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Expression of Cyclooxygenase-2, Prostaglandin-F2α-synthase and receptor during recrudescence of spermatogenesis following downregulation using a slow release GnRH agonist implant in the dog – preliminary results

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Enzymes involved in the prostaglandin pathway, like cyclooxygenases (PTGS) and Prostaglandin-F2α synthase (PGFS), are considered to play an important role for male fertility. The observations that both enzymes had been shown to be expressed in Leydig cells (LC)¹ and that arachidonic acid has a stimulatory effect on LC-derived testosterone production, resulted in the hypothesis of both factors being involved in regulation of Steroid acute regulatory (StAR) protein. Application of a slow release GnRH agonist implant in the dog results in downregulation of testicular endocrine function – indicated by basal testosterone concentrations - including downregulation of the whole steroidogenic apparatus with StAR being the “bottle neck”.² ³ Consequently, the model of the downregulated canine testis and its recrudescence seems to be valuable to study if PTGS2, PGFS and Prostaglandin-F2α-receptor (PTGFR) are differentially expressed during downregulation and subsequent recrudescence possibly indicating a putative role for steroidogenesis or autocrine regulation of LC. To induce downregulation, sexually mature, healthy male Beagle dogs were treated with a GnRH-agonist implant containing 18.5 mg azagly-nafarelin. After 5 months the implant was removed at downregulation and 3-4 dogs were castrated at 3-week intervals (week 0, 3, 6, 9, 12 and 24). 5 untreated adult (CG) and 3 untreated juvenile dogs (JG) served as controls. To study the state of downregulation in more detail, additionally 3 dogs were treated for 5 months with an implant containing buserelin (PG) and castrated thereafter. Conventional RT-PCR and semiquantitative RT-qPCR were performed using primers against the canine PTGS2, PGFS and PTGFR. To test for the protein localization of PTGS2, immunohistochemistry (IHC) was performed using a PTGS-2 Monoclonal Antibody. On the mRNA level, expression of PTGS2, PGFS and PTGFR was proven for all the samples, but no significant differences could be identified between downregulated samples and different states of testicular recrudescence. IHC revealed specific immunopositive staining against PTGS in the cytoplasm of Leydig cells (LC) of all samples. Compared to the other samples, the staining intensity was subjectively most pronounced in the juvenile dogs (JG). Additionally, specific immunopositive staining against PTGS2 was also found in Sertoli cells (SC) within the tubules in all samples. The present study confirms the presence of PTGS2, PGFS und PTGFR in the canine testis on mRNA and PTGS2 on protein level. Different to our hypothesis, neither PTGS2 nor PGFS/PTGFR were differently expressed on mRNA level using whole testicular homogenate. Investigating pure interstitial tissue obtained by laser-assisted cell picking might reveal further insights into the mRNA expression, as well as objective computer-assisted evaluation of the PTGS IHC staining might shed light into a possible posttranscriptional regulation.

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