

## **Proteomic analysis of follicular fluid in carriers and non-carriers of the Trio allele for high ovulation rate in cattle**

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Follicular fluid provides a microenvironment for oocyte development and serves as a signaling medium for systemic and intrafollicular communication between cells. In previous work a major gene for high ovulation rate in cattle was confirmed and mapped to chromosome 10; subsequent gene expression analysis strongly implicated SMAD6, an inhibitor of the transforming growth factor-beta/bone morphogenetic protein (TGF-beta/BMP) signaling pathway, as the responsible gene. To increase understanding of the gene's role in affecting ovulation rate, proteomic analysis was performed to characterize differences in proteins in follicular fluid between the carriers and non-carriers of the allele. We hypothesized that proteins may be differentially expressed between the genotypic groups. A total of four non-carrier and five carrier females were used in an initial study with four and six additional non-carrier and carrier females, respectively, used in a validation study. Emergence of the follicular wave was synchronized and the ovaries containing the dominant follicle(s) were extracted by ovariectomy for follicular fluid collection. A hexapeptide ligand library was used to overcome the masking effect of high abundance proteins and to increase the detection of low abundance proteins in tandem mass spectrometry (LC-MS/MS). A total of 763 proteins were identified in the initial study; after correcting for multiple comparisons, only two proteins, Glia-derived nexin precursor (SERPINE2) and Inhibin beta B chain precursor (INHBB), were significantly differentially expressed (false-discovery rate < 0.05). In a replicate study of analogous design differential expression of these two proteins was confirmed ( $p < 0.05$ ). A total of 752 proteins were observed in the validation study, 472 of which were observed in the initial study and the remainder unique to the validation study. Joint analysis of results from the two studies was performed by combining p-values for proteins with consistent differential expression across studies using the weighted-Z method. Three additional proteins were consistently differentially expressed between genotypes at a false discovery rate < 0.05 (Phospholipase A1 member A precursor (PLA1A), Serine protease 23 precursor (PRSS23), and Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 precursor (PLOD1)). Previous studies have indicated that expression of SERPINE2, INHBB and PLOD1 is increased by TGF-beta/BMP signaling; their reduction in follicular fluid from carrier animals is consistent with the ~9-fold overexpression of SMAD6 which is inhibitory to this pathway.