ABSTRACTS

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Vitrification of domestic cat oocytes – Studies on viability assessment and cytoskeletal changes

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OBJECTIVES AND METHODS: Most of the 36 wild felid species are classified as threatened, vulnerable or endangered due to poaching and habitat loss. Assisted reproduction techniques (ART), like in vitro fertilization, which use gametes collected from live or dead animals, and embryo transfer are crucial for wild animal recovery programs. Cryopreservation of domestic cat oocytes is an important part of ART. However, studies to date have not been very successful at recovering viable cryopreserved cat oocytes. This study used different methods of assessment to improve our understanding of the processes during cat oocyte cryopreservation. Vitrification is a non-equilibrium freezing method that prevents water from building ice crystals that may damage cells. Minimal volume vitrification, as it is in many other species (1), could be a very useful tool for the cryopreservation of feline gametes. Our preliminary study was conducted in order to establish vitrification solution components and the duration of each step of the vitrification procedure (2). In two experiments, both immature and in vitro matured oocytes were cryopreserved by vitrification. Vitrification solution (VS) consisted of 20% EG, 20% DMSO, 20 % FCS, 1.5 M trehalose with or without addition of 10% Ficoll PM-70. In experiment 1, immature and in vitro matured oocytes were vitrified with a use of Cryoloop technique in both types of VS. Subsequently in vitro matured oocytes were vitrified in VS with Ficoll PM-70 with the use of Cryotop Device (Kitazato BioPharma Co., Ltd. Fuji, Shizuoka Japan). After storage in liquid nitrogen (LN), oocytes were thawed, immature oocytes were denuded and all oocytes were stained for viability assessment with propidium iodide (PI, 200µg/ml) and fluorescein diacetate (FDA, 10 µg/ml) and assessed under an epifluorescence microscope. The cytoplasm of live oocytes appeared green (FDA positive) and dead oocytes were characterized by overall red colorization (PI). In experiment 2, frozen-thawed immature and in vitro matured oocytes were analyzed by three-color fluorescence staining using a modified protocol after Luciano et al. (3). Oocytes were fixed in 2% formaldehyde PBS, permeabilized using 0.2% Triton X-100 and immunostained for microtubules with a FITC-anti-α-tubulin monoclonal antibody (1/50), followed by microfilament staining by incubation in TRITC-labeled phallolidin (3 µg/ml). DNA was stained with DAPI (1 µg/ml). Oocytes were examined with a laser-scanning confocal microscope and classified into patterns A (normal), B (slightly abnormal) or C (abnormal) according to morphological appearance of non-cryopreserved oocytes (3). All data were compared with chi-square test.

RESULTS: In experiment 1 statistically significant differences were found between post-thaw viability of immature oocytes vitrified with and without Ficoll PM-70, 41% (27/65) and 25% (8/32), respectively. No differences were found in the survival of mature (52%, 32/62) and immature 41% (27/65) oocytes after vitrification with Ficoll PM-70. Also the different vitrification devices had no impact on survival rate with 52% (32/62) and 51% (33/65) viable oocytes on Cryoloops and on Cryotops, respectively. In experiment 2, 32% (38/120) of in vitro matured oocytes and 28% (28/101) of immature oocytes did not show any cytoskeleton damage, which was significantly different from the control group where normal cytoskeleton appearance was found in 63% (17/27) of in vitro matured oocytes and 55% (25/45) of immature oocytes.

CONCLUSIONS: The FDA/PI staining is a quick and effective method to assess post-thaw viability of vitrified feline oocytes. According to our data, Ficoll PM-70 essentially improves the oocyte survival upon vitrification. There was no difference in the post-thaw viability of mature/immature and Cryotop/Cryoloop preserved oocytes. Cytoskeleton examination by an established procedure allows the assessment of subcellular damages after freezing. This is a very useful tool for further optimization of vitrification protocols.

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