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Effect of Coenzyme Q-10 on sperm motility, plasma membrane integrity, acrosomal integrity and mitochondrial membrane potential of stallions cryopreserved spermatozoa

R. Lançoni, E.C.C. Celeghini, C.P.T. Carvalho, G.B. Zoca, L.N.G. Oliveros, L. Batissaco, R.P. Arruda

FMVZ-USP - Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Pirassununga, SP, Brasil.

Coenzyme Q-10 plays a crucial role in cellular bioenergetics acting as a cofactor in the electron transport chain in the mitochondria (respiratory chain), and is therefore essential for the production of energy in ATP form. In addition to acting as a carrier for electrons and protons in the mitochondria, its reduced form plays a potent lipophilic antioxidant role and is able to "recycle" and "regenerate" other antioxidants such as tocopherol and ascorbate. Therefore, the objective of this study was to analyze the effect of CoQ-10 on sperm motility and plasma membrane and acrossomal integrity, and mitochondrial membrane potential of stallion's cryopreserved spermatozoa. Five ejaculates from five stallions (n=25) were used. Each ejaculate was separated into three treatments: a) control freezing extender (BotuCrio®- Botupharma, Botucatu, SP, Brazil); b) CoQ-10 1 mM and c) CoQ-10 50 µM (added at the respective concentrations to the same extender used in the control treatment). Then, the semen was cryopreserved in automated TK 3000® (Uberaba, MG, Brazil) system and analyzed post-thawing. The analyzed variables were total motility (MOT), progressive motility (MOTPR) and percentage of rapid cells (RAP), which were assessed using the CASA system (HTM-IVOS, version 12.3, Hamilton Thorn Research, Beverly, Massachusetts, USA). In addition, plasma membrane integrity (propidium iodide and *Hoechst* 33342), acrosomal (FITC-PSA) and mitochondrial membrane potential (JC-1) were assessed using fluorescent probes. Cells were classified as the percentage of completely intact cells, I.E., those having intact plasma membrane, intact acrosome and high mitochondrial membrane potential (PIAIH); percentage of cells with intact plasma membrane (IPM); percentage of cells with intact acrosome (IA); and percentage of cells with high mitochondrial membrane potential (HMMP). Data were tested for the normality of residues, and comparisons of the treatments were performed by the MIXED procedure of the SAS program (version 9.3), the differences between the treatments were given by the Tukey test. A significant difference was considered when $P \leq 0.05$. The results of the characteristics of MOT, MOTPR and RAP were similar between treatments. Regarding membranes integrity, the CoQ-10 1 mM addition showed higher ($P<0.05$) percentage of PIAIH cells (31.86 ± 2.00), IPM (32.64 ± 2.05) and HMMP (32.12 ± 1.97) when compared to the control group (27.88 ± 2.35; 29.00 ± 2.49; 28.26 ± 2.39, respectively). Therefore, it is concluded that the addition of CoQ-10 at 1 mM concentration into the freezing extender is better to the membranes integrity preservation of equine cryopreserved spermatozoa.

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