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Proliferation of spermatogonia from prepubertal cat testicular tissue after vitrification

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The cryoprotectants are organic solvents whose function is to protect cells against damage caused by extreme reduction of tissue temperature during vitrification. There are no established vitrification protocols for the testicular tissue in cats\textsuperscript{1}. The technique of AgNOR, which consists of silver staining of nucleolar organizer regions (NORs) allows the analysis of cell proliferation rate after the process of vitrification\textsuperscript{2}. Therefore, the aim of this study was to compare the rate of cell proliferation in testicular tissue from prepubertal cat after vitrification with different cryoprotectants associations. Five pairs of testicular prepubertal cats were used. The fragments were distributed in the control group and experimental groups containing associations of cryoprotectants dimethyl sulfoxide (DMSO)/ethylene glycol (EG) and DMSO/glycerol (GLY). Testicular fragments of the control group were fixed in Bouin's solution and processed to obtain the histological sections. For vitrification of the experimental groups, the fragments were subjected to equilibrium (each cryoprotectant at 1.4M) and vitrification (each cryoprotectant at 2.8M) solution with a final concentration of 5.6M cryoprotectant in vitrification solution. Then each fragment was subjected to a solid surface vitrification and the vitrified fragments were transferred to the cryotubes and stored in liquid nitrogen for up to 1 week. The samples were thawed at room temperature and water bath at 37 °C for one minute each and subjected to immersion baths with decreasing solutions of sucrose for removal of cryoprotectant. Then the fragments were fixed, processed and stained similarly to the control group. The obtained histological slides were stained by AgNOR technique for quantifying the NORs. The fragments were stained with silver 50% solution associated with a colloidal solution containing 2% gelatin in the ratio 2:1. Ten cells / field in 10 random fields of each fragment in each group were counted, amounting to a total count of 500 cells per experimental group. The counting of NORs was performed by observing the black color dots within the nucleoli of spermatogonia at a 1000x magnification in an optical microscope. For comparison among groups, the data were log transformed and proportions found were submitted to ANOVA, followed by Tukey's test to compare the averages of NORs in each group (P <0.05). The control group had a higher proliferation rate (3.85 ± 1.81) per cell than all other groups studied. The DMSO/EG group (2.17 ± 1.55) showed a cell proliferation significantly lower than the group DMSO/GLY (3.24 ± 1.91). The spermatogonia can be grown in vitro after vitrification and the obtained sperm used for artificial insemination\textsuperscript{3}. A higher rate of cell proliferation is a greater possibility of viable cells after the process of vitrification\textsuperscript{2}. The association of the cryoprotectant DMSO/GLY showed higher activity spermatogenic cells from prepubertal cats and is effective to use prepubertal cat testicular tissue vitrification protocols.