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

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Declaration

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Abbreviations

11βHSD2	11βhydroxysteroid 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_2R	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	periconceptional undernutrition
PKA	protein kinase A
POMC	pro-opiomelanocortin
PR	placental restriction
ST-1	Nonapeptide of pro-opiomelanocortin spaning the cleavage point between adrenocorticotropic 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, $CRHR_1$, 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 $CRHR_1$ 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 $CRHR_1$ and the proportions of pituitary cells with combinations of antisera were quantified.

Four subpopulations of corticotrophs were identified, which expressed either: POMC+ACTH+$CRHR_1$, ACTH+$CRHR_1$, POMC+ $CRHR_1$ or POMC-only. There was a significant decrease in the proportion of pituitary cells expressing POMC+ACTH+$CRHR_1$ 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+$CRHR_1$ in early gestation was reduced. PR resulted in a significantly higher proportion of corticotrophs expressing POMC+ACTH+$CRHR_1$ during the prepartum period.

This work represents the discovery of the differential expression of POMC, ACTH and $CRHR_1$ 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.