Post-natal expression of cytochrome P450, family 26 genes in canine testis
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Cytochrome P450 (CYP) enzymes are a diverse group of catalysts that consist of several members in humans and animals. Cytochrome P450 26 class is involved in the metabolism of retinoic acid (RA), rendering this classical ligand inactive through oxidation. Spermatogenic or oogenic fate is dictated by the signals from the gonadal environment, in addition to the genetic mechanisms of sex determination. Gonadal environmental stimuli are primarily mediated by the selective exposure of RA ligand. Retinoic acid, therefore, plays a critical role in germ cell development. Cytochrome P450 26B1 degrades RA in the embryonic testis, preventing STRA8 expression, thereby delaying meiosis. Cytochrome P450 26B1 is thus required for the maintenance of the undifferentiated state of male germ cells during embryonic development, inducing arrest in the G0 phase of the cell cycle and preventing meiotic entry. Cytochrome P450 26B1 is considered a major catabolizing enzyme in embryonic and adult testes. However, CYP26A1, CYP26B1 and CYP26C1 efficiently metabolize all-trans-RA to polar aqueous soluble metabolites. Therefore, we investigated the expression pattern of CYP26A1, CYP26B1 and CYP26C1 in canine testes. The objective of this study was to elucidate the gene expression of these enzymes in young, peripubertal and adult dog testes and to substantiate the gene expression pattern by protein localization of these enzymes in adult testis.

Gene expression patterns of CYP26A1, CYP26B1 and CYP26C1 were studied in young (N = 8), peripubertal (N = 6) and adult (N = 8) testes of mixed, medium-sized breeds using a real time polymerase chain amplification technique. SYBR green chemistry was employed, relative changes of gene expression in the young animal were calculated after normalization with the endogenous control, canine beta actin and the related fold changes were analyzed by ANOVA using 2 delta delta Ct values to ascertain statistical significance of any differences in gene expression. Protein localizations were examined using immunohistochemistry. These enzymes were tagged 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.

Cytochrome P450 26B1 expressions were more abundant in young, peripubertal testes and adult testes compared to the mRNA expressions of CYP26A1 and CYP26C1. CYP26B1 expression was significantly lower at the peripubertal age. This finding implies that CYP26B1 is the major metabolizing enzyme in canine testis that controls the testicular RA level and spermatogenesis. Interestingly, lower expression of CYP26B1 at the peripubertal age supports its critical role in initiation of meiosis at puberty. Higher expressions at adult age suggest that other putative regulators also participate in maintaining the RA level in adult testis (in addition to CYP26B1) to ensure the continuous production of sperm. On immunohistochemistry images, CYP26B1 was mainly confined to the peritubular epithelial cells and interstitial area. Lower target signals were also observed for CYP26A1 and CYP26C1. The protein localization supports the functionality of the enzyme in the adult testis.

Keywords: Dog, testis, spermatogenesis, CYP26 family