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

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# Table of Contents

Declaration.............................................................................................................................xi
Acknowledgements..............................................................................................................xiii
Abbreviations .....................................................................................................................xv
Abstract.............................................................................................................................. xvii

Chapter 1: Introduction ........................................................................................................ 1
  1.1 Physiological roles of the HPA axis ................................................................. 1
    1.1.1 Role of glucocorticoids in fetal development ....................... 3
  1.2 Pituitary anatomy.............................................................................................. 5
    1.2.1 Identifying corticotrophs ......................................................... 5
  1.3 ACTH biosynthesis......................................................................................... 7
    1.3.1 Post-translational processing of POMC................................. 9
    1.3.2 Regulation of POMC processing by CRH and glucocorticoids .......... 11
    1.3.3 POMC processing during vesicle maturation ................. 11
    1.3.4 Unstimulated corticotroph secretion .................................. 12
    1.3.5 Regulation of corticotroph activity by CRH, AVP and cortisol .......... 13
  1.4 Corticotroph heterogeneity ................................................................. 16
    1.4.1 Heterogeneity of the response to CRH, AVP and cortisol amongst corticotrophs .......... 16
    1.4.2 Morphological heterogeneity amongst corticotrophs .......... 21
    1.4.3 Heterogeneity of POMC processing amongst corticotrophs 22
1.5 Dynamic models known to alter corticotroph phenotype and
function ........................................................................................................... 23

1.5.1 Fetal development .............................................................................. 23

1.5.1.1 Pituitary organogenesis ............................................................... 23

1.5.1.2 Ontogenic changes in the corticotroph population .............. 24

1.5.1.3 Ontogenic changes in the morphologically heterogeneous
subpopulations of corticotrophs ............................................................... 25

1.5.1.4 Ontogenic changes in the biological actions of precursors
at adrenal cortex ....................................................................................... 25

1.5.1.5 Ontogenic changes in the ratio of ACTH1-39 to its
precursors in the fetus ........................................................................... 26

1.5.1.6 Ontogenic changes in the regulation of corticotrophs by
CRH, AVP and glucocorticoids ............................................................... 28

1.5.2 Perturbations ..................................................................................... 30

1.5.2.1 Fetal exposure to maternal glucocorticoids ..................... 30

1.5.2.2 Fetal stress response ....................................................................... 31

1.5.2.3 Ontogenic changes in fetal HPA axis response to stress 31

1.5.2.4 Long term effects of inappropriate exposure of the fetus to
glucocorticoids ....................................................................................... 32

1.5.2.5 Maternal periconceptional undernutrition ......................... 33

1.5.2.6 Placental restriction of nutrient supplies to the fetus ...... 35

1.6 Models of corticotroph subpopulations ............................................. 36

Chapter 2: Method development and validation ............................... 43

2.1 Introduction .......................................................................................... 43
2.2 Methods ................................................................. 45

2.2.1 Animals ............................................................... 45

2.2.1.1 Pituitary Collection and Processing ..................... 45

2.2.1.1.1 Tissue collected for western blotting ............... 45

2.2.1.1.2 Tissue collected for immunohistochemistry ......... 46

2.2.2 Western Analysis ............................................................. 46

2.2.3 Immunohistochemistry .................................................. 48

2.2.3.1 Bleaching ................................................................. 48

2.2.3.1.1 Chemical Reduction of Autofluorescence .......... 48

2.2.3.1.2 Photo-bleaching ................................................... 48

2.2.3.1.3 Autofluorescence measurements ..................... 49

2.2.3.1.4 Optimal bleaching protocol .......................... 50

2.2.3.2 Antigen Retrieval ..................................................... 50

2.2.3.2.1 Optimal antigen retrieval .............................. 51

2.2.3.3 Antibody binding ...................................................... 52

2.2.3.4 Controls ................................................................. 52

2.2.3.4.1 Preabsorption and replacement ..................... 53

2.2.3.4.2 Primary omission control .............................. 53

2.2.3.4.3 Secondary antisera specificity ...................... 53

2.2.4 Imaging ................................................................. 54

2.2.4.1 Qualitative imaging .............................................. 54

2.2.4.1.1 Intracellular localisation .............................. 54

2.2.4.2 Quantitative imaging .......................................... 55

2.2.5 AnalySIS module ....................................................... 55
2.2.5.1 Calibration ................................................................. 57
2.2.5.2 Grey scale threshold .................................................. 57
2.2.5.3 Colocalisation by subtraction ....................................... 61
2.2.5.4 Counting cells ......................................................... 63
  2.2.5.4.1 Determining cell size for cytoplasmic stains .......... 68
2.2.5.5 Validation against manual counts ................................. 68
2.2.6 Data analysis ............................................................... 69

2.3 Results .............................................................................. 69
  2.3.1 Western Analysis .......................................................... 69
  2.3.2 Bleaching ...................................................................... 71
    2.3.2.1 Sodium Borohydride ................................................. 71
    2.3.2.2 Optimal Globe ........................................................ 72
    2.3.2.3 Optimal Duration .................................................... 73
  2.3.3 Antigen Retrieval (AR) ................................................... 74
  2.3.4 Controls ....................................................................... 77
  2.3.5 Colocalisation ............................................................... 80
  2.3.6 Quantification of Corticotroph subpopulations ............... 82
    2.3.6.1 Corticotroph Cell Size and Cluster Size ................... 82
    2.3.6.2 Validation of automated method against manual counts . 84

2.4 Discussion ........................................................................ 85
  2.4.1 Corticotroph subpopulations ........................................ 86
  2.4.2 POMC and ACTH antisera specificity ................................ 89
  2.4.3 Intracellular localisation of antigens .............................. 90
    2.4.3.1 Cytoplasmic localisation of CRHR1 .......................... 91
6.2 Differential expression of POMC, ACTH and CRHR1 .......... 158

6.2.1 Corticotroph subpopulations ........................................ 160

6.2.1.1 POMC processing ...................................................... 160

6.2.1.2 CRHR1 expression ................................................... 162

6.2.1.3 Transdifferentiation between corticotroph subpopulations .......................... 168

6.3 Roles of corticotroph subpopulations .................................... 169

6.3.1 Inhibitory corticotrophs .............................................. 169

6.3.2 Stimulatory corticotrophs ............................................. 171

6.4 Response of corticotroph subpopulations to suboptimal intrauterine environments .................................................. 172

6.5 Conclusions ........................................................................ 175

Appendix A: Solutions ............................................................. 179

A.1 5x Phosphate Buffered Solution (PBS) ............................ 179

A.2 Antigen Retrieval Buffers .................................................. 179

A.2.1 Citric Acid Buffer (100mM) ........................................... 179

A.2.2 Acetic Acid Buffer (10mM) .......................................... 180

A.2.3 HEPES Buffer (10mM) .................................................. 180

A.2.4 Trisma-Base Buffer (10mM) ......................................... 181

A.3 Antibody Diluent ................................................................ 181

Appendix B: AnalySIS module codes ...................................... 183

B.1 Detection of positive areas in grey scale images .................. 183

B.2 Quantification of individual cells with multiple labels .......... 189

Bibliography ............................................................................ 203
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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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>11βHSD2</td>
<td>11βhydroxysteroid dehydrogenase type 2</td>
</tr>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>AR</td>
<td>antigen retrieval</td>
</tr>
<tr>
<td>AVP</td>
<td>vasopressin</td>
</tr>
<tr>
<td>BP</td>
<td>bandpass</td>
</tr>
<tr>
<td>CRH</td>
<td>corticotropin releasing hormone</td>
</tr>
<tr>
<td>CRHR1</td>
<td>corticotropin releasing hormone receptor 1</td>
</tr>
<tr>
<td>CRHR2</td>
<td>corticotropin releasing hormone receptor 2</td>
</tr>
<tr>
<td>CLIP</td>
<td>corticotrophin-like intermediate lobe peptide</td>
</tr>
<tr>
<td>Cy</td>
<td>cyanine</td>
</tr>
<tr>
<td>DAPI</td>
<td>4',6-diamidino-2-phenylindole, dihydrochloride</td>
</tr>
<tr>
<td>GR</td>
<td>glucocorticoid receptor</td>
</tr>
<tr>
<td>HMW</td>
<td>high molecular weight</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
</tr>
<tr>
<td>irACTH</td>
<td>immunoreactive adrenocorticotropic hormone</td>
</tr>
<tr>
<td>JP</td>
<td>joining peptide</td>
</tr>
<tr>
<td>LMW</td>
<td>low molecular weight</td>
</tr>
<tr>
<td>LP</td>
<td>longpass</td>
</tr>
<tr>
<td>LPH</td>
<td>lipotrophin</td>
</tr>
<tr>
<td>MC2R</td>
<td>melanocortin 2 receptor</td>
</tr>
<tr>
<td>MSH</td>
<td>melanocyte stimulating hormone</td>
</tr>
<tr>
<td>RHPA</td>
<td>reverse haemolytic plaque assay</td>
</tr>
<tr>
<td>RIA</td>
<td>radioimmunoassay</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>RIPA</td>
<td>radioimmunoprecipitation assay</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PC1</td>
<td>prohormone convertase 1</td>
</tr>
<tr>
<td>PC2</td>
<td>prohormone convertase 2</td>
</tr>
<tr>
<td>PCUN</td>
<td>periconceptional undernutrition</td>
</tr>
<tr>
<td>PKA</td>
<td>protein kinase A</td>
</tr>
<tr>
<td>POMC</td>
<td>pro-opiomelanocortin</td>
</tr>
<tr>
<td>PR</td>
<td>placental restriction</td>
</tr>
<tr>
<td>ST-1</td>
<td>Nonapeptide of pro-opiomelanocortin spanning the cleavage point between adrenocorticotropic hormone and β-lipotrophin</td>
</tr>
<tr>
<td>$V_{1b}$</td>
<td>Vasopressin receptor 1b</td>
</tr>
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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 adrenocorticotropic (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 periconceptional 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 periconceptional
nutrient restriction and placental restriction at the level of individual corticotrophs.