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Optimizing follicular integrity in feline ovarian tissue during vitrification

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Ovarian tissue cryopreservation followed by tissue culture is a promising approach to preserving the fertility of biomedical models and endangered species. Successful preservation of primordial follicle integrity and function was achieved recently in our laboratory in fetal bovine tissues [1]. The objective of the present study was to explore a similar approach in post-natal ovarian tissue of domestic cats – a key model species for wild felids. A series of experiments was conducted to identify appropriate vitrification variables. Specifically, we investigated the impact of exposure time to equilibration solution (ES; 7.5% dimethyl sulfoxide (DMSO) + 7.5% ethylene glycol (EG) + 20% fetal bovine serum (FBS) in base medium; 10 or 25 min) and vitrification solution (VS; 15% DMSO + 15% EG + 20% FBS in base medium; 2 or 10 min), presence of sucrose (0 or 0.5 M) in VS, exposure temperature (room temperature (RT) or 4°C), base medium for ES, VS and warming solutions (Dulbecco’s PBS or Eagle MEM with Hank’s salts). Ovaries were collected from 11 peri-pubertal cats (3 to 6 months of age) at local spay clinics. Ovarian cortical pieces were obtained by isolating the cortex and dissecting it into 1 x 1 x 0.2 mm³ pieces. The cortical pieces then were threaded onto 30 G needles (8 pieces per needle, one needle per treatment group per animal replicate), exposed to ES then VS in one of the conditions mentioned above, plunged directly into liquid nitrogen, and stored for ≥ 24 h in liquid nitrogen. At warming, the needles were transferred quickly to a sucrose gradient at 37°C. The cortical pieces then were cultured in our standard laboratory conditions for 0, 1 or 7 days to determine the ability of the tissue to recover from cryoinjury. For each treatment group and at each time point, two cortical pieces underwent histological preparations for haematoxylin and eosin staining to determine the mean percentage of normal follicles. Each treatment group had 2 to 4 animals with 2 technical replicates each. The fresh controls maintained a constant level of normal morphology (P ≥ 0.05) throughout the culture period (D.0: 62.5%; D.1: 48.2%; D.7: 53.8%). Cortical pieces were exposed to ES for 10 min, then to VS with or without sucrose for 2 or 10 min prior to vitrification. Those exposed to VS with sucrose for 10 min had the highest percentage (P < 0.05) of normal follicles throughout the culture period (D.0: 43.4%; D.1: 12.1%; D.7: 19.5%), while those exposed to VS without sucrose for 10 min had the lowest (D.0: 9.8%; D.1: 0.2%; D.7: 1.0%). Then, exposure to ES for 25 min followed by 10 min in VS with sucrose drastically decreased normal morphology (D.0: 33.8%; D.1: 0.7%; D.7: 0%). Substituting MEM for DPBS further improved morphology in tissue exposed to VS for 10 min both with sucrose (D.0: 49.0%; D.1: 22.9%; D.7: 22.2%) and without sucrose (D.0: 32.7%; D.1: 21.6%; D.7: 3.7%). Decreasing the exposure temperature from RT to 4°C improved follicular morphology for tissue exposed to VS with sucrose for 10 min (D.0: 58.2%; D.1: 37.8%; D.7: 1.6%), but exposure to VS without sucrose for 10 min at 4°C resulted in the best overall follicular morphology (D.0: 51.4%; D.1: 31.8%; D.7: 24.2%). This last treatment group was the only one to have percentages of normal follicles not significantly different from the fresh control at all time points (P ≥ 0.05). In conclusion, the optimal feline ovarian tissue vitrification protocol included exposure to ES and VS for 10 min each at 4°C, using MEM as the base medium. This protocol provides a solid foundation on which to optimize ovarian tissue cryopreservation in the domestic cat and to investigate the molecular effects of vitrification.