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Lipid content assessment of feline oocytes in vitro maturation (IVM)

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Resumo

The domestic cat has been considered an important experimental model for the application of reproductive biotechnologies on aiming the conservation of endangered felids. The intracellular lipid content and composition have an important role in oocyte viability during cryopreservation. Some studies have already demonstrated an increase in lipid content within extended IVM time duration in some species, such as bovine and swine. The aim of this study was to investigate the time course of lipid accumulation during IVM in feline oocytes. For reaching that, oocytes were recovered from feline ovaries obtained in elective surgeries and, after selection, transferred to IVM (TCM-199 hepes, 4 mg/mL BSA, 0.2 mM piruvate, 50 ug/ mL penicillin-streptomycin, 0.5 ug/mL FSH, and 1 ug/mL estradiol, at 37 °C, in maximum humidity) for 24, 28 and 32 h. For lipid content evaluation, oocytes from the three experimental groups (G24, G28, and G32), plus immature oocytes (GI), were fixed in 4% paraformaldehyde solution for 40 min and stored in phosphate-buffered saline at 4 °C. Then, all structures were stained with Oil Red O solution (Sigma Chemical Co.). Oocytes were washed in a 50% ethanol solution for 2 min, stained for 15 min in Oil Red O solution and washed three times, for five min each, in 50% ethanol solution. After, they were kept for five min in distilled water before being evaluated. Images of each structure were captured using phase-contrast microscope and evaluated for the stained area fraction using Image J software (NIH Image, Bethesda, MD, USA). The lipid content results were submitted to ANOVA. The Tukey test was used for comparison among groups. A total of 37 oocytes were used (GI: n=7/ G24: n=11/ G28: n=11/ G32: n=8), which were obtained in three replicates. The oocytes from GI had a lower (P<0.05) lipid content compared to those of G28 and G32 (46.4%a x 72.3%b x 74.7%b, respectively), and similar to 24 h (67.7%a), although the lipid content had increased about 50% in the latter. Considering specifically the three IVM timepoints (24 h, 28 h, 32 h), no difference (P>0.05) was observed in lipid content. It was concluded that a significant increase in lipid content can be observed in oocytes after 28 h of IVM, suggesting that like in other species, IVM also causes lipid accumulation in feline oocytes.

Keywords: domestic cat, IVM, lipids, oocyte.