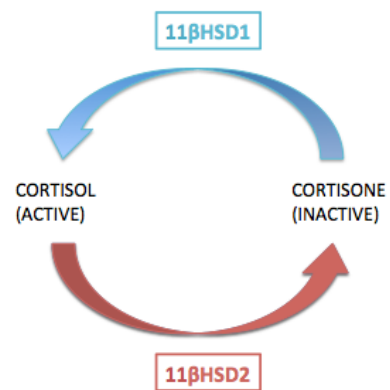


## **The expression of 11 $\beta$ HSD1 in the human myometrium**

### **Introduction:**

Preterm birth, which is defined as birth before 37 weeks gestation is the leading cause of neonatal morbidity and mortality.<sup>1</sup> It occurs in around 8 – 10% of pregnancies<sup>2</sup> and is associated with an increased risk of various neurodevelopmental disorders and other neonatal health complications arising from underdeveloped organ systems. 70% of preterm births occur as a result of spontaneous preterm labour, and many of the underlying pathological processes associated with this are still poorly understood.<sup>1</sup>

The production of foetal cortisol has important roles during pregnancy and labour. Foetal cortisol, which increases towards the end of gestation, promotes foetal organ maturation (in particular, of the lungs) and initiates labour. Cortisol increases the synthesis of various prostaglandins that are involved in stimulating events in the laboring cascade such as myometrial contraction, cervical ripening and membrane rupture.<sup>2</sup>



The bioavailability of cortisol is dependent on the activity of two enzymes: 11 $\beta$ -Hydroxysteroid dehydrogenase 1 (11 $\beta$ HSD1), which converts inactive cortisone to active cortisol and 11 $\beta$ -Hydroxysteroid dehydrogenase 2 (11 $\beta$ HSD2), which catalyzes the opposite reaction. Recently in the Norman lab, it was found that in the murine uterus, 11 $\beta$ HSD1 expression increases as gestation advances and then significantly decreases in labour (unpublished). Although expression was predominantly seen in the endometrium, 11 $\beta$ HSD1 activity was thought to also be present in the myometrium. Although the role of 11 $\beta$ HSD1 in the myometrium has not been explored in depth, it seems logical that the increase in 11 $\beta$ HSD1 towards the end of pregnancy triggers the initiation of labour by generating more cortisol. Examining the expression of 11 $\beta$ HSD1 in the term and preterm human myometrium will potentially further our understanding of the underlying pathologies of preterm labour.

### *Hypotheses:*

In the human, the expression of 11 $\beta$ HSD1 is:

- higher in the preterm myometrium compared to the term myometrium
- lower in the laboring myometrium compared to the non-labouring myometrium

### *Aim:*

To examine the expression of 11 $\beta$ HSD1 mRNA and protein in the term and preterm human myometrium.

### **Study Design:**

Samples of myometrial tissue used were selected from four groups of pregnant women: term laboring (TL), term non-labouring (TNL), preterm laboring (PTL) and preterm non-labouring (PTNL). Samples of labouring myometrium were obtained from biopsy

during emergency caesarian sections and samples of non-labouring myometrium from elective caesarian sections. The samples were then frozen, stored in RNAlater® stabilising reagent or fixed in NBF (neutral buffer formalin).

### Methods:

1. Immunohistochemistry (IHC):

This technique was used to localise 11 $\beta$ HSD1 protein in the myometrial samples. A non-purified sheep 11 $\beta$ HSD1 antibody was chosen after trialing both the non-purified and purified antibody at varying dilutions (eg 1:2000, 1:4000, 1:8000, 1:16,000) as it gave the clearest image with the least background staining.

2. Real time quantitative polymerase chain reaction (RT-qPCR):

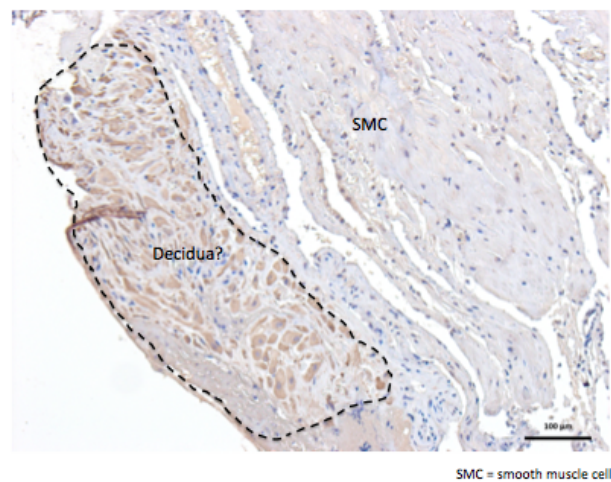
This method was used for 11 $\beta$ HSD1 mRNA quantification. Beta-actin was used as the reference 'housekeeping' gene.

3. Western Immunoblotting:

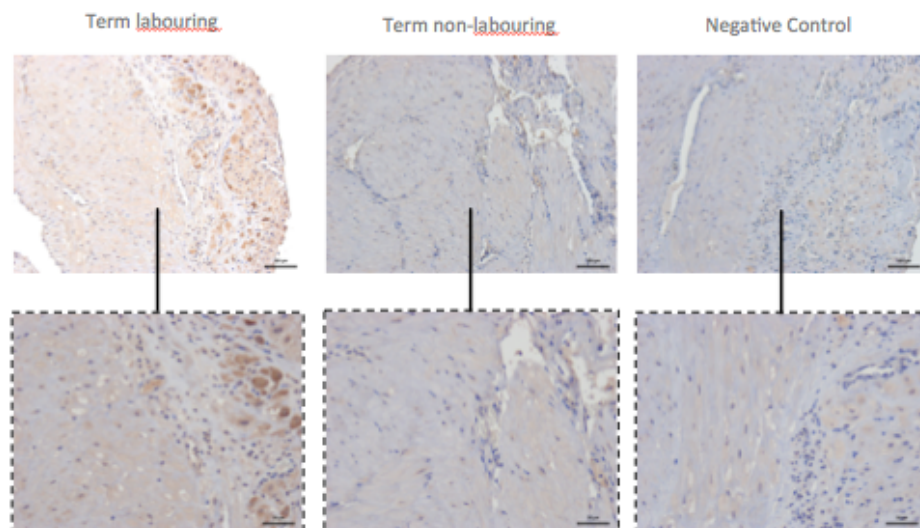
This was used for 11 $\beta$ HSD1 protein quantification. Alpha tubulin used as a loading standard and a TNL sample of decidua was also used as the positive control. Fluorescent secondary antibodies were used to allow digital visualization of results.

### Results:

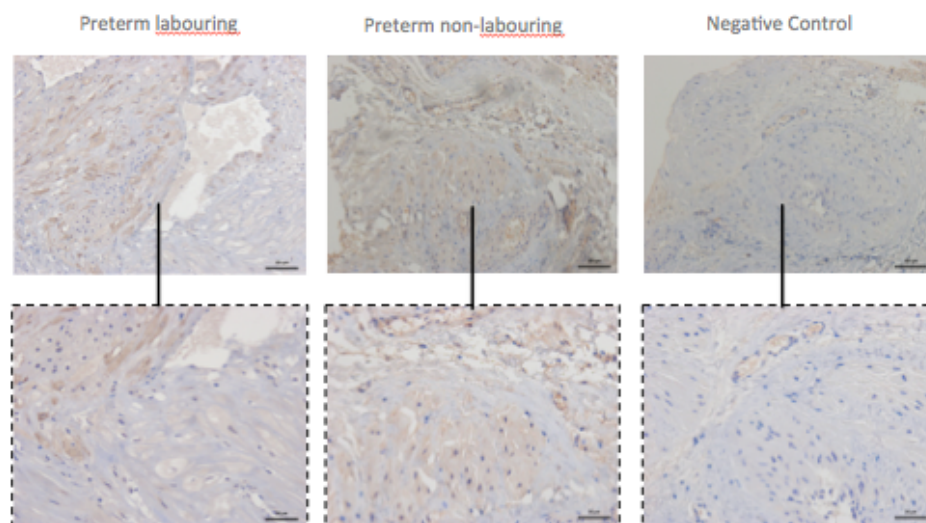
1. *IHC - 11 $\beta$ HSD1 localisation:*



**Figure 1:** IHC image of a TNL sample

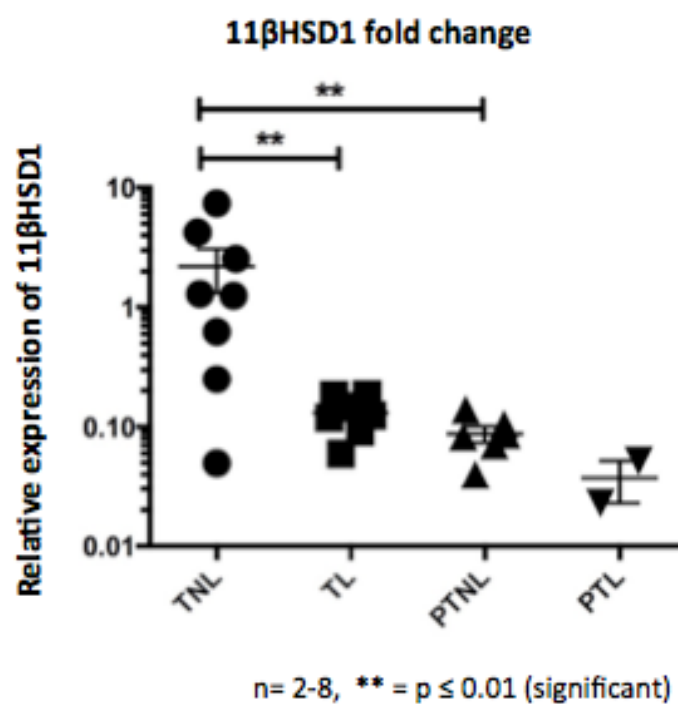


**Figure 2:** Comparison of IHC images of Term labouring and non-labouring samples



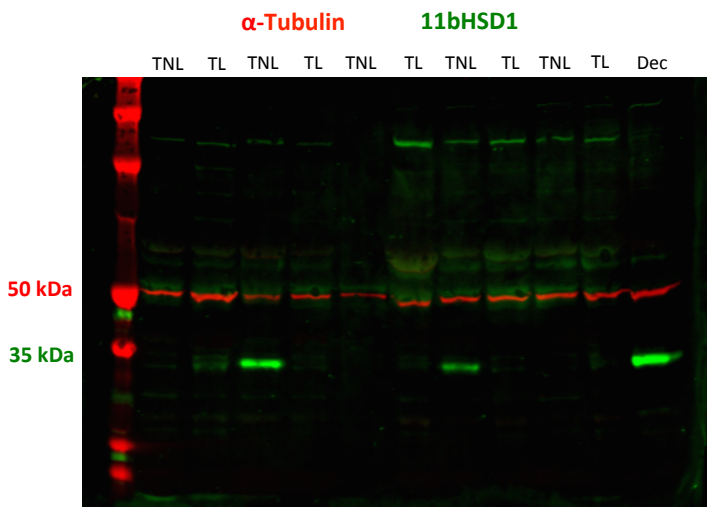
**Figure 3:** Representative samples of IHC images of preterm myometrium

## 2. RT-qPCR - $11\beta$ HSD1 mRNA quantification

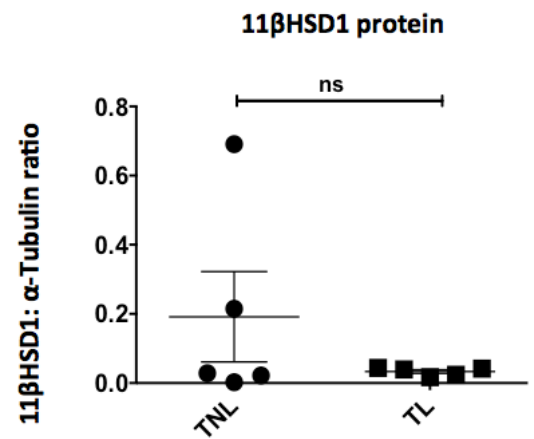


**Figure 4:** Relative expression of  $11\beta$ HSD1 mRNA to Beta-Actin (reference gene)

### 3. Western Blot - 11 $\beta$ HSD1 protein quantification



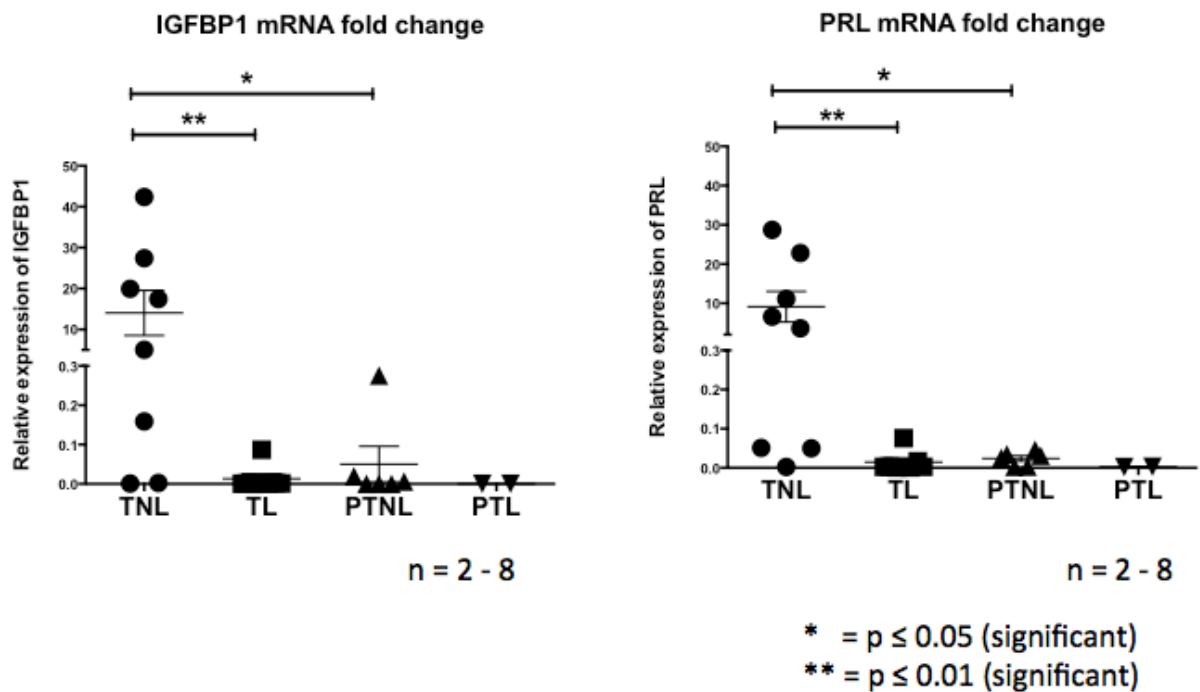
**Figure 5:** Digital image of Western Blot results. Far left: Reference ladder. Far right: positive control (TNL decudua sample). Red banding at 50kDa indicates the loading standard  $\alpha$ -tubulin. Green banding at 35kDa indicates expression of 11 $\beta$ HSD1 protein.



t-test,  $p=0.2599$ , ns= not significant  
n = 5

**Figure 6:** Graph displaying analysed results from western blot gel.

### 4. Decidua marker gene mRNA quantification



**Figure 7:** Graphs displaying analysed results from IGFBP1 and PRL RT-qPCR.

## Discussion:

Immunohistochemistry images of the myometrial samples did not appear to show convincing 11 $\beta$ HSD1 protein expression in the smooth muscles of the myometrium. The antibody used appeared to quite 'sticky' and resulted in heavy non-specific brown background staining. However, in a number of the samples more cell specific cytoplasmic staining could be seen in certain patches of cells (see Fig 1). Due to the shape and arrangement of these cells, it was suspected that these are decidual cells (cells of the pregnant endometrium).

Results from RT-qPCR found that the relative expression of 11 $\beta$ HSD1 mRNA was significantly greater in the TNL group compared to the TL group and both preterm groups (See Fig 4). Analysis of the results from the Western blot found that only two samples (both TNL) showed an increased 11 $\beta$ HSD1 protein:  $\alpha$ -tubulin ratio (see Fig 6), and this was consistent with the banding seen on the Western gel (see Fig 5). However, the overall 11 $\beta$ HSD1:  $\alpha$ -tubulin ratio in the TNL group was not significantly greater than in the TL group.

These varied results led us to consider the possibility that 11 $\beta$ HSD1 mRNA and protein expression in the samples originated from decidual cells and not from the smooth muscle cells of the myometrium. Due to the way that the myometrial samples are collected during biopsy, it is not feasible to fully exclude the possibility of decidual cell contamination.

In order to investigate this further, a second RT-qPCR was carried out, using the same myometrial samples but this time detecting for marker genes specific to decidual cells: IGFBP-1 (Insulin-like growth factor binding protein 1) and PRL (prolactin).<sup>3</sup> The relative expression of mRNA for both marker genes was found to be significantly greater in the TNL group compared to TL, PTNL and PTL groups (see Fig 7). This trend mirrors the expression of 11 $\beta$ HSD1 found in both the first RT-qPCR and the Western blot.

The RT-qPCR results for samples of TNL myometrium with the highest 11 $\beta$ HSD1 mRNA expression were compared and it was found that these samples also showed high expression of both IGFBP-1 and PRL mRNA (see Fig 8). Interestingly, 2067 and 2084 were the two samples that showed 11 $\beta$ HSD1 banding on the Western blot gel (see Fig 9). These findings support the theory that 11 $\beta$ HSD1 activity in the samples originates from residual decidual cells and not from the myometrial smooth muscle cells.

TNL samples with the highest mRNA concentration

	TNL samples			
Gene	2076	2067	2084	2063
11 $\beta$ HSD1	+++	++	++++	+
IGFBP1	+++	++	+++	++
PRL	+++	++	+++	+

+ is the concentration of mRNA based on Ct values

Figure 8



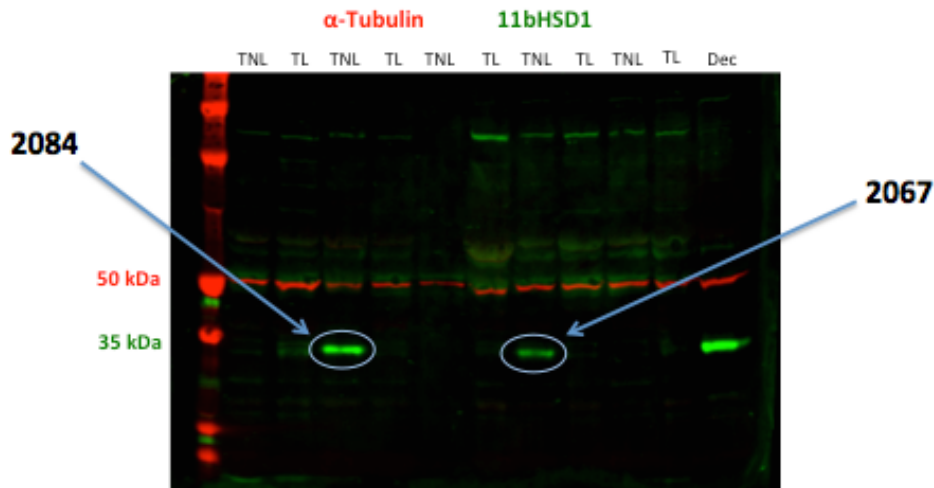


Figure 9

### Conclusions:

1. 11 $\beta$ HSD1 is unlikely to be expressed in the smooth muscle cells of the term and preterm myometrium.
2. Further experiments are needed to confirm the origin (decidua?) of highly expressed 11 $\beta$ HSD1 mRNA and protein in some of the myometrial samples.

### Future Work:

It would be interesting to investigate further into the expression of 11 $\beta$ HSD2 in the myometrium. As this catalyzes the opposite reaction to 11 $\beta$ HSD1 - converting active cortisol back into inactive cortisone, does expression of this enzyme change during labour? Although a good quality 11 $\beta$ HSD2 antibody is currently unavailable, 11 $\beta$ HSD2 mRNA expression was briefly looked at during the project. RT-qPCR gave interesting results and it was found that 11 $\beta$ HSD2 mRNA expression in both term and preterm non-labouring tissue was significantly higher compared to term laboring samples (see Fig 10).

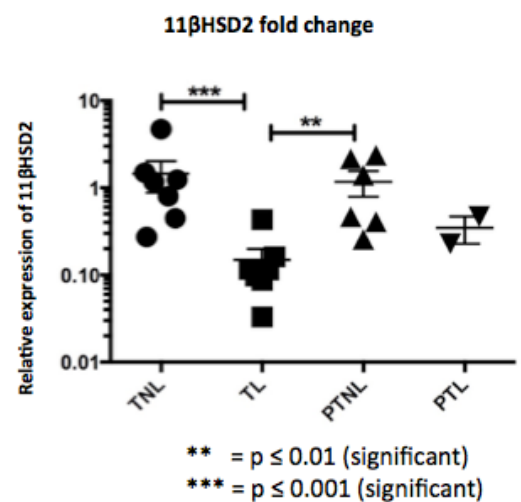


Figure 10

### Acknowledgements:

I would like to thank Sofia Makieva and Sara Rinaldi for supervising me and helping me throughout the project and also to Professor Jane Norman for giving me the opportunity to carry out this project. I would also like to thank the Society for Reproduction and Fertility for kindly granting me this vacation scholarship and funding this work.

**References:**

1. Romero R, Dey S, Fisher S. Preterm labor: One syndrome, many causes. *Science*. **2014**;345(6198):760-765.
2. Li X, Zhu P, Myatt L, Sun K. Roles of glucocorticoids in human parturition: A controversial fact? *Placenta*. **2014**;35(5):291-296.
3. Sugawara K, Hamatani T, Yamada M, Ogawa S, Kamijo S, Kuji N et al. Derivation of human decidua-like cells from amnion and menstrual blood. *Scientific Reports*. **2014**;4.