POOLING OF LENS EPITHELIAL CELLS IS REQUIRED FOR RNA EXTRACTION FOR EXPRESSION STUDIES IN CANINE CATARACTS.

Alexandre L. Andrade[1]*, Fernando F. Vissani[1], Natália F. Scaramele[1], Flávia de A. Lucas[1], Flávia L. Lopes[1]

[1]São Paulo State University (Unesp), School of Veterinary Medicine, São Paulo, Brazil.
* Autor para correspondência: al.andrade@unesp.br

Purpose: Cataracts are the main cause of vision loss in dogs. The role of mRNAs and miRNAs in cataracts have not been described in dogs. However, individual lenses may be insufficient for extraction of good quality RNA, required for global expression analysis. We aimed to evaluate the concentration of RNA in individual and pooled lens fragments (n=5/pool), obtained from capsulorhexis of animals with immature and mature cataracts, as well as normal lenses. **Methods:** Fragments (5mm) from the anterior capsules (capsulorhexis) were obtained during phacoemulsification surgery. Individual samples were divided as: immature cataract (n=1), mature (n=5) and control (n=2); and pools were collected from: immature cataracts (n=5), and control (n=5). All samples were snap frozen and total RNA extraction was performed using the miRVANA miRNA Isolation kit (Life Tech®). RNA quantity was evaluated by spectrophotometry (Nanodrop). **Results:** RNA concentrations were 2.3, 2.6, 2,7, 22.0 and 21.7ng/µl, for individual mature cataracts, and 2.6ng/µl and 27.0ng/µl for immature cataract and control, respectively. From the pool of immature and normal lenses, concentrations were 23.2ng/µl and 72.9ng/µl, respectively. Fragments originating from capsulorhexis harbor a small number of lens epithelial cells, and even smaller in pathological conditions, due to cell migration to the posterior capsule, and apoptosis. **Conclusions:** Pooling cataract lenses, obtained by phacoemulsification capsulorhexis, should be considered for RNA extraction intended for global expression analysis.

Key-words: Lens ephithelial cells, RNA, cataract, dog.

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