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Prostaglandin F2α regulation of the mRNA for AP-1 transcriptional factors in porcine corpora lutea: Lack of induction of *JUN* and *JUND* in CL without luteolytic capacity

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Abstract

Porcine CL develop sensitivity to regression by PGF2α, termed luteolytic capacity, about 13 d after estrus. We postulated that PGF2α regulation of AP-1 transcriptional factor expression underlies acquisition of luteolytic capacity. Gilts on Day 9 (estrous cycle) or Day 17 (pseudopregnancy) had CL collected before or after PGF2α treatment with mRNA measured for *FOS*, *FOSB*, *FOSL1, FOSL2*, *JUN*, *JUNB*, and *JUND* and AP-1 target genes *CCL2* and *SERPINE1*. At 0.5h after PGF2α, both Day 9 and Day 17 CL had increased ($P < 0.01$) mRNA for *FOS* (2,225% and 1,817%), *JUNB* (237% and 358%), and *FOSB* (1,060% and 925%). Intriguingly, at 0.5 h after PGF2α there were increased ($P < 0.01$) mRNA encoding *JUN* (1099%) and *JUND* (300%) in Day 17 but not Day 9 CL. At 10 h after PGF2α there was elevated *FOSB* mRNA in Day 17 (771%) but not Day 9 CL and no PGF2α-induced change in *FOS*, *JUN, JUND*, and *JUNB* mRNA in Day 9 or Day 17 CL. Treatment with PGF2α increased mRNA for AP-1-responsive genes, *CCL2*, at 0.5 h (202%) and *CCL2* and *SERPINE1* at 10 h (719% and 1515%) only in Day 17 CL. Thus, many of the fos family of transcription factors are dramatically induced by PGF2α in CL with or without luteolytic capacity. However, PGF only induced *JUN* and *JUND* expression in CL with luteolytic capacity, a finding that may be key for understanding acquisition of luteolytic capacity given that *JUN* is the only AP-1 family member with strong N-terminal trans-activation activity.

Keywords

corpus luteum; luteolysis; ovary

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1. Introduction

Luteolysis involves a decrease in progesterone (P4) production and induction of luteal cell death [1]. In most species, the early CL does not undergo luteolysis, even when challenged with the normal luteolysin, PGF2α [2]. Acquisition of luteal sensitivity to PGF2α has been termed luteolytic capacity [3,4]. The pig CL acquires luteolytic capacity later in the luteal phase (Day 12–13) and well after CL have reached mature size and maximum hormonal secretion [2].

The mechanisms involved in acquisition of luteolytic capacity are largely undefined. Active receptors for PGF2α (FP receptors- *PTGFR*) are present on luteal cells well before acquisition of luteolytic capacity [5,6]. For example, PGF2α induces a decrease in *PTGFR* (FP receptor) and *HSD3B1* (3-beta hydroxysteroid dehydrogenase) mRNA in CL that have or do not have luteolytic capacity [3,4]. Nevertheless, some gene expression pathways are only induced by PGF2α in CL with luteolytic capacity. For example, PGF2α differentially regulates pathways involved in production of PGF2α [3,4], P4 [2,7], endothelin-1 (*EDN1*) [8], chemokine C-C motif ligand 2 (*CCL2*), also known as monocyte chemoattractant protein-1 [9], and estradiol biosynthesis and signaling [10]. Differential regulation of gene expression in CL with luteolytic capacity likely involves differences in activation of transcription factors and signaling pathways.

The activating protein-1 (AP-1) family of transcription factors contain characteristic basic leucine-zipper regions and includes *FOS*, *FOSB*, *FOSL1*, *FOSL2*, *JUN*, *JUNB*, and *JUND* proteins. Genes for AP-1 are immediate early genes that regulate a wide range of physiological responses such as cell death, inflammation, and proliferation [11]. Responses to induction of AP-1 are dependent on specific gene promoter, cell type, and which AP-1 proteins are induced [12]. The Jun family members can homodimerize with other Jun proteins or heterodimerize with Fos proteins or with other basic leucine zipper-containing transcription factors such as activator transcription factor (ATF) family members. In contrast, Fos members do not homodimerize but can only heterodimerize with Jun family members to form active transcription complexes.

The AP-1 proteins have been localized within the pig CL [13]. Also, treatment with PGF2α was found to induce AP-1 proteins in pig CL [14] and in bovine luteal cells via a PKC-dependent MAP kinase pathway [15]. One indication that AP-1 proteins may be differentially regulated in CL without luteolytic capacity is that some AP-1-regulated genes are regulated differently in CL with or without luteolytic capacity. For example, PGF2α increases *CYP19A1* [10] and *CCL2* mRNA [9], and decreases *STAR* mRNA [7] only in CL with luteolytic capacity. All three of these genes (*CYP19A1*, *STAR*, and *CCL2*) are regulated by AP-1 transcriptional complexes [16–20]. Therefore, this study was undertaken to determine whether AP-1 transcription factors are differentially regulated by PGF2α in porcine CL before and after acquisition of luteolytic capacity. We hypothesized that PGF2α would induce AP-1 transcription factors only after acquisition of luteolytic capacity. Alternatively, specific AP-1 transcription factors may be differentially regulated, potentially providing insight into the underlying transcriptional mechanisms associated with acquisition of luteolytic capacity.

2. Materials and Methods

2.1. Chemicals and Reagents

Cloprostenol was purchased from Bayer Corporation (Shawnee Mission, KS), Ketamine was from Fort Dodge Animal Health (Fort Dodge, IO), and Xylazine was from Phoenix Pharmaceuticals (St. Joseph, MO). T7 RNA polymerase, Taq polymerase, Reverse Transcriptase, dNTPs, RNAsin and DNAase I were purchased from Promega (Madison, WI). Molecular weight markers were from Gibco/BRL (Gaithersburg, MD). Magnetight oligo(dt) beads were from Novagen (Madison, WI). Unless otherwise specified, other chemicals and reagents used in these studies were purchased from Sigma (St. Louis, MO).

2.2. Animals

Crossbred gilts (Cambrough × Line 19) 6–8 months of age were obtained from the university herd or purchased from Pig Improvement Company (PIC, Franklin, KY). Animals were kept in individual pens with free access to water and were fed a maintenance diet of corn and soybean meal. For all studies, animals were checked daily for standing estrus with a mature boar. First day of estrus was designated as Day 0. Pseudopregnancy was induced in some gilts with daily injections of estradiol benzoate (2 mg i.m.) on Days 11–15. On the day ovaries were collected, anesthesia was induced with i.m. injection of ketamine (15 mg/kg) and xylazine (0.3 mg/kg). Gilts were intubated and surgical plane of anesthesia maintained with halothane. Ovaries were collected via midventral laparotomy and CL were dissected away from ovarian stroma and either frozen in liquid nitrogen or transported to the laboratory in cold media (M199, 100 IU/mL penicillin, 10 mg/mL streptomycin, 0.1% BSA) for further processing. The Research Animal Resource Center Committee of the College of Agricultural and Life Sciences at University of Wisconsin-Madison approved all procedures performed on animals.

2.3. Experiment I

This experiment examined the acute (0.5 h) in vivo regulation of mRNA for FOS, *FOSB*, *FOSL1*, *FOSL2*, *JUN*, *JUNB*, *JUND*, *SERPINE1* and *CCL2* by PGF2α. On Day 9 after estrus (n=4) or Day 17 of pseudopregnancy (n=4), gilts were anesthetized and one ovary collected (control CL). Following removal of the control ovary, 500 μg of PGF2α (cloprostenol i.m.) was given and the other ovary was collected 0.5 h later (treated CL). Corpora lutea were collected and immediately frozen in liquid nitrogen for later quantitation of specific mRNAs.

2.4. Experiment II

This experiment examined the late (10 h) in vivo regulation of mRNA for *FOS*, *FOSB*, *FOSL1*, *FOSL2*, *JUN*, *JUNB*, *JUND*, *SERPINE1* and *CCL2* by PGF2α. Gilts were checked for estrus daily with a mature boar. Animals were randomly assigned to one of four groups: Day 9 saline (n=5), Day 9 PGF2α (n=4), Day 17 saline (n=5) and Day 17 PGF2α (n=5). On Day 9 of the estrous cycle or 17 of pseudopregnancy, gilts received either saline or PGF2α (500 μg of cloprostenol i.m.) and ovaries were surgically removed 10 h later. Corpora lutea were collected and immediately frozen in liquid nitrogen.

2.5. Isolation of mRNA

Total RNA was isolated using the RNAgents total RNA isolation system (Promega, Madison, WI). Briefly, CL were ground in a mortar and pestle cooled with liquid nitrogen. Approximately 40 mg of tissue was transferred to a fresh tube containing 900 μL of denaturing solution (4M Guanidine thiocyanate, 0.01M Tris [pH 7.5], 0.97% β-Mercaptoethanol) and homogenized for 20 sec using a polytron tissue grinder. Ninety μL of 2 M sodium acetate and 900 μL of phenol/chloroform/IAA were added to the lysate and incubated on ice for 15 minutes. Samples were centrifuged for 20 min at 14,000 RPM in a refrigerated microcentrifuge. Supernate was transferred to a fresh tube and RNA was precipitated with an equal volume of isopropanol and incubated at −20°C for 1 h. Samples were then centrifuged at 14,000 RPM for 10 min to pellet RNA and washed with 1 mL 70% ethanol. RNA pellet was dried and resuspended in 30 μL DEPC-treated water and RNA purity and quantity was measured by absorbance at 260/280 nm in a spectrophotometer.

2.6. Quantification of mRNA using RT-PCR and real-time PCR

Evaluation of specific mRNAs was done using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA as an internal control. Primers for *GAPDH, FOS, FOSB, JUNB, FOSL1, CCL2* and *JUN* were synthesized from published genebank sequences (see Table 1) to produce the expected 285, 599, 129, 387, 377, 308, and 467 bp products. Reverse transcription was carried out with 19 μL of 1X master mix (1X RT buffer, 0.2 mM dNTPs, 100 pmol random primer and 40 U reverse transcriptase) and 1 μL of sample for 1.5 h at 37°C. For PCR, 4 μL of RT reaction were added to 1X PCR master mix (1X thermophilic buffer supplied with enzyme, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM each of forward and reverse primers and 0.5 U Taq DNA polymerase) in a 20 μL final volume and amplified with 25–30 cycles of PCR (95°C: 30 sec, 55°C: 30 sec and 72°C: 30 sec) followed by a final extension at 72°C for 5 min. Reactions were separated on 5% PAGE gel and stained with ethidium bromide. For each sample, the two products were quantified using the Collage imaging system (Fotodyne, Heartland, WI). Values were calculated as the ratio of the gene-specific band intensity/GAPDH band intensity.

Steady-state concentrations of investigated mRNAs for *FOSL2*, *JUND*, and *SERPINE1* were quantified by real-time PCR using a GeneAmp® 5700 Sequence Detection System (PE Biosystems, Foster City, CA) with PCR products detected with SYBR Green I (Molecular Probes, Eugene, OR). Primers for amplification were designed using Primer Express (PE Biosystem, Foster, CA) and are listed in Table 1. Each PCR reaction mix (25 μL) contained 1× PCR Buffer (Promega, Madison, WI) with 1:20,000 dilution of SYBR Green I, 1.5 mM $MgCl_2$, 200 μM dNTP, 250 nM forward primer, 250 nM reverse primer, 2 μL RT products, and 1.25 U GoToTaq polymerase (Promega, Madison, WI) [21]. Thermal cycling conditions were 94°C for 30 sec, followed by 40 cycles at 94° C for 30 sec, 57° C for 30 sec, and 72° C for 30 sec, and finally 72° C for 10 min. Melting curve analyses and agarose gel electrophoresis were performed after real-time PCR reactions to monitor PCR product purity.

The threshold cycle (C_T) numbers were determined for the amplified cDNA for each investigated mRNA and for the housekeeping gene, *ACTB* (known as β-ACT), in each

unknown sample during real-time PCR. The relative quantification of investigated gene expression was evaluated using a standard curve method [22]. For each sample, the amount of investigated mRNA and the housekeeping mRNA (*ACTB*) was determined from the standard curve. Then, the amount of investigated mRNA was divided by the amount of *ACTB* to obtain a normalized mRNA value for each investigated gene.

2.7. Statistical analyses

Results from experiment I (0.5 h) were analyzed by paired t-test. Results for experiment II (10 h) were analyzed by two way analysis of variance (ANOVA) using the general linear model (GLM) procedure of the Statistical Analysis System (SAS). If a positive F-test was detected, means were separated using Fisher's least significant difference test (LSD). A P value < 0.05 was considered significant.

3. Results

3.1. Experiment I

The mRNA for *FOSL1* was not changed by PGF2α treatment in either Day 9 (Control 0.25±0.03; PGF2α 0.31±0.01 band intensity *FOSL1* mRNA/*GAPDH* mRNA) or Day 17 (Control 0.26±0.02; PGF2α 0.25±0.02 band intensity *FOSL1* mRNA/*GAPDH* mRNA) CL. Likewise, levels of *FOSL2* mRNA were not changed in either Day 9 (Control = 2.33±1.00; PGF2α = 2.18±0.58; normalized to *ACTB* mRNA and X 10^{-3} for all values) or Day 17 (Control = 2.06±0.23; PGF2α = 2.02±0.59; normalized to *ACTB* mRNA and X 10^{-3} for all values) CL at 0.5 h after treatment. In contrast to *FOSL1* and *FOSL2* mRNA, the mRNA for *FOS*, *FOSB*, and *JUNB* were dramatically induced ($P < 0.01$) at 0.5 h after PGF2α treatment in both Day 9 and Day 17 CL. The most dramatic example was the increase ($P < 0.01$) in *FOS* mRNA (Figure 1, P < 0.01) that was observed in both Day 9 (22.3-fold increase) and Day 17 (18.2-fold) CL. The basal concentrations of *FOS* mRNA were low (0.04±0.02 and 0.06±0.03 for Day 9 and 17, respectively) prior to PGF2α. Likewise, the basal levels of *FOSB* were low prior to PGF2α treatment (0.05±0.01 and 0.08±0.05 for day 9 an d17, respectively). Treatment with PGF2α acutely induced ($P < 0.01$) *FOSB* mRNA in both Day 9 (10.6-fold) and Day 17 (9.3-fold increase) CL (Figure 2). In contrast, the basal levels of *JUNB* were moderately high under basal conditions (0.51 ± 0.10 and 0.48 ±0.15 for day 9 and 17, respectively). Nevertheless, the concentrations of *JUNB* mRNA were also increased ($P < 0.01$) in both Day 9 (2.37-fold) and Day 17 (3.58-fold) CL (Figure 3).

In contrast to FOS, *FOSB*, and *JUNB*, there was an intriguing difference in the acute PGF2α-induced expression pattern for *JUN* and *JUND* mRNA in CL with or without luteolytic capacity (Figure 4A). Under basal conditions, *JUN* was at low concentrations (0.02 ± 0.003 – Day 9; 0.02 ± 0.004 – Day 17). The low concentration of *JUN* mRNA did not change after PGF2α in Day 9 CL (0.04 ± 0.01 – after PGF2α on Day 9). However, *JUN* mRNA was dramatically increased ($P < 0.01$) by PGF2α in Day 17 CL (11.0-fold increase). *JUND* mRNA concentrations were also increased ($P < 0.01$) by PGF2α in Day 17 CL (~2.5 fold) and not Day 9 CL (Figure 4B)

The mRNAs for two AP-1-responsive genes were also evaluated in this study, *CCL2* and *SERPINE1*. Treatment with PGF2α increased ($P < 0.05$) mRNA for *CCL2* only in Day 17 (2.0-fold), but not Day 9 CL (Figure 5, P < 0.05). However, *SERPINE1* mRNA concentrations were not affected by PGF2α in either Day 9 (Control 6.10± 4.09; PGF2α 9.40±4.26) or Day 17 (Control 6.14±4.25; PGF2α 7.52±2.25) CL at 0.5 h after treatment.

3.2. Experiment II

At 10 h after PGF2α treatment, the mRNA for these AP-1 factors (*FOS*, *JUN*, *FOSL1*, *FOSL2*, and *JUNB*) were not different from mRNA concentrations in Day 9 or Day 17 CL from untreated gilts (Table 2). In contrast to the mRNA for these AP-1 members, there was clear differential regulation of *FOSB* at 10 h after PGF2α treatment (Figure 2). In Day 9 CL, although there had been a dramatic induction of *FOSB* mRNA at 0.5 h after PGF2α treatment, there was no difference in *FOSB* mRNA at 10 h after treatment (0.09 ± 0.03 vs. 0.08 ± 0.03). However, in Day 17 CL (Figure 2B), there were much greater ($P < 0.01$) mRNA concentrations for *FOSB* in CL treated with PGF2α (10 h after treatment) than in control CL (7.7-fold increase). The mRNA concentrations for *JUND* were also higher ($P < 0.05$) in Day 17 than Day 9 CL at 10 h after PGF2α treatment (Table 2).

The concentrations of mRNA for the two AP-1-responsive genes were increased ($P < 0.01$) at 10 h after PGF2α treatment. Expression of *CCL2* mRNA was increased 7.2-fold ($P < 0.01$) at 10 h after PGF2α in Day 17 CL but there was no difference due to PGF2α treatment in Day 9 CL (Figure 5). Similarly, treatment with PGF2α increased ($P < 0.01$) concentrations of mRNA for *SERPINE1* only in Day 17 (15.2-fold) but not Day 9 CL (Table 2).

4. Discussion

This research utilized the porcine CL model which is particularly interesting due to development of luteolytic capacity at a relative late state of luteal development (Day 12–13). However, development of luteolytic capacity occurs so close to the time of natural luteolysis that treatments to evaluate luteolytic capacity can be confounded with the natural luteolytic process [4]. To assure that CL had full luteolytic capacity but had not been exposed to uterine PGF2α, we utilized the pseudopregnant pig model in which treatment with estradiol is used to alter endometrial PGF2α production, similar to what occurs during pregnancy [10].

Acquisition of luteolytic capacity in pigs and other mammals is associated with changes in the PGF2α-induced transcriptional regulation of key genes involved in luteal function. It is logical that key transcriptional factors, such as the AP-1 factors, are not induced in CL without luteolytic capacity and that this would result in lack of changes in gene expression following PGF2α. However, many of the AP-1 transcriptional factors were dramatically induced by PGF2α in both the CL with (Day 17 pseudopregnant) as well as without (Day 9) luteolytic capacity. For example, *FOS* increased more than 20-fold after PGF2α treatment of the early porcine CL but yet this CL does not undergo regression in response to PGF2α. Similarly, *FOSB* and *JUNB* were also dramatically induced following PGF2α treatment. This is the first report of such a dramatic induction of genes following treatment with

PGF2α of CL lacking luteolytic capacity and provides clear evidence that PGF2α can produce physiological responses in the early CL. Previous results indicated that receptors for PGF2α were abundantly present in the early CL [5,6] and that some genes were down-regulated by PGF2α in the early CL [3,4]. From our results it is clearly evident that PGF2α activates at least some of the intracellular signal transduction pathways associated with luteolysis in the early CL. Nevertheless, induction of these members of the AP-1 transcription complex appears to be insufficient to induce the luteolytic cascade. Thus, an intriguing paradox is that many of the critical genes associated with luteolysis are not activated by PGF2α in the early CL in spite of a clear activation of many intracellular regulators, such as *FOS*, *FOSB*, and *JUNB*.

The present study provides evidence that an early step in this differential regulation of gene expression may involve induction of the key AP-1 transcription factors, *JUN* and *JUND*. There was a dramatic difference in PGF2α-induction of *JUN* and *JUND* mRNA concentrations with this induction only occurring in CL with luteolytic capacity. In addition, there was an unexpected difference in *FOSB* expression at 10 h after PGF2α treatment with PGF2α induction of *FOSB* only in CL with luteolytic capacity. Differences in mRNA expression of AP-1 factors may translate into differences in expression of a variety of luteolysis-related gene products such as the AP-1-responsive genes, CCL2 and SERPINE1, which were induced by PGF2α only in Day 17 CL with luteolytic capacity. Nevertheless, a key limitation was that only mRNA was evaluated in this study. Obviously, this experimental approach can introduce the concept that regulation of AP-1 proteins, and particularly members of the jun family, could be key to molecular regulation of luteal resistance, however analysis of proteins and functional assays of AP-1 regulated transcription will be required to validate or invalidate the importance of these pathways. Transcriptional regulation and DNA binding studies were beyond the scope of the present study.

The fundamental role of an increase in transcription of fos and jun family members in activation of the AP-1 transcriptional complex has been extensively demonstrated in previous studies [12]. It is also clear that both fos and jun family members must be present to allow transcriptionally competent heterodimers to form [11,12,23]. Of particular interest to our research findings, upregulation of *JUN* activity has a critical role in induction of AP-1 transcriptional activity [24]. All of the Jun proteins have a DNA binding domain and a basic leucine-zipper region that allows heterodimerization with fos family members [12,24]. However, *JUN* is unique in having an N-terminal trans-activation domain whereas, *JUNB* and *JUND* exhibit only weak trans-activation activity [24]. The unexpected finding in our research of a lack of induction of *JUN* mRNA in CL without luteolytic capacity, although other elements of the AP-1 transcriptional complex have been induced, provides strong associative data for a key role of this protein in luteolytic capacity. Thus, given the potential importance of AP-1 activation in inducing the cellular pathways involved in luteolysis, the lack of *JUN* induction in the early CL may be crucial to preventing specific AP-1 mediated transcriptional events in the early CL.

Induction or lack of induction of *JUN* has been found to be crucial for other types of cellular regression [11,23,25]. For example, overexpression of *JUN* induces cell death in vitro [26]. Conversely, removal of *JUN* by either expression of a dominant negative form of *JUN* [27]

or specific inactivation of *JUN* in the central nervous system using a cre/lox system [28] delayed or completely abolished injury-induced neuronal cell death. These results highlight the central role of *JUN* in normal cell death mechanisms and are consistent with the idea that *JUN* expression may be fundamental to initiation of the luteolysis process.

A number of other AP-1 proteins, in addition to *JUN*, also seem to be critical in cell death [11,23]. For example, evidence is accumulating that an AP-1 response element is critical for induction of FasL-mediated cell death in T-cells [29] and hepatocellular carcinoma cells [30]. Luteolysis is an apoptotic process [31] with Fas/FasL implicated in luteolysis-related cell death in rat [32] and bovine [33] CL. Thus, it seems likely that AP-1 also has a critical role in the FasL-mediated cell death associated with luteolysis. In particular, *FOSB* was found to be critical for the induction of cell death in T-cells [29]. Our results are consistent with these findings. Expression of *FOSB* remained elevated at 10 h after PGF2α treatment of Day 17 CL when the initial stages of luteal cell death would be observed (between 6–12 h after cloprostenol; [31]). Thus, specific and acute induction of *JUN* as well as sustained induction of *FOSB* is clearly associated with cellular capacity to respond to PGF2α with complete regression of the CL, or luteolytic capacity.

Unfortunately, the results of this in vivo study do not allow determination of the mechanisms that underlie the differential expression of AP-1 transcriptional factors in CL with or without luteolytic capacity. Given the vital role of P4 in luteal function [34–36], it is possible that high intraluteal concentrations of P4 could regulate induction of AP-1 proteins. Supplementation of pregnant rats with P4 blocks parturition and blocks the normal induction of *FOS*, *FOSB*, *FOSL1*, *FOSL2*, and *JUNB* mRNA occurring in the uterus at parturition [37]. However, a number of AP-1 proteins (*FOS*, *FOSB*, *JUNB*) were induced by PGF2α in the presence of high P4 in either Day 9 or Day 17 CL. In addition, *JUN* and *JUND* were induced in the Day 17 CL (Figure 4) in spite of high intraluteal P4 concentrations [4]. It seems possible that the sustained induction of *FOSB* could be related to lowered P4 because intraluteal P4 had dramatically decreased by 10 h after PGF2α treatment in Day 17 but not Day 9 CL [4]. However, the induction of *JUN* and *JUND* at only 0.5 h after PGF2α was not associated with changes in intraluteal P4 and is much more likely to be related to differences in factors present in the DNA promoter/enhancer region of *JUN* and *JUND* in Day 9 compared to Day 17 CL. The lack of sustained elevation in mRNA for *FOS*, *JUN* and *JUNB* at 10 h after PGF2α is probably due to autoregulatory mechanisms designed to rapidly terminate AP-1 signaling [38,39]. In the case of *JUN*, we speculate that once the luteolytic cascade is initiated continued induction of *JUN* is unnecessary. In vitro evaluations are likely to be required in order to elucidate the molecular mechanisms that underlie the switch during acquisition of luteolytic capacity that produces *JUN* and *JUND* responsiveness to PGF2α treatment.

We also chose to evaluate the effect of luteolytic capacity on 2 AP-1 regulated genes, one that has previously been evaluated in the CL, *CCL2*, and the other that has not been previously examined, *SERPINE1*. Luteolysis has clearly been associated with induction of *CCL2* in many species [9,40,41]. In the bovine CL, *CCL2* is induced only after acquisition of luteolytic capacity [9]. Likewise, we observed a similar response in the porcine CL with a dramatic and rapid increase in *CCL2* mRNA only in CL with luteolytic capacity. Expression

of *CCL2* is regulated by AP-1 proteins in many cell types [42] including luteal cells [16]. A simplified scenario could be that lack of AP-1 activation due to lack of *JUN* induction leads to lack of *CCL2* induction in CL without luteolytic capacity. A lack of *CCL2* induction in the early CL could prevent premature recruitment and activation of immune cells in the CL and subsequent luteolysis. Similarly, the serine protease inhibitor E1 (*SERPINE1*) is regulated by AP-1 promoter activity [43]. This gene was induced only in CL with (Day 17) but not without (Day 9) luteolytic capacity. SERPINE1 may be a critical regulator of the tissue remodeling occurring during structural degradation of the CL by modulating the activity of tissue plasminogen activator [44,45].

In addition, two of the genes that were previously found to be differentially regulated in CL with versus without luteolytic capacity are also clearly regulated by AP-1 promoters. The AP-1 proteins are transcriptional repressors of *STAR* gene [19,20]. In addition, *CYP19A1* is induced by binding of AP-1 proteins to the aromatase gene promoter [18]. These 2 genes appear to only be regulated by PGF2α in CL with luteolytic capacity. The mRNA and protein for STAR is decreased only in CL with luteolytic capacity [7]. In addition, PGF2α specifically induces expression of *CYP19A1* mRNA only in CL with luteolytic capacity [10]. Thus, pathways that allow induction of AP-1 transcriptional pathways by PGF2α, most likely due to PGF2α-induced expression of *JUN* and/or *JUND* mRNA, may have a fundamental role in development of the cellular pathways associated with luteolysis.

In summary, this study has used an in vivo model to clearly demonstrate that PGF2α acutely and dramatically induces the mRNA expression of specific luteolysis-related AP-1 transcription factors, such as *FOS*, *FOSB*, and *JUNB*, even in CL that will not undergo luteolysis. However, the lack of acute induction of mRNA for *JUN* and *JUND*, and possibly sustained *FOSB* mRNA expression, may prevent AP-1-mediated gene expression events and may represent a physiologically important functional lesion underlying luteolytic capacity.

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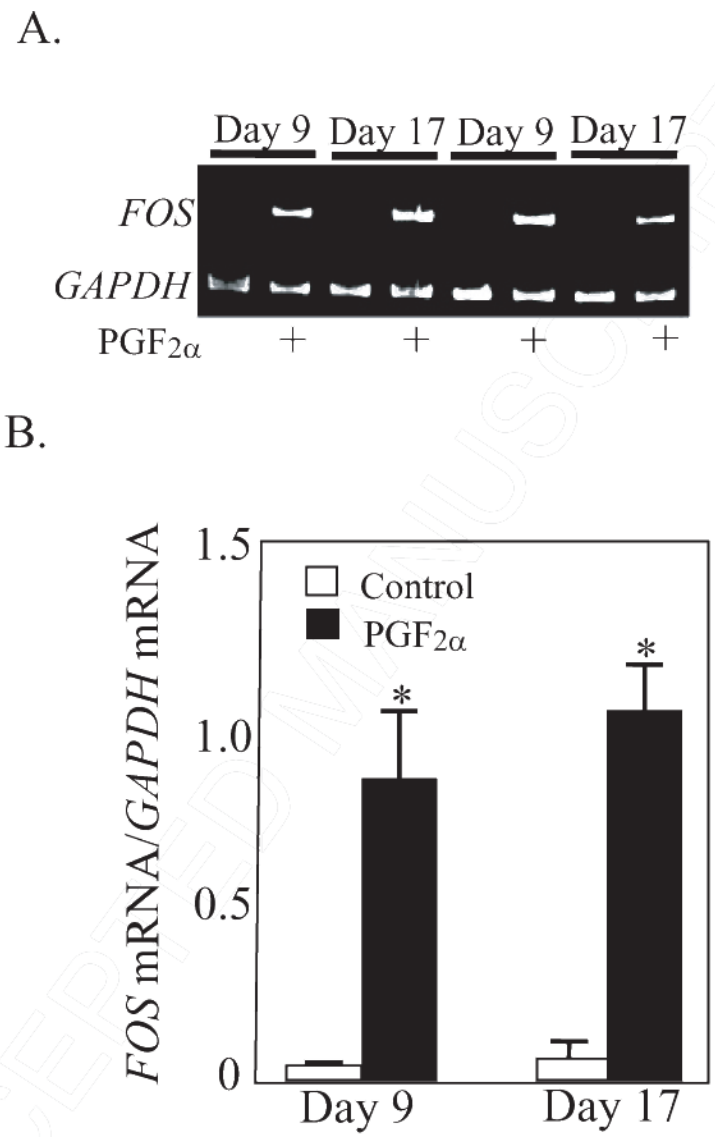

Figure. 1.

A. Representative gel photos of PCR products for *FOS* and *GAPDH* mRNA at 0.5 h after treatment with PGF2α. B. Steady state concentrations of *FOS* mRNA (ratio of *FOS* mRNA/ *GAPDH* mRNA) at 0.5 h after PGF2α treatment in CL collected from gilts on Day 9 (n=4/5 per group) and Day 17 (n=5 per group) of pseudopregnancy. *Denotes differences from paired control, $P < 0.01$.

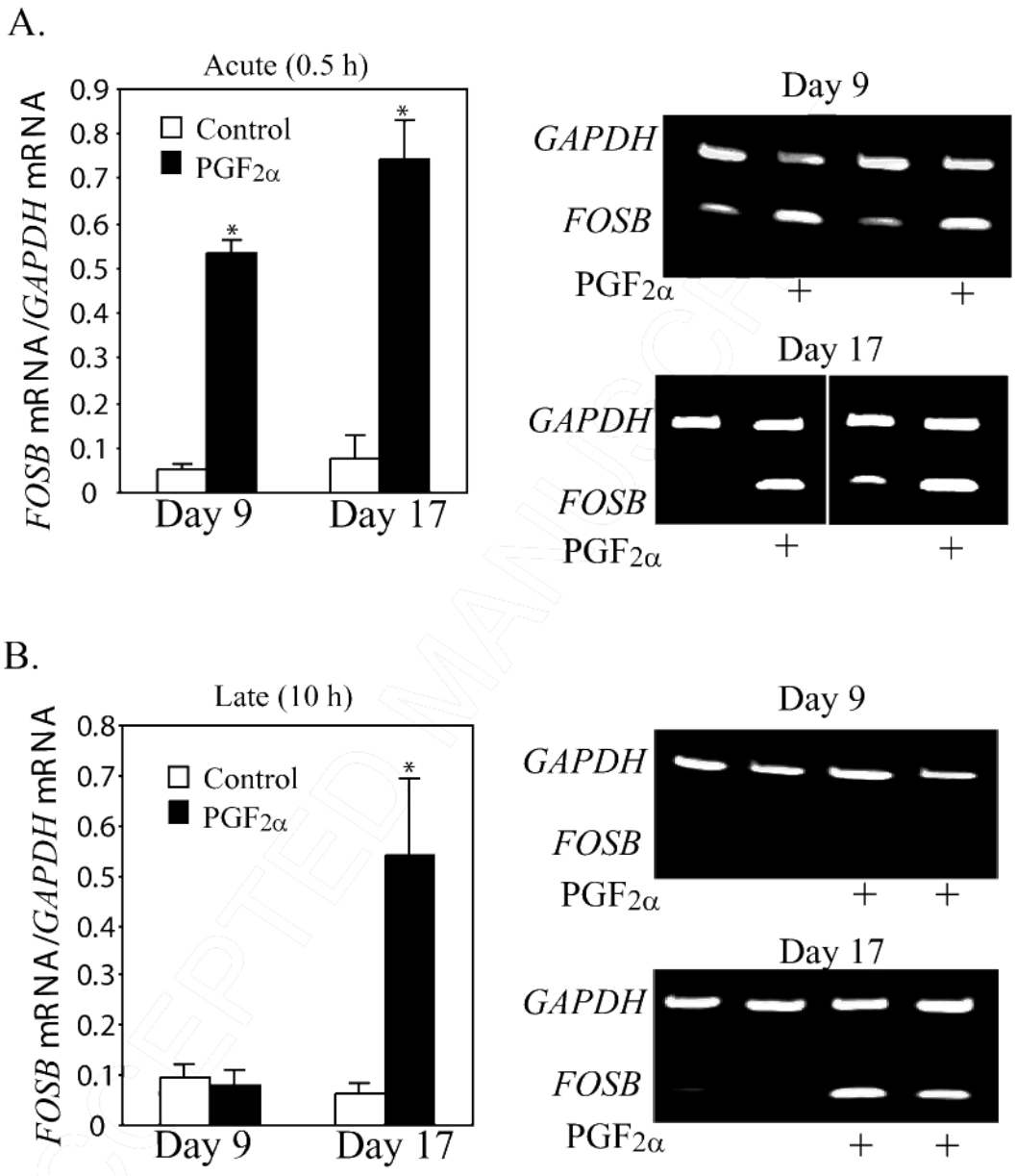

Figure. 2.

Steady state concentrations of *FOSB* mRNA (ratio of *FOSB* mRNA/*GAPDH* mRNA) at (A) 0.5 h and (B) 10 h after PGF2α treatment in CL from gilts on Day 9 (n=4/5 per group) or Day 17 (n=5 per group). Representative gel photos of PCR products for *FOSB* and *GAPDH* mRNA are shown on the right. *Denotes differences from control, $P < 0.01$.

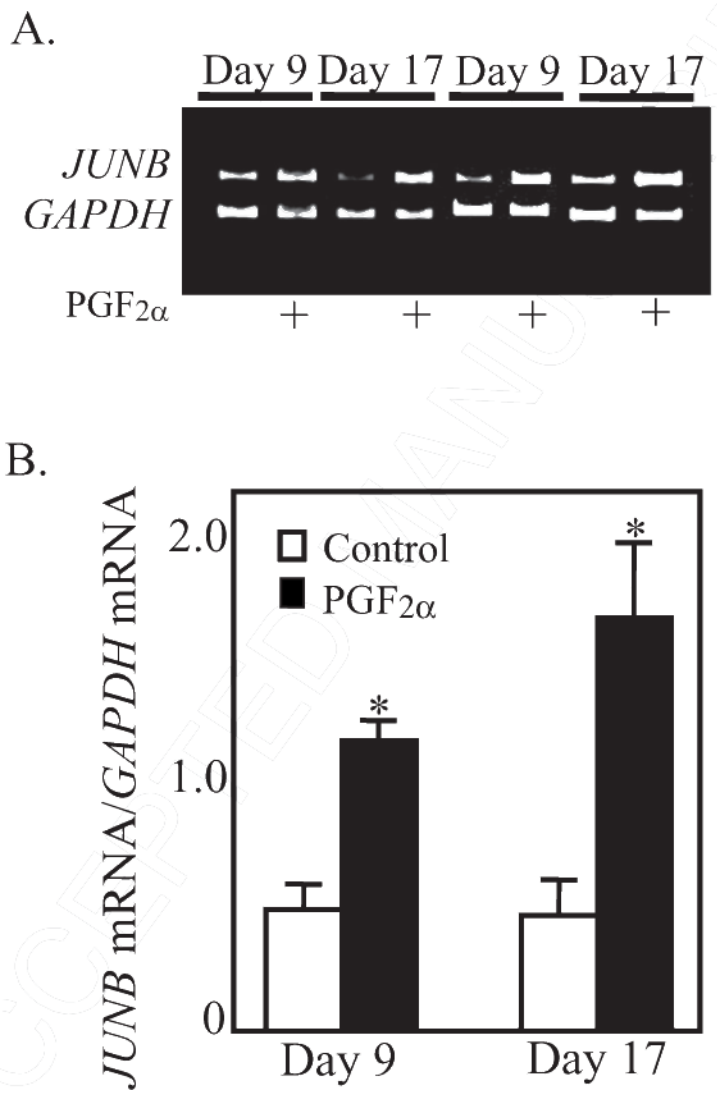

Figure. 3.

A. Representative gel photos of PCR products for *JUNB* and *GAPDH* mRNA. B. Steady state concentrations of *JUNB* mRNA (ratio of *JUNB* mRNA/*GAPDH* mRNA) at 0.5 h after PGF2α treatment in CL from gilts on Day 9 (n=4/5 per group) or Day 17 (n=5 per group). *Denotes differences from paired control, $P < 0.01$.

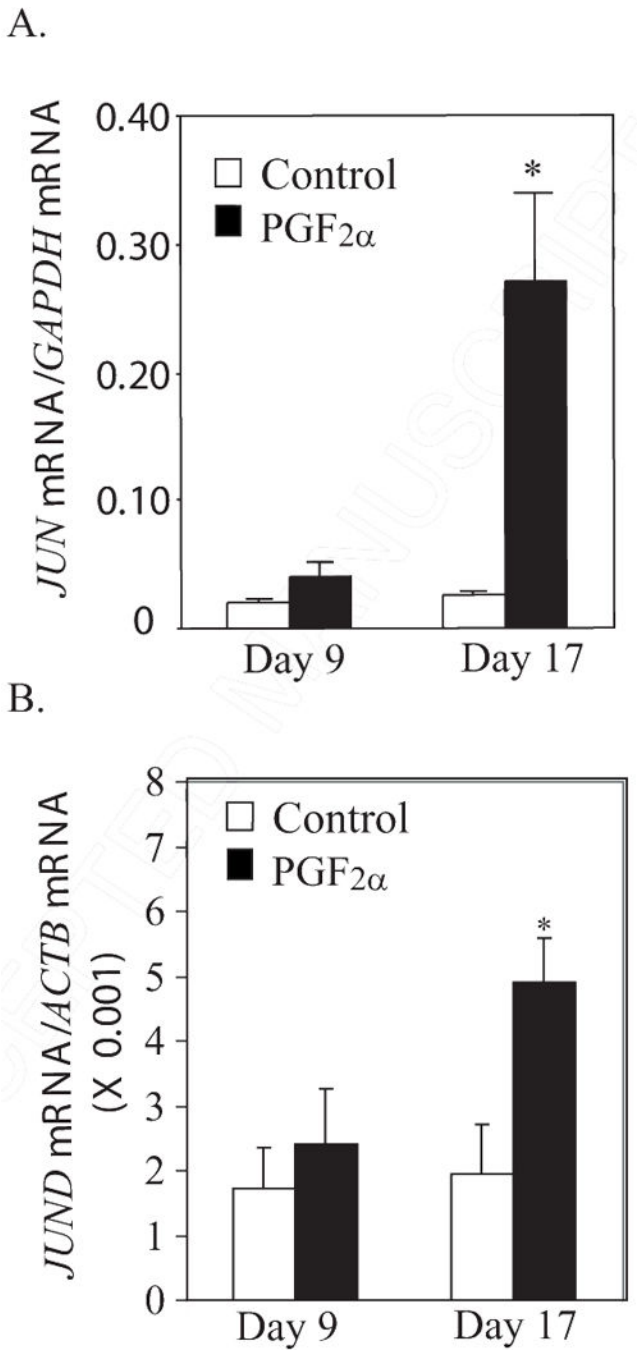

Figure. 4.

A. Steady state concentrations of *JUN* mRNA (ratio of *JUN* mRNA/*GAPDH* mRNA) at 0.5 h after PGF2α treatment in CL from gilts on Day 9 (n=4/5 per group) or Day 17 (n=5 per group). B. Steady state concentrations of *JUND* mRNA (normalized to *ACTB* mRNA) at 0.5 h after PGF2α treatment in CL from gilts on Day 9 (n=4/5 per group) or Day 17 (n=5 per group). *Denotes differences from paired control, $P < 0.01$.

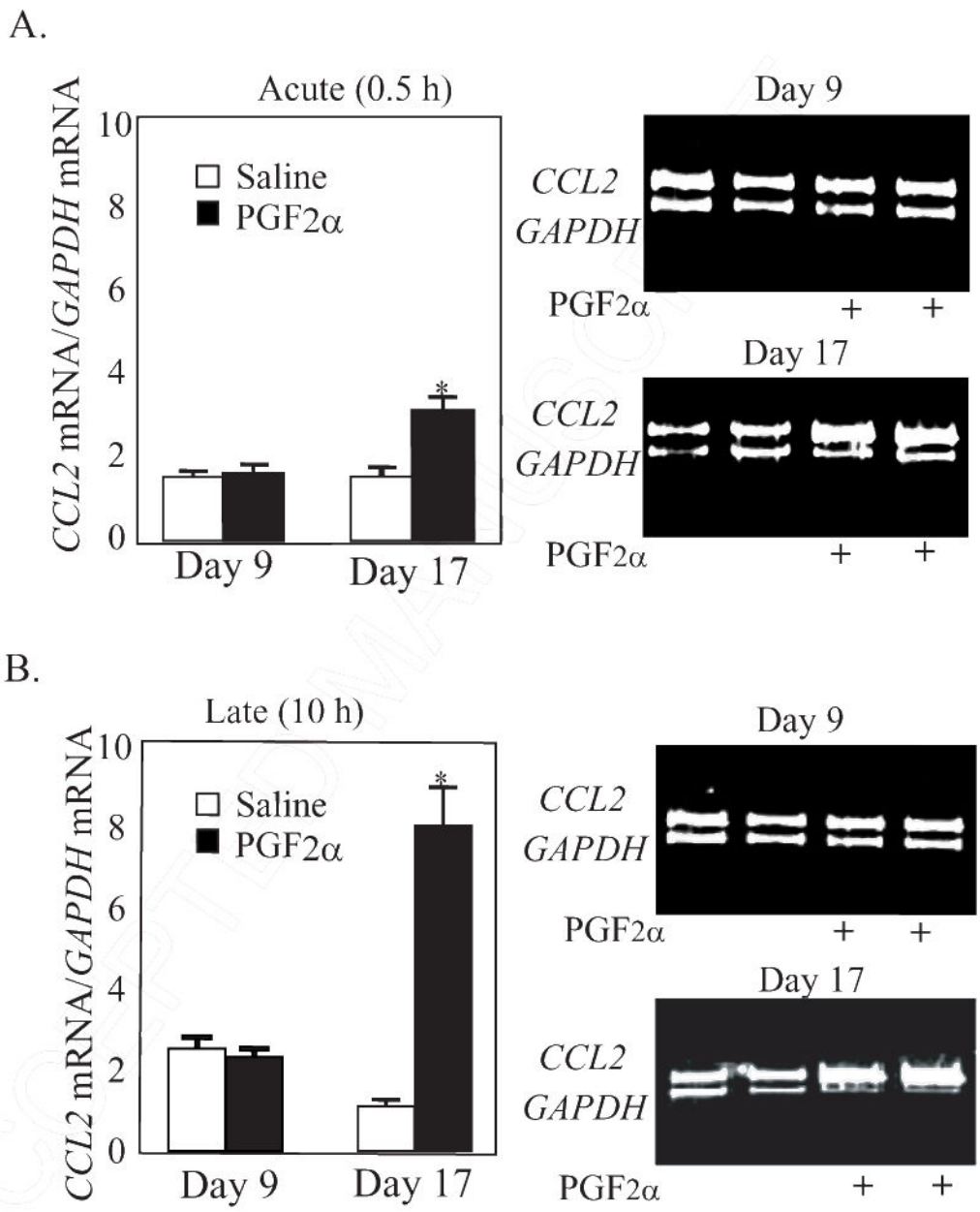

Figure. 5.

Steady state concentrations of *CCL2* mRNA (ratio of *CCL2* mRNA/*GAPDH* mRNA) at (A) 0.5 h and (B) 10 h after PGF2α treatment in CL from gilts on Day 9 (n=4/5 per group) and Day 17 (n=5 per group). Representative gel photos of PCR products for *CCL2* and *GAPDH* mRNA are shown on the right. *Denotes differences from paired control, $P < 0.05$.

Table 1

Primer sequences used for PCR. All sequences are 5′ to 3′.

Gene	Forward	Reverse	Reference
FOS	GGAAAGGAATAAGATGGCTG	AGTCTGCTGCATAGAAGG	AJ132510
FOSB	CCGGGCATGAGTGGCTACAG	CGTCTCCTCTCGGGGTCTCCT	AF120155
FOSL1	GAGGAGCGCCGCCGAGTAAG	CAGGCTGGGGGTGAAAGGAG	X16707
FOSL2	CAGCATTGCTGGGGGCTTCTA	TGATTGGTCCCCGCTGCTACT	X16706
JUN	TTCGCGGTCGCTGGTGAGGA	GGGTCGGCGTGGTGGTGATG	S83515
JUNB	CTACACGACTACAAACTCCT	GGTGTCACGTGGTTCATCT	BC009466
JUND	CGTTGGTTGTGTGTGTGTGTG	CAGGAATGTGGACTCGTAGCA	NM_005354
CCL2	TGAAGGTCTCTGCAGCCCTC	AGTCAGGCTTCAAGGCTTCG	X79416
SERPINE1	TTGCCCTTGTGTGCTTGTTAG	AAAGAGAGGAGCAATGGGGTT	NM_213910
GAPDH	ATTGCCCTCAACGACCACTT	ACATGACGAGGCAGGTCTCC	X94251
ACTB	CCCAGCACGATGAAGATCAAG	AGAAGCATTTGCGGTGGACGA	AY550069

Table 2

Effects of PGF2α on mRNA concentrations for AP-1 factors in Day 9 and Day 17 porcine CL at 10 h after treatment.

	Day 9		Day 17	
mRNA	**Saline**	PGF2α	**Saline**	**PGF2α**
FOS[*]	0.09 ± 0.05	0.06 ± 0.01	0.07 ± 0.03	0.12 ± 0.08
FOSL1[*]	0.25 ± 0.01	0.27 ± 0.02	0.25 ± 0.03	0.28 ± 0.02
FOSL2 (× 10^{-3})[#]	2.14 ± 1.06	1.83 ± 0.55	1.80 ± 0.27	2.67 ± 0.33
JUN[*]	0.02±0.004	0.02±0.003	0.02±0.004	0.05±0.012
JUNB[*]	0.52±0.07	0.49±0.03	0.62±0.13	0.67±0.18
JUND (× 10^{-3})[#]	1.82 ± 0.81^{ab}	1.38 ± 0.24^{a}	2.04 ± 0.55^{ab}	3.33 ± 0.49^{b}
SERPINE1 (× 10^{-3})[#]	0.70 ± 0.19^{a}	1.63 ± 0.50^{a}	0.66 ± 0.30^{a}	10.0 ± 3.40^{a}

[*] Values are mean ± SEM of pixel intensity of specific mRNA/*GAPDH* mRNA.

[#] Values are mean ± SEM of specific mRNA from real-time PCR/ACTB mRNA.

a, b, c Groups within a row without common superscripts are different ($P < 0.05$).