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Effect of storage media and storage time on histological and ultrastructural changes in dog epididymal cells

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The recovery and freezing of sperm from the epididymides of testes obtained either from dead animals (post-mortem recovery) or after orchiectomy is a viable option for preserving male gametes and thus for maintaining germ banks. On many occasions, the only option for salvaging gametes is to transport the testes and epididymides to a laboratory that is equipped for sperm processing. Previous data have show sperm quality upon recovery after various days of epididymal storage at 4°C or 5°C decreases as storage interval increases. In addition epididymal storage media affect sperm quality in cat but not in dogs. In cats, morphological changes in epididymal cell were higher when the storage time increases. Likewise an effect of storage media was showed in feline. The aim of this study was to assess histological and ultrastructural (UL) changes in principal cells of dog epididymides (EPI) stored at 4°C for 24, 48 or 72 h in two different media. The hypothesis was that the stored media do not modify the autolytic process, however the storage time yes. Testes of 13 dogs (n = 13) aged between 2 and 7 years, were obtained. The animals participated in a voluntary program for control of urban feline reproduction in a pet public shelter. Four right epididymides of 4 dogs were fixed immediately after orchiectomy (0 h). Eighteen epididymides of nine dogs were stored at 4°C in two different media. After bilateral orchiectomy, the left testis with adjacent epididymis from each animal was placed in a TRIS egg yolk extender (TEY) and the right in saline solution (SAL) supplemented with penicillin at 100 IU/mL and stored at 4 °C. Testes and epididymides from each animal placed in each medium were allocated to one of three storage times (24, 48 or 72 h) and were evenly distributed among treatments (3 animals/treatment time). The tissue samples from the cauda epididymis were fixed by immersion in 2% glutaraldehyde in phosphate buffer pH 7.3 for 2 hours at 4 °C. Following primary fixation, specimens were post-fixed for 1 h in 1% osmium tetroxide (OsO\textsubscript{4}), dehydrated through a graded series of ethanol solution, cleared in acetone and embedded in epoxy resin. Semi-thin sections (1 µm) were stained with toluidine blue for light microscopy (1000x). The ultrathin sections (90 nm) from selected areas were cut using a Reichert-Jung ultramicrotome. After then ultrathin sections were mounted on 200-mesh copper grid and uranyl acetate and lead citrate were used as contrast. The sections were then examined with a JEM-1200 EX (Jeol) transmission electron microscope at 80 kV. In all samples principal cells of epididymal epithelium were observed. In histological study, according to the nuclear features (NF) and stereocilia morphology (SM) samples were scored from 0-3. As well in the ultrastructural study according the nuclear chromatin (NC) and SM samples were scored from 0-3. In addition on photomicrographs ultrastructural morphometry was used to calculate the nuclear number (NN), nuclear area (NA) and mitochondrial area (MA) using Image J 1.43e (National institutes of Health, USA). Data were analyzed by Mixed procedure of SAS\textsuperscript{®}. In the histological study, parameters changed with time but not with media. A significant effect of time was observed (P<0.01), the score of morphological changes was higher when the stored time increase. In the ultrastructural study, NC and SN change with time (P<0.01) but not with media. In addition, MN, NA and MA did not change with media or time (P>0.05). Our results agree with previous data in which storage media no affect dog epididymal sperm parameters in epididymides stored at 4°C.