THE IMPACT OF THE PERICONCEPTIONAL AND PREIMPLANTATION ENVIRONMENT ON ADRENAL DEVELOPMENT AND STEROIDOGENESIS IN THE FETAL SHEEP

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Addendum:

1. Section 2.1, page 7, last sentence: This statement applies to the sheep rather than the human.
2. Section 2.1, page 52, line 12: "...have found that offspring exposed..."
3. Section 2.1, page 53, line 23: "...which have investigated..."
4. Section 2.4.3, page 87, line 8: "...this suggests that there may be a specific effect..."
5. Section 2.4.5.3, page 91, lines 19-20: "...have been found to occur only after 112 days of gestation (Wallace, 1948) it may be possible that..."
6. Section 2.4.5.3, page 92, second last line: "...occurs plays a part..."
7. Section 3.1, page 95, second last line: "...which have investigated..."
8. Section 3.2.1, page 96, line 15: comment added "The same animals were used in Chapter 3 as in Chapter 2."
9. Section 3.2.7, page 100, line 5: "...5 minutes prior to CRH..."
10. Figure 3.4, page 107: added "# denotes a significant decrease in fetal P_aO_2."
11. Figure 3.13, page 120: "# denotes a significant increase in plasma cortisol concentration compared to pre-infusion values" deleted and replaced by: "Different alphabetical subscripts denote mean values, which are significantly different."
12. Section 3.4.4, page 128, last line: "...that twins had a greater ACTH and cortisol concentrations..."
13. Section 3.4.5, page 130, third last line: "...which suggests..."
14. Section 4.3.3, page 146, first line: "on the" deleted
15. Figure 5.4, page 189: "Fetal plasma ACTH concentration in singletons at 116 – 145 days of gestation"
16. Section 5.4.2.2.2, page 206, line 12: "...absence of serum. Unfortunately..."
17. Section 6, page 209, line 15: "It is also not known..."
18. Section 6.3, page 215, line 4: "...in Chapter 5 provides important..."

DECLARATION

This body of scientific work contains no material that has been accepted for the award of any other degree or diploma in any other University or Tertiary Institution. To the best of my knowledge and understanding, this thesis contains no material previously published or written by any other person, except myself and where due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the Barr Smith Library, being available for loan and photocopying.

Signed: ……

Date: ……

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The journey of a PhD can be very challenging with its highs and lows, but I feel that the entire experience has made me a stronger person and helped me define who I am today. It undoubtedly has been a long road and I am thankful to have come to the end. Along the path there was much support and help from many people and I won't attempt to list them all here, but I am certain you know who you are.

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used in these studies. I am also grateful to the members of the Turretfield Research Centre who played a crucial role in running the nutritional protocols and the care and maintenance of the animals.

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To my husband John, yes I will say it again, it is done. This thesis is dedicated to you. Now that this heavy weight has been lifted we can go on and celebrate life!

COMMONLY USED ABBREVIATIONS

A B C

ad libitum	to any desired extent
AC	abdominal circumference
ACTH	adrenocorticotrophic hormone
AI	artificial insemination
ANOVA	analysis of variance
ART	assisted reproductive technologies
ATP	adenosine triphosphate
AUC	area under the curve
AVP	arginine-vasopressin
11-βHSD-2	11beta-hydroxyl steroiddehydrogenase
bp	base pairs
cDNA	complementary deoxyribonucleic acid
CNS	central nervous system
CR	crown rump
CRH	corticotropin-releasing hormone
CYP17	cytochrome P450 17alpha-hydroxylase

D E F G

DMD	differentially methylated domain
DNA	deoxyribonucleic acid
dsDNA	double stranded deoxyribonucleic acid
EDTA	ethylenediamine tetraacetic acid
ET	embryo transfer
GR	glucocorticoid receptor

GIFT	gamete intrafallopian transfer

H I J K

h	hour(s)
Hb	arterial haemoglobin content
HPA axis	hypothalamo-pituitary-adrenal axis
HS	human serum
ICR	imprinting control region
ICSI	intracytoplasmic sperm injection
IGFs	insulin-like growth factors
IGF1	insulin-like growth factor 1
IGF2	insulin-like growth factor 2
IGF1R	insulin-like growth factor type 1 receptor
IGF2R	insulin-like growth factor type 2 receptor
i.m.	intramuscular
i.v.	intravenous
IVC	*in vitro* culture
IVF	*in vitro* fertilization
IVM	*in vitro* maturation
IVP	*in vitro* production

L M N O

LOS	Large Offspring Syndrome
LH	lateral hypothalamic area
MAP	mean arterial blood pressure
MC2R	melanocortin type 2 receptor (ACTH receptor)
ME	metabolisable energy
MER	metabolisable energy requirements

min	minute(s)
MOET	multiple ovulation embryo transfer
mRNA	messenger ribonucleic acid
NAC	non-amplification control
ncRNA	non-coding ribonucleic acid
NS	no serum
O_2 content	arterial oxygen content

P Q R S

PAT	perirenal adipose tissue
PCO_2	arterial partial pressure of carbon dioxide
PCUN	periconceptional undernutrition
PG	prostaglandin
PGF	prostaglandin F 2 alpha
PGHS-II	prostaglandin H synthase type II
PM	post mortem
PO_2	arterial partial pressure of oxygen
POMC	proopiomelanocortin
PVN	paraventricular nucleus
rRNA	ribosomal ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SEM	standard error of the mean
SOF	synthetic oviductal fluid
SPSS	statistical package for social sciences
SSC	cytochrome P450 side chain cleavage
StAR	steroidogenic acute regulatory protein

T U V W X Y Z

TGF	transforming growth factor beta
ZIFT	zygote intra-fallopian transfer

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ABSTRACT

Experimental and clinical studies provide evidence that perturbations and manipulation of the *in vivo* and *ex vivo* nutritional environment during the periconceptional period alters the development of the fetal hypothalamo-pituitary-adrenal (HPA) axis and gestation length. In particular periconceptional maternal undernutrition results in an earlier prepartum activation of the fetal HPA axis and adrenal development whereas culturing embryos *in vitro* in the presence of human serum is associated with delayed parturition in the sheep. It is not clear, however, whether the effects resulting from periconceptional undernutrition are due to the impact of undernutrition acting on the development of both the oocyte and embryo or on just the early embryo. It is also not known how culturing embryos *in vitro* in the absence or presence of human serum impacts on the prepartum activation of the HPA axis and adrenal development. Lastly, the intra-adrenal molecular mechanisms by which changes in the *in vivo* or *in vitro* nutritional environment of the early embryo alter HPA development have not been fully investigated.

This thesis provides evidence for the first time which suggests that periconceptional undernutrition may differentially target components of the fetal HPA axis depending on exposure to undernutrition during specific periconceptional time periods. Specifically, periconceptional undernutrition alters fetal adrenal growth and development whilst undernutrition during the preimplantation period alone is sufficient to alter the development of the fetal anterior pituitary in late gestation.

A further novel finding of this thesis is that when embryos were cultured *in vitro* in a defined medium fetal plasma ACTH concentration significantly increased in singletons whereas relative adrenal weight and adrenal CYP17 mRNA expression significantly increased in both singleton and twins in late gestation. This suggests that this embryo culture system affects adrenal growth and development, independent of fetal number and importantly, that there is an early activation of the HPA axis in the singleton fetus in late gestation. Interestingly, addition of serum to the *in vitro* culture media reverses the effects of culturing embryos *in vitro* in the absence of serum and the mechanism(s) by which restoration of fetal adrenal development occurs may involve the intra-adrenal IGF system.

In summary, alteration of the development of the fetal HPA axis appears to be dependent on specific periconceptional time windows of poor nutritional exposure and type of culture media to which an embryo is exposed to.