

Review

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## Regulation of intraluteal production of prostaglandins

Milo C Wiltbank\*<sup>1</sup> and Joseph S Ottobre<sup>2</sup>

Address: <sup>1</sup>University of Wisconsin, Madison Dairy Science Department, Madison, WI 53706, USA and <sup>2</sup>Ohio State University, Department of Animal Sciences, Columbus, OH 43210, USA

Email: Milo C Wiltbank\* - wiltbank@calshp.cals.wisc.edu; Joseph S Ottobre - Ottobre.2@osu.edu

\* Corresponding author

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### Abstract

There is clear evidence for intraluteal production of prostaglandins (PGs) in numerous species and under a variety of experimental conditions. In general, secretion of PGs appears to be elevated in the early corpus luteum (CL) and during the period of luteolysis. Regulation of intraluteal PG production is regulated by a variety of factors. An autoamplification pathway in which PGF-2alpha stimulates intraluteal production of PGF-2alpha has been identified in a number of species. The mechanisms underlying this autoamplification pathway appear to differ by species with expression of Cyclooxygenase-2 (Cox-2) and activity of phospholipase A2 acting as important physiological control points. In addition, a number of other responses that are induced by PGF-2alpha (decreased luteal progesterone, increased endothelin-1, increased cytokines) also have been found to increase intraluteal PGF-2alpha production. Thus, regulation of intraluteal PG production may serve to initiate or amplify physiological signals to the CL and may be important in specific aspects of luteal physiology particularly during luteal regression.

### Introduction

Although progesterone is the major luteal hormone, the CL also produces a number of other substances including prostaglandins (PGs) and oxytocin. The PGs are of particular interest because of their potential autocrine/paracrine actions within the CL. The involvement of PGF<sub>2α</sub> in regression of the CL in many species makes it likely that there is a role for intraluteal PGF<sub>2α</sub> production in luteal regression. In addition, functional roles for PGs in the early CL have also been postulated [1,2]. In this review the general pathways in PG production will be reviewed (Section I) before discussion of the evidence for and the timing of intraluteal PG production in various species (Section II). Although many factors can regulate intraluteal PG production, amplification pathways within the CL that are induced by PGF<sub>2α</sub> (Section III) or other factors (Section IV) will be emphasized in this review. Some speculations on the physiological role of intraluteal PG production are

also provided (Section V). Other excellent reviews have been previously published on regulation of intraluteal PG production [1,2] that contain material not discussed in this review.

### General Pathways for Production of Prostaglandins

Prostaglandins are derivatives of membrane phospholipids that regulate diverse physiological processes such as pregnancy, ovulation, luteolysis, inflammation, gastric secretion, and blood flow. The substrates for PG synthesis are arachidonylated phospholipids, such as plasmalogen phospholipids, such as plasmalogen phospholipids, and alkylacyl glycerophosphorylcholine. Prostaglandin biosynthesis begins with the liberation of arachidonic acid from these membrane phospholipids. This step is primarily catalyzed by the hormone-responsive enzyme cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) [3]. The cPLA<sub>2</sub> is one member of a larger family of

enzymes organized into 11 groups (I-XI) that also include various secreted forms of PLA<sub>2</sub> [4]. An increase in free intracellular calcium can cause activation of cPLA<sub>2</sub> by binding to an amino-terminal domain causing translocation to cellular membranes, particularly to the nuclear envelope and endoplasmic reticulum, where it can hydrolyze arachidonic acid from membrane phospholipids (see review by Gijon and Leslie [5]). However, one study did not detect translocation of cPLA<sub>2</sub>, but found that active or inactive cPLA<sub>2</sub> was randomly distributed in the cytoplasm [6]. Phosphorylation by MAP kinases of Ser 505 [7] can also contribute to full activation of cPLA<sub>2</sub>. In some cell types, activation of PKC and/or inhibition of certain phosphatases with okadaic acid also contribute to cPLA<sub>2</sub> activation [5]. Recent findings indicate that there is sustained cPLA<sub>2</sub> activity during luteolysis in the pseudopregnant rat [8].

Free arachidonic acid is converted to PGH<sub>2</sub> by the enzyme cyclooxygenase (Cox). This is generally considered the rate-limiting step in PG production and commits arachidonic acid to the PG synthesis pathway. There are two enzymatic steps in the conversion of arachidonic acid to PGH<sub>2</sub>. First, a cyclooxygenase step that catalyzes the conversion of arachidonic acid to PGG<sub>2</sub> and a second peroxidase step that reduces PGG<sub>2</sub> to PGH<sub>2</sub>. At least two isoforms of Cox exist, Cox-1 and Cox-2 that catalyze conversion of arachidonic acid to PGH<sub>2</sub> through a similar catalytic site and mechanism. A third Cox isoform, Cox-3, was postulated [9] and has been recently isolated and characterized [10]. This enzyme is derived from the Cox-1 gene but with retention of intron 1 in the mRNA. The physiological function of this third isoform or other Cox isoforms has not yet been determined, but Cox-3 may be the elusive target for acetaminophen action [10]. Cox-1 is characterized by constitutive expression in many tissues and may regulate various homeostatic functions such as arterial blood pressure [11] and gastric epithelium function [12]. Cox-2 is inducible in many tissues and has been found to regulate PG production during many acute responses such as inflammation [13]. Many non-steroidal anti-inflammatory drugs such as aspirin and indomethacin inhibit both Cox-1 and Cox-2; there are now Cox-2 specific inhibitors available commercially and for research purposes. Regulation of Cox-2 expression has been clearly demonstrated in the CL, as discussed below.

There is no clear evidence that we are aware of that arachidonic acid requires specific transport proteins to reach the Cox enzymes; however, there appears to be utilization of different arachidonic acid pools by Cox-1 and Cox-2 within the same cell. Antisense Cox-2 RNA treatment of murine macrophages blocks endotoxin-induced PGE<sub>2</sub> production, but not arachidonic acid release. The constitutively present Cox-1 enzyme in these cells was unable to

use this pool of endotoxin-stimulated arachidonic acid [14]. Surprisingly, addition of exogenous arachidonic acid to the media could be utilized by Cox-1, but not by Cox-2. In mouse mast cells, cytokine treatment (IL-10 + IL-1β + Kit ligand) induces Cox-2 and PGD<sub>2</sub> production, while IgE plus hapten-specific antigen induces Cox-1-mediated PGD<sub>2</sub> production that is not affected by the presence of Cox-2 [15]. These results in macrophages and mast cells indicate that Cox-1 and Cox-2 utilize different pools of arachidonic acid. The subcellular localization of Cox-1 and Cox-2 differ and this may be important for the differential use of substrate by these enzymes. Intense Cox-2 immunostaining is observed on the nuclear membrane, while Cox-1 immunostaining is equally localized to the ER and nuclear membrane [16]. Using a histofluorescence method for determining Cox activity, Morita et al. [16] found Cox-1 activity associated mainly with the ER, while Cox-2 activity was mostly in the nucleus. Intriguingly, as mentioned above, activated cPLA<sub>2</sub> also localizes to perinuclear membranes. Thus, activated cPLA<sub>2</sub> and Cox-2 may be in close subcellular proximity and this may explain why cPLA<sub>2</sub>-released arachidonic acid is utilized by Cox-2 while exogenously-added arachidonic acid is utilized by Cox-1 (reviewed in [5]). Subcellular distributions and differential utilization of arachidonic acid has not yet been experimentally evaluated in the CL.

After conversion of arachidonic acid to PGH<sub>2</sub> there can be production of a wide variety of PGs according to the particular PG synthase enzymes that are present. The PGF-synthase enzyme has been cloned and is a member of the aldo-keto reductase family of enzymes that includes 20α-hydroxysteroid dehydrogenase [17]. PGF-synthase mRNA has been identified in the CL but PGF<sub>2α</sub> treatment did not alter steady-state PGF-synthase mRNA concentrations [18]. Many cells, including luteal cells, have been found to produce multiple PGs that may have differential actions [19]. In luteal cells from day 17 pseudopregnant pigs, there is induction of luteal production of both PGF<sub>2α</sub> and PGE<sub>2</sub> by treatment with PGF<sub>2α</sub> [20]. This is surprising because PGF<sub>2α</sub> is considered to be luteolytic while PGE<sub>2</sub> is considered to be luteotropic. Thus, although differential expression of PG synthase enzymes seems like a potential mechanism to direct production of specific PGs, we have not yet found evidence of this differential regulation in the luteal cell literature.

Intraluteal metabolism of PGF<sub>2α</sub> could also be a physiologically regulated event. It has been known for many years that there is an enzymatic activity termed PGE<sub>2</sub>-9-ketoreductase that can convert PGF<sub>2α</sub> to PGE<sub>2</sub> or alternatively PGE<sub>2</sub> to PGF<sub>2α</sub> [21]. Similar to PGF-synthase, this enzyme was found to be part of the aldo-keto reductase family of enzymes [22]. Surprisingly, a pure preparation of the 20α-hydroxysteroid dehydrogenase enzyme was

found to also contain PGE<sub>2</sub>-9-ketoreductase activity [22]. Similarly, the sequences of the mRNA for these 2 enzymes are identical [23] suggesting that the enzyme that inactivates progesterone may also inter-convert PGs within the CL. Nevertheless, a functional role for PG conversion activity by this enzyme in the CL has not been determined. PGF<sub>2α</sub> can also be metabolized to the inactive PG, 13,14-Dihydro-15-Keto-PGF<sub>2α</sub> (PGFM) by the enzyme 15-hydroxyprostaglandin dehydrogenase (PGDH) [24]. Local metabolism of PGs has been reported in many tissues including the CL [25]. We found that PGDH mRNA was abundant in the pig CL [20]. In the sheep CL, PGDH activity is greatest in the early CL and during maternal recognition of pregnancy, both times when the CL is relatively resistant to PGF<sub>2α</sub> action [25]. This suggests that PGDH expression may have a luteoprotective role in the CL by inactivating any PGF<sub>2α</sub> that is produced; however, this hypothesis will require further experimental evaluation.

Prostaglandins have both hydrophobic and hydrophilic domains that should make it difficult for these compounds to traverse cellular membranes. Indeed, cellular transporters of PGs have been identified. These PG transporters contain 12 transmembrane domains and are part of the organic anion transport class of transmembrane proteins [26]. Expression of these transporters in *Xenopus* oocytes or HeLa cells allows entry of PGs into these cells [26]. Cell that synthesize PGs (as determined by Cox expression) also have substantial expression of the PG transporter [27] and this may be important for release of the synthesized PGs. Similarly, it seems likely that expression of PG transporters may be required to allow secretion of PGs from luteal cells; although this idea has never been tested.

Intraluteal actions of PGs are likely to be mediated through the plasma membrane PG receptors that are pharmacologically designated by their major PG ligand. For example, the receptors that bind PGF<sub>2α</sub> with high affinity have been designated FP receptors; whereas, the receptors that bind PGEs with high affinity are designated as EP1, EP2, EP3, and EP4. The FP receptor mRNA is induced in bovine granulosa cells within 1 d after the LH surge and is expressed at more than 100-fold greater concentration in the CL than in any other tissue [28]. Binding to the plasma membrane FP receptor probably requires an extracellular location for PGF<sub>2α</sub> to cause activation. There also appears to be expression of EP3 receptor in the CL; however, it is present at much lower concentrations than luteal FP receptor [28]. Conversely, intracellular PGs could potentially interact with specific peroxisome proliferator activated receptors (PPARs). The PPARs act as transcriptional factors and PPAR $\gamma$  has been found to be activated by PG<sub>2</sub>, a metabolite of PGD<sub>2</sub> [29]. It seems

likely that regulation of the transport of PGs through the plasma membrane (extracellular vs. intracellular) could be a mechanism for regulation of functional effects by luteal cell-derived PGs. This manuscript will primarily discuss the luteal production of PGs; however, a complete understanding of luteal PG action must also consider the diversity of distinct PG receptors linked to varying intracellular effector systems within the CL.

### Changes in Intraluteal PG Production During the Luteal Phase

As mentioned above, the corpora lutea of various species have the capacity to synthesize PGs (rats [19], rabbits [30], pigs [31-34], sheep [18,35], cows [36,37], horses [38], rhesus monkeys [39], cynomolgus monkeys [40], women [41]). Investigators have assessed the pattern of change in luteal PG production throughout the luteal phase in a number of different species. The objective of these experiments was frequently to determine if changes in luteotropic or luteolytic PGs coincided with developmental or regressive changes in luteal function. Often this was done by surgically removing the corpora lutea at three to four different stages of the cycle or pseudopregnancy. These stages represented luteal developmental (early luteal phase), maximal function (mid-luteal phase), and luteal regression (late luteal phase). Dispersed cells or tissue samples were incubated to assess their capacity to produce the various prostaglandins. Luteotropic PGs examined were PGE<sub>2</sub> and PGI<sub>2</sub> (as reflected by its major metabolite 6-keto-PGF<sub>1α</sub>). The luteolytic PG examined was PGF<sub>2α</sub>. Below we will discuss some experiments of this nature in various species.

#### Rats

Prior to examining luteal production of PGs in rats, studies were designed to examine how luteal concentrations of PGs varied throughout pseudopregnancy. Luteal concentrations of PGF<sub>2α</sub> increased as the luteal phase progressed from day 7 to 13 of pseudopregnancy in rats [42]. Luteal concentrations of PGE were greatest on day 11 and decreased by day 13. Luteal production of PGF<sub>2α</sub> and PGE<sub>2</sub> increased from day 7 to 10 and declined to day 13 of pseudopregnancy [19]. These increases in PG production coincided with the onset of decreased luteal progesterone production as the CL transitioned from day 7 to 10. Luteal production of 6-keto-PGF<sub>1α</sub> did not change over time.

#### Pigs

Porcine luteal tissue was collected from the slaughterhouse and staged on the basis of morphological and histological criteria. Corpora lutea were assigned to the following groups: early luteal (day 3-6), mid-luteal (day 7-14), and late-luteal (including corpora albicantes, day 15-19) [33]. Production of PGF<sub>2α</sub> and PGE<sub>2</sub> decreased from the early to the mid-luteal phase and rebounded in

the late luteal phase. The rise in PGF production from the mid to late luteal phase supports an earlier study from the same authors [34]. Using pigs whose stage of the estrous cycle was carefully monitored, Guthrie and Rexroad studied changes in luteal prostaglandin production in CL collected on day 8, 12, 14, 16, and 18. They also observed an increase in PGF<sub>2α</sub> production as the corpus luteum transitioned from mid to late luteal phase [31].

### Cows

Early bovine CL produced the highest levels of PGI<sub>2</sub> (as reflected by its major metabolite 6-keto-PGF<sub>1α</sub>) and PGF<sub>2α</sub> compared to mid- and late cycle [36]. Similar results were observed by Rodgers et al. [43]. These patterns of change in luteal production over time were reflected in the initial prostaglandin content. The ratio of the initial content of PGF<sub>2α</sub>:6-keto-PGF<sub>1α</sub> increased as the cycle progressed [36]. Koybayashi et al. [44] used an intraluteal microdialysis system to study production of luteal prostaglandins *in vivo* during days 3–6 of the estrous cycle. They found that luteal secretion of PGF<sub>2α</sub> and PGE<sub>2</sub> was elevated on day 3 and was relatively diminished on days 4–6. Consistent with these results on PG secretion, they also found that luteal concentrations of Cox-2 mRNA were greater in the early than in the mid to late luteal phase. In an experiment by Grazul et al. [45], patterns of luteal production of PGF<sub>2α</sub> did not vary significantly by stage of cycle. DelVecchio et al. [46-48] compared luteal production of prostaglandins collected at two stages of the estrous cycle. They found that luteal cells derived from late bovine CL produced more PGE<sub>2</sub> and PGF<sub>2α</sub> than those derived from mid-cycle CL.

### Horses

Corpora lutea were collected from mares on days 4–5, 8–9, and 12–13 [38]. Production of PGF, PGE<sub>2</sub>, and 6-keto-PGF<sub>1α</sub> was highest in corpora lutea collected in the early luteal phase. PGE<sub>2</sub> production increased from CL collected on days 8–9 to days 12–13, but production of the other prostaglandins did not change over this interval. The ratio of PGF:PGE<sub>2</sub> increased from days 4–5 to days 8–9, and remained higher than early in the luteal phase on days 12–13.

### Non-human old world primates

In the rhesus monkey [49], luteal production of PGE<sub>2</sub> and 6-keto-PGF<sub>1α</sub> was highest in the early luteal phase, decreased in mid-luteal phase, and remained suppressed in late luteal phase. The notion of a luteotropic role for PGE<sub>2</sub> and PGI<sub>2</sub> in primate CL was supported by significant positive correlations between luteal production of P and that of PGE<sub>2</sub> and 6-keto-PGF<sub>1α</sub> (the stable metabolite of PGI<sub>2</sub>). Concentrations of PGF<sub>2α</sub> decreased from early luteal phase to mid-luteal phase, but in contrast to the luteotropic PGs, rebounded in late luteal phase to levels

observed in early luteal phase. No differences were observed in the ratio of PGE<sub>2</sub>/PGF<sub>2α</sub> during the luteal phase, but there was a tendency for an increase in the ratio of PGF<sub>2α</sub>:6-keto-PGF<sub>1α</sub> from early luteal phase to mid-luteal phase, similar to the results from studies in cattle [36].

In the cynomolgus monkey, luteal production of PGF<sub>2α</sub> was highest in the early luteal phase, and there was no apparent rebound in the late luteal phase as was observed in the rhesus monkey [40]. Luteal production of PGE<sub>2</sub> was high in the early luteal phase, decreased in the mid-luteal phase, and rebounded in late luteal phase to levels observed in early luteal phase.

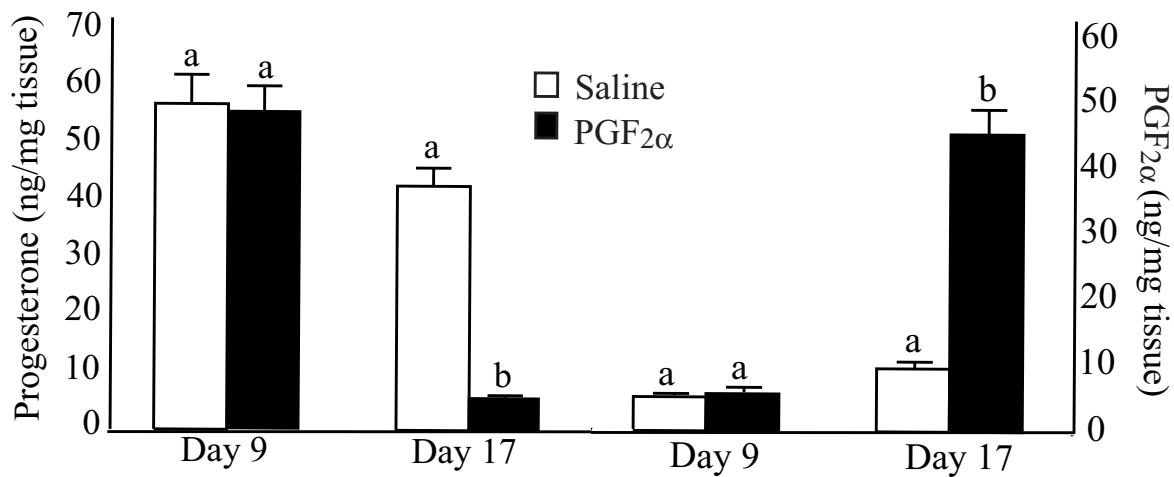
### Humans

Prior to studying luteal PG production, the content of PGs in the human CL was considered. In two experiments [50,51] there was a significant increase in luteal PGF<sub>2α</sub> concentration as the CL progressed from the mid to late luteal phase. Swanston et al. [52] also observed an increase in luteal content of PGF<sub>2α</sub>, but this increase occurred as late CL became corpora albicantes. Patwardhan and Lanthier [41] observed an increase in PGF content as the CL progressed from early to mid-luteal phase, and the high level was maintained during the late luteal phase.

Recently, PG production by human CL of different stages was assessed [53]. In this study, only two stages were considered, mid-luteal phase (days 5–9 after ovulation) and late luteal phase (days 10–14 after ovulation). Luteal production of PGF<sub>2α</sub>, PGE<sub>2</sub>, and 6-keto-PGF<sub>1α</sub> were significantly higher in the mid vs. late luteal phase.

To evaluate relative changes in PG synthetic enzymes over time, human CL were studied using immunocytochemistry [54]. Cyclooxygenase and PGF<sub>2α</sub> synthase were reported to qualitatively increase from early to mid-luteal phase and to further intensify by late luteal phase. Prostaglandin I<sub>2</sub> synthase appeared to be most prevalent in mid-luteal phase CL when compared to the other two stages examined. These data are consistent with the notion that luteal production of luteolytic PG increases as the luteal phase progresses in the woman. The data support the studies mentioned above with respect to luteal content of PGF<sub>2α</sub> [41,50-52], but they are not consistent with the PG production data of Friden et al. [53].

In assessing these types of studies, it can be seen that differences exist in the patterns of luteal PG production across species and experiments. However, with some exceptions, there are similarities that can be noted. First of all, luteal production of luteolytic and luteotropic PGs is often high in the early luteal phase. Many have suggested



**Figure 1**

Secretion of (A) progesterone and (B) PGF<sub>2α</sub> from tissues from gilts on day 9 of the estrous cycle or day 17 of pseudopregnancy treated *in vivo* with a PGF<sub>2α</sub> analog (Cloprostenol) 10 hours prior to ovary removal. Progesterone and PGF<sub>2α</sub> were measured in media following 2 hour incubation of collected porcine luteal tissue. A, B – statistically different ( $P < 0.05$ ) within day (from [20]).

that the luteotropic PGs may play a role in luteal development, and it is well known that young corpora lutea are not sensitive to the luteolytic effects of PGF<sub>2α</sub>. Additionally, in many studies/species there is either an increase in production of PGF<sub>2α</sub> as the CL ages from mid- to late luteal phase, or there is an increase in the ratio of PGF<sub>2α</sub>:PGE<sub>2</sub> or PGF<sub>2α</sub>:6-keto-PGF<sub>1α</sub>. As such, the balance of luteolysin to luteotropin often favors the luteolysin in the late luteal phase. Thus, a combination of changes in luteal production of luteotropic and luteolytic PGs and changes in the responsiveness of luteal tissue to these PGs may play a role in controlling luteal function in various species.

#### Regulation of Intraluteal PG by PGF<sub>2α</sub>

One of the most intriguing aspects of luteal PGF<sub>2α</sub> production is that there appears to be an autoamplification loop such that treatment of luteal cells with PGF<sub>2α</sub> induces production of PGF<sub>2α</sub> by luteal cells. In initial reports of this phenomenon, researchers treated sheep [35] or pigs [55] with cloprostenol (PGF<sub>2α</sub> analog) *in vivo*, removed the corpora lutea, and then incubated luteal slices *in vitro*. In these studies treatment *in vivo* with a PGF<sub>2α</sub> analog dramatically increased the *in vitro* production of PGF<sub>2α</sub>. Similarly, treatment of large luteal cells *in vitro* with PGF<sub>2α</sub> or

activation of PGF<sub>2α</sub> second messenger pathways (free calcium, activation of PKC) can increase PGF<sub>2α</sub> production *in vitro* [18,20]. As shown in Figure 1, this increase in PGF<sub>2α</sub> production only occurs in CL with luteolytic capacity (from pigs on day 17 of pseudopregnancy) and not in CL without luteolytic capacity (day 9 of the estrous cycle) [20]. Thus, there is clear evidence for an intraluteal positive feedback pathway for PGF<sub>2α</sub> production and this pathway appears to be associated only with CL that undergo regression after a single treatment with PGF<sub>2α</sub>.

There are probably multiple intracellular mechanisms involved with PGF<sub>2α</sub>-induced production of intraluteal PGF<sub>2α</sub>. There is evidence that PLA<sub>2</sub> activity is increased during luteolysis [56]. Treatment with PGF<sub>2α</sub> dramatically increases free intracellular calcium in large luteal cells [57] and free calcium will bind to cPLA<sub>2</sub> causing translocation to the nuclear membranes. In addition, PGF<sub>2α</sub> treatment activates MAP kinases [58] and activates PKC [59], both intracellular effectors that can contribute to activation of cPLA<sub>2</sub>. There was no detectable increase in luteal cPLA<sub>2</sub> mRNA after *in vivo* treatment with PGF<sub>2α</sub> [60], suggesting that luteal changes in cPLA<sub>2</sub> were probably mediated by protein activation rather than transcriptional regulation. Thus, treatment of CL with PGF<sub>2α</sub> is likely to cause

translocation and activation of cPLA<sub>2</sub> through a number of key intracellular pathways allowing liberation of free arachidonic acid from membrane phospholipids. Treatment *in vivo* with PGF<sub>2α</sub> also dramatically increases expression of Cox-2 mRNA and protein [18,20,61]. *In vitro* treatment of large luteal cells with PGF<sub>2α</sub> or activators of intracellular effector systems that are activated by PGF<sub>2α</sub> also dramatically increased Cox-2 mRNA, protein, and PGF<sub>2α</sub> production [18,20]. Thus, PGF<sub>2α</sub> will activate both of the key rate-limiting steps in PGF<sub>2α</sub> production by activating the cPLA<sub>2</sub> protein and by inducing Cox-2 activity.

The molecular mechanisms involved in PGF<sub>2α</sub> induction of Cox-2 have been analyzed. There was about a 40-fold induction of reporter gene expression following treatment of transfected large luteal cells with PGF<sub>2α</sub> [62]. This induction could be inhibited by a specific inhibitor of PKC, myristoylated pseudosubstrate for PKCα and β. Inhibitors of calcium calmodulin kinase and the MAP kinase pathways had no effect on PGF<sub>2α</sub> induction of Cox-2. We have found that there are three key DNA response elements in the 5' flanking DNA region that act synergistically to regulate induction of Cox-2 by PGF<sub>2α</sub> in ovine large luteal cells [62]. The most critical element is an E-box region that is about 50 bp upstream of the transcription initiation site. Thus, PGF<sub>2α</sub> acting through PKC and an E-box DNA element, specifically increases Cox-2 gene transcription.

The mechanisms underlying the stage-specific regulation of this autoamplification pathway remain unresolved in spite of recent studies using a variety of luteal models. In the bovine CL, PGF<sub>2α</sub> induces expression of Cox-2 mRNA only in CL with luteolytic capacity (day 11 of estrous cycle). In the bovine CL without luteolytic capacity (day 4), treatment with PGF<sub>2α</sub> induces a number of cellular responses, indicating that the PGF<sub>2α</sub> does activate FP receptors in the early CL, but there is no induction of Cox-2 mRNA [61,63]. In non-luteinized granulosa cells, activation of protein kinase (PK) A by cAMP was a potent stimulator of Cox-2 gene expression; whereas, PGF<sub>2α</sub> had no effect on Cox-2 expression. However, after 7 days of luteinization during culture, PGF<sub>2α</sub> became a potent stimulator of Cox-2 expression, similar to the timing observed *in vivo* [64]. In ovine granulosa cells, Cox-2 mRNA and PGF<sub>2α</sub> secretion were also induced by activation of the PKA but not the PKC pathway [62]. In contrast in ovine large luteal cells, Cox-2 mRNA and PGF<sub>2α</sub> production were primarily stimulated by PKC and not PKA. Wu and Wiltbank used bovine granulosa cells to evaluate the changes in expression of the Cox-2 promoter linked to a luciferase reporter during luteinization and acquisition of luteolytic capacity [65]. In non-luteinized granulosa cells, activation of PKA but not PKC dramatically increased Cox-2 promoter-driven expression. However, after 8 days

of luteinization the promoter became responsive to PKC and PGF<sub>2α</sub> but not to PKA activation. Intriguingly, the induction of the Cox-2 promoter by either PKA (non-luteinized granulosa cells) or PKC (luteinized granulosa cells) was mediated by the E-box element mentioned above. Tsai et al., also found that human granulosa-lutein cells, only display this PGF<sub>2α</sub> autoamplification pathway after 8 days of luteinization [66]. In early granulosa-lutein cells (day 2 of culture) there is no increase in Cox-2 in response to PGF<sub>2α</sub> treatment. However after 8 days of luteinization, PGF<sub>2α</sub> dramatically induced Cox-2 mRNA and inhibited progesterone production, indicating the acquisition of luteolytic capacity develops over time in culture.

In contrast to the studies cited above using bovine, ovine, and human luteal or granulosa-lutein cells, studies with the pig CL have not found an association between luteal Cox-2 expression, luteal PGF<sub>2α</sub> production, and luteolytic capacity [20]. *In vivo* treatment with PGF<sub>2α</sub> induced expression of Cox-2 mRNA and protein in either CL without luteolytic capacity (day 9) or with luteolytic capacity (day 17). However, there was no increase in luteal PGF<sub>2α</sub> production in day 9 CL (Figure 1) in spite of the dramatic induction of Cox-2 mRNA. We were unable to determine the rate-limiting step that prevented induction by PGF<sub>2α</sub> of intraluteal PGF<sub>2α</sub> production in the early pig CL [20].

The physiological significance of this autoamplification pathway has not yet been clearly defined but it would allow small amounts of PGF<sub>2α</sub> from the uterus to induce a dramatic increase in intraluteal PGF<sub>2α</sub> production. Intraluteal PGF<sub>2α</sub> production may be crucial for complete luteolysis. In an elegant series of recent experiments, Griffeth et al. [67], found that intraluteal treatment with the PG synthesis inhibitor, indomethacin, prevented the decrease in luteal weight that is normally associated with luteal regression; although, the decrease in luteal progesterone production was not affected. Intriguingly, they also reported that in hysterectomized ewes, doses of PGF<sub>2α</sub> (1 mg and 3 mg) that caused luteal regression also induced peaks of PGFM at 12–36 hours after PGF<sub>2α</sub> treatment. These peaks of PGFM were not of uterine origin and may have originated from the CL.

### Other Regulators of Intraluteal PG Production

It appears that secretion of PGF<sub>2α</sub> and progesterone are interrelated. Treatment of CL that have luteolytic capacity with exogenous PGF<sub>2α</sub> inhibits progesterone production. Conversely, treatment of bovine luteal cells with progesterone decreased PGF<sub>2α</sub> production in a dose-dependent fashion [68]. However, progesterone may affect intraluteal PG production differently in CL with or without luteolytic capacity. Okuda and Skarzynski [1] reported that blockade of the progesterone receptor with a highly

selective progesterone antagonist, onapristone, inhibited  $\text{PGF}_{2\alpha}$  production by bovine luteal cells from the early CL (Days 4–5); whereas, onapristone treatment increased  $\text{PGF}_{2\alpha}$  production by bovine luteal cells from mid-cycle CL (Days 8–12). Thus, the effect of progesterone in regulating intraluteal PG production may shift from stimulatory to inhibitory during luteal development.

Cytokines are also potent stimulators of luteal  $\text{PGF}_{2\alpha}$  production. Interleukin-1 $\beta$  and tumor necrosis factor- $\alpha$  dramatically stimulated  $\text{PGF}_{2\alpha}$  production by cultured bovine luteal cells [37,69,70]. The acute stimulation of  $\text{PGF}_{2\alpha}$  production has been postulated to occur through activation of cPLA<sub>2</sub> [70-72]. The chronic effects (72 h) of interleukin-1 $\beta$  may be mediated by upregulation of Cox [70]. In human luteinized granulosa cells, interleukin-1 $\beta$  was also found to increase prostanoid synthesis, increase Cox-2 mRNA, and decrease Cox-2 mRNA degradation [73]. Interferon- $\gamma$  has also been found to stimulate luteal  $\text{PGF}_{2\alpha}$  production, but only after 72 h of treatment [74]. Of particular importance, all of the stimulatory effects of these three cytokines on  $\text{PGF}_{2\alpha}$  production could be inhibited by simultaneous treatment with progesterone [37,71,74]. Although cytokines increase  $\text{PGF}_{2\alpha}$  production, it does not appear that  $\text{PGF}_{2\alpha}$  production is required for cytokine-mediated inhibition of progesterone production or induction of cell death because indomethacin treatment did not prevent these cytokine actions [74,75].

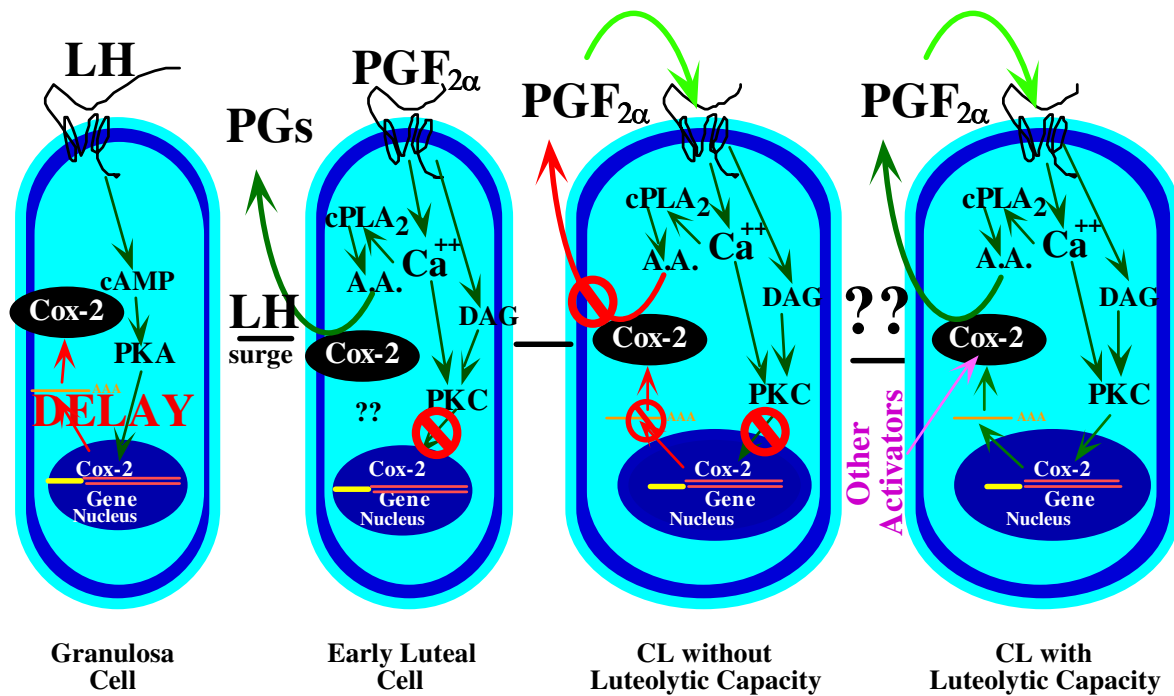
Another peptide that appears to be critical in luteolysis is endothelin-1 (ET-1; see Milvae [76] for review). Treatment with ET-1 decreases progesterone production from bovine luteal cells *in vitro* or *in vivo*, an effect blocked by a specific ETA receptor antagonist [77,78]. In bovine microdialyzed CL, ET-1 was effective at decreasing progesterone only after CL were exposed to  $\text{PGF}_{2\alpha}$  [79]. Moreover, there appears to exist an intraluteal amplification pathway between  $\text{PGF}_{2\alpha}$  and ET-1. Treatment with  $\text{PGF}_{2\alpha}$  induces ET-1 mRNA and protein in bovine CL [80], and conversely treatment with ET-1 will induce  $\text{PGF}_{2\alpha}$  production by luteal cells [81]. Thus, ET-1, as well as certain cytokine peptides, has complex but critical relationships with  $\text{PGF}_{2\alpha}$  and progesterone during luteolysis. Further research will be required to define the precise temporal sequence and intracellular mechanisms involved in the interrelationships of these peptides with the actions and production of intraluteal  $\text{PGF}_{2\alpha}$  and progesterone. Estradiol-17 $\beta$ , oxytocin, noradrenaline, and nitric oxide have also been found to stimulate intraluteal PG production (reviewed in [1]), and these could serve to amplify signals that reach luteal cells from extraluteal or intraluteal sources.

### Possible Physiological Role of Intraluteal PG production

Figure 2 shows a simplified model for the regulation of PG production in the CL. The LH surge dramatically induces Cox-2 expression in the granulosa cells, and there is a subsequent increase in intrafollicular PG production. This induction is mediated by the cAMP/PKA intracellular effector system and there is a delay between the LH surge and Cox-2 expression [82]. The molecular mechanisms causing this delay in expression are unclear but this allows PG production to occur only a few hours before the time of ovulation [83]. This induction of Cox-2 is essential for ovulation as evidenced by the lack of ovulation in Cox-2 knock-out mice [84]. Binding of  $\text{PGE}_2$  to the EP2 receptor appears to be essential for normal ovulation [85].

Within 2 days after the LH surge there is an induction of FP receptors in the CL [82]. The presence of FP receptor on the luteinized granulosa cells (large luteal cells) would allow response to  $\text{PGF}_{2\alpha}$ . However during this early time period, treatment with  $\text{PGF}_{2\alpha}$  does not cause luteolysis. As reviewed above, the CL of most species produce large amounts of PGs during the early luteal phase. The physiological importance of intraluteal PG production in the early CL is not clear. Okuda and Skarzynski [1] suggested that since luteal production of  $\text{PGF}_{2\alpha}$  is high in the early luteal phase of the cow, the early CL may be desensitized to  $\text{PGF}_{2\alpha}$ . If so, this would have to be a very specific desensitization, since  $\text{PGF}_{2\alpha}$  has been shown to stimulate a number of events in the early CL [63]. Treatment with indomethacin and sodium meclofenamate during the early to mid-luteal phase caused a decrease in circulating progesterone, suggesting a luteotropic role of luteal PGs [86,87]. However, it is also possible that one of these inhibitors, meclofenamate, had other direct effects on luteal cAMP or progesterone production that were independent of inhibition of PG production, as suggested by Zelinski-Wooten et al. [88]. Alternatively, perhaps luteotropic PGs, produced by the CL, protect the CL from luteolytic  $\text{PGF}_{2\alpha}$  during the early luteal phase. In addition, CL without luteolytic capacity appear to lack the pathways that induce intraluteal  $\text{PGF}_{2\alpha}$  production, suggesting that lack of these pathways is critical to protect the CL from luteolysis during the early luteal phase. Intraluteal PG production in the early CL may have numerous other physiological functions such as blood flow regulation, intercellular communication, or cellular differentiation that have not yet been clearly examined.

The physiological role of intraluteal  $\text{PGF}_{2\alpha}$  production in the luteolytic cascade has been frequently discussed. In species without involvement of the uterus in luteal regression it seems likely that intraluteal production of  $\text{PGF}_{2\alpha}$  may be a critical part of the luteolytic mechanism. In primates, as in other species, changes in the responsiveness



**Figure 2**

A model for the regulation of PG production during different stages of luteal differentiation. In the granulosa cell of the preovulatory follicle there is very low PG production and low expression of Cox-2. The LH surge induces Cox-2 expression through the protein kinase A (PKA) pathway but with a delay in expression depending upon the species [83]. In the early luteal cell there is high PG production that is stimulated by pathways that have not yet been defined. It is also possible that high Cox-2 protein has been left after the dramatic induction of Cox-2 after the LH surge. In the early luteal cell and in the CL without luteolytic capacity (these 2 stages may overlap), there are  $PGF_{2\alpha}$  receptors but  $PGF_{2\alpha}$  does not stimulate increased intraluteal PG production (shown by red lines). In addition,  $PGF_{2\alpha}$  does not induce other activators of PG production, such as decreased progesterone secretion, increased endothelin-1 production, or increased cytokine production. Unknown mechanisms cause the CL to acquire luteolytic capacity. After acquisition of luteolytic capacity, treatment with  $PGF_{2\alpha}$  increases intraluteal PG production. Activation of cytosolic phospholipase  $A_2$  (cPLA $_2$ ) by increased free intracellular calcium concentrations provides arachidonic acid (A.A.) substrate to the induced Cox-2 enzyme. Although not shown, these events are likely to be localized to the nuclear membrane. Intraluteal  $PGF_{2\alpha}$  production activates an autoamplification loop in the mature CL due to  $PGF_{2\alpha}$ -induced Cox-2 expression and  $PGF_{2\alpha}$  induction of other activators of Cox-2 expression.

of the corpus luteum to PGs as the corpus luteum ages may be as important as changes in luteal production of PGs [89,90]. In species with uterine-dependent regression of the CL, it seems clear that the luteolytic factor secreted by the uterus is  $PGF_{2\alpha}$ . However, the small amounts of uterine  $PGF_{2\alpha}$  could be dramatically amplified by intraluteal  $PGF_{2\alpha}$  production. As discussed above, this could be through direct effects of  $PGF_{2\alpha}$  on Cox-2 expression and induction of other  $PGF_{2\alpha}$  biosynthesis pathways (Section III). In addition,  $PGF_{2\alpha}$  would decrease luteal progesterone production and increase luteal cytokine and endothe-

lin production; changes that have all been found to increase luteal  $PGF_{2\alpha}$  production (Section IV). Thus, luteal  $PGF_{2\alpha}$  production could serve to initiate (primates and possibly other species with uterine-independent luteal regression) or amplify (species with uterine-dependent luteal regression) the luteolytic cascade. The intriguing preliminary report by Griffeth et al. [67] raises the possibility that extraluteal signals (e.g., uterine  $PGF_{2\alpha}$ ) may initiate the inhibition of progesterone production that accompanies luteal regression, but that the increase in intraluteal  $PGF_{2\alpha}$  production may be critical for the struc-



tural demise of the CL. In addition, intraluteal PGE<sub>2</sub> production is increased to a similar extent as PGF<sub>2α</sub> production during luteolysis [91]. Similarly, we have consistently found intraluteal PGE<sub>2</sub> and PGF<sub>2α</sub> production to be highly correlated (LA Anderson and MC Wiltbank, unpublished results). It seems likely that increased production of both PGs is mediated by the increased Cox-2 and PLA<sub>2</sub> activity associated with luteolysis. A possible physiological role for increased intraluteal PGE during luteolysis is not clear; indeed, the PGE increase seems counterintuitive given the reported luteal protective effects of PGEs [92]. It seems clear that in spite of the substantial scientific progress that has been achieved in this research area in the past few years, there still remain numerous physiological and molecular questions related to intraluteal PG production.

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