

# Expression of RNA Binding Proteins DND1 and FXR1 in the Porcine Uterus during the Estrous Cycle and Early Pregnancy

## A.S. Leaflet R2815

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### Summary and Implications

Pig uterine endometrium undergoes significant transcriptional and translational reorganization during the course of the estrous cycle and during early pregnancy establishment. The expression of two RNA binding proteins, DND1 and FXR1, was analyzed from samples of endometrial tissue taken on different days of either the estrous cycle or gestation. Expression of FXR1 is significantly affected by day of estrous cycle, while DND1 expression is not. Using immunohistochemistry DND1 protein expression was demonstrated to occur primarily in luminal epithelial cells of the uterine endometrium.

### Introduction

MicroRNA (miRNA) are known to influence the mRNA and protein abundance through post-transcriptional gene regulation (PTGR) following interactions with the 3'UTR that lead to translation inhibition and/or mRNA degradation. Interactions between miRNA silencing complexes and additional RNA binding proteins have the ability to influence the outcome of miRNA:mRNA interactions. Dead end homolog 1 (DND1) and fragile-X mental retardation homolog 1 (FXR1) are RNA binding proteins capable of influencing miRNA mediated regulation of mRNA stability and translation. The binding of DND1 to target mRNA near miRNA targeting sequence inhibits miRNA PTGR, while the binding of FXR1 to its target mRNA improves mRNA translational efficiency. Our working hypothesis is that RNA binding proteins DND1 and FXR1 are expressed in the uterine endometrium during the estrous cycle and early pregnancy of the pig and can impact PTGR of specific mRNA.

### Materials and Methods

Cyclic, cross-bred sows were observed for estrous behavior daily in the presence of an intact boar and the onset of estrus was designated as day 0 of the estrous cycle. Hysterectomy, followed by isolation of the uterine endometrium was conducted on day 0, 5, 10, 12, 14, and 18 of the estrous cycle and on days 10, 12, 14, and 18 of early gestation (n = 4 animals per day/pregnancy status). The mRNA levels of FXR1 and DND1 were quantified

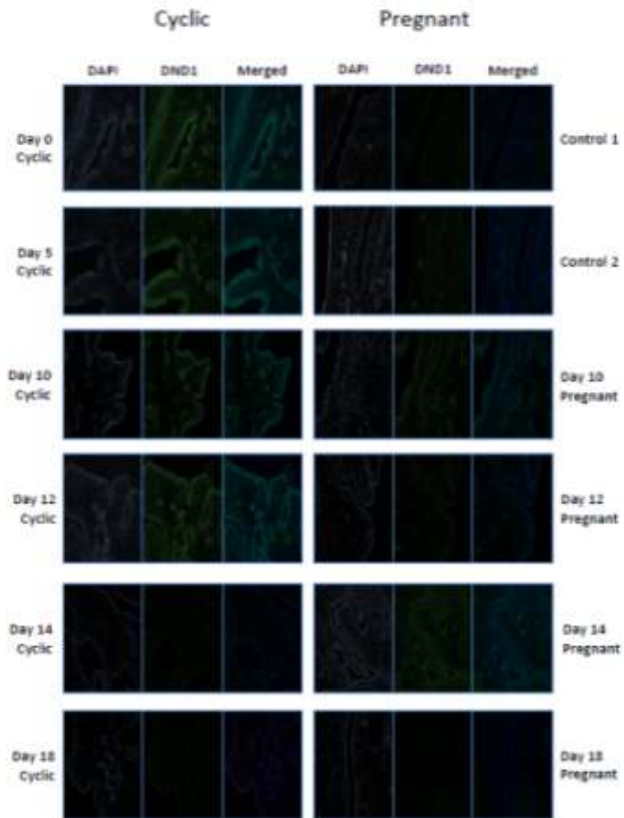
using the QuantiTect SYBR-Green RT-PCR Kit (Qiagen). Cycle threshold ( $C_T$ ) values were normalized to 18S ribosomal RNA, an endogenous control. Significance ( $P < 0.05$ ) was determined by probability differences of least squares means. Sectioned slides of uterine tissue were incubated with mouse anti-DND1 primary antibody (Millipore) in 5% BSA followed by using a fluorescent anti-mouse secondary antibody. Exposure time for microscope images was established using three negative controls: 1) mouse IgG in place of primary antibody, 2) without primary antibody, and 3) without secondary antibody (Figure 1).

### Results and Discussions

Abundance of FXR1 mRNA was significantly affected by day of the estrous cycle but not by pregnancy status. The abundance of FXR1 mRNA is lowest during the mid to late luteal phase of the estrous cycle corresponding to the loss of progesterone receptor in the luminal and glandular epithelium. Transcript abundance of DND1 was not affected by day of the estrous cycle or pregnancy status. Immunohistochemistry staining demonstrated DND1 protein is localized to the luminal and glandular epithelium during days 0 through 12 (Figure 1). The expression of DND1 protein was lowest during days 14 and 18 of the estrous cycle and pregnancy compared to earlier stages of the estrous cycle. The lack of correlation between DND1 mRNA and protein expression suggest that DND1 protein abundance may be subject to posttranscriptional regulation during the estrous cycle in pigs.

### Acknowledgements

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**Figure 1.** DND1 protein localizes to the luminal and glandular epithelium of the uterine endometrium.