Abstract

Interactions between proteins and saccharide moieties play an indispensable role in mammalian reproduction as they stand behind of such processes as maturation and mutual recognition of gametes and sperm oviductal reservoir formation. In my dissertation thesis I focused on activities of glycosidases from bovine and porcine follicular fluids and their changes connected with follicle development. Activities of five glycosidases were detected in tertiary and preovulatory follicles in both species. The most active enzymes were α-L-fucosidase in cow and α-D-mannosidase in sow and both enzymes also demonstrated the most pronounced increase in their activities during follicle maturation. Interestingly, both α-L-fucose in cow and α-D-mannose in sow were described as saccharides responsible for the formation of the sperm oviductal reservoir and we offered a hypothetical mechanism of synchronisation between sperm release from their reservoir with the time of ovulation based on a surge of activities of corresponding follicular glycosidases through the oviduct. Subsequently, it was demonstrated that β-D-galactosidase and α-D-mannosidase affect sperm-zona pellucida binding in pig, as they both decrease interaction between sperm receptors for zona pellucida and zona pellucida. This may explain the observation that maturation changes of zona pellucida induced by follicular fluid lead to lower level of polyspermic fertilisation.

For the sake of a better characterisation of studied glycosidases, I developed red native electrophoresis - a novel electrophoretic method suitable for enzyme separation according to their molecular weight and subsequent visualisation of their activities directly in gel. Red native electrophoresis revealed several isoenzymes of detected glycosidases, some of which seemed to be of follicular origin.

In the next part of my dissertation thesis, I analysed antimicrobial properties of follicular, oviductal and uterine fluids and demonstrated that oviductal fluid is the most potent in inhibiting of the growth of *E. coli*. In attempt to identify compounds responsible for observed antimicrobial properties, I first narrowed the search into molecular weight range of 3 500 - 30 000 and subsequently identified histones H2A type 2-C, H2B type 1-K, H3.3, and H4 as the putative antimicrobial agents in bovine oviductal fluid. Their role was further strongly confirmed by inhibition of antimicrobial properties of fluids by adding antibodies against histones.

And finally, I studied secretions of Cowper's glands. In bull, I concentrated on its role within ejaculate and demonstrated that it increases semen viscosity, decreases the rate of sperm release from ejaculate and enhances binding of seminal proteins to sperm surface. All these observations can be explained by the fact that bovine Cowper's gland secretion positively affects aggregation of seminal protein.

In boar, Cowper's gland secretion forms a seminal plug in the cervix of sow after copulation preventing thus a semen back flow and ensuring its paternity. However, we demonstrated that uterine fluid from the sow in the oestrous phase of the reproductive cycle is capable of rapid proteolytic degradation of the plug in contrast with fluid from dioestrous sow. We also detected several serine and metalloproteases present in uterine fluid, which are putative agents responsible for the plug degradation. In the course studies on the porcine seminal plug, we also developed a novel method of dissolving of highly glycosylated mucus matrix under native conditions using a buffered boric acid solution.