

Effect of Z-VAD-FMK on in Vitro Viability of Dog Follicles

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Abstract : Mammalian ovaries contain thousands of follicles that eventually degenerate or die after culture in vitro. Caspase-3 is a key enzyme that regulating cell death. Our objective was to examine the influence of anti-apoptotic drug Z-VAD-FMK (pan-caspase inhibitor) on in vitro viability of dog follicles within the ovarian cortex. Ovaries were obtained from prepubertal (age, 2.5-6 months) and adult (age, 8 months to 2 years) bitches and ovarian cortical fragments were recovered. The cortices were then incubated on 1.5% (w/v) agarose gel blocks within a 24-wells culture plate (three cortical pieces/well) containing Minimum Essential Medium Eagle - Alpha Modification (Alpha MEM) supplemented with 4.2 µg/ml insulin, 3.8 µg/ml transferrin, 5 ng/ml selenium, 2 mM L-glutamine, 100 µg/mL of penicillin G sodium, 100 µg/mL of streptomycin sulfate, 0.05 mM ascorbic acid, 10 ng/mL of FSH and 0.1% (w/v) polyvinyl alcohol in humidified atmosphere of 5% CO₂ and 5% O₂. The cortices were divided in six treatment groups: 1) 10 ng/mL EGF (EGF V0); 2) 10 ng/mL of EGF plus 1 mM Z-VAD-FMK (EGF V1); 3) 10 ng/mL of EGF and 10 mM Z-VAD-FMK (EGF V10); 4) 1 mM Z-VAD-FMK; 5) 10 mM Z-VAD-FMK and (6) no EGF and Z-VAD-FMK supplementation. Ovarian follicles within the tissues were processed for histology and assessed for follicle density, viability (based on morphology) and diameter immediately after collection (Control) or after 3 or 7 days of in vitro incubation. Comparison among fresh and culture treatment group was performed using ANOVA test. There were no differences ($P > 0.05$) in follicle density and viability among different culture treatments. However, there were differences in this parameter between culture days. Specifically, culturing tissue for 7 days resulted in significant reduction in follicle viability and density, regardless of treatments. We found a difference in size between culture days when these follicles were cultured using 10 mM Z-VAD-FMK or 10 ng/mL EGF (EGF V0). In sum, the finding demonstrated that Z-VAD-FMK at the dosage used in the present study does not provide the protective effect to ovarian tissue during in vitro culture. Future studies should explore different Z-VAD-FMK dosages or other anti-apoptotic agent, such as surviving in protecting ovarian follicles against cell death.

Keywords : anti apoptotic drug, bitches, follicles, Z-VAD-FMK

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