Effects of Melatonin on Stallion Sperm Motility and Viability *in Vitro*

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Recent research has shown that melatonin has positive effects on sperm viability during cryopreservation. Therefore, melatonin could potentially be included in commercial stallion semen extender to aid in the survival and function of fresh cooled spermatozoa. The objective of this study was to determine effects of melatonin on stallion sperm motility and viability during 48 h of storage at 5°C. In four separate trials, ejaculates from three stallions were collected and diluted to a final concentration of 500 million sperm/ejaculate with a skim milk-based extender, without antibiotics, supplemented with 0, 0.1, 1.0 or 10.0 mM of melatonin and stored at 5°C for 48 h. Total motility (TM), progressive motility (PM), track velocity (VCL), straight line velocity (VSL) and smoothed path velocity (VAP) were evaluated by Computer-Assisted Semen Analysis (CASA) at 0, 24 and 48 h of storage. An eosin-nigrosin stain was used to subjectively evaluate the live/dead ratio of spermatozoa at 0, 24 and 48 h of storage. Data were analyzed using the PROC MIXED procedure of SAS. TM, PM, VCL and sperm viability decreased over time ($P < 0.05$); however, there were no differences in these variables due to melatonin concentrations at any time point. At 24 and 48 h, semen extended with 10mM of melatonin had significantly decreased VCL and VAP compared to control ($P < 0.05$). VSL and VAP decreased from 0 to 24 h, but there were no differences in VSL between 24 and 48 h. Overall, addition of melatonin to stallion semen extender did not affect sperm motility and viability during storage at 5°C.