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Apoptosis in canine corpus luteum during spontaneous and prostaglandin-induced luteal regression

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Abstract

Spontaneous luteal regression and prostaglandin-induced luteolysis in bitches were evaluated by measuring the apoptotic index for DNA fragmentation and the relative level of Bax gene expression in ovaries removed from nine untreated nonpregnant bitches at selected times during diestrus and in nine pregnant bitches after 1 day of administering abortive doses of a PGF-analog gel formulation given intravaginally at selected times during gestation. Nonpregnant diestrus was divided into three periods (early, mid and late) based on vaginal cytology and plasma progesterone concentration. Pregnant bitches were treated with a PGF-analog gel at corresponding stages of pregnancy (early, mid and late) and evaluated by ultrasound. Another eight pregnant bitches were similarly studied and serum progesterone concentrations were determined after 1, 2, 3 or 4 days of PGF-analog gel. Corpora lutea obtained by ovariohysterectomy were analyzed for apoptotic internucleosomal DNA fragmentation relative to that in a control cell line (U937), using an apoptotic DNA ladder kit and gel electrophoresis and for relative expression of the pro-apoptotic Bax gene by RT-PCR and electrophoresis. In nonpregnant bitches, the DNA fragmentation apoptotic index was greater in late than in early diestrus ($P < 0.01$). The index after 1 day of PGF-analog gel was higher in early pregnant bitches than in early diestrus bitches ($P < 0.05$); it was highest in midpregnancy ($P < 0.05$). The degree of apoptosis was related to the number of times PGF-analog gel was administered. Bax mRNA was detected in the corpus luteum (CL) and Bax expression increased from early to middiestrus in nonpregnant subjects ($P < 0.05$). Potential elevation in Bax due to PGF-analog gel treatment in pregnancy was only significant in relation to normal diestrus during early pregnancy ($P < 0.01$). In conclusion, we inferred that the effects of endogenous or exogenous prostaglandin on CL life span in bitches involved increases in apoptotic activity and that increased apoptosis was implicated in normal luteal regression in nonpregnant bitches.

Keywords: Corpus luteum; Apoptosis; Prostaglandin; Pregnancy; Luteolysis

1. Introduction

In the bitch, luteal progesterone secretion persists for all but approximately the last day of the 65 days of pregnancy, and persists for a similar but more variable period (50–80 days) in the nonpregnant bitch [1]. Luteal structure and function, luteotrophic requirements and mechanisms of luteolysis and luteal regression have been studied extensively in several domestic animals species [2]. However, for dogs, there is limited information on the luteotrophic requirements for progesterone secretion at various stages of the cycle or of pregnancy and little is known about the process of slow, progressive luteal regression in nonpregnant bitches [1]. In pregnant bitches, the abrupt prepartum

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luteolysis is apparently the consequence of large amounts of $PGF_{2\alpha}$ released by the pregnant uterus shortly before parturition [1]. Both luteinizing hormone (LH) and prolactin (PRL) have been considered luteotrophic in dogs [1]. More recently, Hori et al. [3] suggested that maintenance of the mature CL is mainly a function of PRL alone.

Exogenous $PGF_{2\alpha}$ was luteolytic when administered frequently in relatively large amounts, compared to doses required in most other species. The luteotrophic hormone requirements of the early developing canine CL remains unclear; there is evidence that both LH and PRL are required for normal progesterone secretion, although there appears to be a degree of semi-autonomy [1,4]. The early developing CL is very resistant to the effects of exogenous $PGF_{2\alpha}$ or PRL-lowering doses of dopamine agonist [1,4]. After 30 days, $PGF_{2\alpha}$ treatments or suppression of LH or PRL activity can render progesterone concentrations nondetectable (or nearly so) [4]. The slow regression of the canine CL after 40 days typically lasts longer in the nonpregnant bitch, because it is not interrupted by the acute luteolysis associated with parturition in pregnant bitches [5,6].

Some reports show that in the bitch, as in other mammals, the physiological involution of the CL is associated with apoptosis [7–9], a highly conserved and genetically controlled cell death program that starts in response to a variety of signals and involves the activation of specific genes (e.g. Bax gene). It involves well organized morphological and biochemical processes, characterized by membrane blebbing, cell shrinkage and DNA fragmentation (with oligonucleosome formation and the appearance of the DNA ladder) and the formation of apoptotic bodies. The BAX protein is widely known as a pro-apoptotic Bcl-2 family member that, when overexpressed, can trigger apoptosis in multiple cell types. The Bcl-2 family, comprising both pro and anti-apoptotic proteins, is a central-regulator of the programmed cell death mechanism. In fact, these proteins, by inserting into organelle membranes, influence membrane permeability, serve as docking sites for other proteins and interact with other proteins, including other Bcl-2 family members [10]. Although DNA degradation is regarded as part of the cell death program process in apoptotic cells, the apoptotic events and their timing during luteal regression and/or acute luteolysis in dogs are still not well characterized, despite intense investigation [9].

In dogs, as in several other mammalian species, exogenous $PGF_{2\alpha}$ is considered to be a primary luteolytic agent that causes an abortifacient decline in luteal progesterone production, similar to what occurs naturally prior to parturition, although the exact mechanism by which $PGF_{2\alpha}$ elicits its response in the CL remains unclear [11]. The action of $PGF_{2\alpha}$ is likely mediated via specific $PGF_{2\alpha}$ receptors located on the plasma membrane of luteal cells, as observed in the CL of the ovarian cycle in domestic ruminants [12,13]. In the bitch, the efficacy of exogenous $PGF_{2\alpha}$ in inducing abortion is not only dose- and frequency-dependent, but is also related to the stage of pregnancy. Higher doses are needed to induce a complete luteolysis in early than in late pregnancy, despite the occurrence of more severe side effects [14]. The clinical side effects after $PGF_{2\alpha}$ administration could be related to the action of acetylcholine (Ach), through an increase in the concentration of the cholinergic neurotransmitter, probably via an inhibition of Ach-esterase activity, or through direct action on a cyclase receptor [15].

In preliminary studies, we observed that daily vaginal deposition of a PGF-analog gel formulation was able to induce abortion within 4–5 days of treatment during the midluteal phase [16,17]. In this paper, we studied the effects of the vaginal administration of a PGF-analog gel on CL regression in the pregnant bitch and on the onset of CL apoptosis, in comparison to apoptotic activity in similarly aged CL from nonpregnant bitches. The occurrence of programmed cell death was evaluated by visualizing the DNA ladder indicative of programmed cell death and by quantification of the expression of the early pro-apoptotic Bax gene.

2. Materials and methods

2.1. Animals, treatments and hormone assays

Corpora lutea were obtained by elective surgery. Twenty-six crossbred bitches enrolled in this study were healthy, aged 1.5–9 years and weighing 5–30 kg. Each bitch was classified regarding reproductive condition (pregnant diestrus versus nonpregnant diestrus) and stage of cycle (early, mid and late luteal phase), on the basis of their reproductive history, vaginal cytology (Harris–Shorr staining) and plasma progesterone concentrations measured by a radioimmunoassay with a sensitivity of 0.03 ng/mL (Diasorin I^{125} labeled progesterone, Diasorin, Milan, Italy). Pregnancy diagnosis was made by ultrasonography (Sonomed, Concept/MCV, 6.5 MHz MCX) and gestational sac, fetal membranes, fetal growth, movements and cardiac activity were evaluated.

To prepare the PGF-analog gel formulation, 60 g of triacetine (Sigma, Milan, Italy) were mixed with 37.5 mg of luprostiol (Prosolvin, Intervet, Milan, Italy) a synthetic analog of $PGF_{2\alpha}$ and the mixture added to 4 g of fumed