

**Impact of Environmental Factors on the
Development of Corticotroph Subpopulations in the
Fetal Sheep Pituitary.**

A thesis submitted for the degree of Doctor of Philosophy

to

The University of Adelaide

November, 2007

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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.

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Farrand K, McMillen IC, Tanaka S, Schwartz J. (2006) Subpopulations of corticotrophs in the sheep pituitary during late gestation: effects of development and placental restriction. *Endocrinology*. 147(10): 4762-71.

Acknowledgements

The work described in this dissertation would not have reached this form without the unending support of my family and friends, my mentors and mentees – each have provided guidance and inspiration when needed.

An infinitely huge thank you to my Mum and Tony, your patience and belief in me has made this doctorate achievable and I look forward to being able to give you so much more when this stage is completed. We have all been through this drama together, and we will all be graduating together as a team. To my closest, dearest friends, Leonie, Olivia and Cass, I know the big rocks should always be put into the box first, but thank you for putting up with me getting this wrong so many times.

Of course, my most special thanks go to my primary supervisor, Jeff Schwartz, who not only made this work possible, but who picked me up off the ground time and time again, to keep going, to see the light and to become the sunshine. And to my cosupervisor, Caroline McMillen, your strength and success will always be an inspiration to me as I move through life. It has become very clear to me now that I have moved to another institution, that the unique blend of close-knit support and ever rising standards of my supervisors, and the faculty of the Discipline of Physiology, particularly Michael Roberts and Pat Buckley, has provided me with an exceptionally good training ground. I know that I am very lucky to have been mentored by you all.

The substantial significance of the pro-opiomelanocortin antibodies to my research is clearly evident throughout this thesis and I will always be in the

debt of Shigeyasu Tanaka for his generous donation of this resource. In addition to countless students and staff who helped with the animal work, I would like to thank Sarah Williams and Severence MacLaughlin for their donation of tissues from the animal models of suboptimal uterine environments used in this dissertation. I am also grateful to the impeccable organisation of Laura O'Carroll and Anne Jurisevic, who managed the collection of new tissues for this dissertation and provided me with a wealth of tissues and records to investigate. The teams of the Rodgers laboratory and Adelaide Microscopy Services have generously provided their expertise, time, resources and wise words to shape the investigations in this thesis. In particular I would like to thank John Terlet, Meredith Wallwork, Peter Self, Lyn Waterhouse and Angus Netting for welcoming me into your haven on many a Friday evening.

To all of my students, most especially Eva Szarek, through teaching I have learnt, through mentoring I have grown, and you have made this possible. Our discussions have given me the most inspirational insights into the underlying mechanisms and associations between all things.

And to the team at UQ, David Adams, Phil Poronnik, Lesley Lluka, Roger Moni and Mick McManus who have graciously given me the time, and the reason, to finalise the degree.

I would also like to acknowledge the financial support of the National Health and Medical Research Council; project grants that grow large, diverse teams of collaborators have certainly had a positive impact on my induction into the world of research.

Abbreviations

11 β HSD2	11 β hydroxysteroid dehydrogenase type 2
ACTH	adrenocorticotropic hormone
AR	antigen retrieval
AVP	vasopressin
BP	bandpass
CRH	corticotropin releasing hormone
CRHR ₁	corticotropin releasing hormone receptor 1
CRHR ₂	corticotropin releasing hormone receptor 2
CLIP	corticotrophin-like intermediate lobe peptide
Cy	cyanine
DAPI	4',6-diamidino-2-phenylindole, dihydrochloride
GR	glucocorticoid receptor
HMW	high molecular weight
IgG	immunoglobulin G
irACTH	immunoreactive adrenocorticotropic hormone
JP	joining peptide
LMW	low molecular weight
LP	longpass
LPH	lipotrophin
MC ₂ R	melanocortin 2 receptor
MSH	melanocyte stimulating hormone
RHPA	reverse haemolytic plaque assay
RIA	radioimmunoassay

RIPA	radioimmunoprecipitation assay
PBS	phosphate buffered saline
PC1	prohormone convertase 1
PC2	prohormone convertase 2
PCUN	periconceptual undernutrition
PKA	protein kinase A
POMC	pro-opiomelanocortin
PR	placental restriction
ST-1	Nonapeptide of pro-opiomelanocortin spanning the cleavage point between adrenocorticotrophic hormone and β -lipotrophin
V _{1b}	Vasopressin receptor 1b

Abstract

The prepartum surge in fetal plasma cortisol, essential for the maturation of organs in mammals and the normal timing of parturition in some species, including sheep, may result from an increase in the molar ratio of adrenocorticotropin (ACTH) to pro-opiomelanocortin (POMC) in the fetal circulation. Related to this, the cleavage of POMC to ACTH by the enzyme, prohormone convertase 1 (PC1), may be influenced by corticotrophin releasing hormone (CRH) stimulation. Accumulating evidence suggests that the capacity of individual corticotrophs to process POMC to ACTH may vary and individual corticotrophs are differentially responsive to CRH. It is not known, however, if there are separate corticotroph subpopulations in the fetal sheep pituitary which can be identified by differential colocalisation of POMC, ACTH and the CRH receptor 1, CRHR₁, nor if changes in the relative proportions of such subpopulations play a role in the molecular mechanisms underlying the overall changes in pituitary function described previously during gestation and in response to suboptimal uterine environments. To investigate these hypotheses, it was first necessary to develop novel methods for the simultaneous immunohistochemical labelling of POMC, ACTH and CRHR₁ in individual cells on sections of fetal sheep pituitary. In addition, I developed and validated an automated method to categorise and count individual cells to increase the quantitative power of this study.

Pituitary tissue was collected from control fetuses at 53-55 (n=6), 63-85 (n=6), 110 (n=4), 139-141 (n=4) and 144-145 (n=6) days gestation. Two

animal models, known to alter pituitary function in the fetal sheep, were used to investigate corticotrophic adaptations to suboptimal uterine environments. For the maternal periconceptual undernutrition (PCUN) model, maternal feed was reduced to 70% of maintenance requirements from at least 45 days before to 7 days after mating and fetal tissues were collected at 53-55 days gestation (n=7). For the placental restriction (PR) model, the majority of the placental attachment sites were removed in five ewes before mating and fetal tissues were collected at 140 (n=4) and 144 (n=4) days gestation. Pituitary sections were simultaneously labelled with antisera raised against full length POMC, ACTH and CRHR₁ and the proportions of pituitary cells with combinations of antisera were quantified. Four subpopulations of corticotrophs were identified, which expressed either: POMC+ACTH+CRHR₁, ACTH+CRHR₁, POMC+ CRHR₁ or POMC-only. There was a significant decrease in the proportion of pituitary cells expressing POMC+ACTH+CRHR₁ between 53-55 and 65-85 days gestation, before an increase at 110 days gestation and a further marked decrease between 139-141 and 144-145 days gestation. In fetuses from the PCUN group, the proportion of pituitary cells expressing POMC+ACTH+CRHR₁ in early gestation was reduced. PR resulted in a significantly higher proportion of corticotrophs expressing POMC+ACTH+CRHR₁ during the prepartum period.

This work represents the discovery of the differential expression of POMC, ACTH and CRHR₁ in individual corticotrophs of the fetal sheep pituitary and the first insights into the pituitary adaptations to periconceptual

nutrient restriction and placental restriction at the level of individual corticotrophs.

