Changes in Sialylation of Sperm Glycocalyx during in Vitro Capacitation

Pavla Postlerova[1,2]; Natalie Kalcicova[2]; Veronika Kraus[1]; Barbora Klusackova[2]; Petra Secova[3]; Jana Antalikova[3]

1. Laboratory of Reproductive Biology, Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic
2. Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Czech Republic
3. Laboratory of Reproductive Physiology, Institute of Animal Biochemistry and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovak Republic

Glycocalyx plays an important role in sperm cell physiology and its changes, especially during the capacitation process, can lead to sperm preparation for fertilization. The level of sialylation is crucial for mature spermatozoa giving the sperm a negative charge and protecting them from the immune system within the female reproductive tract. Sialylation may affect sperm motility, participate in the sperm oviductal reservoir formation and in the sperm binding to the *zona pellucida*. The aim of this work was to find differences in sialylation of sperm glycocalyx before and after *in vitro* capacitation using biotin-labelled lectins. Using indirect fluorescence, we detected the most significant changes in the flagellum after capacitation of boar spermatozoa showing a decrease in the MAL-II (recognizing α2-3 sialylated Galβ1-3GalNAc) and SNA (recognizing α2-6 sialylated LacNAc) lectin binding signals. On the acrosome, an increase in percentage of spermatozoa with MAL-II and SNA lectin signals was observed only in the apical part of the acrosome. Unlike pig sperm, the SNA lectin does not recognize any glycans on bull spermatozoa before and after capacitation. MAL-II lectin labelled bull sperm flagella with the same intensity before and after *in vitro* capacitation. However, a weak non-uniform signal in the head region begins to appear on capacitated spermatozoa. Using MAL-II and SNA lectins in Western blot analysis, we detected a significant decrease in the number of various sialoglycoproteins after capacitation indicating the removal of sialic acids or the sialoproteins from the boar sperm. Similar to fluorescent labelling, an increase in sialylation detected by MAL-II of some bull sperm proteins is visible on Western blots. Our results confirmed desialylation of the boar sperm surface and redistribution of sialoglycoproteins on the sperm acrosome after *in vitro* capacitation for subsequent binding to the oocyte. The detection of sialylation, especially on the tail of boar sperm, could thus serve as a marker for the evaluation of capacitation status, and in addition, it appears that sialylation on the sperm surface is species-specifically different.

Acknowledgments: This work was supported by the Grant Agency of the Czech Republic (GA22-31156S), VEGA-2/0074/24, the support of the Institute of Biotechnology (RVO: 86652036), and project BIOCEV (CZ.1.05/1.1.00/02.0109) from the ERDF.