The effects of EC-Oxyrase® and coconut water on equine sperm cryopreservation

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The use of milk and egg yolk in semen extenders raises the possibility of xenobiotic contamination and increases variability between batches. Alternative sources of lipoproteins could replace animal byproducts. Oxidative damage is thought to be a major cause of damage occurring during cryopreservation. EC-Oxyrase® (Oxyrase, Inc, Mansfield, OH) is an E. coli origin membrane-derived enzyme system that removes oxygen from liquids. We hypothesized that: 1) post-thaw semen parameters and pregnancy rates would not be different in coconut water treated samples compared with egg yolk treated samples and, 2) the use of EC-Oxyrase would improve post-thaw sperm motility and membrane integrity and decrease lipid peroxidation.

Experiment 1: Three ejaculates each from five stallions were frozen using EquiPlus semen extender (MOFA Global, Verona, WI) in one of four treatments: 2% egg yolk (EY), 2% coconut water (CW), 2% egg yolk with 8.69% EC-Oxyrase (EYO), or 2% coconut water with 8.69% EC-Oxyrase (CWO). Fresh semen was added to a basic extender (EquiPlus part A) lacking CW or EY and progressive motility (PM) was measured using a computer assisted sperm analysis system (SpermVision, MOFA Global, Verona, WI). A portion of the ejaculate was evaluated with flow cytometry using propidium iodide, annexin, and BODIPY probes to assess apoptosis, membrane integrity, and lipid peroxidation, respectively. Samples were frozen according to EquiPlus instructions at a concentration of 200x10^6 cells per mL using a controlled rate freezer (Planer, Middlesex, UK) and then thawed for 30s in a 37°C water bath. A post-hoc comparison between all treatments was done using a Bonferroni adjustment for multiple comparisons. CW showed better post-thaw PM than EY (dropping 51.4% and 56.3%, respectively, P<0.05). There were no differences in apoptosis observed among groups. Membrane damage increased in EY, EYO, and CWO when compared to CW (P<0.05). No differences were seen in membrane oxidation between EC-Oxyrase and control samples.

Experiment 2: One ejaculate was divided into two aliquots and frozen using the same method as in Experiment 1 in either coconut water (CW) or egg yolk (EY) extender. Mares (n = 12) were randomly assigned to either CW or EY, inseminated at 24 and 42 h after deslorelin injection (when in estrus with a follicle greater than 35mm) with thawed semen (50 x 10^6 PMS/dose), and examined for pregnancy 14 d after ovulation. The pregnancies were terminated immediately after diagnosis and the trial was repeated with the alternate treatment in each mare. Each mare thus served as her own internal control in this crossover experimental design. Statistical analysis of the crossover data used Prescott and McNemar tests and employed exact inference using Crossover-1 statistical software (Cytel Software Corporation, Cambridge, MA). More CW mares became pregnant (11/12) than the EY mares (6/12) (P = 0.0373). In both experiments, CW performed better than EY in preserving semen parameters and fertility (P<0.05). EC-Oxyrase did not seem to positively affect semen parameters (P>0.05).

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