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Prostaglandin synthesis enzymes gene transcription in bitches with endometritis

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Endometritis is a major cause of infertility in many domestic species. However, until now the pathogenesis of the endometritis in the bitch is unclear\textsuperscript{1}. In addition, molecular mechanisms regulating the uterine microenvironment during diestrus in the bitch have not been studied yet. The aim of this study was to evaluate the gene transcription pattern of prostaglandin (PG) synthesis enzymes (cyclooxygenase [COX2], PTGES-1 and PGFS) in the endometrium of bitches with or without endometritis. Thirty mixed breed bitches in diestrus, aged between 1-5 years, and weighing between 10 and 30 kg were used. The bitches were undergoing a thorough clinical and reproductive examination. The diestrus was determined based on the history provided by the owner and confirmed in each bitch based on ovarian structures, serum P\textsubscript{4} (SP) and vaginal cytology (VC). The experiment had the approval of the IACUC of FCV UNLP (40-4-14 B). Before the ovariohysterectomy (OVX) a VC and a blood sample were taken. All blood samples were centrifuged and stored at -20 °C until P\textsubscript{4} was measured by chemiluminescence immunoassay (Elecsys\textsuperscript{®}, Progesterone II; Roche, Mannheim, Germany). After OVX, uterine biopsy samples were collected from the middle part of both horns. Then, endometrial epithelium was collected using the cytobrush-method and mRNA analysis was performed by real-time RT-PCR. Ribosomal protein L27 gene was chosen as the housekeeping gene. Primers sequences were: COX2 (FW 5´-GTATGAGCACAGGTATTTGACATGA3´, RV 5´-AATTCGGTGTTGACGTTT-3´), PTGES-1 (FW 5´-CAGAGCCCACCGGAATGAA3´, RV 5´-GGAAGAAGACGAGGAAGTGCAT-3´) and PGFS/AKR1C3 (FW 5´-GCTAGAGCCTTCAACGAGA-3´; RV 5´-AGGCTGCTCAGAGTCCATG-3´). Data were analyzed with Mann-Whitney-Wilcoxon U-test using the SAS\textsuperscript{®} software. Uterine condition was identified by endometrial biopsies (normal endometria [n=11; NE], acute endometritis [n=10; AE] and chronic endometritis [n=9; CE]). The COX2, PTGES-1 and PGFS/AKR1C3 mRNA expression in bitches with and without endometritis was similar (4.6±1.3 vs. 2.3±1.0, p<0.11; 4.6±1.8 vs. 4.5±3.1, p<0.20; 21.4±8.8 vs. 13.1±8.4, p<0.45, respectively). Except for PGFS/AKR1C3, gene transcription of COX2 and PTGES-1 was significantly increased in AE compared with NE (16.1±8.4 vs. 13.1±8.4, p<0.29; 7.1±2.2 vs. 2.3±1.0, p<0.01; 6.6±3.0 vs. 4.5±3.1, p<0.10, respectively). In addition, except for PGFS/AKR1C3, gene transcription of COX2 and PTGES-1 was significantly increased in AE compared with CE (16.1±8.4 vs. 26.7±15.9, p<0.18; 7.1±2.2 vs. 1.9±0.8, p<0.01; 6.6±3.0 vs. 2.4±1.5, p<0.05, respectively). In contrast, no differences were found for COX2, PTGES-1 and PGFS/AKR1C3 mRNA expression in the samples of NE compared with CE (2.3±1.0 vs. 2.4±1.5, p<0.45; 4.5±3.1 vs. 4.5±3.1 vs. 2.4±1.5, p<0.46; 13.1±8.4 vs. 26.7±15.9, p<0.38, respectively). These findings are in agreement with the results obtained by Silva et al., who found higher expression of PG synthesis enzymes genes in pyometra compared to normal endometria in the bitch\textsuperscript{2}. The presented results are confirmatory and supportive for results by Silva et al., 2009\textsuperscript{2}.
