ABSTRACTS

ISCFR 2012

July 26-29, Whistler, Canada

7th International Symposium on Canine and Feline Reproduction

In a joint meeting with

EVSSAR 2012

15th Congress of the European Veterinary Society for Small Animal Reproduction

Editors: Gary England, Michelle Kutzler, Pierre Comizzoli, Wojciech Nizanski, Tom Rijsselaere and Patrick Concannon

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Molecular insights in the biosynthesis and degradation of canine placental prostaglandins: Expression and biological function of prostaglandin F2α-synthase (PGFS) and 15-prostaglandin dehydrogenase (15PGDH)

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OBJECTIVES AND METHODS: Amongst many still not fully understood issues of the canine reproductive endocrinology, the origin of the PGF2α output that can be seen at the time of the prepartal luteolysis remains one of the most interesting questions [1]. Canine specific PGF2α-synthase (PGFS) sequence has been cloned and characterized for the first time in our laboratory [2]. Recently its placental expression and cellular localization have been shown, revealing the highest PGFS-mRNA expression during the post-implantation and mid-gestation stages of pregnancy [3]. Surprisingly, there was a prepartal decrease observed [3] which led us to speculate on possible regulatory mechanisms involved. These could include the post-transcriptional regulation of PGFS gene expression resulting in low peripheral PGF2α concentrations prior to prepartal luteolysis. On the other side the increased prepartal PGF2α output could be a result of an increased substrate turnover leading to the decreased PGF2α-mRNA levels at concomitantly increased expression of Cox2 [3]. However, due to the lack of a canine-specific and/or cross-reacting PGFS antibody these questions remained unanswered. In order to substantiate any functional conclusion, here, the expression of PGFS-was assessed at the protein level.

The biological availability of PGF2α can be regulated at the level of 15PGDH activity that catabolizes the reduction of PGF2α to its metabolite, the 15-ketodihydro-PGF2α (PGFM) [4]. In contrast to other species, there is no information available on the expression and regulation of 15PGDH during pregnancy and labour in the dog. To get some new insights in the possible regulatory mechanisms of the prostaglandin provision its spatio-temporal expression was qualified and quantified at the mRNA and protein level in canine tissues throughout pregnancy and at prepartal luteolysis. Therefore utero/placental compartments from bitches during the pre-implantation, post-implantation and mid-gestation period of pregnancy and during the prepartal luteolysis were used.

Canine-specific PGFS and 15PGDH antibodies were generated and the expression and cellular localization of PGFS and 15PGDH were investigated; western blot (WB) and immunohistochemistry (IHC) were applied. Additionally, in situ hybridization (ISH) and real time PCR were performed in order to better characterize the 15PGDH expression.

RESULTS: As revealed by WB analysis, the PGFS expression was strongly time-related with highest protein levels detected during post-implantation and at mid-gestation with a prepartal decrease observed thereafter. At the cellular level strong immunoreactive signals were observed in superficial uterine glands throughout gestation and in trophoblast cells at the feto-maternal contact zone during placentation. 15PGDH expression was strongly upregulated from the pre-implantation until mid-gestation, followed by a strong prepartal decrease. All changes in the 15PGDH expression were more pronounced at the protein level. 15PGDH was mainly localized in the epithelium of deep and superficial uterine glands. At the protein level only weak signals were observed in the fetal trophoblast cells. Biochemical properties of recombinant PGFS and 15PGDH proteins towards their specific substrates were confirmed after the transient expression of cloned canine PGFS and 15PGDH in heterologous systems.

CONCLUSIONS: The possible involvement of PGFS in the processes of implantation and placentation is suggested from the significantly increased expression of PGF during earlier stages of pregnancy. The increased availability of 15PGDH during this time, its co-localization with the expression of PGFS, and especially, the decreased expression during prepartal luteolysis suggest its role as a possible local regulator of placental prostaglandin provision.