

Abstract

The recent technology development of instrumentation as well as sample preparation and computational techniques involved in mass spectrometry-based proteomics allows for tailoring the applied methodology specifically for the investigated problem. The aim of the work presented in this thesis was the development of a proper quantitative proteomic workflow for the oocyte quality assessment on the basis of changes in human follicular fluid (hFF) proteome in women undergoing the in vitro fertilization procedure. A considerable number of diversely conducted research studies already focused on exploring the hFF proteome; however, no explicit protein biomarkers were introduced to infertility treatment procedures. The complex data obtained from a large-scale clinical study conducted with sufficient depth of quantitative analysis and a relevant statistical design could point to novel oocyte quality biomarkers. Thus, two distinct label-free quantitative proteomic workflows were created, optimized, and tested for this task in a series of experiments presented in three published studies. The Triple Quad-TOF workflow was designed to be fast and low cost/resource demanding. At the same time the proteome coverage was extended using the SWATH-MS method allowing for the application of multiple fractionation strategies in the spectral library construction without the loss of quantitative measurement accuracy. On the other hand, the Quad-Orbitrap workflow provided comprehensive and sensitive analysis in each single measurement at a higher expense of time and resources. The absolute concentrations of all analyzed proteins were calculated using Total Protein Approach. The utility of both workflows was examined in small-scale clinical studies, which simultaneously generated biomarker candidates of oocyte maturity and competence of blastocyst development. The information obtained in the course of all the conducted experiments allows new insight into the proteome and peptidome landscape of hFF. The good compatibility of the results obtained by both workflows allows to choose a suitable methodology of a future clinical study according to the specific purpose of planned research and available facilities.