Gene expression of retinoic acid receptors in post-natal canine testis
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Canine spermatogenesis is a complex and tightly controlled cell differentiation process, producing mature spermatozoa from spermatogonia stem cells. The role of dietary vitamin A and retinoid acid signaling for normal testicular development and spermatogenesis has been recognized for many years. Retinoic acid (RA), which is the active metabolite of vitamin A, binds to and activates nuclear RA receptors (RARA, RARB, and RARG) for the desired downstream functions in target tissues. Although there is little published research concerning testis-specific RARs, a few previous gene ablation studies in mice suggested that RA signals through RARA either in the germ cells or in the supporting cells are necessary for normal testicular function and thus for spermatogenesis. Hence, the objective of this study was to elucidate the gene expression of three major isomers of RARs (RARA, RARB and RARG) in young, peripubertal and adult canine testis and to identify their protein localization in adult testis.

The gene expression of RARA, RARB and RARG was analyzed in young (N = 8), peripubertal (n = 6) and adult (n = 8) testes of mixed-breed, medium-sized dogs using real time polymerase chain amplification technique. A non-specific SYBR chemistry approach was employed to detect target DNA sequences using specific set of primers for RARA, RARB and RARG. Relative quantification of RARB and RARG gene expression to RARA gene expression in three aged groups was calculated following normalization with the endogenous control, canine beta actin. Related fold changes were analyzed by ANOVA using 2ΔΔCT values to ascertain statistical significance of any differences in gene expression. Protein localization in adult testes was visualized using two-step immunohistochemistry. These receptors were labeled with primary antibodies on frozen adult testes sections. The ligand-primary antibody complex was then tagged with FITC-conjugated secondary antibodies. Images were captured using a white light laser confocal microscope.

RARA gene expression was highly abundant in young, peripubertal and adult testis compared to the mRNA expressions of RARB and RARG (p < 0.05). On immunohistochemistry images, RARA protein localization was more intense when compared to RARB and RARG protein localization. Together, gene expression data and protein localization images from this study suggest that RARA plays a critical role in RA signal transduction for the normal function of spermatogenesis in the canine testis.

Keywords: RAR, RA signaling, spermatogenesis, testis, dog