

IPD Project Details

Project ID: IPD4918

Project Title: Identification of Potential Protein Biomarkers for Early Detection of Pregnancy in Cow Urine uses 2D DIGE and Label Free quantitation

Description: Background: An early, reliable and noninvasive method of pregnancy diagnosis is a prerequisite for efficient reproductive management in dairy industry. The early detection of pregnancy also helps to reduce the calving interval and rebreeding time which is beneficial for farmers and dairy industries. The aim of this work to identify potential biomarker for pregnancy detection at earlier stages (16-25 days). To achieve this goal, we performed differential in gel electrophoresis (DIGE) and label free quantitation (LFQ) for identification of protein which have significant differential expression during pregnancy. Results: DIGE experiment revealed eleven differentially expressed proteins out of which nine proteins were up regulated having fold change ≥ 1.5 . The LFQ data analysis gave 202 differentially expressed protein out of 30 proteins were up-regulated and 40 down regulated having significant fold change ≥ 1.5 and ≤ 0.6 respectively. Further bioinformatic analysis showed that majority of proteins was involved in regulation of leukocyte immunity, endopeptidase inhibitor activity, regulation of peptidase activity and polysaccharide binding. Conclusion: To the best of our knowledge, this is first report on identification of differentially expressed proteins in urine of cows during various time points of pregnancy using DIGE and LFQ. In our investigation, we have discussed functional significance of few selected proteins such as A2HS, MBP, GRP, IGFBP-II, SERPIN, Vitamin D binding protein etc which were differentially expressed and actively involved in pregnancy associated events such as embryo implantation, establishment and maintenance of pregnancy. Thus, we have identified a set of potential protein biomarkers for early detection of pregnancy.

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Sample Preparation: In- Solution Digestion For in-solution digestion, 20 μ g of pooled samples (n=6) from non- pregnant and pregnant cows (0,16,22 &35 days) were collected on different days of pregnancy was processed. In – solution digestion method was performed as reported earlier with slight modification (1 J. Adachi, In brief , 45 mM DTT in 50mM NH_4HCO_3 was used to reduces disulfide bonds followed by alkylation

of cysteine residues using 10 mM IAA in 50 mM MNHCO₃.

Peptide Separation: Digestion was carried out overnight using trypsin (1:20) (modified sequencing grade; Promega, USA) at 37°C. The reaction was subsequently stopped with 10% TFA, peptides vacuum dried, desalted by zip tip and stored at -80°C.

Protein Characterization: Data Processing and Bioinformatics analysis MS data were analyzed using MaxQuant (12 Cox and Mann, 2008) software version 1.5.0.8 and searched with UniProt Bos taurus and Bubalus bubalis database along with common contamination sequences. Database search was performed in MaxQuant environment integrated with Andromeda. For searching, the enzyme specificity was set to trypsin with the maximum number of 2 missed cleavages. The precursor mass tolerance assigns to be 0.07 Da for the first search and 0.006 Da for the main search. Mass tolerance for matching peaks to theoretical ion series was set to 40 ppm. The false discovery rate (FDR) for PSM, protein, and site decoy fraction was set to 1%. The search included variable modifications of protein N-terminal acetylation, methionine oxidation and carbamidomethylation of cysteines was searched as a fixed modification. The maximal number of modifications per peptide was set to be 6. The minimum peptide length of 6 was set, and the 'peptide requantification' function was enabled. To validate and transfer identifications across different runs, the 'match between runs' option in MaxQuant was enabled with a retention time window of 0.7 minute and an alignment time window of 20 min. Subsequent bioinformatics analysis were performed using Protein ANalysis THrough Evolutionary Relationships (PANTHER) to compare the GOBP, GOCC, GOMF and GOPC. The obtained PANTHER (Paul et al., 2003) data was further analyzed and graphs were prepared using MS Excel 2007.

Experiment Type: Shotgun proteomics, Gel-based experiment

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue, deamidated residue

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