

# Non-invasive diagnosis of intra-amniotic infection and preterm birth from proteomic analysis of vaginal fluid

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## Objective

To identify non-invasive predictors of intra-amniotic infection (IAI) and preterm birth (PTB), we conducted a systematic analysis of the vaginal fluid proteome.

## Study design

Vaginal fluid samples from a prospective observational cohort of 284 women in preterm labor at 20-34 weeks' gestation were analyzed. IAI was defined as a positive amniotic fluid (AF) culture and/or AF interleukin-6 >2000 pg/mL. PTB was defined as  $\leq 34$  weeks. Vaginal fluid proteome analysis was performed using fluorescence 2D gel analysis, multidimensional liquid chromatography tandem mass spectrometry (2D LC-MS/MS) and label-free quantification. Pair-wise comparison was performed using  $\div 2$  tests and statistical significance determined after adjusting for multiple comparisons. Immunoassays were used for accurate quantification and evaluated using Receiver Operating Characteristic (ROC) curves and logistic regression.

## Results

Of 284 subjects, 153 (54%) delivered at  $\leq 34$  weeks and 56 (37%) of these had IAI. There were no significant differences in demographic or reproductive factors between PTB/IAI, PTB/no IAI and term groups. Vaginal fluid proteome analysis revealed 154 unique proteins. Label-free quantification identified 16 proteins differentially expressed ( $p < 0.05$ ) in PTB and IAI. Trend analysis (term, PTB, PTB/IAI) showed 8 proteins as significant ( $p < 0.05$ ), including immunoregulators (calgranulins, L-plastin, lysozyme, lactoferrin, leukocyte elastase), acute phase proteins (alpha-1-acid glycoprotein, profilin, matrix metalloproteinase-9) and high abundance AF proteins (insulin-like growth factor binding protein1 and vitamin D binding protein). Preliminary analysis of 4 potential biomarkers showed good discrimination with area under (AU)ROCs from 0.64 to 0.82. Logistic regression increased discriminant ability (AUROC > 0.85).

## Conclusion

Vaginal fluid proteome analyses identified novel predictors of IAI and PTB. These could provide a sensitive, non-invasive test to diagnose PTB complicated by IAI.

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