Bacteriological quality of frozen-thawed stallion epididymal sperm
M. Ciccarelli, a D. Diaz-Campos, b, c A.J. Campbell, a A. Tibary a
 aComparative Theriogenology, Department of Veterinary Clinical Sciences and bDepartment of Veterinary Microbiology and Pathology, cWashington Animal Disease Diagnostic Laboratory, Washington State University, College of Veterinary Medicine, Pullman, WA

Stallion epididymal sperm collection and freezing is a valuable tool to preserve genetics from superior stallions after castration or euthanasia. Retrograde flushing of the cauda epididymis is the most common and efficient technique to collect sperm. This process may expose samples to contamination. In the present study we evaluated the bacteriology of samples collected using this technique. We hypothesized that retrograde flushing of the cauda epididymis with a commercial extender will result in minimal contamination making this frozen-thawed spermatozoa safe for use in mares. The objective of this study was to determine the risk for contamination of stallion epididymal spermatozoa collection and to identify the bacteria most likely isolated. Epididymal sperm was harvested from 13 stallions following closed castration. The samples were cryopreserved using a standard technique for ejaculated semen involving dilution in INRA 96® (antibiotics listed: penicillin [27 mg/L], gentamycin [76 mg/L], amphotericin), centrifugation, and re-suspension of the pellet in a freezing extender (EZ Freezin LE® that contains ticarcillin disodium). All samples had a final concentration of 400x10⁶ spz/mL and frozen in 0.5 mL straws. One straw per stallion was thawed and the contents cultured for aerobic and anaerobic bacteria. Conventional culture media was inoculated, incubated at 37°C and examined for seven days. Bacterial identification was achieved by the use of Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) technology. One sample (7.7%) showed high number of mixed aerobic bacteria (Bacillus spp, coagulase negative Staphylococcus spp, alpha and beta Streptococcus spp) suggesting a high-level environmental contamination. Anaerobic cultures revealed moderate contamination in 6 of 13 stallions (46%) with non-pathogenic bacteria. The most common isolates were Lactobacillus spp, Peptostreptococcus spp and Propionobacterium spp. Bacterial contamination occurs frequently in ejaculates of stallions collected by artificial vagina. Antimicrobials present in the extender help to reduce this contamination. In this study, the use of antibiotic-containing extender for flushing and freezing seemed to be sufficient to control the contamination with pathogenic aerobic bacteria. The presence of anaerobic bacteria, though non-pathogenic, in 46% of the samples is interesting and warrants further investigation. In conclusion, harvesting epididymal spermatozoa with antibiotic-containing extenders seems to be a safe technique as far as risk for contamination of the uterus upon use for artificial insemination.

Keywords: Cryopreservation, artificial insemination, equine.