IPD Project Details

Project ID: IPD4034

Project Title: Vaginal secretory proteome dynamically changes across the early and mid-trimesters of pregnancy

Description: This study investigates the dynamic alterations in high vaginal fluid (HVF) proteome and its correlation with physiological changes during progression of term pregnancy. The HVF samples were collected at three time points as defined as V1 (6-12 weeks), V2 (18-20 weeks) and V3 (26-28 weeks) and SWATH-MS strategy were applied to profile changes in protein expression at early and middle stage of pregnancy. Using in-house generated HVF-specific protein library, 61 proteins (>1.5 fold at V2/V1 or V3/V1, q-value < 0.05) changed as a function of gestational age. The stage-specific expression pattern of these proteins was mainly associated with the biology of cervical remolding, fetal development and microbial defense.

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Sample Preparation: The maternal HVF was collected using swab from the enrolled participants. All samples were buffer exchanged with 100 mM ammonium bicarbonate (pH 8.0)through a 3 kDa MWCO membrane (Amicon Ultra, Millipore).

Peptide Separation: Trypsin were used for proteins digestion at 20:1 protein to enzyme ratio. The digested samples were desalted using C18 fast-flow tips (Pierce Thermo Fisher scientific) and spiked with iRT (Biognosys, Switzerland). Samples were vacumm dried and resuspended in 0.1% formic acid for LC-MS/MS analysis using an TripleTOF 5600 (SCIEX)in DDA or DIA (SWATH-MS) mode. Validation of selected proteins were performed using MRM assay on a QTRAP 6500 mass spectrometer coupled with LC. Precursor was selected using SRM-Atlas, NIST human and in house generated spectral library. MRM data were analysed in skyline and MultiQuant software.

Protein Characterization: MaxQuant analysis software (version 1.6.1.0) with default settings were used for identification of peptides and proteins from DDA MS files. Reference spectral library was generated using Spectronaut (Biognosys) with default settings. All DIA datasets (.wiff files) were converted to HTRMS format before analysis

and Spectronaut (version 11) software was used for retention time normalisation and targeted data extraction from the DIA MS files. The protein quantitation and peptide quantities were calculated as a mean intensity within the XIC peak area of the respective fragment ions at MS2. The protein CVs were calculated based on the summed intensities of their respective peptides.

Experiment Type: SRM/MRM

Species: Homo sapiens

Tissue: Vaginal fluid (bto:0003084)

Cell Type: Vaginal fluid

Disease: Unknown

Instrument Details: Q TRAP (MS:1000187)

Protein Modifications: No PTMs are included in the dataset

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