Exogenous and endogenous retinoic acid modulates meiosis-associated genes expression in canine testis, an in-vitro model
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Pharmacological approaches to the control of spermatogenesis in dogs are required for the long-term solution to the population explosion amongst dogs. The objectives were 1) to investigate the regulation of meiosis-associated and male germ cell-related genes (STRA8, SYCP3, DMC1, DMRT1 and DAZL) following exogenous administration of retinoic acid (RA) and after modulation of endogenous RA by a CYP26B1 inhibitor in an in-vitro canine testis model; and 2) to compare the effect of increased endogenous RA by inhibition of the enzyme CYP26B1 to the consequences of direct administration of exogenous RA. Testes of five healthy, medium-sized dogs of mixed breed were used for the organotypic cultures. The dogs were about seven months old. Testes cultures were carried out following a previously described procedure. All trans-RA at 2μM final concentration, CY26B1 inhibitor R115866 at 1μM final concentration and the control, dimethyl sulphoxide (DMSO) were administered to the testes cultures and the cultures were maintained for 24 h. Subsequent to these in-vitro treatments, real time PCR was performed to analyze the meiosis-associated genes expressions in the testis by ANOVA using 2^-∆∆Ct values to ascertain statistical significance of any differences in gene expressions (see figure).

Genes STRA8, DAZL and DMRT1 were significantly up-regulated as a result of the direct and indirect increase of RA in the testis, after the exogenous administration of all trans RA and CYP26B1 inhibitor. Up-regulation of STRA8 was very prominent compared to DAZL and DMRT and the drastic up-regulation of STRA8 was also observed with CYP26B1 inhibitor. Since DAZL encodes a germ cell-specific RNA binding protein, required for the induction of STRA8 and initiation of meiosis, we might see the expression differences temporally with the stage of spermatogenesis. DMRT1 is a unique gonad and stage specific transcription factor, directly activates STRA8 and has a temporal influence on its expression. No significant differences were found with the early meiotic markers, SYCP3 and DMC1 with RA, CYP26B1 inhibitor and vehicle treatments. In conclusion, pharmacological intervention of canine spermatogenesis pertinent to RA signaling is plausible and the effect of modulation of spermatogenesis differs upon the types of pharmacological targets such as agonists, antagonists and inhibitors. Temporal and spatial influences on spermatogenesis should also be considered.

Keywords: Dog, spermatogenesis, meiosis-associated gene, retinoic acid, CYP 26B1

![Graph showing mRNA expression of STRA8, SYCP3, DAZL, DMRT1, DMC1 with different treatments](image-url)