Abstract

Boar's sperm differs with many macro- and microscopic parameters from the sperm of other animal species. This causes necessity of the storage of boar's semen intended to insemination purposes in the unfrozen form. Thereby the sperm stored in the temperatures 15 – 17ºC keeps fertilization abilities only through a few days (1 – 5) because of the progressive degradation of spermatozoa as the result of unfavorably working factors of the external environment.

The aim of this work was to develop a method for a production of a stable antioxidant enzyme preparation intended to extend the fertilization ability of a boar semen stored in the unfrozen form.

The experimental material was: edible garlic (*Allium sativum L.*), which was used for isolation of a plant superoxide dismutase (SOD), enzymes of animal origin – SOD from bovine erythrocytes and catalase (CAT) from bovine liver, used for the enzyme conjugates creation and sperm for biological tests collected from boars of the breed of PBZ (Polska Biała Zwisłoucha).

Plant SOD, cheaply and efficiently isolated from the garlic, was present in obtained preparations in the form of active monomer with a molecular weight of about 14 kDa. The use of a two-stage chromatographic purification procedure allows to obtain a homogeneous preparations containing 4-fold purified enzyme with the activity of 3101 U_{SOD}/mg protein. In the case of two-step chromatographic purification of catalase from bovine liver the similar procedure allows to obtain a 1.65-fold purified homogeneous preparation with the activity of 9330 U_{CAT}/mg protein. Such purified CAT has a molecular weight of about 125 kDa and is an active dimer.

The reaction of CAT and SOD conjugation was performed using the dextran aldehyde (AD). The mixture of final products CAT-AD-SOD was fractionated over a molecular sieve. The presence of protein and enzymatic activities in the same fraction confirmed the presence of a conjugate. The CAT and SOD conjugates were present in a variable molar ratio lying in the range from 0.08 to 1.00. The largest group of compounds represented connections in which two monomers of CAT were bind with one monomer of SOD, and conjugates, in which one monomer of CAT was bind with one monomer of SOD.

Free enzymes SOD and CAT as well as CAT-AD-SOD conjugates were closed in liposome structures composed of lecithin and cholesterol (Lec/Chol). The best efficiency of the protein encapsulation in the structure of Lec/Chol liposomes were obtained for a higher content of cholesterol (Lec/Chol 6:4, mol/mol), in a solution with a higher content of protein (5 mg/ml), using the procedure of FAT-MLV-$VET_{400\ nm}$. In all cases the enzymes remained active. Enzymosomes thus obtained, stored at 4°C, remained stable for at least 2 months.

To investigate the antioxidant efficacy of prepared enzymosomes boar's semen collected routinely for insemination, which, unlike the sperm of other species cannot be stored in a frozen form, because of its higher sensitivity to low temperatures, was selected. To 1 ml of a boar's sperm suspended in the Bio'dil extender enzymosomes of the activity of 200 U_{CAT}, 150 U_{SOD} and in the case of conjugate 200/150 U_{CAT}/U_{SOD} were added. Samples thus prepared were stored at 17°C for 12 days. After completion of the 4^{th}, 8^{th} and 12^{th} day of incubation in each sample respiratory activity of the sperm was measured. The measurements were done with the help of a Clark's oxygen electrode. The results confirmed the negative effect of time on the motion activity of boar spermatozoa stored for 12 days, and showed that these changes can be associated with the process of loosing of the integrity of inner mitochondrial membrane of spermatozoon midpiece.

Enrichment of a being in commercial use extenders with the containing CAT-SOD conjugates enzymosomes protects the sperm from damage and conserves spermatozoa motion activity, not disturbing activities of enzymes of its mitochondrial electron transport system.