Animal Reproduction

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The effect of resveratrol during immature oocyte vitrification on the mitochondrial activity in feline

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Resumo

The use of a domestic cat as an experimental model for wild felids has provided advances in the investigation of reproductive physiology and gamete cryopreservation. One of the main challenges of oocyte vitrification is to reduce the cooling damage, such as mitochondrial dysfunction, caused by low mitochondrial membrane potential. This study aimed to evaluate the effect of resveratrol, as an antioxidant, and to identify the best moment for its exposure during vitrification of immature feline oocytes on mitochondrial activity. For this, oocytes were recovered from feline ovaries obtained in elective ovariosalpingohisterectomy surgeries. Oocytes presenting homogeneous cytoplasm and surrounded by, at least one layer of cumulus cells, were selected and vitrified in eight replicates, according to the groups: control (CONT), vitrified oocytes without resveratrol exposure; pre-exposure (PRE), oocytes subjected to resveratrol exposure before vitrification; or post-exposure (POST), oocytes subjected to resveratrol exposure after vitrification. Exposure was carried out for 90 min in TCM 199 supplemented with 1 mmol/L of pyruvate, 4 mg/mL of BSA, 100 uL/mL of penicillin-streptomycin, and 1 uM of resveratrol, at 38.5 °C, 5% CO2 in atmospheric air and maximum humidity. Oocytes from CONT remained for the same time and medium (without resveratrol) after warming. In addition, two experimental groups containing fresh oocytes were also assessed: exposed (FRESH-R) or not (FRESH) to resveratrol. After vitrification, oocytes were warmed, denuded, and incubated with 0.5 nM Mitotracker Green to assess mitochondrial activity. During the evaluation under the fluorescence microscope, pictures of each oocyte were taken and the fluorescence intensity was measured using the ZEN 3.5 Blue Edition software (Carl Zeiss Microscopy, Jena, Germany). The mitochondrial activity was obtained from the ratio of the fluorescence intensity and the total area in each oocyte. The data obtained were normalized and submitted to ANOVA. The SNK test was used for comparison among groups, at a significance level of 5%. Both vitrified (n=62) and fresh (n=39) oocytes were evaluated. The CONT showed higher ($P<0.05$) mitochondrial activity (0.537a) when compared to the other groups. Regarding the vitrified groups treated with resveratrol, there was no difference ($P>0.05$) between PRE (0.369b) and POST (0.300b,c). The groups FRESH-R (0.192c,d) and FRESH (0.180d) did not differ ($P>0.05$), as well as, POST and FRESH-R. In conclusion, resveratrol reduced the mitochondrial activity of vitrified feline immature oocytes when compared to the control group. However, it did not affect the mitochondrial activity of fresh oocytes, suggesting that appropriate culture conditions lead to lower mitochondrial activity.

Keywords: Cryopreservation, mitochondria, antioxidant

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