Proceedings of the 8th International Symposium on Canine and Feline Reproduction

ISCFR

June 22-25, 2016
Paris, France

In a joint meeting with the XIX EVSSAR Congress

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Estradiol production by cyclical and gestational canine luteal cells in cell culture
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The similarity between the luteal phase of cyclic and pregnant bitches is one of the most intriguing aspects in the reproductive physiology of this species yet differences in luteal cell physiology exist. In order to investigate estrogen production by cyclical and gestational luteal cells cultured in vitro, we performed ovariohysterectomy in bitches on cyclic (n=20, control group) and gestational (n=20, experimental group) diestrus at different timepoints (10, 20, 40 and 60 days after the pre-ovulatory LH surge, being n=10 animals per timepoint (5 control and 5 experimental). The corpora lutea (CL) of control (CG) and experimental (EG) group were isolated and enzymatically digested in solution containing DMEM High glucose (D5671; Sigma-Aldrich, Germany) and collagenase type 1 (1 mg/ml; C0130; Sigma Aldrich, USA) for one hour at 37° C. The resulting contents were filtered (70 µm filter), centrifuged for three times (1500, 1200 and 900 rpm, for 10 minutes at 20°C respectively). The final pellet was resuspended in DMEM supplemented with antibiotics (penicillin and streptomycin), antifungal (amphotericin), α- glutamine 2% (M4655; Sigma Aldrich, USA) and bovine fetal serum 5% (F1051; Sigma Aldrich, Germany). These solution containing luteal cells were distributed on 24 well plates (20 x 10⁴ cells/ well) and incubated at 37 ° C under humidified atmosphere (5% CO2). A plate containing only culture medium without cells served as control solution at every moments of collection. Culture medium containing luteal cells of EG and CG were collected after 36 (stage 1), 48 (stage 2) and 60 (stage 3) hours of culture and stored at -20°C. Estradiol production was measured by radioimmunoassay methodology and reading on gamma Wizard 2 counter (Perkin Elmer, Waltham, Massachusetts, USA) after validation of the medium using matrix integrates (R squared=0.962, and p<0.0001). The statistical analysis was performed using ANOVA (p<0.05) in SAS PROC GLM. It was observed that estradiol production by luteal cells in culture did not differ between CLs with age 10, 20 and 60 days after LH pre-ovulatory surge between both CG and EG in all analyzed moments (p>0.05). However, CL of CG with 40 days (after LH pre-ovulatory surge) presented a higher production (p=0.042) of estradiol (342 pg/ml) when compared to CLs of EG with the same age (51.51 pg/ml) in all moments analyzed. This estradiol production by CG was about nine times higher than EG. The differentiated production of estradiol by cyclical CL in cultivation is an event never reported. Previous studies regarding estradiol serum production in vivo are not fully conclusive, since some authors obtained variation in the production of E2 between pregnant and non pregnant animal, and others not 1. This increased production of estradiol by cyclical luteal cells may be an indication that perhaps the role of E2 in luteotrophic complex non-pregnant animals is of great importance and differentiated in relation to pregnant animals.