Does cell enrichment influence the gene expression of Sertoli cells, Leydig cells and spermatogonia cells specific markers in canine testis?
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Sertoli cells, Leydig cells and spermatogonia actively proliferate and differentiate after birth in all mammalian species and the enrichment of these cells are considered stable in adulthood. These cells' enrichment were well studied in mice. At day 35, complete spermatogenesis is observed in mice. Spermatogonia increase in number until the day 21 and Sertoli and Leydig cells amplify in numbers during first three weeks. These cell numbers are essentially constant from day 35 in mice. Cell enrichment occurs in the canine testis until puberty. Several genes are associated to testicular function and spermatogenesis. Our hypothesis was that spermatogenesis-associated genes expressions are not enhanced with the mere increase of these cells numbers. To accomplish the objective, we investigated cell specific markers such as FSHR and AMH (Sertoli cell specific), LHR and INSL3 (Leydig cell specific) and THY1 and CDH1 (spermatogonia specific) in immature and mature canine testis.

Testes of four biological replicates of immature and mature groups were processed to elucidate cell specific markers. Complementary DNA was synthesized from 1 µg of total RNA. Real-time PCR was performed using specific primers. Threshold cycles (C_T) were used to analyze mRNA expressions. Fold comparisons were made between immature and mature testes. The normalized threshold cycles data were analyzed by ANOVA, using 2^ΔΔCT to ascertain statistical significance of any differences in mRNA expressions.

Gene expressions of these cells' specific molecular markers were down-regulated (P<0.05) in adult canine testis in our investigation. Albeit, there is obvious enrichment of these cells from the immature dog testis to the mature dog testis, the cells' specific markers were not enriched from immature testes to mature testes in this study. The results supports that the gene expressions do not directly correlate with the mere increase of the cell numbers during post-natal development, but changes in gene expressions warrant functional significance.

Key words: Spermatogonia, Sertoli cells, Leydig cells, testis, dog