Abstract

Insemination of female livestock is currently one of the most important biotechnological methods. In case of pigs, the most optimal way to store boar semen is to use it in a liquid state.The reason for this is the specific structure and sensitivity to cold shock of the boar sperm cell membrane, and the susceptibility of cell structures to oxidative disruption caused by reactive oxygen species. The purpose of this study was to analyse the functional and structural changes occurring in spermatozoa during storage of boar semen in a liquid state, taking into account the evaluation of oxidative disorders of spermatozoa, as well as to determine the effects of the addition of superoxide dismutase (SOD) and catalase (CAT) to the extenderon the quality of stored semen. The material for the study was semen obtained from 52 Duroc boars (n = 72 ejaculates) stored for 7 days in Vitasem LD extender (Megapor, Spain). The protective effect of antioxidants on spermatozoa was validated by adding the following variants of SOD concentrations to the extender: 25 IU/mL, 50 IU/mL and 75 IU/mL, and CAT: 100 IU/mL, 200 IU/mL and 300 IU/mL. The functional and structural changes in spermatozoa were analysed on the 1st, 3rd, 5th and 7th day of semen storage based on computer-assisted assessment of sperm motility, HOS-test, fluorochrome staining with SYBR-14/PI, JC-1, BODIPY, DCFH-DA and assessment of total antioxidant capacity.

During the first stage of the study, it was found that semen storage time exerted a significant influence on the analysed sperm motility indices, cell membrane integrity and sperm mitochondrial activity. It was demonstrated that during the storage period membrane lipid peroxidation and intracellular H_2O_2 generation intensify in spermatozoa, with a decrease in total antioxidant capacity in their extracellular environment. The study revealed a correlation between sperm oxidative disorders and the other assessed quality indices of the stored semen. In the following part of the study, it was found that, with respect to the control group, the quality indices of the stored semen developed most favourably in the extenders with the addition of 25 IU/mL and 50 IU/mL of SOD and 100 IU/mL and 200 IU/mL of CAT. On the whole, these experimental variants showed a higher percentage of motile spermatozoa and spermatozoa with an integral cell membrane and active mitochondria. In addition, a smaller proportion of spermatozoa with membrane lipid peroxidation and intracellular production of H_2O_2 was recorded in the extenders with the addition of the enzymes.

The results obtained indicate that the quality of boar semen deteriorates with storage time, which may be due to the production of excessive amounts of reactive

oxygen species. These changes appear to be compensated for by the addition of antioxidant compounds to the extender, which may contribute to improving the efficiency of boar semen storage methods.

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