The expression of 11βHSD1 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. It occurs in around 8 – 10% of pregnancies 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.

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.

The bioavailability of cortisol is dependent on the activity of two enzymes: 11β-Hydroxysteroid dehydrogenase 1 (11βHSD1), which converts inactive cortisone to active cortisol and 11β-Hydroxysteroid dehydrogenase 2 (11βHSD2), which catalyzes the opposite reaction. Recently in the Norman lab, it was found that in the murine uterus, 11βHSD1 expression increases as gestation advances and then significantly decreases in labour (unpublished). Although expression was predominantly seen in the endometrium, 11βHSD1 activity was thought to also be present in the myometrium. Although the role of 11βHSD1 in the myometrium has not been explored in depth, it seems logical that the increase in 11βHSD1 towards the end of pregnancy triggers the initiation of labour by generating more cortisol. Examining the expression of 11βHSD1 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 11βHSD1 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 11βHSD1 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 11βHSD1 protein in the myometrial samples. A non-purified sheep 11βHSD1 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 11βHSD1 mRNA quantification. Beta-actin was used as the reference ‘housekeeping’ gene.

3. **Western Immunoblotting:**
   This was used for 11βHSD1 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 - 11βHSD1 localisation:**

![IHC image of a TNL sample](image1)

*Figure 1: IHC image of a TNL sample*

![Representative samples of IHC images from myometrium](image2)

*Figure 2: Representative samples of IHC images from myometrium*
2. **RT-qPCR - 11βHSD1 mRNA quantification**

![Figure 3: Representative samples of IHC images of preterm myometrium](image)

![Figure 4: Relative expression of 11βHSD1 mRNA to Beta-Actin (reference gene)](image)
3. **Western Blot - 11βHSD1 protein quantification**

![Western Blot Image](image)

**Figure 5:** Digital image of Western Blot results. Far left: Reference ladder. Far right: positive control (TNL decidua sample). Red banding at 50kDa indicates the loading standard α-tubulin. Green banding at 35kDa indicates expression of 11βHSD1 protein.

![11βHSD1 protein](image)

**Figure 6:** Graph displaying analysed results from western blot gel. t-test, \( p=0.2599 \), ns = not significant. \( n = 5 \)

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 11βHSD1 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 11βHSD1 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 11βHSD1 protein: α-tubulin ratio (see Fig 6), and this was consistent with the banding seen on the Western gel (see Fig 5). However, the overall 11βHSD1: α-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 11βHSD1 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). 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 11βHSD1 found in both the first RT-qPCR and the Western blot.

The RT-qPCR results for samples of TNL myometrium with the highest 11βHSD1 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 11βHSD1 banding on the Western blot gel (see Fig 9). These findings support the theory that 11βHSD1 activity in the samples originates from residual decidual cells and not from the myometrial smooth muscle cells.
Conclusions:

1. 11βHSD1 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 11βHSD1 mRNA and protein in some of the myometrial samples.

Future Work:

It would be interesting to investigate further into the expression of 11βHSD2 in the myometrium. As this catalyzes the opposite reaction to 11βHSD1 - converting active cortisol back into inactive cortisone, does expression of this enzyme change during labour? Although a good quality 11βHSD2 antibody is currently unavailable, 11βHSD2 mRNA expression was briefly looked at during the project. RT-qPCR gave interesting results and it was found that 11βHSD2 mRNA expression in both term and preterm non-labouring tissue was significantly higher compared to term laboring samples (see Fig 10).

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References: