DESCRIPTION FOR THE GENERAL PUBLIC

One of the main problems facing the extensive application of frozen-thawed boar semen is the inherent poor post-thaw sperm quality. It is envisaged that manipulation of the biochemical structure of the sperm membrane will render spermatozoa less susceptible to the cryo-induced stresses. Hence, the application of specific protein systems of the seminal plasma to improve membrane stability is a novel approach that could improve semen freezability in individual boars. Identification of these protein systems that facilitate cryo-tolerance through their interactions with the sperm membranes during the cooling and freezing-thawing process will be the main objective of this research project.

In this research project different variants of modifications of the of the sperm membrane structure during cryopreservation of boar semen will be conducted. Fractionated seminal plasma (Sephacryl column, Sephacryl S- 200 HR) comprising different protein fractions will be exposed to sperm samples for 1,5h at room temperature, and then the mixture will be held in BTS extender (Beltsville Thawing Solution) for 1h at 17°C. The cooled samples will be frozen in a controlled programmable freezer (Ice Cube 14M), using a standard cryopreservation protocol. Besides routine semen assessments, biochemical changes in spermatozoa will be assessed with fluorescent and biochemical methods at different stages of the cryopreservation procedure. The computer-assisted sperm analysis (CASA) system will be used to analyze the sperm kinematic parameters. Furthermore, subtle changes in the sperm plasma membrane integrity will be assessed with SYBR-14, in combination with propidium iodide (PI), whereas the sperm acrosome status will be monitored with the fluorescein isothiocyanate-conjugated peanut agglutinin (FITC-PNA) staining technique, using cytometric or fluorescent microscopic analysis. The functional and energy status of the sperm mitochondria will be evaluated with the JC-1/PI assay, in conjunction with the measurements of ATP content, using the bioluminescence method. Apoptotic-like changes in spermatozoa will be monitored with an apoptotic marker, the fluorescent dye, Yo-Pro-1, whereas the lipid peroxide mediated damage to the sperm membranes will be determined by the measurements of the levels of malondialdehyde (MDA) with the thiobarbituric acid assay. Scanning electron microscopy will be performed to provide valuable information about the ultrastructural and morphological alterations in spermatozoa at different stages of the cryopreservation procedure, Proteomic studies of the fractionated and nonfractionated seminal plasma will include SDS-PAGE followed by densitometry analysis to identify the components of the protein fractions which may be responsible for the varying levels of cryo-tolerance among the boars during the cooling and freezing-thawing processes. Furthermore, variations in the seminal plasma and sperm membrane proteome will be analyzed by two-dimensional gel electrophoresis (2D-PAGE), followed by MALDI-ToF/ToF mass spectrometry, whereas the expression of membrane proteins of spermatozoa, exposed to different components of the fractionated seminal plasma during cooling and freezing-thawing, will be verified by the western-blot analysis. In addition, the antioxidant status in the seminal plasma will be characterized by the analysis of the level of the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx), and total antioxidant status (TAS).

It is envisaged that the identification and characterization of the proteins fractionated seminal plasma and the sperm membrane proteins will allow to detect the level of sperm cryo-tolerance among individual boars. Furthermore, this project will provide more-in-depth study regarding the role of seminal plasma and sperm membrane protein systems in the cryo-tolerance of boar spermatozoa, which is required to elucidate the factors responsible for differences in their cryo-susceptibility. Understanding the interactions of these systems with the seminal plasma protein will allow the improvement in the cryopreservation technology of boar semen for AI stations in Poland, which may serve as an impulse to create regionalized cryo-banks. Additionally, such major improvement in the cryopreservation technology of boar semen may offer new prospective to the commercial pig AI industry. The project results will be disseminated as widely as possible at national and international scientific conferences, seminars, and will be published in the journals cited in the Journal Citation Reports (JCR).