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Evaluation of follicular and tissue viability in domestic dog ovaries cryopreserved by vitrification.

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Introduction. Oocyte from domestic dogs and Mexican wolves maintain viability after vitrification and warming [1], but to our knowledge no reports have been published regarding ovarian tissue viability after cryopreservation. Studies have used domestic dogs as an experimental model. Our objective was to evaluate follicular and tissue viability in dog ovaries cryopreserved by vitrification.

Material and Methods. Ovaries were obtained from adult bitches after routine ovariohysterectomy in a local veterinary clinic. Only ovaries without CL were used, and both ovaries from the same dog were used; however, the cortex of each ovary was removed using a different technique. One ovary was cut in half and medullar content was gently removed using a scalpel (Group A). A template was placed on top of the other ovary, covered so that the template was gently pressed, and a scalpel was used to slice the cortex (Group B) [2]. In both methods, the slices were placed in HEPES at room temperature until 4 slices from each ovary were cut and ready to be processed. One slice from each ovary was processed for histological evaluation. Remaining samples were vitrified as previously described [3]. Briefly, samples were placed in a Falcon tube containing 10mL of equilibration solution for 25 minutes and then transferred to another Falcon tube, containing 10mL of vitrification solution for 15 minutes. Each fragment was placed on aluminum foil for better cold conduction, immersed directly into liquid nitrogen and placed in labelled cryovials. For thawing, slices were removed from the cryovials, immersed in thawing solution for 3 min and then immersed in a 0.5M Sucrose solution for 5 minutes, both at 37°C [3]. One slide from each method was processed for histological evaluation, and the remaining slices were cut into smaller pieces, placed in a 96-well plate containing 200µL of growth media, and maintained in an incubator (5% CO2 at 38.5°C). Every other day 100 µL of media was removed and the same volume of fresh media was replaced. After 7 days of culture, samples were processed for histological evaluation. Follicular viability was evaluated according to follicular morphology. Follicles with cytoplasm damage, picnotic nucleus or both were considered non-viable. Tissue viability was evaluated by arterial and venous damage. For statistical analysis Fisher’s Exact Test was used, with 2x2 contingency tables. For group comparison we used non-parametric Fisher’s Kruskal-Wallis. Data were considered different when p < 0.05.

Results. Fresh samples from Group A had better viability (p=0.0002) than frozen-thawed and incubated (p=0.013) samples. However, there was no difference between frozen-thawed and incubated samples or in follicle integrity among groups. In Group B fresh samples had better viability than incubated samples (p=0.0002), but there was no difference between fresh and frozen-thawed samples. Frozen-thawed samples were more viable than incubated samples (p=0.0311). There was a difference between fresh and incubated regarding follicle integrity (p=0.0159). Group B frozen-thawed samples had better preservation than group A (p=0.0373).

Conclusions. There is a loss of viability in dog ovaries after cryopreservation. Using the template method for preparing ovarian tissue [2] for freezing resulted in better post-thaw ovarian viability.

References