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PROCEEDINGS

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EFFETTI DI UN NUOVO ANTIIOSSIDANTE SULLA QUALITÀ POST-CONGELAMENTO DELLO SPERMA DI STALLONE
EFFECTS OF A NOVEL ANTIOXIDANT ON POST-THAW QUALITY OF STALLION SEMEN

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Introduction. Several studies have been performed during the last decades to improve the post-thaw quality of stallion semen, but yet the cryopreserved sperm is not as fertile as the fresh semen. This has been attributed to many factors, including damages to the sperm membrane particularly due to lipid peroxidation (LP), occurring during the freezing/thawing process (Watson, 1995). Therefore the ability of the freezing extender to preserve from oxidative stress is a key element in determining the post-thaw sperm quality.

Objectives. The aim of this experiment was to evaluate the effects of EC-Oxyrase, an Escherichia coli membrane preparation acting as antioxidant in the freezing extender, on post-thaw equine sperm quality.

Materials and methods. Five stallions of proven fertility, aged between 5 and 15 years, were used for this experiment. Semen was collected using an artificial vagina (Model Missouri). Each stallion was collected for three times at 48 h intervals. Half of the gel-free volume of semen was diluted 1:1 with the pre-warmed centrifuge medium and the remaining half with the pre-warmed centrifuge medium added with EC-Oxyrase. The two treatment samples were centrifuged at 400xg for 15 min (Schulman et al., 2003). The two supernatant-free sperm pellets were re-suspended in the cryopreservation extender and in the cryopreservation extender added with EC-Oxyrase, respectively. The extended semen was loaded in 0.5 ml French straws and frozen in a programmable freezer attending the following descending curve: -0.5°C/min from +24°C to +4°C, -10°C/min from +4°C to -15°C, -15°C/min to -120° (Backman et al., 2004). Post-thaw quality was evaluated by the following parameters: a) motility, evaluated by a phase contrast microscope at 200x, b) morphology, by eosin-nigrosin staining, c) vitality and membrane integrity, by FITC-PNA and PI fluorescent staining, d) mitochondrial membrane potential, evaluated through the JC-1 fluorescent probe. Statistical analysis was performed by the GLM procedure of SAS program.

Results. A significant decrease was observed in the progressive sperm motility (PSM) of post-thaw stallion sperm in comparison with the fresh semen (P<0.001). EC-Oxyrase improved significantly the PSM after thawing in comparison with the control extender (P<0.05). The morphological abnormalities were significantly less in semen extended in EC-Oxyrase in comparison with the sperm cryopreserved in control extender (P<0.05). Furthermore no significant differences were recorded between spermatozoa frozen in Oxyrase or in control extenders in vitality, membrane integrity and mitochondrial membrane potential.

Conclusions. EC-Oxyrase is able to improve equine post-thaw PSM reducing the O2 level in the cryopreservation extender, therefore reducing the oxidative damage to the membrane lipids. The absence of significant differences in membrane integrity indicates that sperm motility may be a more sensitive indicator of oxidative stress and is inhibited before any LP effects could be detectable by the markers used. Reactive Oxigen Species decrease sperm motility earlier than LP reducing ATP levels (Baumber et al., 2000). EC-Oxyrase protects the spermatozoa also against the mechanical stresses, source of secondary morphological abnormalities, engendered by centrifugation and osmotic volume excursions. Further studies are needed to detect if the EC-Oxyrase protection could depend also by an interaction between Escherichia coli membrane fragments and spermatozoa membranes.

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References


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