The Role of Vitamin D Metabolism in the Timing of Birth

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INTRODUCTION

Figure 1: Global Burden of Preterm Birth in 2010 (Blencowe et al. 2012)

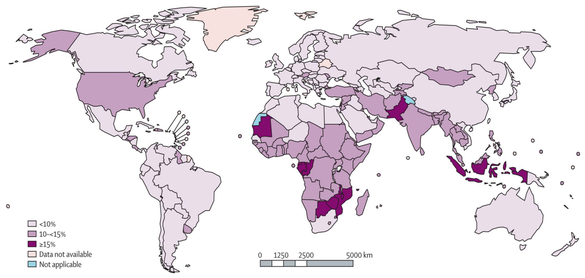
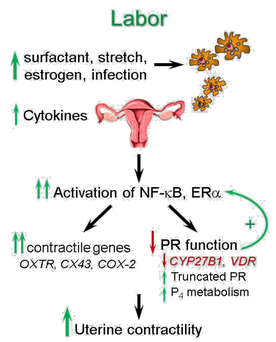


Figure 2: Mechanisms for Progesterone/PR Regulation of Uterine Quiescence during Pregnancy and for the Induction of Uterine Contractility Leading to Term and Preterm Labor (Hardy et al. 2006; Renthal et al. 2010, amended)

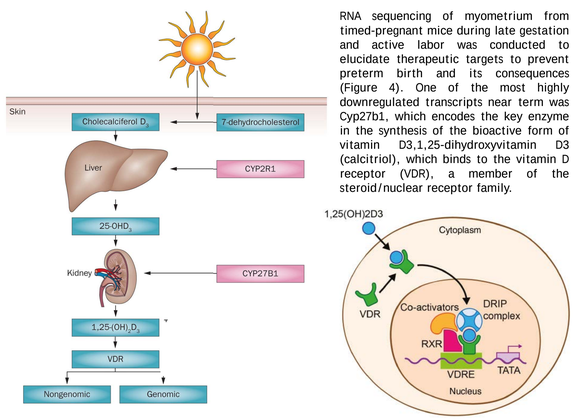
Labor is associated with increased levels of proinflammatory cytokines within fetal and maternal reproductive tissues. This activates inflammatory transcription factors (e.g. NF-κB) that enhance expression of genes encoding contraction-associated proteins (CAP), including oxytocin receptor (OXTR), connexin-43 (CX43), and cyclooxygenase 2 (COX-2). By contrast, uterine quiescence is maintained through most of pregnancy by increased circulating progesterone (P_4) and enhanced progesterone receptor (PR) activity, which silence expression of proinflammatory mediators and CAP genes.

Studies in humans and in animal models have led to the concept that parturition in all species is initiated by a concerted series of biochemical events that impair PR function and block its ability to maintain myometrial quiescence. As a result, progestin treatment of pregnant women who are at risk of delivering prematurely has only a negligible effect to prevent PTB.



In this study, we provide new and exciting evidence that a mechanism whereby P_4/PR maintains uterine quiescence during pregnancy is through induction of the vitamin D signaling pathway via upregulation of 1α-hydroxylase (CYP27B1) and vitamin D receptor (VDR). Thus, the loss of PR function near term may compromise the action of vitamin D to block inflammatory pathways, leading to labor.

Figure 3: Vitamin D Metabolism (Jensen 2014; Nandi et al. 2014)

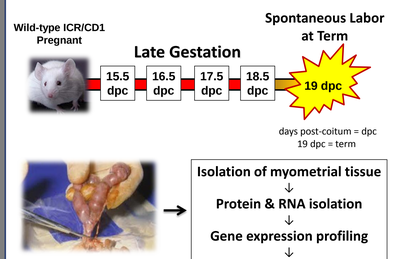


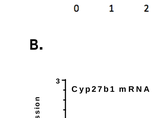
OBJECTIVES

- To identify novel genes and mechanisms that maintain uterine quiescence during pregnancy and promote the initiation of term and preterm labor
- To characterize the gestational changes and regulation of CYP27B1 and VDR and define their underlying mechanisms of action during pregnancy and labor

MATERIALS & METHODS

1. Spontaneous Labor Model





(Montalbano 2016)

Isolation of myometrial tissue
↓
Protein & RNA isolation
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Gene expression profiling
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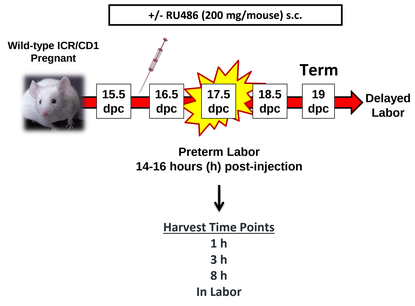
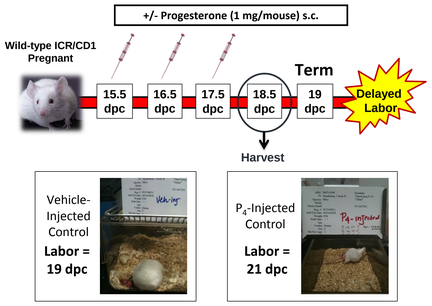
RNA sequencing

2. Preterm Labor Model: RU486-Induced

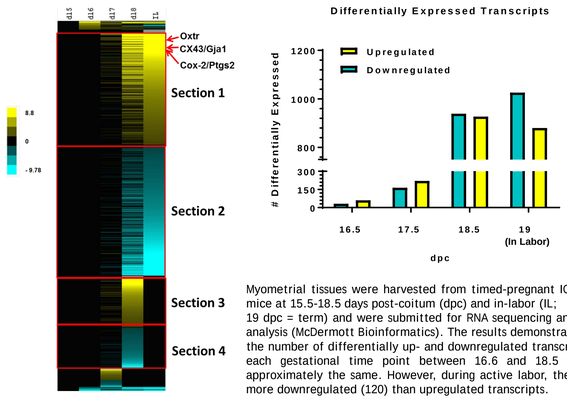


3. Delayed Labor Model: Progesterone-Induced



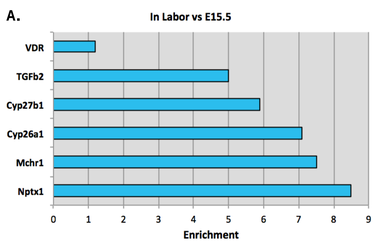
RESULTS

Figure 4: RNA Sequencing: Myometrial Transcripts



Myometrial tissues were harvested from timed-pregnant ICR/CD1 mice at 15.5-18.5 days post-coitum (dpc) and in-labor (IL; 19 dpc = term) and were submitted for RNA sequencing and data analysis (McDermott Bioinformatics). The results demonstrate that the number of differentially up- and downregulated transcripts at each gestational time point between 16.6 and 18.5 dpc is approximately the same. However, during active labor, there are more downregulated (120) than upregulated transcripts.

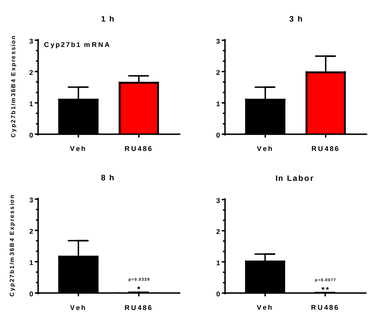
Figure 5: Cyp27b1 mRNA Expression Significantly Decreases in Mouse Myometrial Tissues Near Term



(A) RNA sequencing identified Cyp27b as one of the most highly downregulated transcripts during labor, as compared to 15.5 dpc levels.
(B) Cyp27b1 mRNA decreased by ~70% at 18.5 dpc and by 95% during labor, and **(C)** VDR expression decreased by 25% during labor, as compared to 15.5 dpc.

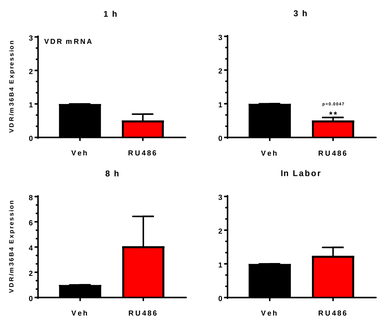
RT-qPCR was used to validate the gene expression levels of Cyp27b1 and VDR in an independent series of myometrial tissues. Cyp27b1 mRNA expression decreased significantly at 18.5 dpc ($P < 0.05$) and IL ($P < 0.001$), as compared to 15.5 dpc. VDR mRNA levels decreased in laboring tissue by 25% as compared to 15.5 dpc. All cohorts contained n ≥ 10 mice per time point. Values are mean ± SEM. Statistically significant differences were calculated using one-way ANOVA with Dunnett's post-hoc test. ***$P < 0.0001$; *$P < 0.01$

Figure 6: RU486 Treatment of 16.0 dpc Mice Causes a Significant Decrease in Cyp27b1 mRNA Expression Near Term



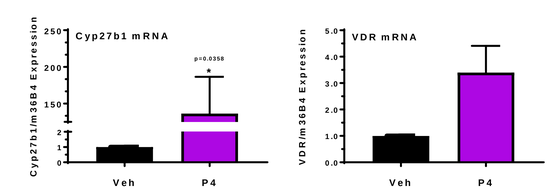
Myometrial tissues isolated from RU486-treated mice 1 h, 3 h, and 8 h post-injection and during active labor (following delivery of a single pup) were analyzed for **(Figure 6)** Cyp27b1 and **(Figure 7)** VDR mRNA levels using RT-qPCR.

Figure 7: RU486 Treatment of 16.0 dpc Mice Has No Effect on VDR mRNA Expression Near Term



Matched pairs analysis showed that the mouse myometrium 8 h post-RU486 injection and in labor manifested a highly significant reduction in Cyp27b1 expression, compared to vehicle-injected controls ($P < 0.0001$). All cohorts contained n ≥ 3 mice per time point per treatment. Values are mean ± SEM. Statistically significant differences were calculated using the Student's *t*-test. **$P < 0.01$; *$P < 0.05$

Figure 8: P_4-injected Mice at 18.5 dpc Manifest Significantly Higher Cyp27b1 mRNA Expression Levels in Myometrium than Vehicle-injected Controls

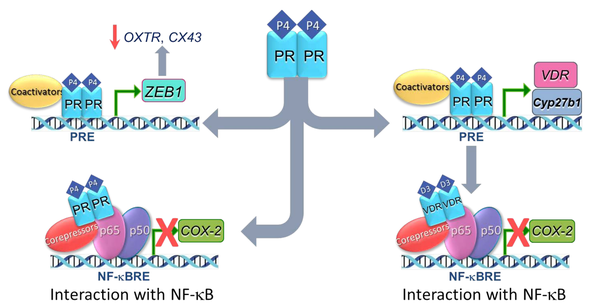


WT ICR mice were injected s.c. with progesterone (P_4) or vehicle (veh) as illustrated in the "Delayed Labor Model" in the Materials & Methods section. Myometrial tissues were collected at 18.5 dpc from both P_4 and vehicle treated mice. All cohorts contained n ≥ 3 mice per treatment. Values are mean ± SEM. Statistically significant differences were calculated using the Student's *t*-test. *$P < 0.05$

CONCLUSIONS

- We postulate that Cyp27b1 and VDR are key P_4/PR target genes in the pregnant myometrium that act cooperatively with P_4/PR to maintain myometrial quiescence via their anti-inflammatory actions.
- The decline in PR function near term, accompanied by a parallel decline in Cyp27b1/VDR may contribute to increased inflammatory gene expression that leads to myometrial contractility and labor.
- These studies may provide important insight into the potential regulation and role of local expression of Cyp27b1 in maintaining uterine quiescence during pregnancy.
- We suggest that the vitamin D pathway serves an important role in maintaining myometrial quiescence and preventing fetal rejection during pregnancy. Thus, calcitriol may provide a safe and effective treatment for prevent of preterm birth.

Figure 9: P_4/PR Inhibits Proinflammatory & Contractile Genes in Myometrium, in part, by Enhancing the VDR Pathway



FUTURE RESEARCH

- Immunohistochemistry to determine the cellular localization of Cyp27b1 and VDR in mouse myometrium
- Immunoblotting of nuclear and cytoplasmic fractionations of mouse myometrial tissues for Cyp27b1 and VDR
- ELISA for calcitriol levels in myometrium during late gestation
- Gene expression levels of CYP27B1 and VDR in uterine tissues from women in-labor and not-in-labor at mid- and late gestation

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