ABSTRACTS

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

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INTRODUCTION: 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. The conditions (time, temperature and environment) under which epididymides are handled could cause important changes in the viability of sperm samples. Cat epididymal sperm parameters are strongly affected by the epididymal storage time and media (1, 2). Even sperm cells can survive for some time in the epididymides of dead animals changes related to body decomposition affect spermatozoa quality (3). It is very likely that the autolytic changes within the epididymides could be responsible for this reduction in sperm parameters (1). As a result of the dead in cell occur degenerative changes by action of endogenous enzymes derived largely from lysosomes. This is a process of autolysis post-mortem. This phenomena takes place in all cells post-mortem inducing histological and ultrastructural changes (4). The aim of this study was to assess histological and ultrastructural changes in principal cells of cat epididymides stored at 4 °C for 24, 48 or 72 h in two different media. The hypothesis was that epididymides stored in TRIS egg yolk would have delayed epithelial cell autolysis decreasing cells morphological changes.

MATERIALS AND METHODS: Testes of 13 cats (n = 13) aged between 0.6 and 3 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 cats were fixed immediately after orchiectomy (0 h). Eighteen epididymides of nine cats 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 (1) 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 (OsO4), 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. The histological study was done on the semi-thin sections and the ultrastructural study was done on photomicrographs taken from the ultrathin sections. In histological study, according to the nuclear features (NF) and stereocilia morphology (SM) samples were scored from 0-3. As well in ultrastructural study according the nuclear chromatin (NC) and SM samples were scored from 0-3. In addition on study, according to the nuclear features (NF) and stereocilia morphology (SM) samples were scored from 0-3. In addition on study, according to the nuclear features (NF) and stereocilia morphology (SM) samples were scored from 0-3. The nuclear changes observed (P<0.01), the score of morphological changes was higher when the stored time increase. When we study the effect of stored media, morphological changes were higher in SAL compared with TEY (P<0.01). In the ultrastructural study, NC and SM changed with time and media as the histological study (P<0.01). In addition NN and NA change with time (P<0.004; P<0.001) but not with media. Conversely, MN and MA did not change with media or time (P>0.05).

DISCUSSION: The results of previous studies that have examined sperm quality upon recovery after various days of epididymal storage at 4°C or 5°C have shown that sperm parameters decreases as storage interval increases (1,2). This observation could be related with development of the autolytic process on the epididymides (1,2). In agreement with these studies, our results show that the storage time increased changes in epididymal cell morphology. The histological and ultrastructural features of epididymal principal cells in non stored organs (0 h) agree with those previously described by others authors (6,7). Likewise morphological and ultrastructural changes observed in stored epididymides are in agreement with cells changes related with the cell death process (4). In the histological study, our result show that cell morphological
changes were higher in epididymides stored compared with non-stored organs and these changes were higher when the storage time increases. Our result agree with data obtained in previous studies which found that the acrosome integrity, motility, velocity and vital stain were lower in sperm recovery of epididymides stored 72 h at 4°C compared with cells recovery of organs stored 24 h. On the other hand, Tittarelli et al. (1), found that sperm recovered from epididymides stored in TEY had higher sperm parameters that those recovered from organs stored in SAL. In conclusion these result show that TEY preserved the epididymal epithelial cell better than SAL and therefore this finding could improve the sperm quality recovery of stored epididymides in TEY.


(2) Gañán N, Gomendio M, Roldan E.R.S. Effect of storage of domestic cat (Felis catus) epididymides at 5 8C on sperm quality and cryopreservation Theriogenology 2009; 72 1268–1277.


