Summary

In this work, we took advantage of modern proteomics in order to characterize intraamniotic infection and inflammation related changes in the proteome of amniotic fluid from preterm birth patients. Proteins with altered levels could subsequently serve as potential biomarkers for timely recognition of intraamniotic infection and inflammation.

Due to the extremely high complexity and high dynamic range of amniotic fluid proteome, we first developed a technique - CysTRAQ - which enables proteome complexity reduction based on cysteinyl peptide capturing and features a multiplexed protein quantitation across four samples. The developed method was subsequently applied into the comparative proteomic analyses of amniotic fluid.

Our study included patients with both principal phenotypes of spontaneous preterm birth - spontaneous preterm labour with intact membranes as well as preterm premature rupture of membranes - in order to provide a comprehensive insight into the proteomic background of the infectious and inflammatory processes occurring in amniotic fluid. By employing our CysTRAQ approach in combination with additional fractionation and separation techniques, we managed to describe a remarkable number of the amniotic fluid proteins. Owing to the quantitation feature of CysTRAQ, we were also able to quantify the differences between samples, where intraamniotic infection and inflammation was confirmed or ruled out, respectively.

In our results, the preterm premature rupture of membranes cohort showed a considerably higher degree of proteome dysregulation with regard to the presence of intraamniotic infection and inflammation compared to the spontaneous preterm labour patients. In both cohorts, we observed major changes in antimicrobial peptides, protease inhibitors and acute inflammatory phase signaling molecules. In the preterm premature rupture of membranes cohort, we described a dysregulation of a complex web of proteases as well as of their respective inhibitors. In the spontaneous preterm labour cohort, on the other hand, proteins related to neutrophil degranulation were among the most obviously dysregulated. Noteworthy, we observed profound changes in proteins related to neutrophils extracellular traps. Proteins constituting these structures were found to be dysregulated in both preterm premature rupture of membranes as well as in spontaneous preterm labour cohort and include histone proteins, neutrophil defensins and azurocidin. According to our knowledge, this work is the first one to suggest the presence of neutrophil extracellular traps in amniotic fluid during intraamniotic infection and inflammation.