Abstract

Glycosidases are enzymes that hydrolytically cleave polysaccharide structures and have fundamental importance above all for processing the glycocalyx on the surface of the sperm during the fertilization process, so that the sperm can finally successfully interact with the *zona pellucida* and fuse with the oocyte membrane. The bachelor thesis investigates the activities of several important glycosidases (β-galactosidases, α-galactosidases, α-mannosidases and neuraminidases) in mixed samples of reproductive fluids, specifically it is epididymal fluid, seminal plasma and oviductal fluid, originating from the domestic pig. Another sample in which the activity was measured of the aforementioned enzymes was ejaculated sperm. The activities of the given enzymes were determined using fluorescent substrates in slightly acidic and neutral pH. In addition to determining specific activity of individual enzymes, this bachelor's thesis was enriched with qualitative information regarding glycoproteins in male and female reproductive fluids and ejaculated sperm lysate. Glycoproteins are the dominant component of the sperm glycocalyx and many of them also represent the corresponding naturally occurring substrates for the above-mentioned glycosidases. Based on electrophoretic separation followed by staining, the overall protein profile of all samples was determined with samples of late and early follicular fluid. They were subsequently detected through a lectin binding study of a carbohydrate structure containing sialic acid, galactose and α-linked mannose. Also was carried out direct immunodetection of neuraminidase, one of the enzymes whose specific activity was determined. The result of the detection of glycoproteins was the discovery of glycosyl proteins compose the significant part of the total protein profile. Furthermore, it was possible to indicate the effect of the activity of the studied enzymes on the surface of the sperm traveling to the egg in the reproductive fluids with which the sperm is in close contact during its journey. Another surprising finding was the fact that a certain was found between the rate of specific activity of some of the enzymes in the samples and the representation of the corresponding carbohydrate structures on glycoproteins in the same samples.