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Effect of LDL on stallion sperm motility after cryopreservation
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Cryopreservation and subsequent thawing places several stressors on cells, the most detrimental include ice crystal formation, osmotic changes, temperature shock and physical manipulation. Spermatozoa of different species tolerate the cryopreservation process differently. For example, stallion spermatozoa do not tolerate the freezing process as well as bull or human spermatozoa. Current methods to improve freezability include altering the freezing media, freezing process and thawing protocol. Egg yolk improves post-thaw quality of spermatozoa, and it has been suggested that the cryoprotectant properties of egg yolk are found in the low density fraction which is primarily composed of low density lipoprotein (LDL). Low density lipoprotein has been shown to improve post-thaw quality of dog, bull, boar and ram spermatozoa. Simply adding clarified egg yolk plasma has been shown to improve stallion freezing quality. 1

Thus, the goal of this project was to determine if the addition of LDL to freezing media at different concentrations could improve the post-thaw motility of stallion spermatozoa compared to that of semen frozen with clarified egg yolk plasma. Two extenders were used: INRA 96 (IMV, Maple Grove, MN) and a modified Kenney extender. Four stallions of three breeds ranging from 4-18 years of age were each collected eight times for a total of 32 collections. Each collection was divided into 10 aliquots and cryopreserved using standard methodology in INRA 96 and a modified Kenney extender with clarified egg yolk or 2,4,6,8% LDL added. Egg yolk was clarified by centrifugation and LDL was extracted from fresh chicken eggs by a combination of centrifugation and induced salt formation. 2 Post-thaw motility of the samples was assessed by computer assisted sperm analysis (CASA) and the data were analyzed using the SAS mixed procedure method. Post-thaw progressive motility did not differ significantly (P > 0.1) between extenders containing LDL and clarified egg yolk. A significant reduction (P < 0.03) in total motility post-thaw was observed in extenders containing 6 and 8% LDL from clarified egg yolk. When semen extenders were analyzed separate from each other the addition of LDL to the INRA extender did not (P > 0.1) significantly affect total motility. However, when the modified Kenney extender was used with higher levels of LDL, it significantly reduced (P < 0.02) total motility post-thaw compared to clarified egg yolk. It is clear that higher levels of LDL were not beneficial to post-thaw motility in this study.

Keywords: Low density lipoprotein, stallion, cryopreservation, CASA

References