Immunolocalization of matrix metalloproteinase-2 and membrane type 1 matrix metalloproteinase in canine testes
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Matrix metalloproteinase-2 (MMP-2) activity is important for spermatogenesis. Membrane type 1 (MT1)-MMP controls proMMP2-activation in Sertoli cells. This study was designed to demonstrate immunolocalization of MMP-2 and MT1-MMP in canine testes. It was postulated that MT1-MMP and MMP-2 are localized in Sertoli cells and germ cells at various stages of spermatogenesis.

Testes from healthy dogs (N = 12; > 2 years of age) were sectioned and fixed in modified Davidson’s solution for at least 24 h. Immunohistochemistry was performed and 10 fields per specimen were evaluated (400 ×) in a semi-quantitative manner for the analyses of immuno-reactivity of MMP-2 and MT1-MMP staining. Slides were scored as the estimated area in proportion (P; scale 0 to 4) and intensity of positive-staining cells (I; scale 0 to 4). The total score (TS) was the sum of P and I. For determination of MT1-MMP and MMP-2 gene expression, testicular samples were homogenized, purified, total RNA extracted, complementary DNA synthesized and PCR was performed.

Cytosol staining of MT1-MMP showed a significant distribution (TS=8) in Sertoli cells and spermatogonia, while no staining of MT1-MMP (TS=0) was observed in spermatocytes, spermatids and elongated spermatids. Cytosol staining of MMP-2 was concentrated mainly (TS=8) in the perinuclear region of round spermatids, representing the region of developing Golgi organelles. Interestingly, MMP-2 was located at the tips of elongated spermatids (TS=8) while spermatocytes and spermatogonia showed negative staining (TS=0). Both amplified amplicons of MT1-MMP and MMP-2 were detected by PCR and were observed on agarose gel electrophoresis. Similar results were found in specimens from all dogs.

In conclusion, this is the first report demonstrating the presence of MT1-MMP in Sertoli cells and spermatogonia, and MMP-2 in round and elongated spermatids in dogs.

Keywords: MT1-MMP; MMP-2, Sertoli cells, acrosome development, canine testis