

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**Ewe nutrition during pregnancy:
Effects on the development of
twin fetuses**

A thesis presented in partial fulfilment of the
requirements for the degree of

Master of AgriScience in Agriculture

At Massey University, Palmerston North
New Zealand

Natalia de la Paz Martín

2011

ABSTRACT

Martín, N.P. (2011). Ewe nutrition during pregnancy: Effects on the development of twin fetuses. A thesis presented in partial fulfilment of the requirements for the degree of Master of AgriScience in Agriculture. At Massey University, Palmerston North, New Zealand.

This study set out to investigate the effects of dam nutrition during pregnancy on the anatomical development of twin fetuses, with particular focus on the fetal mammary gland. Ewes were fed at 3 different levels in early pregnancy (day 21 to 50, Low (L_{D21-50}) vs. Medium (M_{D21-50}) vs. High (H_{D21-50})) and 2 different levels in mid- to late-pregnancy (day 50 to 140, Medium ($M_{D50-140}$) vs. High ($H_{D50-140}$)). At D140, 58 twin-bearing ewes were euthanised, and dam and fetal organs were collected and weighed.

H_{D21-50} ewes were heavier than L_{D21-50} and M_{D21-50} ewes at D50. At D140, $H_{D50-140}$ ewes were heavier, in better condition score and gained more weight than $M_{D50-140}$. Ewe nutrition in either period had no effect on the total placental membranes weight, gravid uterus weight, total placentome number or their level of eversion at D140. Nutritional treatments in both early and mid- to late-pregnancy failed to affect fetal weight or general size measurements (crown-rump length, girth circumference, femur or fore-leg length). The *semitendinosus* muscles from L_{D21-50} - $H_{D50-140}$ fetuses were heavier than L_{D21-50} - $M_{D50-140}$ and H_{D21-50} - $H_{D50-140}$ after adjustment for fetal weight. Fetuses from L_{D21-50} dams had lighter mammary glands compared to the M_{D21-50} and H_{D21-50} fetuses, and these differences remained after adjustment for fetal weight. Maternal nutrition affected other organs and glands, including thyroids, liver, brain and ovaries.

The results indicate a critical window of early mammary gland development between days 21 to 50 of gestation, as the fetal mammary glands for the group restricted in early gestation remained lighter, independent of fetal weight or size. A larger cohort of these animals has been kept to monitor their lifetime performance. This work has the potential to change current farming practices and possible review of the fundamentals of human nutrition and health.

ACKNOWLEDGEMENTS

This section is dedicated to those who made this work possible. It hasn't been easy, but I made it thanks to all of you.

First of all, I am very grateful to the International Sheep Research Centre, Massey University, for giving me the opportunity to be part of this project, and to the National Research Centre for Growth and Development, for providing financial support for the project and giving great learning opportunities such as the writing retreat at the end of last year.

A big thanks goes to my main supervisor Paul Kenyon. Paul, thanks for the guidance, patience, encouragement, dealing with my 'I have one question' and fixing my spanglish (only in pencil). I did learn a lot and enjoyed doing so throughout these 2 years. Thanks!

Many thanks to Hugh Blair and Patrick Morel, my co-supervisors, who assisted with the planning, statistical analysis and interpretation, writing and giving ideas to work on. Thanks also to the IVABS team and students involved in this long-term project, particularly Sarah, Maria and Amy, with whom sharing and discussing ideas was a great help. Thanks to AgServices staff as well, for taking care of the animals and sharing my everyday's work.

Muchas gracias to my family and friends back in Argentina, for being in touch and making me part of your life in the distance, for your love and support. Gracias also to the people of the Sheep Unit at the Faculty of Agronomy (UBA), especially to Ana. I wouldn't be here if it wasn't for all of you!

Thanks to my kiwi friends and gracias to the Latin family over here, for so many mates, asados, barbecues, talks, music and laughs together. You are great! And especial thanks to Belen, Javier and Ronaldo, for helping with my student life.

Finally, a million thanks to Grant. Thanks for being so genuine and always having a smile for me, thanks for your constant support, hard work and making my days so wonderful. I'm very lucky to have you in my life and I hope we will share many many more years together.

TABLE OF CONTENTS

ABSTRACT	I
ACKNOWLEDGEMENTS	II
TABLE OF CONTENTS.....	III
LIST OF FIGURES.....	V
LIST OF TABLES.....	VII
CHAPTER 1 INTRODUCTION.....	1
CHAPTER 2 LITERATURE REVIEW	3
2.1 FACTORS INFLUENCING THE FETAL DEVELOPMENT	3
2.1.1 <i>Dam live weight and body condition</i>	4
2.1.2 <i>Dam parity</i>	5
2.1.3 <i>Environment</i>	6
2.1.4 <i>Photoperiod</i>	6
2.1.5 <i>Shearing</i>	6
2.1.6 <i>Birth rank of the lamb</i>	7
2.2 NUTRITION DURING PREGNANCY	8
2.2.1 <i>Stages of Gestation</i>	10
2.2.2 <i>Peri-conception</i>	12
Effects on fetal growth, body weight, dimensions, organs and placenta	13
2.2.3 <i>Early Pregnancy (days 0-50)</i>	18
Effects on fetal growth and body weight.....	18
Effects on fetal dimensions	19
Effects on organ size	25
Effects on placenta.....	31
2.2.4 <i>Mid (days 50-100) and Late (days 100-150) Pregnancy</i>	33
Effects on fetal growth, body weight and dimensions	33
Effects on fetal organ size.....	37
Effects on placenta.....	40
2.3 SUMMARY AND CONCLUSIONS FROM THE LITERATURE REVIEW	41
CHAPTER 3 MATERIALS AND METHODS	43
3.1 BACKGROUND INFORMATION.....	43
3.2 THE PRESENT STUDY	44
3.3 MEASUREMENTS	44
3.3.1 <i>Pasture</i>	44

3.3.2 Dam.....	45
3.3.3 Fetus.....	45
3.3.4 Placenta.....	45
3.4 STATISTICAL ANALYSIS.....	46
CHAPTER 4 RESULTS	49
4.1 PASTURE.....	49
4.2 EWE	49
4.2.1 Ewe live weight	49
4.2.2 Change in ewe live weight	50
4.2.3 Ewe condition score.....	50
4.2.4 Ewe carcass weight, GR depth and abdominal fat weight.....	50
4.2.5 Ewe organs weight, mammary gland weight and uterine and placental weight.....	50
4.3 FETUS	54
4.3.1 Fetal size measurements	54
4.3.2 Fetal organ and system measurements.....	54
Main organs and glands.....	54
Head and brain.....	55
Reproductive system	56
Weight ratios.....	57
4.4 PLACENTA	69
4.4.1 Per ewe.....	69
4.4.2 Per fetus.....	69
CHAPTER 5 DISCUSSION.....	73
CHAPTER 6 CONCLUSION	77
REFERENCES	79
APPENDIX	85

LIST OF FIGURES

Figure 2-1: Regulation of mammalian fetal growth. Intrauterine growth is regulated by genetic, epigenetic, and environmental factors. These factors affect placental growth and therefore the availability of nutrients for fetal growth. Source: Wu <i>et al.</i> (2006)	3
Figure 2-2: Relationship between the weight (kg) of Welsh Mountain (○, n=59) and Mule (●, n=34) ewes and the birth weight (kg) of their singleton offspring. Linear regression indicated a significant effect of ewe weight on weight of the lamb (F=100, P<0.001, R ² =0.20). Source: Gardner <i>et al.</i> (2007).....	4
Figure 2-3: Relationship between the weight (kg) of ewes at mating and birth weight (kg) of twin lambs (R ² =0.012; y=3.76 + 0.0201x ± 0.005). Source: Kenyon <i>et al.</i> (2004).	4
Figure 2-4: Fetal/maternal weight ratio (%) of lambs at term split according to low (<2), medium (2-3.5) or high (>3.5) body condition score ewes. Values are mean ± S.E.M. for individual data points for low (n=29), medium (n=122) or high (n=15) body condition score of ewes at mating. Differing superscripts indicate statistical difference at P<0.001. Source: Gardner <i>et al.</i> (2007).	5
Figure 2-5: Least-squares means for birth weights of lambs relative to parity of ewes. After Gootwine and Rozov 2006. Source: Gootwine <i>et al.</i> (2007).	5
Figure 2-6: Fetal/maternal weight ratio (%) of lambs at term split according to singleton or twins. Values are mean ± S.E.M. for individual data points of singleton (n=95) and twin (n=50) lambs. Statistical differences are *P<0.001. Source: Gardner <i>et al.</i> (2007).....	7
Figure 2-7: Birth weights and perinatal survival rates for lambs (n=4781) born to Afec-Assaf ewes (Volcani Center, Israel), according to litter size. After Gootwine and Rozov 2006. Source: Gootwine <i>et al.</i> (2007).	7
Figure 2-8: Relationships between fetal weight and day of gestation (based on a polynomial regression). Level of nutrition from day 70 to 140 are Low (1.5x maintenance) and High (2.0x maintenance). Source: Rattray <i>et al.</i> (1974).....	8
Figure 2-9: Differential fetal growth trajectories resulting in similar birth weights. The graph shows three exaggerated hypothetical growth trajectories: rapid growth in the first half of pregnancy with slowing thereafter (---); a similar initial trajectory followed by a period of slow	

growth before there is intrauterine catch-up growth (·{-}); initially slower growth with acceleration in the last half of gestation (-). Source: Bloomfield *et al.* (2006). 9

Figure 2-10: Growth of the foetus and placenta during pregnancy in Florida Native ewes (n=4 per age group). After Bazer *et al.*, unpublished results. Source: Gootwine *et al.* (2007)..... 10

Figure 2-11: Summary of the main developmental windows, organs affected and different long-term effects in the offspring after maternal nutrient restriction at defined stages of the reproductive cycle in sheep. CV, cardiovascular system. Adapted from: Symonds *et al.* (2007; 2010). 11

Figure 2-12: Critical periods during gestation in sheep for the expression of effects of maternal under-nutrition (0.5×maintenance vs. 1.0×maintenance) on fetal ovarian development. After McEvoy and Robinson 2002. Source: Robinson *et al.* (2006)..... 11

Figure 2-13: Schematic representation of lifetime mammary development. The width of the arrow represents qualitatively the size of the gland. Proposed critical windows, points at which altered development could have a long-term effect, are shown by text boxes. Source: Knight & Sorensen (2001). 11

Figure 2-14: Depiction of the cellular events involved in the ontogenesis of muscle fibres (light grey), brown adipose tissues (BAT; dark grey) and white adipose tissues (WAT; white) in cattle. Major variation factors and putative interactions between tissues and/or regulation are indicated. Source: Bonnet *et al.* (2010). 12

Figure 2-15: Overview of embryonic mammary development in mice. Source: Cowin & Wysolmerski (2010). 28

Figure 2-16: Effect of increased maternal food intake over the final half of gestation on relative fat mass, the brown adipose tissue specific uncoupling protein (UCP) 1 and the abundance of the prolactin receptor (PRLR) in the newborn sheep. Mothers were either fed to 100% of total metabolisable energy requirements (open boxes) or *ad libitum* (i.e. 150% of metabolisable energy requirements; closed boxes). After Budge *et al.* (2000). Source: Symonds *et al.* (2010). 38

Figure 3-1: Trial design and timeline 44

LIST OF TABLES

Table 2-1: The effects of maternal nutritional manipulation starting prior to mating on fetal growth, weight and metabolism. Adapted from: Kenyon (2008) and van der Linden (2010). ...	14
Table 2-2: The effects of maternal nutritional manipulation starting prior to mating on fetal dimensions.....	15
Table 2-3: The effects of maternal nutritional manipulation starting prior to mating on fetal organs. Adapted from: Brameld & Daniel (2008).	16
Table 2-4: The effects of maternal nutritional manipulation starting prior to mating on the placenta.....	17
Table 2-5: The effects of maternal nutritional manipulation starting in early pregnancy on fetal weight, post-natal growth and metabolism. Adapted from: Kenyon (2008) and van der Linden (2010).	21
Table 2-6: The effects of maternal nutritional manipulation starting in early pregnancy on fetal dimensions.....	23
Table 2-7: The effects of maternal nutritional manipulation starting in early pregnancy on fetal organs. Adapted from: Kenyon (2008), Brameld & Daniel (2008) and van der Linden (2010). ..	29
Table 2-8: The effects of maternal nutritional manipulation starting in early pregnancy on the placenta.....	32
Table 2-9: The effects of maternal nutritional manipulation starting in mid pregnancy on fetal weight and post-natal growth. Adapted from: Kenyon (2008).	35
Table 2-10: The effects of maternal nutritional manipulation starting in late pregnancy on fetal growth, weight, post-natal growth and metabolism. Adapted from: Kenyon (2008) and van der Linden (2010).....	36
Table 2-11: The effects of maternal nutritional manipulation starting in mid or late pregnancy on fetal dimensions.....	36
Table 2-12: The effects of maternal nutritional manipulation starting in mid or late pregnancy on fetal organs. Adapted from: Brameld & Daniel (2008).	39
Table 2-13: The effects of maternal nutritional manipulation starting in mid or late pregnancy on the placenta.	40

Table 4-1: Effect of nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on pasture quality (ME MJ/kg DM) and pre- and post-grazing masses (kg DM/ha). Source: Kenyon <i>et al.</i> (2011).....	49
Table 4-2: Effect of nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on ewe live weight (kg) on Days -1, 21, 50 and 137. ..	51
Table 4-3: Effect of nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on ewe condition score on Days -1, 21, 50 and 137. ...	52
Table 4-4: Effect of nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on ewe carcass weight (kg), abdominal fat weight (g) and GR depth (mm) on Day 140.	53
Table 4-5: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the fetal size measurements (body weight, <i>semitendinosus</i> muscle weight, crown-rump length (CRL), girth length, fore and hind-leg lengths and femur length) on Day 140.	58
Table 4-6: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the fetal size measurements (<i>semitendinosus</i> muscle weight, crown-rump length (CRL), girth length, fore and hind-leg lengths and femur length) on Day 140, adjusted for fetal body weight.....	59
Table 4-7: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the fetal organs (liver, kidneys, spleen, lungs, heart and heart fat) on Day 140.	60
Table 4-8: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the fetal organs weight (liver, kidneys, spleen, lungs, heart and heart fat) on Day 140, adjusted for fetal body weight.....	61
Table 4-9: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the fetal glands (thymus, thyroid, pancreas and adrenals) on Day 140.	62
Table 4-10: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the fetal glands weight (thymus, thyroid, pancreas and adrenals) on Day 140, adjusted for fetal body weight.	63

Table 4-11: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the fetal head measurements and the weight of the brain and pineal gland at Day 140.64

Table 4-12: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the fetal head measurements and the weight of the brain and pineal gland on Day 140, adjusted for fetal body weight.65

Table 4-13: Effect of nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the weight data of the fetal reproductive organs (testes, ovaries and mammary gland) on Day 140.66

Table 4-14: Effect of nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the fetal reproductive organs weights (testes, ovaries and mammary gland) on Day 140, adjusted for fetal body weight.67

Table 4-15: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the weight ratios between Brain and Fetus, Liver and Fetus, Liver and Brain, and Fetus and Placentomes on Day 140.68

Table 4-16: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on total caruncle number, placentome number and empty caruncle number, per fetus, on Day 140.70

Table 4-17: Effect of the fetal sex (female vs. male) and nutritional treatments during D21-50 (Low (L) vs. Medium (M) vs. High (H)) and D50-140 (Medium (M) vs. High (H)) on the weight of the placentomes (total and grouped in A, B, C and D type) per fetus, on Day 140.71

