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Expression of cyclooxygenase 1 and 2 in the canine corpus luteum during diestrus

Mariusz Pawel Kowalewski [a,*], Gerhard Schuler [a], Anja Taubert [b], Eva Engel [a], Bernd Hoffmann [a]

[a] *Clinic for Obstetrics, Gynecology and Andrology of Large and Small Animals, Justus-Liebig-University Giessen, Frankfurter Strasse 106, D-35392 Giessen, Germany*
[b] *Institute for Parasitology, Justus Liebig-University Giessen, Rudolf-Buchheim Strasse 2, 35392 Giessen, Germany*

Abstract

In the dog, unlike most other domestic animal species, corpus luteum (CL) life span is not affected by hysterectomy. Only in pregnant dogs, during the immediate prepartum decline of progesterone, does PGF2α clearly seem to act as an endogenous luteolytic agent. Whether endogenous PGF2α plays a role in the slow regression of the corpora lutea of the nonpregnant cycle is not known. To test for possible paracrine/autocrine effects of locally produced PGF2α, luteal expression of the key rate-limiting enzymes in prostaglandin biosynthesis, i.e. cyclooxygenase 1 and 2 (Cox1 and Cox2), was examined in dogs during diestrus, including the periods of CL formation, as well as early and late CL regression. Corpora lutea were collected by ovariohysterectomy from nonpregnant bitches 5, 15, 25, 35, 45 and 65 days after ovulation. On the mRNA-level, expression of Cox1 and Cox2 was tested by qualitative and quantitative, Real Time (Taq Man) RT-PCR; on the protein level, expression of Cox2 was studied by immunohistochemistry. The mRNA for Cox1 and Cox2 were detected at all stages of diestrus. Expression of Cox1 was lowest on Day 5 (ovulation = Day 0) and higher and nearly constant thereafter. Expression of Cox2-mRNA was distinctly cycle related and highest on Day 5; it decreased by Day 15 and remained constantly low until Day 65. Immunohistochemistry localized expression of Cox2 in the cytoplasm of luteal cells. Staining was restricted to Days 5 and 15, with stronger signals on Day 5. These data suggested that increased expression of Cox2 is associated with luteal growth and development and not luteal regression. Furthermore, the expression of Cox1 more likely reflected activity of a housekeeping gene.

Keywords: Cyclooxygenase 1 and 2; Cox; Corpus luteum; Luteolysis; Dog

1. Introduction

Domestic dogs are monoestrous and predominantly nonseasonal breeders, with several months of anestrus between active reproductive phases [1,2]. In contrast to other domestic animal species, luteal function is almost identical in pregnant and nonpregnant females, except that pregnant animals attain baseline concentrations of progesterone earlier, owing to the immediate prepartum decline of this hormone [3]. Progesterone production of follicular origin commences at the end of pro-estrus, reaching peripheral plasma concentrations of about 5 ng/mL at the time of ovulation [4]. Formation of the corpora lutea (CL) is indicated by the continuing increase of peripheral progesterone concentrations, which in general reach maximum values during the first 20 days of diestrus. This period is followed by a continuous decline of progesterone for 1–3 months,

* Corresponding author. Tel.: +49 641 99 38704; fax: +49 641 29328.
E-mail address: bernd.hoffmann@vetmed.uni-giessen.de (M.P. Kowalewski).

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eventually reaching concentrations <1 ng/mL, indicating onset of anestrus [5]. In contrast with polyestrus species (e.g. cattle, sheep, pigs), where cyclicity is maintained due to the periodic production of a luteolysin (PGF2α) of endometrial origin, canine corpora lutea exhibit their inherent life span during diestrus and normal ovarian function is observed following hysterectomy [6,7]. Regardless, there is continuing discussion regarding the role of PGF2α in respect to its involvement in the control of luteal function in the dog; this is largely related to two observations. Firstly, the immediate prepartum decline of progesterone in the dog coincides with an increase of PGF2α [8,9]. Across species, this prepartum release of PGF2α seems to be the major trigger for initiation of uterine contractility leading to onset of labor [10]. However, in some animal species maintaining pregnancy through luteal progesterone, e.g. cattle, this prepartum PGF2α increase was also responsible for induction of luteolysis [11,12] and application of a cyclooxygenase inhibitor prevented this effect [12]. Also in the dog, application of exogenous PGF2α during diestrus or pregnancy induced luteal regression [13,14]. The hypothesis of an active role of PGF2α was also supported by the observation that blocking prepartal PGF2α-synthesis by applying the cyclooxygenase inhibitor indomethacin extended the average duration of pregnancy. However, because high doses (>5 mg/kg BW/day) were needed, it was concluded that peripheral prostaglandin concentrations do not fully reflect PGF2α at the level of the Cl and that local PGF2α might be a major factor contributing to luteolysis [15]. This hypothesis was particularly supported from observations in nonpregnant dogs, where any role of PGF2α would not involve uterine PGF2α [6,7,16]. The mechanisms by which PGF2α causes luteolysis are still under discussion [17]. However, as shown in the cow [18], capillary endothelial cells express receptors for PGF2α, and PGF2α likely acts directly on these cells, leading to reduction in capillary density, thereby reducing blood flow; likewise apoptosis of capillary endothelial cells might be induced, possibly mediated by endothelin-1 [19].

Luteal prostaglandin production was reported in ruminants, primates and the rat [20–23] and both stimulating and inhibiting effects have been observed in the cow and rat [24,25]. Hence, in order to test for the capacity of the canine CL to produce prostaglandins, we examined expression of cyclooxygenase (Cox, prostaglandin G/H synthase; PGHS), a key rate-limiting enzyme in prostaglandin biosynthesis, in CL obtained during CL formation and during early and late luteal regression. Cyclooxygenase has two sequential catalytic activities, one that catalyzes the conversion of arachidonic acid to PGG_2 and a peroxidase activity that produces PGH_2, a common precursor for the synthesis of all PGs [26]. At least two isoforms of Cox exist, Cox1 and Cox2. Cox1 is characterized by a constitutive expression in many tissues and is thought to be responsible for the synthesis of prostanoids necessary for physiological functions. Cox2 is generally undetectable in most tissues, but can be induced in response to inflammatory reactions, growth factors and tumor promoters; furthermore, gonadotropins modulate the expression of Cox2 [27]. Thus, in the present study expression of both enzymes, Cox1 and Cox2, was assessed.

2. Materials and methods

2.1. Animals and collection of tissue

Healthy and sexually mature Beagle bitches were assigned to the experiment. Ovarian function was monitored by determining progesterone [7] in 2–3-day intervals; according to Concannon et al. [3], the day of ovulation (Day 0) was defined as the day when plasma progesterone concentration reached 5 ng/mL. Groups of five bitches each were ovariohysterectomized on Days 5, 15, 35, 45 and 65 after ovulation except that six bitches were done on Day 25. The CL were separated from the surrounding ovarian tissue. For preservation of RNA, the CL were initially embedded in Tissue tec® O.C.T. (Vogel, Giessen) and stored at −80 °C. For handling reasons, this procedure was replaced during the course of the experiments by incubating the CL over night in RNAlater® (Ambion Biotechnologie GmbH, Wiesbaden), an aqueous reagent for stabilization of cellular RNA (ratio mass:volume = 1:6). Final storage was again at −80 °C until further use.

For immunohistochemistry (IHC), tissue samples were fixed for 24 h in 10% neutral phosphate buffered formalin. After washing in phosphate buffered saline (PBS) and subsequent dehydration in a graded ethanol series, tissue samples were embedded in a paraffin substitute (Histo-Comp, Vogel, Giessen).

2.2. Reverse transcription (RT)-polymerase chain reaction (PCR)

For CL embedded in Tissue tec® O.C.T., approximately 15 sections (approximately 15 μm thick) were