# Molecular Characterisation of Primary Wool Follicle Initiation in Merino Sheep

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June, 2009

This thesis is submitted in partial fulfilment of the award of PhD in the Discipline of Agricultural and Animal Science, School of Agriculture, Food and Wine,

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# Table of Contents

**Abstract** ..... ii

**Declaration** ..... iv

**Acknowledgements** ..... v

**Abbreviations** ..... vii

**Chapter 1 Introduction and Literature Review** ..... 2

**1.1 Introduction** ..... 2

**1.2 Structure and Development of the Wool Follicle** ..... 4

**1.2.1 Structure and Function of Mammalian Skin** ..... 4

**1.2.2 Wool Follicle Types** ..... 5

**1.2.2.1 Primary Follicles** ..... 6

**1.2.2.2 Secondary and Secondary-Derived Follicles** ..... 6

**1.2.2.3 Secondary to Primary Ratio (S/P ratio)** ..... 8

**1.2.3 Molecular Basis of Primary Follicle Neogenesis** ..... 8

**1.2.3.1 First Dermal Signal: Formation of the Epidermal Placode** ..... 9

**1.2.3.2 First Epidermal Signal: Formation of the Dermal Condensate** ..... 13

**1.2.3.3 Down growth of the epidermal placode** ..... 13

**1.2.3.4 Formation of the dermal papilla** ..... 14

**1.2.3.5 Formation of the Follicle Bulb** ..... 14

**1.2.3.6 Inner Root Sheath (IRS)** ..... 15

**1.2.3.7 Outer Root Sheath (ORS)** ..... 16

**1.2.3.8 Follicle Accessory Structures** ..... 16

**1.2.3.8.1 Sebaceous Gland** ..... 16

1.2.3.8.2 Sweat Glands (Primary Follicles Only) .................................... 17

1.2.3.8.3 Arrector Pili Muscle (Primary Follicles Only).......................... 17

1.3 Stem Cells in the Skin ........................................................................... 17

1.3.1 Embryonic vs Adult Stem Cells ................................................................ 18

1.3.2 Epidermal Stem Cells ................................................................................ 19

1.3.3 Dermal Stem Cells...................................................................................... 19

1.3.4 Division and Maintenance of Stem Cell Populations ................................ 20

1.3.5 Location of Stem Cells in the Hair/Wool Follicle ...................................... 21

1.3.5.1 Mechanisms of Follicle Initiation: Reaction Diffusion Theory vs. Founder Cell Theory.................................................................... 22

1.3.5.2 Dermal Condensates: Proliferation, Migration or Both?.................. 24

1.4 Summary .................................................................................................... 25

1.5 Project Aims and Hypothesis ..................................................................... 26

Chapter 2 General Materials and Methods ................................................ 28

2.1 Materials ..................................................................................................... 28

2.2 Foetal Tissue Collection .............................................................................. 28

2.3 RNA isolation and purification .................................................................. 28

2.4 Estimation of RNA concentration and assessment of RNA integrity ............ 29

2.5 RT-PCR of isolated RNA for cDNA production ............................................ 29

2.6 Optimisation of PCR conditions for isolation of sheep cDNA sequences....... 30

2.7 Analysis of PCR products by agarose gel electrophoresis.............................. 30

2.8 Preparation of PCR template and automated sequencing .............................. 31

2.9 Optimisation of quantitative RT-PCR reaction conditions............................. 31

Chapter 3 Foetal Skin Series and Histology ................................................ 34

3.1 Introduction ................................................................................................. 34

3.2 Specific Methods ........ 34
3.2.1 Matings ........ 34
3.2.2 Generation of Foetal Skin Series ........ 34
3.2.3 Crown Rump Length Measurements ........ 35
3.2.4 Processing, Embedding and Sectioning ........ 36
3.2.5 Haematoxylin and Eosin Staining ........ 37
3.2.6 SacPic Staining ........ 38
3.2.7 Histology Images ........ 39
3.2.8 Statistical Analysis ........ 39
3.3 Results ........ 40
3.3.1 Foetal Measurements ........ 40
3.3.1.1 Foetal Data Comparison with a Previous Study ........ 43
3.3.2 Histological Characterisation ........ 45
3.4 Discussion ........ 48
3.4.1 Foetal Sample Series ........ 48
3.4.2 Histological Characterisation ........ 51
3.5 Conclusion ........ 52

Chapter 4 : Quantitative PCR Analysis of Whole Skin ........ 54
4.1 Introduction ........ 54
4.1.1 Normalisation of qRT-PCR data: geNorm ........ 55
4.1.2 Candidate Genes for qPCR Analysis of Wool Follicle Initiation ........ 56
4.1.2.1 Actin-based Fibroblast Migration ........ 56
4.1.2.2 Skin Stem Cell Markers ........ 56
4.1.2.3 Cell Proliferation ........ 58
4.1.2.4 Tumor Necrosis Factor Signalling in Hair Follicle Development ........ 59
4.1.2.5 Sonic Hedgehog Signalling in Hair Follicle Development ........ 60

4.2 Aim ... 62

4.3 Specific Methods ... 62

4.3.1 qRT-PCR ... 62

4.3.2 geNorm Analysis and Normalisation of qPCR data ... 63

4.3.3 Statistical Analysis of Relative Expression Patterns ... 64

4.4 Results ... 64

4.4.1 RNA extractions ... 64

4.4.2 geNorm Analysis ... 66

4.4.3 Gene Expression Analysis ... 70

4.4.3.1 Migration Markers ... 70

4.4.3.2 Stem Cell Markers ... 72

4.4.3.3 Proliferation Markers ... 74

4.4.3.4 Tumor Necrosis Factor Signalling Pathway Members ... 76

4.4.3.5 Sonic Hedgehog Signalling Pathway ... 82

4.4.3.6 Gene Expression Correlations ... 84

4.5 Discussion ... 85

4.5.1 Gene Expression Analysis of Candidate Migration Markers ... 87

4.5.2 Gene Expression Analysis of Candidate Stem Cell Markers ... 88

4.5.3 Gene Expression Analysis of Candidate Cell Proliferation Markers ... 89

4.5.4 Ectodysplasin Signaling During Primary Wool Follicle Initiation ... 90

4.5.5 Gene expression analysis of Sonic Hedgehog and Patched-1 during primary follicle development ... 94

4.5.6 Differences in Gene Expression between the Midside and Rump ... 95

4.6 Conclusion ... 97

Chapter 5 : Laser Capture Microdissection of Foetal Sheep Skin ... 99

5.1 Introduction ... 99

5.2 Aim ... 100

5.3 Specific Methods ... 100

5.3.1 Frozen tissue sectioning and fixation ... 100

5.3.2 Haematoxylin and Eosin staining of frozen sections for laser capture microdissection ... 100

5.3.3 Laser Capture Microdissection Protocol ... 101

5.3.4 RNA extraction from laser captured tissue ... 102

5.3.4.1 TRIZOL and RNAqueous Micro Extraction Protocols ... 102

5.3.4.2 Qiagen RNeasy Micro Extraction Kit ... 103

5.3.5 Estimation of RNA quality and quantity ... 103

5.3.6 Reverse Transcription of RNA ... 104

5.3.7 Cells Direct One-Step Kit ... 104

5.4 Results ... 104

5.4.1 Optimisation of Sectioning and Staining for Laser Capture Microdissection ... 104

5.4.2 Optimisation of RNA extraction and RT-PCR of Laser Capture Microdissected Material ... 107

5.4.3 Optimisation of Slide Type for Laser Pressure Catapulting ... 112

5.4.4 Laser capture microdissection and qRT-PCR analysis of follicle regions vs. non-follicle regions of foetal sheep skin. ... 116

5.5 Discussion ... 121

5.6 Conclusion ... 129

Chapter 6 General Discussion ... 131

6.1 Introduction ... 131

6.2 Future Work ... 145

6.3 Conclusion ... 147

**Appendix I: General Solutions, Buffers and Stains....................................................... 149**

**Appendix II: Primers.............................................................................................. 153**

**References............................................................................................................. 155**

# Abstract

Primary wool follicles are initiated in the skin of sheep foetuses at approximately day 50 of gestation as the result of complex reciprocal molecular interactions between the mesenchyme and overlying epithelium. The lifetime wool production potential and fibre diameter of the Merino sheep is dependent on the total number of follicles initiated *in utero*. Understanding the molecular events that surround primary wool follicle initiation may provide approaches to enhance or manipulate this process in order to maximise the profitability of wool production enterprises.

In order to study the morphological and molecular changes occurring during early wool follicle development, a foetal skin series spanning primary follicle initiation was generated. Foetal skin was sampled from the shoulder, midside and rump of four foetuses at 8 time points between day 43 and day 68 of gestation. Histological characterisation of the shoulder skin samples revealed that primary epidermal placodes emerged at around day 53, dermal condensates were visible from day 57 and downgrowth of the follicle began at day 68. An equation relating age of the foetus (day of gestation post AI) and crown-rump length, specific to Merino foetuses, was developed for use in future studies of this nature.

Molecular markers of fibroblast migration, epidermal and dermal stem cells and cell proliferation were selected to test the hypothesis that dermal condensates are initiated at discrete sites beneath the epidermis as a result of a combination of migration and arrangement of multipotent pre-papilla cells. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis of *RAC1* and *RHOa* (migration markers), *β1-integrin* and *alkaline phosphatase* (stem cell markers), *proliferative nuclear cell antigen* and *cyclinB1* (proliferation markers), *patched-1*, selected tumor necrosis factor (TNF) signalling molecules and eleven reference genes was conducted using midside and rump skin samples from each of four foetuses from the 8 time points. geNorm analysis of the reference and target genes revealed that the migration markers *RAC1* and *RHOa* along with *GAPDH* were the most stably

expressed genes in this sample series. Significant changes in mRNA expression were detected for *β1-integrin*, *alkaline phosphatase*, *patched-1* and the TNF members *EDA*, *EDAR*, *TROY* and *TRAF6*. Many of these significant differences in expression coincided with key morphological events. Significant differences in expression were also detected between the midside and rump samples for numerous transcripts.

Laser capture microdissection (LCM) was implemented for analysis of the target transcripts within particular structures of foetal sheep skin. Frozen tissue sectioning, staining, LCM, RNA extraction and cDNA synthesis were optimised for qRT-PCR analysis of endogenous controls and selected TNF transcripts. Several RNA extraction methods and reverse transcription approaches were trialled to ensure optimum extraction and reverse transcription efficiency for this tissue type. Exogenous mRNA transcripts were also incorporated prior to RNA extraction and reverse transcription to track reaction efficiency between samples. A comparison of different slide types revealed that laser pressure catapulting from membrane slides was an absolute requirement for foetal skin tissue studies. Follicle regions (including the epidermal placode and dermal condensate) and the adjacent non-follicle regions were laser captured from foetal skin, and the mRNA expression levels of *patched-1* and selected TNF members was compared. Preliminary qRT-PCR analysis using this technique revealed that *EDAR*, *TROY* and *PTCH1* mRNA levels were higher in the follicle regions than the non-follicle regions.

The TNF signalling pathway appears to play an important role in primary wool follicle initiation and patterning at different sites on the body. Spatial differences in expression of some of these regulators may be involved in initiating different types of follicles. The molecular events surrounding primary wool follicle initiation also show a high degree of conservation between sheep, humans, and mice. Considering the high degree of DNA sequence conservation as well as the histological, signalling and cycling similarities between sheep and humans, sheep may represent a better model for the study of human hair follicle initiation and disease than the currently used mice and rat models.

# Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

Date

Hayley Ann McGrice

# Acknowledgements

First and foremost I would like to thank Australian Wool Innovation for their scholarship and research funding. Without your financial support this thesis would not have been possible.

A loud and resounding thank you must go to my supervisors Dr Cynthia Bottema, Dr Greg Nattrass and Prof Philip Hynd. Cindy you were always there for me and I can't thank you enough for everything you have done for me over the last four years. The speed at which you read and returned my chapters to me was absolutely amazing and the lengths that you went to in order to support me during my PhD candidature were above and beyond the call of duty, so thank you, from the bottom of my heart. To my technical supervisor Greg, thank you for listening to and answering my silly questions over and over again and for all your advice and assistance in the lab, especially in the large scale qRT-PCR experiment design and undertaking. The chats we had over a quite beer on a Friday afternoon were probably the most valuable in terms of understanding and interpreting the "disco" science. Phil, thank you for nurturing my love of writing on the whiteboard, you have a unique gift for helping people to build and get excited about crazy hypotheses. Your wordsmith skills were also greatly appreciated when writing the conference abstracts and of course, this thesis. The three of you were an excellent supervision team and I greatly appreciate all your support.

Special thanks to Dr Simon Bawden for initially creating the project, awakening my interest in wool research and for your continued guidance and advice during an extremely challenging PhD candidature. Thank you also to Dr Stephanie Dunn and Clive McLaughlin from the SARDI molecular team for your technical advice, assistance and friendship, I think we had about as much fun as anyone could have in a molecular lab!

To Dr Michelle Hebart and A.Prof Wayne Pitchford, thank you a thousand times over for your assistance with the statistical analysis and interpretation, stats are not my strong point and I definitely could not have done this without you.

A special mention must also go to Natasha Edwards, Dr Melanie McDowall, and the Roseworthy wool group. Thank you for all your friendship, laboratory assistance and constructive feedback when preparing conference presentations as well as helping me to build explanations for the results along the way.

To all the farm staff at Turretfield Research Centre, thank you for your expertise and assistance with the animal sampling and for your friendship.

To my best friend and office buddy Dr Rebecca Forder, we laughed and we cried together as we rode the PhD rollercoaster. Thank you for being an excellent shoulder to cry on, an open sympathetic ear, someone to laugh with and an excellent role model when it came to finishing this thesis. Thanks also for assisting me with the tissue collection and for your help with the thesis formatting, not to mention the baby sitting that helped get me over the line. On that note, thank you also to Nana Sue and Uncle Gary John for your assistance with Colby care; babies and thesis writing do not mix! I could not have done this without the Forder's.

Thank you also to Tone for being there for me during the emotionally challenging animal sampling and for putting up with the stress fuelled outbursts at various times along the way. Twas a long PhD road for us both!

Last but by no means least, special thanks goes to my Mum and Kylie, you guys were there for me when everything went wrong, you both helped me get back on my feet, and for that I will forever be in your debt. I nearly gave it all up and it was your support and strength that got me through the roughest patch of my life. Thank you for all the babysitting too mum, you're a class 1A mum and Nana.

Thank you all from the bottom of my heart!

# Abbreviations

| | |
|---|---|
| °C | degrees Celsius |
| µl | microlitres |
| µm | micrometres |
| ACTB | β-Actin |
| ALP | Alkaline phosphatise |
| BCC | basal cell carcinoma |
| BMP | bone morphogenic proteins |
| bp | base pairs |
| CAT | chloramphenicol transferase |
| CD34 | CD34 antigen |
| Cdc42 | Cell Division Cycle related family member 42 |
| cDNA | complementary DNA |
| CDK-1 | cyclin dependent kinase 1 |
| CIDR | controlled internal releasing device |
| cm | centimetres |
| COLL3AIII | collagen type 3AIII |
| CoV | coefficient of variation |
| CRL | crown rump length |
| $C_t$ | cycle threshold |
| CYC B1 | cyclin B1 |
| d | day |
| EDA | ectodysplasin A1 |
| EDAR | ectodysplasin A1 receptor |
| EDARADD | ectodysplasin A1 receptor death domain |
| EDTA | ethylenediaminetetraacetic acid |

| | |
|---|---|
| EM | epithelial:mesenchymal |
| FD | fibre diameter |
| FGF | Fibroblast growth factor |
| FGFR | FGF receptor |
| GAPDH | glyceraldehyde 3-phosphate dehydrogenase |
| GLI1 | GLI family zinc finger 1 |
| GSP | gene specific primers |
| GTP | guanosine-5'-triphosphate |
| hrs | hours |
| IKK | IκB kinase complex |
| IRS | inner root sheath |
| KRT5 | keratin-5 |
| LCM | laser capture microdissection |
| LEF/TCF | lymphoid enhancer-binding factor/T cell factor |
| LPC | laser pressure catapulted |
| LUC | luciferase |
| mA | milliamps |
| MIB-1 | mind bomb-1 |
| mins | minutes |
| mM | millimolar |
| mm | millimetres |
| NF-κβ | nuclear factor-kappa-beta |
| ng | nanograms |
| nm | nanometres |
| nmol | nanomoles |
| OCT | optimal cutting temperature |
| oligo dTVN | oligonucleotide dTNV combination |

| | |
|---|---|
| PALM | photo-activated localisation microscopy |
| PCNA | proliferative cell nuclear antigen |
| PBS | phosphate buffered saline |
| PCR | polymerase chain reaction |
| PTCH1 | patched-1 |
| qRT-PCR | quantitative reverse transcriptase PCR |
| RAC1 | ras-related C3 botulinum toxin substrate 1 |
| RHOa | Ras homolog gene family, member A |
| RLT buffer | RNeasy lysis buffer |
| RO | reverse osmosis |
| RPL19 | Ribosomal protein large 19 |
| RT | remnant tissue |
| SAS | statistical analysis software |
| sec | seconds |
| SEM | standard error of the mean |
| SHH | sonic hedgehog |
| S:P ratio | secondary to primary ratio |
| TE buffer | Tris-EDTA buffer |
| TGF-β2 | Transforming growth factor –beta 2 |
| TNF | Tumor necrosis factor |
| TRAF | Tumor necrosis factor associated factors |
| TROY | tumor necrosis factor receptor superfamily, member 19 |
| V | volts |
| WNT | wingless-type |
| XEDAR | X-linked ectodysplasin receptor |
| YWHAZ | tyrosine 3-monooxygenase /tryptophan 5-monooxygenase activation protein, zeta polypeptide |

This thesis is dedicated to my mum Jan and my daughter Colby

Thank you for teaching me the value of education mum,

may I instil the same important values in Colby.