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MOTILITY AND MEMBRANE INTEGRITY OF EJACULATED BOVINE SPERMATOZOA EXTENDED AND CRYOPRESERVED IN *L*-CARNITINE TRIS-EGG YOLK EXTENDER

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ABSTRACT

This study was conducted to examine if *L*-carnitine supplementation in the Tris-egg-yolk extender can improve the motility characteristics, membrane integrity, and in vitro fertilization potential after cryopreservation of ejaculated bovine spermatozoa. It was carried out on January to May 2013 at the University of Wisconsin-Madison. *L*-carnitine at final concentrations of 0.5, 1, 10, and 30mM were added to Tris-egg yolk extender where semen was suspended at $100x10^6$ sperm cells/mL. Tris-egg yolk without carnitine served as control. Motility characteristics and functional integrity of membrane were examined by computer assisted sperm analysis and hypo-osmotic swelling test at 2, 6, and 24 hour at room temperature. The post-thaw effect of *L*-carnitine was likewise assessed. Each treatment suspension was cryopreserved in Tris-egg yolk with 7% glycerol, stored in liquid nitrogen and subjected to computer assisted sperm analysis and hypo-osmotic swelling test post-thawing. Lastly, the fertilization potential of frozen semen treated with *L*-carnitine was used for *in vitro* fertilization and cleavage and blastocyst development were assessed. *L*-carnitine was demonstrated to improve the motility characteristics in a concentration-depended manner; 1mM concentration is efficient but high concentration beyond 30mM had cytotoxic effect. A similar trend was observed on membrane integrity although significant difference was not evident. After IVF, the use of 1 mM *L*-carnitine resulted in significantly higher cleavage rate (92.4% vs. 82.8%) suggesting that *L*-carnitine supplementation at low concentration in Tris-egg yolk extender improves motility and fertilization potential of bovine sperm cells.

Key words*:* bovine semen, computer assisted sperm analysis, L-carnitine

INTRODUCTION

Semen is still the cheapest component of artificial breeding both in the Southeast Asian and other regions with artificial insemination (AI) remains the most implemented reproductive biotechnology and cryopreservation the most important procedure in order to ensure viability of male gamete and guarantee the success of AI. However, the current available methods to preserve semen as a genetic resource and its successful dissemination via AI and other assisted reproductive technologies (ARTs) are still sub-optimal (Rodriguez-Martinez, 2012a, b).

During cryopreservation, sperm cells are being subjected to stress and studies done by Chatterjee and Gagnon (2001) proved that an increase in lipid peroxidation levels during cryopreservation and thawing affects the motility of the frozen-thawed spermatozoa. Oxygen free