Butylated hydroxytoluene (BHT) improved semen quality and sperm DNA of frozen-thawed Arabian stallions preserved in modified INRA-82 extender

Amal Aboelmaaty¹, Islam El-Seadawy¹, Mohamed Kotp¹, Hazem Aldebakey ¹, Heba Hozyen¹, Diya El-Badry², Abeer M. Anwer³, Tsvetan Tsvetkov⁴, and Teodora Daneva⁴

June 16, 2023

Abstract

Background: Alpha tocopherol is one of the non-enzymatic lipophilic antioxidants. Butylated hydroxytoluene (BHT) is a synthetic analog that possesses similar modes of actions in protecting the cryopreserved sperms. Objectives: This study hypothesized that a certain concentration of any antioxidant is suitable for improving the post-thaw semen quality of stallions. Study Design: Case control study. Methods: To determine this concentration, a synthetic antioxidant similar to vitamin E in potency and scavenging oxidative stress power in concentrations of 0.0, 0.25, 0.50, 1.0, 2.0, 4.0 mM/ml were added to semen extender. The post-thaw sperm progressive motility at 0, 1h, 2h, 3h, the sperm viability index, the plasma membrane integrity tested by the hypo-osmotic selling test (HOST), the acrosome integrity, non-fragmented DNA, % of DNA in the comet head, % of DNA in the comet tail length, and comet tail moment were compared. Results: According to our hypothesis, 1.0 mM BHT was the most suitable concentration that preserved the highest (P<0.0001) post-thaw sperm progressive motility at 0.0, 1h, 2h, 3h., the highest viability index (P<0.0001), plasma membrane and acrosome integrities (P<0.0001), non-fragmented DNA (P<0.001), and % of DNA in the comet head (P<0.001), the lowest DNA % in the comet tail (P<0.001) and comet tail moment (P<0.0001), and the shortest (P<0.0001) comet tail length. Main limitations: Collection of semen for research from registered Arabian Horse was the main obstacle for conducting this research. In conclusion, the concentrations of BHT around 1.0 mM/ml (0.5, 2.0mM/ml) proved better post-thaw semen characteristics, but BHT in concentrations >2.0mM/ml indicated the worst of all concentration which kept the lowest semen quality than non-supplemented control.

Hosted file

main_1_mM_BHT__non-blinded.docx available at https://authorea.com/users/621099/articles/649774-butylated-hydroxytoluene-bht-improved-semen-quality-and-sperm-dna-of-frozen-thawed-arabian-stallions-preserved-in-modified-inra-82-extender

¹National Research Centre

²Agriculture Research Center

³Agriculture Research Center, GIZA

⁴Institute of Biology and Immunology of Reproduction Acad Kiril Bratanov Bulgarian Academy of Sciences