



## SRF VACATION SCHOLARSHIP REPORT 2017

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<b>Degree Title and year of study:</b>	BA Natural Sciences Graduated 2017		
<b>Supervisor's Name:</b>	Alison Forhead	<b>Supervisor's Department and Institution:</b>	Department of Physiology, Development, and Neuroscience University of Cambridge
<b>Project Title:</b>	Role of thyroid hormones in adrenal development and steroidogenesis in the ovine fetus		

### **Briefly outline the background and aims of the project** (*max 200 words*)

Development of the fetal adrenal gland and secretion of glucocorticoids (cortisol) from the zona fasciculata near term are essential for normal maturation of the fetus and survival of the neonate at birth. In addition, in sheep, the rise in plasma cortisol concentration seen in the fetus near term is responsible for initiating the onset of parturition.

Thyroid hormones also promote fetal maturation and thyroid hormone deficiency *in utero* prolongs gestation in sheep. Preliminary studies have shown that hypothyroidism before birth reduces fetal plasma cortisol concentration near term. The effects of thyroid hormones on adrenal development and steroidogenic pathway expression in the fetus, however, are unknown.

The **aim of this project** was to investigate the effects of hypothyroidism on adrenal gland volume and zonal composition, and the expression of key genes in the steroidogenic pathway, in the sheep fetus during late gestation.

Total adrenal volume, and percentage zonal composition of cortex (zona glomerulosa and fasciculata) and medulla, were determined by stereological methods in control and thyroid-deficient (TX) sheep fetuses near term. The mRNA abundance of key receptors and enzymes in the steroid hormone and catecholamine biosynthetic pathways was quantified in the adrenal cortex and medulla using quantitative real-time PCR.

### **Did the project change from that proposed in the application? If so, what changes were made and why?** (*max 100 words*)

The project originally proposed to investigate only the mRNA abundance of receptors and enzymes in the steroidogenic pathway in the fetal adrenal cortex. However, upon analysis of the stereological data, an unexpected increase in the volume of the adrenal medulla was observed in the hypothyroid fetuses. Therefore, the project was expanded to analyse the expression of key enzymes in the catecholamine biosynthesis pathway in the adrenal medulla. In addition, the expression of insulin-like growth factors (IGF) and their receptors were investigated as a potential mechanism for overgrowth of the adrenal medulla in the hypothyroid fetus.

### **What were the main results/findings of the project?** (*max 300 words*)

The project focused on three **hypotheses**:

1. Thyroid hormone deficiency in the sheep fetus causes a reduction in plasma cortisol concentration by (a) decreasing the size of the zona fasciculata and (b) suppressing the mRNA abundance of key steroidogenic genes in the adrenal cortex.

2. The reduction in plasma cortisol concentration in the hypothyroid fetus reduces the expression of

phenol-N-methyl transferase enzyme (PNMT), which converts noradrenaline to adrenaline in the adrenal medulla.

3. The overgrowth of the adrenal medulla in the hypothyroid fetus is associated with an increase in mRNA abundance of IGFs and their receptors.

### **Main findings**

#### *Plasma hormone concentrations*

Plasma thyroxine and triiodothyronine were reduced to undetectable concentrations in the TX, compared to sham, fetuses. Plasma cortisol levels were significantly lower in TX compared to sham fetuses.

#### *Structure of the adrenal gland*

There was no significant difference in adrenal mass or volume, when expressed as absolute values or relative to fetal bodyweight, between the sham and TX fetuses. In the TX fetuses, a significant decrease in zona fasciculata percentage volume, and a significant increase in medulla percentage volume, were observed compared to the sham fetuses (Figure 1). When adjusted for fetal body weight, only the relative adrenal medulla mass remained significantly greater in the TX fetuses.

#### *mRNA abundance*

In the TX fetuses, mRNA abundance in the adrenal cortex was significantly reduced for the steroidogenic enzymes CYP11A1, CYP17 and 3 $\beta$ HSD, and the cholesterol transport protein StAR. The reduction in CYP11B1 mRNA abundance seen in the TX fetuses was close to significance ( $p=0.06$ ), and there was no effect of hypothyroidism on ACTH receptor mRNA abundance in the adrenal cortex. The medullary mRNA abundance of PNMT and the IGF receptors did not differ between sham and TX fetuses, but increments in IGFI and IGFII were observed in the adrenal medulla of TX fetuses.

### **What do you conclude from your findings? (max 150 words)**

The results of this project confirm the first hypothesis that hypothyroidism *in utero* impairs the normal development of the structure and steroidogenic capacity of the adrenal gland, with consequences for the circulating concentration of cortisol, in the sheep fetus.

In contrast, catecholamine biosynthesis in the adrenal medulla assessed by mRNA abundance of PNMT is not affected by thyroid hormone deficiency before birth. The overgrowth of the adrenal medulla in hypothyroid fetuses may be due to upregulation of IGF mRNA abundance.

These findings demonstrate the importance of thyroid hormones in the development of the structure and function of the fetal adrenal gland. They highlight the complexity of the interactions between glucocorticoids and thyroid hormones in the control of fetal maturation near term, with implications for understanding the consequences of prematurity and congenital hypothyroidism.

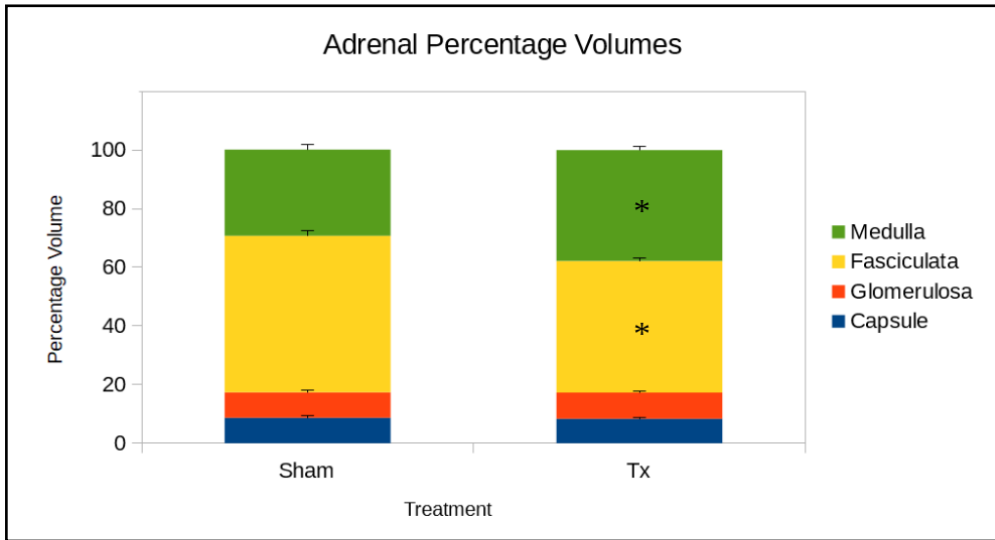
### **How has this experience influenced your thinking regarding your future/ongoing studies, and/or career choice? (max 150 words)**

I have greatly enjoyed both the practical aspects and literature review of this project, which has helped me to confirm my decision to apply for PhD programmes starting in the next academic year. This project has allowed me to gain valuable experience and technical skills in stereology and real-time qPCR, and has expanded my laboratory skill-set overall. It has produced findings which we intend to publish in a high-impact journal, like *Reproduction*.

### **Please use the space below to add any other comments/thoughts about the SRF Vacation Scholarship (max 100 words)**

#### **Student:**

**Supervisor:** We are extremely grateful to the SRF committee for awarding this vacation scholarship to Isabella. It enabled her to learn new techniques and to further her undergraduate research project to generate results for publication. Isabella made excellent progress over the summer and developed her confidence and independence in the lab. She is now well placed to apply for a postgraduate scholarship.



**Figure 1.** Mean ( $\pm$  SEM) percentage volume of adrenal zones in sham and hypothyroid (TX) sheep fetuses near term. \* significantly different from sham fetuses,  $p < 0.05$ .