Diagnosis and effects of urine contamination on stallion semen cooling
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Contamination of semen with urine (urospermia) is known to affect raw semen quality in stallions. While semen extension has been shown to mitigate the effects of urospermia on raw semen, the effects of urine on the motility of cooled stallion semen remain unknown. Practitioners have no proven means to confirm urine contamination in extended cooled-shipped semen. We hypothesized that urine contamination affects semen motility after cooling and extension, and that semen levels of creatinine, urea and pH can be used to detect urospermia. The objectives of this study were: (i) to assess the effects of variable amounts of urine contamination on total and progressive motility of extended fresh and cooled semen and (ii) to test whether pH, creatinine and urea can detect urine contamination in extended-cooled stallion semen. Eleven reproductively healthy light breed stallions with no known history of urospermia were enrolled in the study. Free catch urine samples were obtained from three of the enrolled stallions, pooled and frozen at -20°C until further use in the study. Each stallion was collected using a phantom and a Missouri artificial vagina at two to three day intervals, and a total of thirty-seven ejaculates were obtained. Each ejaculate was assessed for initial motility using Computer-Assisted Sperm Analysis (CASA, Spermvision Minitube of America, Verona, WI), sperm concentration using an Equine Densimeter (Animal Reproduction Systems, Chino, CA), and semen pH using a LAQUA Twin pH meter (Horiba Instruments, Irvine, CA). The ejaculates were then divided into five 5 ml aliquots, and either 0, 0.25, 1, 1.5, or 5 mls of pooled stallion urine was added. Each semen sample was then reassessed for semen pH, creatinine and urea concentrations and extended with INRA 96 (IMV Technologies, Maple Grove, MN) to a final concentration of 25 million sperm/ml. Total and progressive motility were re-assessed, and the samples were then packaged in Whirl-Paks (Nasco, Fort Atkinson, WI) and stored in commercial semen containers (Equitainer I, Hamilton Research Inc, Ipswich, MA) for 24 hours. At 24 hours, the semen containers were opened and semen motility and pH were assessed for all samples. Two ml aliquots of all samples were frozen for later analysis using an automated analyzer (Beckman Coulter, Pasadena, CA). Statistical analyses were performed using mixed models and when significant, post hoc comparisons were made with LSD (JMP 11, SAS Institute, Cary, NC). There were no stallion effects (p>0.05). As expected for raw semen samples, urea, creatinine and pH increased with urine contamination (p<0.0001). Motility decreased in all samples pre- and post-cooling, with pronounced reduction in groups contaminated with 1 to 5 ml of urine (p<0.05). Creatinine and urea measurements in cooled extended semen enable identification of urine contamination and were different than the control group (p<0.05). There were no differences in pH among the groups after cooling for 24 hours (p>0.05). In conclusion, urospermia not only affected raw semen motility, but small amounts of urine contamination affected motility after 24 hours of cooling. Extension of semen with INRA 96 did not prevent detection of urine contamination using creatinine and urea measurements. However, pH can only be used to detect urine contamination in raw semen and not for cooled-extended semen. This is the first study to demonstrate the use of creatinine and urea to detect urospermia in cooled extended stallion semen.

Keywords: Urospermia, stallion, semen cooling, creatinine, urea