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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Steroid hormones in canine X-linked muscular dystrophy using stable isotope dilution liquid chromatography coupled with tandem mass spectrometry

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The Golden Retriever dog is the ideal animal model for preclinical studies of progressive muscular dystrophy, known as Duchenne muscular dystrophy (DMD); this model is referred to as “Golden Retriever muscular dystrophy (GRMD)” [1]. The aim of this study was to determine steroid hormone concentration profiles in healthy Golden Retriever dogs (control group - CtGR) versus GRMD – gene carrier (CaGR) and affected female dogs (AfGR). Therefore, a sensitive and specific analytical method was developed and validated to determine the estradiol (E2), progesterone (P4), cortisol, and testosterone levels in the canine serum by isotope dilution liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) as previously describe [2]. This multihormone assay was then applied to 153 plasma samples collected from 23 female golden retriever dogs throughout their entire estrous cycle (i.e., anestrous, proestrus, estrus, and diestrous); estrous phase was confirmed by serial vaginal cytology. We observed specific patterns in the concentrations of all hormones that are characteristic of and distinguish the 3 groups across phases of the estrous cycle. It showed statistically significant differences between all groups (P < 0.05), except for P4 and E2 in the comparison between the CtGR and CaGR groups. The main variable in the separation of the first dimension is E2; was observed for the AfGR, mean serum levels between 10 to 20 times higher concentration than observed in samples from CtGR and CaGR; irrespective of the phase of the estrous cycle, the mean concentration of E2 exceeded 1,300pg mL-1 in this group, was different (P < 0.0001) compared with the CaGR, and demonstrated discrete variation through the estrous period. Average concentrations of serum cortisol for CaGR and AfGR ranged between 1000 and 2000pg mL-1 in all estrous periods, but concentrations were different (P < 0.05) between groups during the diestruis. In anestrus and proestrus, P4 concentrations were similar between groups; but, during estrus and diestrus, concentrations of all quantified hormones were significantly different between the CaGR and AfGR groups. Similar trends were observed for testosterone concentrations during estrus, which were significantly different between the CaGR and AfGR groups (P < 0.05). These findings stimulated an important discussion about the correlation of steroid hormones concentrations against clinical signs and development of the pathology of these animals. Data obtained bring new opportunities for hormonal behavior studies in dystrophinopathies and that may affect the quality of life of DMD patients.
