The Antioxidative Effect of MnTBAP on Boar Sperm Cryopreservation

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Antioxidants protect cellular function and structure by neutralizing the oxidative stress caused by increased ROS during sperm freezing. Cryopreservation has been studied with antioxidants such as superoxide dismutase, melatonin, vitamins, polyphenols, carotenoids, and minerals, and demonstrated encouraging results. Many studies have utilized mentioned antioxidants to increase the efficiency of sperm freezing and improve the success rate of artificial insemination and pregnancy. MnTBAP (Manganese(III) meso-tetrakis(4-benzoic acid) porphyrin) is one of the newly synthesized antioxidants that has been reported to show positive effects on sperm morphology and capacitation in human, ram, and stallion. In this study, porcine semen was treated with 0, 50, 100, and 150uM of MnTBAP based on Tris-egg-yolk extender and frozen to determine whether MnTBAP can assist the status of sperm during cryopreservation. After thawing, motility, viability, acrosome integrity, mitochondrial potential (MMP), caspase activity, membrane lipid disorder, DNA fragmentation, capacitation ability, and embryo development were measured. First, motility was assessed using the CASA system (Computer-assisted sperm analysis), and the 100uM treatment group (66.8%) showed the highest motile rate compared to the control group (51.1%), so the remaining analyses were conducted comparing the two groups (P<0.05). The following fluorescence staining was applied to examine the control and 100uM group by fluorescence microscopy, and it was found that the viability stained with SYBR-14 and propidium iodide (PI) was 41.7% vs 62.4% and the acrosome integrity stained with Pisum sativum agglutinin and fluorescein isothiocyanate was 77.9% vs 86.4%, which was a significant difference (P<0.05). In addition, mitochondrial membrane potential (MMP) measured by rhodamine 123 and PI were 46.5% vs 51.9%, the fragmented rate estimated by the Sperm-sus-halomax kit was 63.4% vs 57.4%, and caspase activity detected by staining with sulforhodamine was 30.1% vs 22.9%, which tended to be higher in the treated group but did not show statistical significance. Finally, to investigate the effect on fertilization and embryo development *in vitro*, the straw was dissolved and treated with $2.5x10^7$cells/ml. As a result, the cleavage rate was 77.6% vs 84.1% and the blastocyst rate was 9.7% vs 11.4% (P<0.05). In conclusion, these results suggest that antioxidant treatment has a positive effect on sperm freezing-thawing experiments, which increases fertilization capacity and leads to increased embryo development. This

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