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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Leptin and IGF1 in the adult and prepubertal canine testis
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Leptin (Lep) and insulin-like growth factor 1 (IGF1) have been implicated in the autocrine-paracrine regulation of testicular function in several species by influencing germ cell proliferation and differentiation as well as steroidogenesis. Lep generally showed an inhibitory and IGF1 a stimulatory effect on testosterone production.\textsuperscript{1,2} However, these functions may differ between adult and immature animals.\textsuperscript{1,2} So far, in dogs, only the relationship between testicular tumors and the IGF system has been investigated.\textsuperscript{3} The aim of our study was to elucidate the role of Lep and IGF1 as possible autocrine/paracrine regulators of normal testicular function in sexually mature and prepubertal animals.

Testes were collected during routine neutering from healthy, sexually mature dogs without testicular pathology (n=7, age 2-5.5 years, five mixed breeds and two English greyhounds, body weight 12-26 kg) and from prepubertal dogs (n=7, 8-week-old Australian Cobberdogs, body weight 4.1-6.6 kg). The pups came from 2 different litters (3 and 4 dogs from each litter). For gene expression, semi-quantitative real-time (TaqMan) PCR was performed with canine specific primers and probes for Lep, IGF1 and their receptors (LepR and IGF1R, respectively). GAPDH and cyclophyllin A were used as reference genes. For immunohistochemistry, tissues were fixed in 10% neutral phosphate-buffered formalin and an indirect immunoperoxidase method was applied for protein detection. Statistical analysis was carried out with Student’s t-test. Lep and LepR mRNA expression were similar between prepubertal animals and adult dogs (P≥0.069). Testicular IGF1 as well as IGF1R gene expression was significantly higher in 8-week-old puppies than in sexually mature dogs. In adult animals, Lep immunoreactivity was detected in germ cells with strongest signals in spermatocytes and spermatids. Leydig cells showed sporadic, weak staining. In prepubertal animals, intense positive reaction of Lep was present in Leydig and Sertoli cells. IGF1 and IGF1R were detected in spermatogonia and sporadically in Sertoli cells in the mature testis. IGF1 signals in Leydig cells seemed stronger than IGF1R, which showed sporadic, weak staining. In the puppies, IGF1 and IGF1R staining was noted in Leydig cells and sporadically in gonocytes. Sertoli cells showed weak immunoreactivity for IGF1. Blood vessel media also stained strongly for IGF1R in both groups. In conclusion, the Lep and IGF system may have distinct roles in testicular function of adult and prepubertal animals. In sexually mature dogs, Lep and IGF1 may support spermatogenesis at different steps, and IGF1 may also mediate Leydig cell steroidogenesis. In the immature testis, they may promote proliferation of Sertoli cells, Leydig cells and gonocytes.