Environmental estrogens (natural and synthetic) belong to a group of contaminants called endocrine disrupting chemicals (EDCs). These compounds interfere with a function of endocrine system. They can bind to natural estrogen receptors or block synthesis of endogenous hormones. 17β-Estradiol (BE2) is a natural endocrine disruptor produced more by women than men. Also a synthetic 17α-ethynylestradiol (EE2) belongs to EDCs. It is used as a main part of birth control pills. Together with other endocrine disruptors EE2 is probably responsible for feminization of male animals living in effluents of waste water treatment plants.

The aim of this thesis was to develop a method for determining free 17β-estradiol and 17α-ethynylestradiol available to capacitating sperm. In order to determine a status of BE2 during mouse sperm capacitation *in vitro,* a high performance liquid chromatography (HPLC) method with tandem mass spectrometry (MS/MS) detection was used. A reversed-phase separation mode using a SunFire C_{18} column with a simple mobile phase composed of acetonitrile and water at the ratio 40/60 (v/v) containing 0,1% formic acid in both components was applied. Under the optimized separation conditions, calibration curves for 17β-estradiol and 17α-ethynylestradiol were measured in the concentration range of 1-250 μg/L and 1-200 μg/L, respectively. Limits of detection and quantification for the both analytes were determined as tenths and units of μg/L, respectively. A free as well as bound 17β-estradiol to the bovine serum albumin as an essential compound of capacitation medium can be quantified by the proposed method. Results show that the level of free 17β-estradiol available for mouse spermatozoa during capacitation *in vitro* can be quantified by HPLC method with MS/MS detection. This method represents an important tool to determine the amount of environmental estrogens, such as 17β-estradiol, bound to sperm cells at the specific time point of capacitation *in vitro*. With a respect to long time that was taken by the biological experiments with 17β-estradiol, there were only calibration dependences measured for 17α-ethynylestradiol.