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Responsiveness of intraovarian dog follicles in vitro to epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) depends on donor age
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The influence of growth factors on in vitro survival and growth of ovarian follicles has been shown to be dependent on the gonad donor’s age, at least in the cat. This study investigated the influence of EGF and VEGF on in vitro development of follicles within ovarian cortices recovered from prepubertal versus adult dogs. Ovaries were obtained from prepubertal (age, 4-6 mo, n = 6) and adult (age, 10 mo-4 yr, n = 6) dogs undergoing ovariohysterectomy at veterinary clinics, and pieces cultured for 3 or 7 d in αMEM plus EGF (0 or 10 ng/ml) and VEGF (0, 0.1, or 1 ng/ml). At the end of each incubation, cortices were fixed and processed histologically to evaluate follicle morphology, density, size, distribution, and apoptosis (via TUNEL assay). Fresh cortices obtained from the same dogs served as a ‘non-cultured control’ (NCC) and were evaluated similarly. Data were analyzed using a General Linear Model followed by a least significant difference test. The response of ovarian follicles to growth factor supplementation differed between tissue from prepubertal and adult donors. For prepubertal dogs, proportions of structurally normal follicles in cortices cultured for 3 d in low VEGF (0.1 ng/ml) and EGF (10 ng/ml) were comparable (P > 0.05) to the NCC (mean ± SEM, 54.3 ± 6.5 and 55.5 ± 7.3 vs. 74.8 ± 5.9%, respectively). However, percentages of normal follicles in all culture groups were lower (P < 0.05) than in the NCC after 7 d, with no differences among treatments. Follicle density declined (P < 0.05) in all cultured groups even after 3 d compared to the NCC. Primary and secondary stage follicles appeared to be particularly sensitive to in vitro culture, with proportions of these stages decreasing (P < 0.05) by 7 d incubation regardless of culture conditions. Primary follicle diameter in all cortices cultured for 7 d, except in low VEGF was smaller than NCC. TUNEL analysis demonstrated a sharp increase (P < 0.05) in percentages of apoptotic follicles in all treatment groups compared to the NCC. For adult donors, percentages of structurally-normal follicles decreased (P < 0.05) in all culture treatments at 3 and 7 d of culture compared to the NCC. However, among the growth factor treatments, percentages of normal follicles in cortices cultured in low VEGF and the two VEGF (0.1, 1 ng/ml) and EGF (10 ng/ml) combinations were higher (P < 0.05) than in the absence of growth factors or with EGF alone. Similar to prepubertal dogs, culture reduced (P < 0.05) the density of developing follicles (transition to secondary). Follicle diameter was similar (P > 0.05) to NCC, except for primary and secondary follicles in cortices cultured for 3 d in low VEGF. Unlike prepubertal tissue, TUNEL analysis revealed that high VEGF (1 ng/ml) treatment protected follicles against apoptosis, with the proportion of apoptotic follicles at 7 d (42.2 ± 8.9%) being comparable (P > 0.05) to the NCC (14.2 ± 7.3%). In summary, findings demonstrate that dog ovarian cortex is more highly susceptible than other species like the cat, as percentage of structurally normal follicle after 7 d of culture was lower than cat normal follicle after 14 d of culture reported previously (46.3 ± 7.0 vs. 68.0 ± 5.2%, respectively). Furthermore, the response to growth factor supplementation appeared to depend on donor’s age. Specifically, low VEGF concentration and EGF were beneficial in sustaining follicle structure in prepubertal tissues for up to 3 d of in vitro culture, whereas high VEGF in combination with EGF protected follicles against apoptosis in adult cortices.