr>
<tr>
<td>Weaning Wt. (kg)</td>
<td>56</td>
<td>13</td>
<td>39.40 ± 0.91</td>
<td>37.53 ± 1.43</td>
<td>0.186</td>
<td></td>
</tr>
<tr>
<td>GR Birth to Tailing (g/day)</td>
<td>56</td>
<td>13</td>
<td>0.336 ± 0.01</td>
<td>0.327 ± 0.02</td>
<td>0.517</td>
<td></td>
</tr>
<tr>
<td>GR Birth to Weaning (g/day)</td>
<td>56</td>
<td>13</td>
<td>0.348 ± 0.01</td>
<td>0.337 ± 0.01</td>
<td>0.395</td>
<td></td>
</tr>
<tr>
<td>GR Tailing to Weaning (g/day)</td>
<td>56</td>
<td>13</td>
<td>0.355 ± 0.01</td>
<td>0.341 ± 0.02</td>
<td>0.365</td>
<td></td>
</tr>
</tbody>
</table>

1Predicted means, standard errors and P values from GLMs. P < 0.05 are in **bold**, whereas 0.05 ≤ P < 0.2 in ‘single variant’ models are in *italics.*

The presence of *AA* shows trends (0.05 ≤ P < 0.2) with a higher mean tailing weight (16.29 ± 0.52) and weaning weight (39.40 ± 0.91).
Table 7: Association of *IGF1* exon 3 variants with various growth traits (mean ± SE) within the 2007 and 2009 Progeny combined

<table>
<thead>
<tr>
<th>Trait</th>
<th>n</th>
<th>AA</th>
<th>AB</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Wt. (kg)</td>
<td>133</td>
<td>16</td>
<td>5.09 ± 0.11</td>
<td>5.12 ± 0.26</td>
</tr>
<tr>
<td>Tailing Wt. (kg)</td>
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<td>16.31 ± 0.43</td>
<td>14.64 ± 0.98</td>
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<tr>
<td>Weaning Wt. (kg)</td>
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<td>30.02 ± 0.65</td>
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<td>GR Birth to Tailing (g/day)</td>
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<td>0.271 ± 0.01</td>
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<tr>
<td>GR Birth to Weaning (g/day)</td>
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<td>0.280 ± 0.01</td>
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<tr>
<td>GR Tailing to Weaning (g/day)</td>
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<td>0.279 ± 0.01</td>
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</tbody>
</table>

1 Predicted means, standard errors and P values from GLMs. P < 0.05 are in **bold**, whereas 0.05 ≤ P < 0.2 in ‘single variant’ models are in *italics*.

The presence of AA shows a strong trend (0.05 ≤ P < 0.2) with a higher mean tailing weight (16.31 ± 0.43) and a moderately to strong trend with a higher mean growth rate from birth to tailing (0.271 ± 0.01).
IGF-1 is known for its role in young animal growth and also for its contribution to a range of anabolic processes in adults and mature animals. However, this is the first report of an association between variation in IGF1 and growth in Romney lambs in New Zealand. From the analysis, the presence of the A in the ovine IGF1 gene showed strong trends and associations with growth traits in young lambs, primarily pre-weaning. This finding is consistent with reports in other species; humans (Laron, 2001), cattle (de la Rosa Reyna et al., 2010), sheep wool growth (Zhang et al., 2009), and mice (Efstratiadis, 1998).

In this study, the presence of the A sequence of ovine IGF1 was associated with higher birth weights, weaning weights and growth rates from birth to tailing and birth to weaning. While this synonymous substitution does not result in an amino acid change, it may nevertheless be linked to other nucleotide changes in the coding regions, or to sequence variation elsewhere in the gene. This may affect gene expression and/or the function of IGF1 and hence affect lamb growth.

5.1 IGF1 variation and Birth weight

Within the analysis, IGF1 variant A shows associations with birth weight. In 2007, exon 2 A was associated with an increase of 0.56kg in birth weight (Table 2) and exon 3 variation showed a strong trend for association, with a potential increase of 1.00 kg of birth weight for AB lambs compared to AA lambs (Table 5).

IGF-1 was found to have positive effects on birth weight and 180 days gain from birth to weaning in beef cattle (Moody et al., 1996; Pereira et al., 2005). Additionally, associations of IGF1 with birth weight and GH with weight at first calving at 12 months of ages has been observed in Canchim cattle (Andrade et al., 2008; Grossi et al., 2015). Weight differences in this report are quite large which could have an impact on lamb survival, lamb growth, and occurrence of dystocia.

The single largest influence on the survival of lambs in the first few days of life is their birth weight (Oldham et al., 2011). Foetal growth and birth weight are regulated by genotype of the foetus, maternal genotype, maternal nutrition, litter size and the external environment. Low birth weight is associated with increased neonatal mortality (Gardner et al., 2007). Twin lambs are lighter at birth than singles and female lambs are lighter than males.
The 2007 average birth weight of single lambs was heavier than twin lambs and triplet lambs (6.16 kg vs. 5.10 kg vs. 4.28 kg), respectively, and this was also observed in 2009 (6.56 kg vs. 5.95 kg vs. 5.47 kg), respectively. However, birth weight difference does not account for all the difference in survival between single, twin and triplet born lambs. Restricting the level of nutrition to the pregnant ewe can reduce lamb birth weight depending on the timing and severity of the restriction (Holst et al., 1986). Gardner et al. (2007) reported that maternal body condition at mating is a reflection of her energy intake over at least the 6 - 8 weeks prior to conception, which has a significant effect on the birth weight of her lamb.

In sheep, nutrition during pregnancy should be concerned predominately with the requirements of the developing foetus. Severe under-nutrition can reduce milk production by restricting mammary growth and development (Rattray et al., 1974; Roche, 2007), reducing lamb birth weight and therefore milk withdrawal ability (Peart, 1967) or by depletion of essential body reserves (Peart, 1970). There is concern that under-feeding before lambing might cause pregnancy toxaemia and low lamb birth weights. Over-feeding before lambing could cause bearing trouble and lambing difficulties as well as pasture shortage after lambing. Once in the lactation period, a sufficient supply of nutrients is required for body maintenance and milk production for suckling lambs.

Restricted nutrition during the first 3-4 weeks of lactation reduces milk production, which will cause a drop in BCS and liveweight and subsequently restrict lamb growth. Poor pregnancy nutrition, causing considerable weight loss, has had little influence on subsequent milk production. In the last 2 weeks of pregnancy, it is difficult to increase the body condition score as the feed goes to the lamb. However, once a ewe is producing less milk from post lambing feed restrictions, it spends the rest of its lactation period below her maximum production levels, therefore, a greater amount of milk production losses. It is important that a ewe’s diet is managed well during pregnancy to increase lamb birth weight but to minimise lambing difficulties. Feeding levels have a large impact on milk production post lambing, therefore, ewes need to be fed at high herbage allowances during early lactation to ensure lamb growth rates are maximised.

Year of birth can have significant effects on overall birth weight, producing shifts in singles birth weight, on average, of up to 1.0 kg (Gardner et al., 2007). The 2009 born lambs had no association with IGF1 and birth weight. This could be due to the external environment, climate change, lack of feed, improvements in feed management or overall pregnancy ewe weights. The 2009 average birth weight of lambs was 400 g for singles, 850 g for twins and 1.19 kg heavier than those lambs born in 2007.

Oldham et al. (2011) reported that when restricting the level of nutrition to the pregnant ewe, it reduced lamb birth weight and this effect was dependent on the timing and severity of the
restriction and subsequent nutrition. A loss of 10 kg in ewe liveweight between mating and day 100 of pregnancy reduced lamb birth weight by ~0.3 kg, whereas gaining 10 kg from day 100 to lambing increased birth weight by ~0.45 kg. In this experiment, harsh feed restrictions may have occurred early on in ewe pregnancy during 2007 or nutrition levels increased later on in the ewe pregnancy in 2009. However, Oldham et al. (2011) findings confirm that the effects of poor nutrition up until day 100 of pregnancy could be completely overcome by improving nutrition during late pregnancy. Weather and environmental conditions were not recorded during this experiment, which can result in unfair testing between years, however, year was included as a categorical predictor when measuring the years together.

Birth weight has a direct effect on survival of lambs and the ideal birth weight range appears to be between 3.5 kg and 6.0 kg. The relationship follows a quadratic shape with maximum survival at ~4.5 kg in Romney lambs (Knight et al., 1988). Survival is therefore lower in very small or very big lambs, irrespective of the source of the variation in birth weight. The optimum birth weight for lamb survival of 4.6 ± 0.03 kg was reported in (Knight et al., 1988), this is where there is an uncomplicated natural delivery and neonatal survival is maximised.

Smaller lambs have greater susceptibility to the cold environment. However, lamb death because of cold exposure can be misunderstood because of the interactions with infection, birth weight, dystocia, starvation, birth injury and birth coat, which can predispose lambs to death from cold exposure (Alexander, 1984). Forrest et al. (2003) found the E allele of the β3-adrenergic receptor gene (ADRB3) is associated with survival in sire lines S14 and S15. But in both sire lines the inheritance of a particular sire allele is not associated with a difference in birth weight. The difference in survival must therefore be attributable to something other than birth weight, such as increase brown adipose tissue (BAT) mass (when compared with other tissues) or BAT that is more thermogenically active.

High birth weight is generally associated with complicated labour (dystocia) and maternal death (Gardner et al., 2007). New Zealand meat production systems select for greater muscle growth and meat yield. This is generally accompanied by an increase in the incidence of dystocia, and reduction in lamb survival if these traits are not included in the selection goal, however, this is manageable with increasing fecundity as litter sizes increase. Dwyer and Bunger (2012) found single lambs were significantly more likely to need assistance at delivery than either twin or multiple lambs, male lambs required significantly more assistance to females, and young ewes were more likely to be assisted than older ewes. Overall, unassisted lambs were significantly lighter than assisted lambs (Mean birth weight (kg): unassisted = 4.13 ± 0.02, minor assistance = 4.67 ± 0.04, major assistance = 4.61 ± 0.07, veterinary assistance=4.45 ± 0.24, P<0.001).
It is known that breed differences exist in the rates of dystocia (Dwyer, 2003; Dwyer & Lawerance, 1998; Dwyer et al., 1996; Fahmy et al., 1997) and lamb vigour (Dwyer, 2003; Dwyer & Lawerance, 1998; Dwyer et al., 1996; Slee & Springbett, 1986) in sheep. However, lamb weight or lamb weight relative to ewe weight, does not explain all the variation in birth difficulty in sheep (Dwyer et al., 1996).

Birth difficulty may also be associated with maternal factors, such as reduced pelvic dimensions, resulting in disproportion between mother and young as reported in sheep (Cloete et al., 1998). As well as a number of lamb factors clearly contribute significantly to the incidence of birth difficulty in all breeds (litter size, sex, birth weight and presentation at parturition). Thus, a mismatch between lamb birth weight and maternal size or pelvic dimensions contributes to an increase in birth difficulty in breeds selected for increased muscling characteristics, but is of less importance in breeds selected for maternal and hardiness traits. Previous studies have shown that selection for lamb-rearing ability is associated with an increase in pelvic dimensions in Merinos and Romney sheep (Kilgour & Haughey, 1993; Knight et al., 1988).

Previous studies have demonstrated that twin and triplet lambs which died before three weeks of age took at least twice as long to be born than twin and triplet lambs which survived (Mathias-Davis et al., 2010). This prolonged parturition time was associated with death due to dystocia (Everett-Hincks et al., 2007). As the proportion of multiple-born lambs increases in a flock, the impact of dystocia on survival declines. Birth weight also explains presentation of the lamb, as lambs with one or both legs retracted were significantly heavier and lambs that were breech or were in a head back presentation were significantly lighter, than normally presented lambs (Dwyer & Bunger, 2012). Heavier birth weight was advantageous for lamb vigour and sucking ability, both maximising growth potential and requiring less assistance.

Males appear to grow faster than respective females in utero (Oldham et al., 2011). In addition, male lambs are more likely to die of dystocia (Scales et al., 1986) and twice as likely to be incorrectly presented as females (Dwyer, 2003). Lambs were more likely to survive, regardless of birth weight, when there was more feed on offer for their mothers at the time of lambing. Oldham et al. (2011) found maximum benefit when feed on offer was ~2,000 kg DM per ha at lambing which has previously shown to maximise growth and wool growth in dry sheep (Hyder et al., 2002). The sex ranking of serum IGF-1 concentrations (rams > wethers and ewes) is also in agreement with Roberts et al. (1990) and Mears (1995) for lambs. Roberts et al. (1990) found testosterone has a much greater impact than oestrogen levels, however, gender influence on IGF-1 levels still needs further investigation.
5.2 \textit{IGF1} variation and Weaning Weight

In this study, the presence of exon 2 A showed strong trends with variation in weaning weights during 2009 (39.38 ± 0.90 kg) and both years combined (30.19 ± 0.65 kg) (Table 3 and Table 4, respectively). There is a 9.97 kg difference in weaning weights between 2007 and 2009. This is a substantial difference and very inefficient as the 2009 mean weaning weight for lambs can be sent to the works as prime milk lambs, whereas 2007 lambs would need at least an extra 40 days approximately on farm to gain the extra 9.97 kg (approximately growing at 250 g per day).

Strong trends are also seen in Exon 3 with the presence of AA in 2009 (39.38 ± 0.90 kg) (Table 6). This supports findings from where they found a single nucleotide polymorphism in the promoter region of \textit{IGF1}, that is associated with production traits in several cattle breeds (de la Rosa Reyna et al., 2010). They confirm these associations with three growth traits; weaning weight, weaning weight adjusted to 210 days and pre-weaning weight gain in the Charolais breed.

Birth weight, birth rank and rearing rank can all have an effect on weaning weights (Notter & Brown, 2015). Notter & Brown (2015) found many ewes that produced triplets did not suckle all their lambs, which was due to either lamb death losses or management decisions to reduce the size of the litter by hand rearing them. If the ewe was left with triplets, performance of lambs will have been impacted due to less milk for each lamb.

Not only are heavier weaning weights related to the presence of \textit{IGF1}, the environment has a huge impact on the final weaning weight. Weaning weights are moderately heritable (35%), therefore the remaining 65% of potential high weaning weight is affected by the environment. This can include the quantity and quality of mother’s milk and pastures, and later on, parasites. The older the lamb, the greater proportion of feed will be from pastures therefore the greater the chance of being infested with internal parasites. There was no trend seen with growth rates from tailing to weaning. This can be associated with worm burden as lambs are eating a greater amount of grass than pre-tailing.

Gastrointestinal (GI) parasites cause significant production losses in grazing ruminants throughout the world, particularly in young and in peri parturient sheep, goats and cattle (Sykes, 1994). Parasitism infection in the abomasum or small intestine induces protein deficiency while reducing supply through depression of appetite (Sykes & Greer, 2003). Infection with GI parasites can lead to serious impairment of bone growth in sheep (Sykes et al., 1979).

GI nematodes reduce nutrient availability to the host through both reductions in voluntary feed intake and/or reductions in the efficiency of absorbed nutrients although the underlying mechanisms of the depression in appetite have not been fully elucidated (Dynes et al., 1998). Depression in food consumption in susceptible hosts is a major factor influencing production losses,
reductions of between 10% and 30% are common (Dynes et al., 1991) and severe or even complete inappetence has also been recorded (Bown et al., 1989).

*T. circumcincta* infection reduced growth rates to 50% of that in controls, even frequent treatment with anthelmintic (every 21 days), while preventing the production of eggs, restored only 20% of the lost performance (Coop et al., 1982). Another report demonstrated a 21 day anthelmintic treatment regimen, differences of up to 35% in growth rate between animals on clean as opposed to contaminated pastures (McAnulty et al., 1982). Young lambs have the highest relative metabolisable protein (MP) requirement but are the least resistant and resilient to parasite intake (van Houtert & Sykes, 1996). Poor growth rate due to parasitic infection is still considered by many practitioners and advisors to be a consequence of the inability to digest nutrients. However, the majority of studies do not provide sufficient evidence for a significant effect on the ability to digest and absorb feed energy.

In early lactation, parasites have little effect on lambs as their diet mainly consists of milk. Ewe milk production reduces in late lactation (Treacher & Caja, 2002), therefore, lambs eat a greater proportion of grass. Dynes et al. (1991) found weaned lambs no longer have the additional energy and secondary compounds available from milk, yet those lambs were no worse off under either single or continuous ingestion of gastrointestinal parasites than milk fed flock mates. They also found late weaned lambs compete with ewes for the highest quality components of pasture, thus, nutritional benefits of late weaning are expected to be small. Late weaned lambs are also exposed to higher levels of gastrointestinal parasites due to infected pastures, whereas early weaning places lambs on fresh pastures.

Rather than leaving lambs unweaned in the hope of resilience to parasites, strategies to minimise any post-weaning check and subsequent grazing of high quality pastures with low levels of larval contamination may produce the best system outcomes. To improve the rate of parasite eradication, farmers need to feed nutrient-rich (particularly high MP supply) pastures and supplement which increases the rate of development of resistance.

### 5.3 *IGF1* variation and Growth Rates

The presence of *A* shows associations with weaning weights in Exon 2 during 2009 and both years combined (Table 3 and Table 4, respectively). The birth and rearing type of the lamb has a major influence on pre-weaning growth (Notter & Brown, 2015). As found in this analysis, singles tended to have the highest growth rates and triplets had the lowest growth rates. Wylie et al. (1997) showed that generally higher serum IGF-1 concentrations in single lambs overall, were consistent with a higher nutrient intake from a greater access to milk due to single suckling by their dam.
Muir et al. (2000) also found lamb growth rates from birth to 15 weeks of 343 g/d and 292 g/d for single and twin lambs respectively, these lambs reared by East Friesian crossed with Romney ewes, grew faster and were significantly heavier (P < 0.001) than lambs born to other ewe breeds that have lower levels of milk production. Whilst it is tempting to associate the increased lamb growth with the increased milk production of East Friesian Romney cross ewes, there is evidence that increased lamb growth rates may be also due to lamb genotype (mature size effect) and to hybrid vigour (Muir, unpublished data) for ewe milk production.

Even under the optimum feeding conditions in Muir et al. (2000), it appears that single lambs, without competition for milk by a sibling, obtained about 15% of their nutrient requirements from pasture 6 weeks into lactation. At the same time, twin lambs were required to obtain over 33% of their energy requirements from pasture. However, during early and mid-lactation, lambs are unlikely to have achieved full rumen function and the opportunity for lambs to select high quality, highly digestible pasture components will be critical for maximum growth rate. This emphasises the importance of providing high quality, digestible forage to the ewe and lamb(s) during lactation. It is probably that under any situation where feed is limiting, competition between ewes and lambs for high quality feed will restrict lamb growth. This is likely to occur even at peak lactation with well-fed, high milk producing ewes.

Triplet lambs lacked variation in lamb growth rate and had generally low averages compared to singles and twins in Mathias-Davis et al. (2013) study, suggesting there is a limiting factor controlling triplet lamb growth rate. Studies by Dwyer et al. (2005) indicated that triplet lambs had some placental insufficiency in comparison to other litter sizes which correlated with lamb neonatal vigour. It is possible that this may be influencing the subsequent growth rates of triplet lambs. It is also likely that the increased energy demands on ewes having triplet lambs means ewe intake during gestation and rearing is not sufficient for their lambs to reach their growth potential such that they would benefit from increased feed allocations.

There have been several studies which examined the relationship between ewe body condition score (BCS) and lamb growth rate (Mathias-Davis et al., 2013). In some studies, there was a positive relationship between lamb growth rate and ewe BCS (Gibb & Treacher, 1980; Kenyon et al., 2004), while others reported no association (Litherland et al., 1999). Mathias-Davis et al. (2013) found lamb growth rate was significantly affected by an interaction between pre-lambing BCS and the change in BCS between lambing and weaning. Gibb and Treacher (1980) reported consistent findings, where the growth rate for twin lambs was found to be higher for ewes with an average BCS of 3.2 than for ewes with an average BCS of 2.4. A low BCS during gestation or under-nutrition during mid to late
pregnancy can reduce foetal growth and birth weight (Kenyon et al., 2007). BCS and liveweight of ewes provide tools for improving lamb growth and production, as mentioned earlier.

5.4 Future Investigations

From this analysis, there are a few areas which could be improved for future research. Firstly, this experiment has a reasonably small sample size that may lack the strength of detecting the association between IGF1 and the growth traits. There are also contrasting reports about the consistency of IGF1 and its effect on growth in animals. Therefore, a greater number of lambs (at least 500) would be typed to help create more consistent results. Whilst typing more lambs, I would use more progeny from more than one sire and this includes from a range of years, as 2007 progeny had much slower growth rates (higher birth weights) compared with 2009, giving unreliable results. Assumptions had to be made that either 2007 lambing to weaning was a bad season in general, or 2009 was an exceptional growth season. By increasing the sample size whilst including various environmental factors, the more accurately it will reflect the population.

Bass et al. (1990) found IGF-1 was higher in Texel-sired lambs which is consistent with lower carcass fat content, therefore, higher carcass lean content of the Texel breed. This suggests potential for IGF1 as a marker of lean deposition in general and of eye-muscle in particular. The New Zealand Texel breed are leaner subcutaneously shown in their GR measurements compared with the New Zealand Romney (Clark & Kirton, 1990). They have greater depth of the longissimus muscle, higher muscularity values, shorter, lighter leg bones and more muscle. The New Zealand Romney is also a large, lean sheep but not to the extent of a Texel. They are a dual-purpose breed with equal emphasis on meat and wool. However, Texel have a greater composition of meat on their carcass compared with bone and fat than Romneys do. Therefore, if this experiment was repeated with Texels, there would be the assumption that greater association would be seen with IGF1 and lamb birth weight, weaning weight and pre-weaning growth rates.

Growth is a huge component of a well-finished lamb, but meat, fat and bone yield are too. The quality and quantity of a lamb carcass focuses primarily on the lean meat yield, fat composition depends on the end market and bone composition is insignificant. These three carcass components need to have a good ratio to meet consumer demands. In future, a statistical analysis of carcass composition should be carried out to analyse if accelerated lamb growth rates are consistently associated with greater lean meat to fat ratio.
Chapter 6
Conclusion

Birth weight, growth rate prior to weaning and weaning weight are three growth traits that have an important role in lamb meat production. Therefore, breeding for optimal birth weight and increased weight gains, is a major factor in sheep breeding programmes. Growth rate is related to muscle protein accretion, this being the difference between the rates of muscle protein synthesis and degradation. The higher the protein accretion at a set rate of degradation, the higher the growth rate of the animal.

In summary, the presence of the exon 2 A sequence of NZ Romney IGF1 was associated with higher birth weights, weaning weights and growth rates from birth to tailing and birth to weaning. Of the lambs that are born heavier, the majority will be heavier at time of weaning. With good birth weights through nutrition and genetic selection, lambs will reach slaughter weights sooner.

This finding is consistent with reports in other species; humans (Laron, 2001), cattle (de la Rosa Reyna et al., 2010), sheep wool growth (Zhang et al., 2009), and mice (Efstratiadis, 1998)

Ewe condition plays a significant role in lamb productivity. If level of nutrition was restricted for a pregnant ewe, it could reduce lamb birth weight depending on severity and timing of restriction. Climatic and feed conditions may have resulted in the poor lamb growth rates in 2007. Birth rank, rearing rank, year, sex, and variant all influence birth weight, weaning weight and growth rates. Later on in the lactation period internal parasites influence lamb growth rates and weaning weights.

In spite of abundant research showing contrasting results, this analysis showed associations and strong trends with IGF1 and birth weight, weaning weight and pre-weaning growth rates. These results should improve if this study were to continue with an increased sample size, as it will more accurately reflect the population.

Human IGF-1 concentrations have a moderate to high heritability of 0.4 to 0.6 and cattle GH concentrations are highly heritable. If the heritability of ovine IGF-1 levels were similarly inherited, selection for rapid growth based on plasma IGF-1 concentrations would be beneficial in improving the growth rate of livestock.

For the genetic improvement of animals, using IGF1 as a molecular marker could be integrated as part of the selection criteria to complement genetic evaluations of production traits in sheep and other animals.
The New Zealand sheep industry places a strong emphasis on selection for rapid lamb growth and high meat yield with exceptional carcass quality to obtain premium prices. Inefficient lamb growth for meat production has a significant economic impact on the New Zealand sheep industry and provides a considerable opportunity cost for the industry if affordable solutions can be found. The New Zealand meat industry earned $7.5 billion in export revenue in the year ended March 2016 and this sector has always been a principle driver of New Zealand’s economy. Producing fast growing lambs with lean carcasses is of economic importance for the industry.
## A.1  \textit{IGF1} Transcript Sequences

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**Figure A 1**: \textit{IGF-1} Exon 2 Transcript Sequence
A.2  IGF1 Predicted Protein Sequences

Figure A 2: IGF-1 Exon 3 Transcript Sequence

Figure A 3: IGF-1 Exon 2 Translation to Protein (1-157)
Figure A 4: IGF-1 Exon 3 Translation to Protein (1-182)

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1     ACAAGCCACGCGGTACGGCTCGAGCATCGGAGGAGSGCGCCAGACAGGATCGTGGATG
1     NKFTGYYGSSSRAPFQTV
61    AGFGCTGCTCCGAGGCTGATCTGAGGAGCTGAGATGFACTGTCGCTCCTCAAGG
20    ECCFRSCLRRAGEMLYCAPL
121   CCGCAAGTCGGCCTCACTCCGTGGCCAGCGCACCCAGACCACATGCCCAAGGCTCAGA
40    AAKSARSRVAQRHTDMPKAQ
181   AG
```
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