## Abstract

The present project aims were development of a new sensitive separation method using high performance liquid chromatography with tandem mass spectrometric detection, which enables quantification of selected steroid hormones, namely 17α-ethynylestradiol (EE2) and progesterone (PRG) in M2 fertilizing medium. M2 medium is used during sperm capacitation *in vitro*, which is the physiological process of complex molecular and biochemical changes, essential for obtaining fertilizing ability of male gametes. Optimized separation and detection conditions (column Kinetex EVO $C_{18}$ with mobile phase consisted of 50/50 (*v/v*) acetonitrile/water with 0.1% formic acid in both components) were applied on monitoring of concentration changes of each hormone during time dependent sperm capacitation. The concentration tested for EE2 were 200, 20 and 2 µg/l and for PRG 200 µg/l only. It was found that concentration of free, unbounded EE2 firstly decreases, reaches the minimum in about 60 min of capacitation and then increases again. This trend was observed for all three tested concentrations. Concentration (200 µg/l) of free unbounded PRG decreased during whole capacitation. The following kinetic analysis will be based on the concentration dependences of the free unbound hormones on the capacitation time. The results of kinetic study should help to understand better the interaction mechanism between the sperm and the selected steroid hormones on a molecular level.