Chondroitin Sulfate (From *Holothuroidea*) Increases the Cryopotential of Sperm Cells and Improves Fertilization Rates

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Abstract

The aim of this experiment was to investigate the cytoprotective potential of sea cucumber extract against cryopreserved sperm and semen fertility rate. Commercially available sea cucumber extract (Swanson, USA) was weighed out and placed in 3 glass tubes containing, respectively: 10 mL of water-glycerin solution (WG), water-ethanol (EC), glycerin-ethanol (GE), glycerin-DMSO (DG). Tubes were mixed with vortex, placed in a water bath, and incubated for 16 hours at 40°C. For the experiment, six 3-year-old Simmental bulls of known health status were used, from which semen was collected once a week (for 18 weeks) using an artificial vagina. After an initial assessment of semen quality, the ejaculates were pooled to eliminate individual differences between males, then diluted to a final concentration of 80 x 10^6 sperm/mL and divided into 16 equal samples. Control (C) without additive, the test samples contained 2, 4, 6, 8 and 10 µL WG, 2, 4, 6, 8 and 10 µL WE, 2, 4, 6, 8 and 10 µL GE, 2, 4, 6, 8 and 10 µL DG. Semen was frozen/thawed and assessed for motility, viability, DNA defragmentation, mitochondrial membrane potential and acrosome integrity. It was shown a positive effect of WG and GE extracts on the efficiency of sperm preservation at low temperatures. Established that, depending on the type of prepared extract, the sea cucumber can have both cytoprotective (WG, GE, WE) and cytotoxic (DG) effects. Moreover, too high concentrations of the extract can adversely affect the sperm in terms of parameters such as viability, motility, mitochondrial potential, and acrosome or DNA integrity. The present study, thanks to the use of model animals to study the cytoprotective potential of the sea cucumber extract, proves that it can be a potential candidate for use in semen cryopreservation technology, but further research is needed to optimize the composition of individual types of extracts and their effect on sperm. The highest effectiveness of female fertilization was observed when doses from GE groups (2 and 4) were used for insemination. The results of this analysis prove that the addition of the tested extract may improve the fertilization efficiency.

Keywords: cryopreservation, insemination, male, quality, reproduction