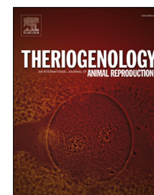
Theriogenology 113 (2018) 34–43

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com



Effect of multiple low-dose $PGF_{2}\alpha$ injections on the mature corpus luteum in non-pregnant bitches

Eyyup H. Ucar [a], Hayrettin Cetin [a], Mehmet O. Atli [b, *]

[a] Adnan Menderes University Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Aydin, Turkey
[b] Dicle University Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Diyarbakir, Turkey

ARTICLE INFO

Article history:
Received 4 October 2017
Received in revised form
23 January 2018
Accepted 23 January 2018

Keywords:
ProstaglandinF2 alpha
Lutelysis
Gene expressions
Corpus luteum
Bitches

ABSTRACT

This study investigated molecular regulation in the canine corpus luteums/corpora lutea (CL) following multiple low-dose prostaglandin F_2 alpha ($PGF_{2}\alpha$) injections in non-pregnant bitches around 30–35 days after ovulation. The CL were obtained by ovariohysterectomy 1 h after the last $PGF_{2}\alpha$ injection. The subjects were divided into the following groups: control (no $PGF_{2}\alpha$ injection, n = 4), one $PGF_{2}\alpha$ injection (injection at 0 h, 1PGF, n = 4), two $PGF_{2}\alpha$ injection (injection at 0 and 8 h, 2PGF, n = 4), and three $PGF_{2}\alpha$ injection (injection at 0, 8 and 24 h, 3PGF, n = 4). In the 1PGF group, the steady-state mRNA levels of an immediate early gene (*NR4A1*) and immune system-related genes (*MCP-1 and IL-8*) increased. *NR4A1* was localized in luteal and endothelial cells. In contrast, *MCP-1* was localized in the luteal tissue between the luteal and endothelial cells. *LHCGR, CYP11A1,* and *StAR* mRNA expression decreased after the second $PGF_{2}\alpha$ injection. *FASLG* increased only after the third $PGF_{2}\alpha$ injection. The mRNA levels of *PTGFR, PGT,* and *PTGS2* decreased as the number of $PGF_{2}\alpha$ injections increased. Immunohistochemistry showed a decrease in StAR protein density as the number of $PGF_{2}\alpha$ injections increased. *BAX* and *CASP3* mRNA expression levels were similar among the groups. Serum progesterone (P_4) levels decreased dramatically after the $PGF_{2}\alpha$ injections but were still higher than the basal level at the end of the study. In conclusion, repeated low-dose $PGF_{2}\alpha$ injections could induce luteolytic mechanisms in the CL of non-pregnant bitches. Furthermore, it can be concluded that, in non-pregnant bitches, some aspects of the molecular regulation of luteolysis in the CL are similar to some aspects of such regulation in other domestic animals.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

The life cycle of the canine corpus luteum/corpora lutea (CL) can be divided into two stages: the early stage (25–30 days after ovulation) and the mature stage (until CL regression). During the early stage, the canine CL is not gonadotropin-dependent and is not responsive to the luteolytic agent, prostaglandin F_2 alpha ($PGF_{2}\alpha$) [1,2], which is consistent with observations of the bovine CL on the first 5 days after ovulation. Factors that support the development and maintenance of the CL at this early stage are prostaglandin-endoperoxide synthase 2 (PTGS2) [3–6] and Prostaglandin E_2 (PGE_2) [5,7]. Following this early stage, the CL becomes mature and sensitive to luteolytic agents. Luteotropic factors, such as prolactin (PRL) and luteinizing hormone (LH), support the CL and sustain progesterone (P_4) production at this mature stage [8,9].

The hormonal regulation of the entire luteal stage of the estrous cycle in pregnant and non-pregnant bitches is similar, with the exception of $PGF_{2}\alpha$ production before parturition. In pregnant bitches, $PGF_{2}\alpha$, which causes lysis of the CL, is produced by the uteroplacental unit before parturition [10]. However, $PGF_{2}\alpha$ is not produced in the endometrium of non-pregnant bitches, and the CL slowly regresses in these animals. This slow regression occurs despite the presence of several luteotropic factors, such as PRL and LH, that support the function of the CL during the mature stage in non-pregnant bitches [2,10]. This indicates that the luteolysis mechanism is rather complicated and varies depending on the pregnancy status of the bitches.

Similar to the formation and development of the CL in bitches, CL regression can also be divided into two phases: functional (decrease in P_4 production) and structural luteolysis (luteal involution) [4]. Due to P_4 secretion decreases gradually, the mature stage of canine CL could be accepted as a long-running the physiological luteolysis. At this stage, the canine CL are responsive to

* Corresponding author. Department of OBGYN, Faculty of Veterinary Medicine, Dicle University, Diyarbakir, Turkey.
E-mail address: moatli@hotmail.com (M.O. Atli).

https://doi.org/10.1016/j.theriogenology.2018.01.018
0093-691X/© 2018 Elsevier Inc. All rights reserved.

exogenous $PGF_2\alpha$; however, in bitches, the administration of $PGF_2\alpha$ at doses high enough to fully occupy $PGF_2\alpha$ receptors (PTGFR) in the CL has serious side effects [11,12]. Therefore repeated injections of low doses of $PGF_2\alpha$ are preferred. To our knowledge, no studies showing the changes in gene expression during multiple low-dose $PGF_2\alpha$ injections in non-pregnant-canine CL have been published. Therefore, in this study, the aim was to investigate the intraluteal molecular changes that follow multiple low doses of $PGF_2\alpha$ in the CL in non-pregnant bitches. The mRNA expression levels of eleven (11) genes were evaluated in each group. The mRNA expression levels of specific classes of genes were measured to test the effect of the $PGF_2\alpha$ injections and the response of the canine CL. The genes included an immediate early gene [nuclear receptor subfamily 4 group A member 1 (*NR4A1*)], steroidogenic genes [steroidogenic acute regulatory protein (*StAR*), cholesterol side chain cleavage enzyme (*P450scc/CYP11A1*), and luteinizing hormone/choriogonadotropin receptor (*LHCGR*)], prostaglandin-related genes [prostaglandin-endoperoxide synthase 1 (*PTGS1*), 2 (*PTGS2*), prostaglandin transporter (*PGT*), and prostaglandin F receptor (*PTGFR*)], immune-related genes [monocyte chemoattractant protein 1 (*MCP-1*), interleukin 8 (*IL-8*), and fas-ligand (*FASLG*)], and apoptosis-related genes [Bcl-2 associated X protein (*BAX*) and caspase-3 (*CASP3*)]. Moreover, we evaluated the cellular localization of two of these genes (*NR4A1* and *MCP-1*) and a protein involved in P_4 synthesis (StAR) using *in situ* hybridization (ISH) and immunohistochemistry (IHC), respectively. The plasma level of P_4 was evaluated by enzyme-linked immunosorbent assay (ELISA). The results are expected to help us better understand the complex luteolysis mechanism in bitches at the molecular level.

2. Materials and methods

2.1. Experimental design

Non-pregnant, owned bitches, various breeds, aged between 1.5 and 5 years old, weight between 15 and 25 kg were included in the present study. Before the study, reproductive history for each bitch was evaluated. The proestrous phase of the estruos cycle of bitches were detected by clinical examination (vulval swelling, serosanguinous vulval discharge, progressive increases in vulval size and turgor). The bitches detected the proestrus phase were taken the shelters until around 30–35 days after ovulation for follow up the continuing the estrous cycle periods and to provide the same care-feeding conditions and to prevent of possibility of mating. Changes in estrus behavior of the bitches were monitored. Clinical examination was supported by vaginal cytological examination. When the estrous cycle period of the bitches were detected as estrous period by clinical and vaginal cytological examination and behavior change, the possible ovulation time interval was determined and were continued clinical and cytological examination until the experiment. In total, 16 bitches around 30–35 days after ovulation, were enrolled in the study and divided into the following groups: control ($n = 4$), one $PGF_2\alpha$ injection (1PGF, $n = 4$), two $PGF_2\alpha$ injection (2PGF, $n = 4$), and three $PGF_2\alpha$ injection (3PGF, $n = 4$) (Dinoprost Tromethamine, Dinolytic, ZOETἑS; 20 µg/kg subcutaneous). In the 1PGF group, the bitches underwent ovariohysterectomy (OHE) 1 h after the $PGF_2\alpha$ injection. In the 2PGF group, the bitches received two injections 8 h apart, and OHE was performed 1 h after the last $PGF_2\alpha$ injection. In the 3PGF group, the bitches were injected at 0, 8, and 24 h and subjected to OHE after the last injection. The bitches in the control group underwent OHE without any prior treatments. The collection time of CL (1 h after $PGF_2\alpha$ injection) was planned by considering an average-time for OHE operations. The ovaries were removed exactly 1 h after injection. Moreover, at that time, our group performed another studies in ewes [13,14] and there we observed the molecular changes in the CL even at 1 h after the $PGF_2\alpha$ injections. Based on these findings we removed the CL 1 h after the last injection. All surgical procedures were performed while the bitches were under anesthesia. Twenty (20) minutes after the last $PGF_2\alpha$ injection, atropin sulfate (0.04 mg/kg subcutane-Atropin 2%-AtaFen) and xylazine (1,1 mg/kg intramuscular-Alfazyne 2%-AtaFen) were administered to bitches for premedication and 10 min later ketamine (10 mg/kg, intramuscular, Alfamyne 10%-AtaFen) were administered for anesthesia.

2.2. Collection of samples

Following the OHE operation, collected CL were cleared off from the surrounding tissues and washed in phosphate buffered solution (PBS). As soon as after CL collection, to prevent self-RNAse activity, pieces of the CL were then minced by a sterile scapel and placed in cryovial and cryovials were immediately snap frozen in liquid nitrogen and kept in −80°C until further analysis. The other pieces of the CL in falcon tubes were incubated in 4% paraformaldehyde (PFA) overnight at 4°C on a shaker. In the next day, the tissues were washed 3 times 10 min each in PBS (pH 7.4, 10 ml) at room temperature. Then the tissues were serially washed in 25%, 50–75%, and 100% methanol, twice with each solution, and kept in 100% methanol at −20°C until further analysis. Protocols for collection of samples was described previously by Atli et al. [15] and Abler et al. [16].

2.3. RNA isolation, RT reaction, and quantitative PCR

About 20 mg of luteal tissue was placed in RNAse-DNAse free homogenizer tube contained Trizol (Invitrogen, USA), and surrounded by ice-water bath and was chopped with a mechanical homogenizer (Slient cruzer M, Heidolph, Germany). Total RNA was then extracted using the manufacturer's protocol (Trizol, Invitrogen, USA). The purity of RNA was evaluated by agarose gel (1%) electrophoresis and optical density at 260/280 nm (NanoDrop ND-2000, Thermo Scientific, Wilmington, DE, USA) was 2 ± 0.1 for all RNA samples. Two µg of total RNA was treated with DNAse I to eliminate possible genomic DNA contamination and RNA was then reverse transcribed in the presence of oligodT primers using the Revert Aid First Strand cDNA Synthesis Kit (Thermo-Fisher Scientific, USA) according to the manufacturer's protocol. To clarify RT specificity, analyses were also done with all components of the RT kit without RNA (RT negative).

Oligonucleotide primers for *NR4A1, PTGFR, PTGS1, PTGS2, PGT, StAR, CYP11A1, LHCGR, BAX, CASP3, FASLG, IL-8, MCP-1* and housekeeping genes (*GAPDH, B-actin, 18S*) were designed by using Primer3 from the sequences in NCBI gene database or from published primer sequence. The primer pair sequences and product sizes are shown on Supplement file, Table 1. All PCR reactions were set up as follows: 5 µl SYBR Green Master Mix (2X), 2.5 pMol of each primer, 0.5 µl cDNA, and ddH2O (*RNAse and DNAse free*) to bring the final volume to 10 µL. Thermal cycling was done by initially incubating the mixture at 50 °C for 2 min with subsequent denaturation at 95 °C for 10 min. This was followed by 40 cycles of denaturation, annealing, and amplification (95 °C 30 s, 60 °C 1 min, 72 °C 30 s). All reactions were done on Applied Biosystems Stepone plus Real-Time PCR System (Foster City, CA, USA). Melting curve analysis was performed as follows: 95 °C for 1 min followed by fluorescence measurement performed at every 1-degree increments between 60 °C and 95 °C. In each run, negative controls with no cDNA template and RT negative controls were included. To verify reaction specificity, amplification products were evaluated after separation on a 2% agarose gel (see supplement file, Table 1). All samples have been assessed in duplicate for each cDNA. From the RNA extraction

Download English Version:

https://daneshyari.com/en/article/8426893

Download Persian Version:

https://daneshyari.com/article/8426893

Daneshyari.com