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—Original Article—

# Prostaglandin $F_{2\alpha}$ Differentially Affects mRNA Expression Relating to Angiogenesis, Vasoactivation and Prostaglandins in the Early and Mid Corpus Luteum in the Cow

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**Abstract.** Administration of prostaglandin (PG) $F_{2\alpha}$ in cattle during the mid-luteal phase (Days 8–12 of the estrous cycle) drastically reduces the plasma progesterone concentrations and the volume of the corpus luteum (CL). However, $PGF_{2\alpha}$ does not induce luteolysis during the early luteal phase (up to Day 5 of the estrous cycle). To characterize the possible distinct difference in acute response to a luteolytic dose of $PGF_{2\alpha}$ administration, we determined various mRNA expressions in the early and mid CL relating to angiogenesis, vasoactivation and PG-related factors at 30 min after $PGF_{2\alpha}$ injection in cyclic cows. The experiments were conducted on Day 4 (early CL) and Days 10–12 (mid CL). Cows were either injected with 500 $\mu$g $PGF_{2\alpha}$ analogue or saline as the control (early CL control, n=5; early CL $PGF_{2\alpha}$ treated, n=5; mid CL control, n=5; mid CL $PGF_{2\alpha}$ treated, n=7). Thirty min after injection of $PGF_{2\alpha}$ or saline, the cows were ovariectomized transvaginally, and the CL tissues were collected from regions designated as the periphery and center of the CL. Administration of $PGF_{2\alpha}$ up-regulated the mRNA expressions of angiogenic-related factors such as vascular endothelial growth factors, vasohibin, fibroblast growth factor 2 and insulin-like growth factor-II in the early CL, whereas $PGF_{2\alpha}$ down-regulated these mRNA expressions in the mid CL. In the vasoactive factors, $PGF_{2\alpha}$ stimulated the mRNA expressions of endothelin-1, angiotensin converting enzyme, endothelial nitric oxide synthase (NOS) and inducible NOS in the periphery area of the mid CL, but not in the early CL. However, $PGF_{2\alpha}$ drastically down-regulated $PGF_{2\alpha}$ receptor mRNA expression in both regions of the early and mid CL. The results indicated a clear difference in the acute action of $PGF_{2\alpha}$ depending not only on the luteal phase (immature *vs.* mature) but also the region (periphery *vs.* center) within the CL at 30 min after $PGF_{2\alpha}$ injection in the cow.

**Key words:** Corpus luteum, Cow, Luteolysis, Prostaglandin $F_{2\alpha}$

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The corpus luteum (CL) is a transient endocrine organ in the ovaries of mammals. The bovine CL rapidly develops within 2–3 days after ovulation, which is accompanied by angiogenesis from the preovulatory follicle. The main function of the CL is to produce the progesterone (P) required for achievement and maintenance of pregnancy [1]. If the cow does not become pregnant, the CL is only functional for 17–18 days, and it must regress within a few days to induce the next chance for ovulation.

In cows, it is well known that prostaglandin (PG) $F_{2\alpha}$ from the uterus on days 17–18 of the estrous cycle is essential to induce regression of the CL [2]. In general, administration of $PGF_{2\alpha}$ in the cow during the mid-luteal phase (Days 8–12 of the estrous cycle; mid CL) drastically reduces the plasma P concentration and volume of the CL. However, the same dose of $PGF_{2\alpha}$ does not induce luteolysis during the early luteal phase (up to Day 5 of the estrous cycle; early CL) [3]. Recently, several studies have focused on the different actions of $PGF_{2\alpha}$ on the systems for steroidogenesis [4], prostaglandin synthesis [5, 6], immune function [7] and vasoactive factors [8–10] between the early CL ($PGF_{2\alpha}$-resistant) and mid CL ($PGF_{2\alpha}$-responsive) in domestic animals. Although $PGF_{2\alpha}$ acutely decreases mRNA expression of 3$\beta$-hydroxysteroid dehydrogenase (3$\beta$-HSD) in both the early and mid CL, $PGF_{2\alpha}$ decreases steroidogenic acute regulatory protein (StAR) mRNA expression in the mid CL but not in the early CL [4], indicating that StAR is a key enzyme to reduce steroidogenesis by $PGF_{2\alpha}$ administration in the cow. Also, the levels of mRNA for cyclooxygenase-2 (COX-2) in the CL elevate after 4 h in response to an injection of $PGF_{2\alpha}$ on Day 11 of the estrous cycle [4]. However, on Day 4 of the estrous cycle, $PGF_{2\alpha}$ does not stimulate COX-2 mRNA after 4 h in the CL [4]. On the other hand, $PGF_{2\alpha}$ drastically decreases $PGF_{2\alpha}$ receptor (FPr) mRNA expression in both the early and mid CL in the cow [4]. These findings suggest that $PGF_{2\alpha}$ clearly exerts actions in both the early and mid bovine CL. Taken together, the mechanism by which $PGF_{2\alpha}$ exerts its luteolytic action in the mid CL, but not in the early CL, still remains unknown.

Many studies have tried to configure the time course of the CL response to $PGF_{2\alpha}$ injection to a period 2 h after or later. However, $PGF_{2\alpha}$ can reach the CL much earlier because *in vivo* administration stimulates oxytocin release from the CL within 5 min [11]. Additionally, we previously reported that administration of $PGF_{2\alpha}$ with a luteolytic dose in the cow induced an acute increase in blood

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flow at the periphery of the CL starting 30 min after treatment during the mid-luteal phase, but not during the early luteal phase [12]. Moreover, we recently reported that more large blood vessels (arteriolovenous vessels) exists in the periphery compared with the center of the matured CL, whereas both the periphery and center of the CL are similarly distributed with capillaries and arteriolovenous vessels in the early CL [13]. Thus, we suggest that this structural change from the early (homogeneous) to the mid (heterogeneous) luteal phase is related to the difference in the CL response to increased blood flow caused by $PGF_{2\alpha}$ observed only in the mature CL [13]. These findings suggest that there are clear differences in the acute action of $PGF_{2\alpha}$ between the early and mid CL at 30 min after administration.

Therefore, to characterize the possible distinct differences in acute response to a luteolytic dose of $PGF_{2\alpha}$ administration, we determined various mRNA expressions in the early and mid CL such as those of angiogenic-, vasoactive-, PG- and steroidogenic-related factors at 30 min after $PGF_{2\alpha}$ injection in cyclic cows.

## Materials and Methods

All experiments were conducted at the Field Center of Animal Science and Agriculture, Obihiro University, and all experimental procedures complied with the Guidelines for the Care and Use of Agricultural Animals of Obihiro University. Multiparous, non-lactating Holstein cows were used for this study; each had at least 2 estrous cycles of normal length (21–23 days) before being used. Luteolysis was induced by intramuscular (i.m.) injection of 500 $\mu$g of the $PGF_{2\alpha}$ analogue (cloprostenol: Estrumate®; Takeda Pharmaceutical, Osaka, Japan), and 100 $\mu$g of GnRH (Conceral®; Takeda Pharmaceutical) was injected i.m. 48 h after the $PGF_{2\alpha}$ injection to ensure ovulation. The day of GnRH injection was designated as Day 0.

### *Experimental design*

The object of the experiment was to determine the acute effects of $PGF_{2\alpha}$ administration on mRNA expression in the periphery and center regions of the CL at the early and mid-luteal phases (immature or early CL *vs.* mature or mid CL). Twenty-two multiparous, non-lactating Holstein cows were used for this study. The experiments were conducted on Day 4 (early CL) and Days 10–12 (mid CL). Cows were either injected with $PGF_{2\alpha}$ or saline as a control (early CL control, n=5; early CL $PGF_{2\alpha}$ treated, n=5; mid CL control, n=5; mid CL $PGF_{2\alpha}$ treated, n=7). Thirty min after injection of $PGF_{2\alpha}$ or saline, luteal blood flow was determined using color Doppler ultrasound, and thereafter, the cows were immediately subjected to transvaginal ovariectomy as reported previously [14]. To examine the local effect of $PGF_{2\alpha}$ in the CL, tissue samples were collected from regions designated as the periphery of the CL and center of the CL in our previous study [15]. Immediately after collection, the samples were placed into a 1.5 ml microcentrifuge tube containing 0.4 ml TRIzol reagent, homogenized and stored at –80 C until analysis.

### *RNA extraction and cDNA production*

The ovary with the CL was collected by ovariectomy, and then total RNA was extracted from CL samples following the protocol of Chomczynski and Sacchi using TRIzol reagent [16] as in our previous study [15]. The extracted total RNA was stored in RNA storage solution (Ambion, Texas, USA) at –80 C until being used for cDNA production. RNA samples were then used to produce cDNA as in our previous study [15]. The synthesized cDNA was stored at –30 C.

### *Real-time reverse transcription-polymerase chain reaction (real-time RT-PCR)*

The following factors were analyzed in the present study: vascular endothelial growth factor (VEGF) 120 and 164, vasohibin, fibroblast growth factor 2 (FGF2), angiopoietin (ANPT) 1 and ANPT2, Tie2 (ANPT receptor), insulin-like growth factor (IGF)-I and IGF-II, endothelin-1 (EDN1), EDN receptor type A (EDNAR) and B (EDNBR), angiotensin converting enzyme (ACE), angiotensin II receptor type 1 (AT1R) and 2 (AT2R), endothelial nitric oxide synthase (eNOS), inducible NOS (iNOS), COX-2, prostaglandin F synthase (PGFS), prostaglandin E synthase (PGES), prostaglandin I synthase (PGIS), FPr, StAR, cytochrome P450 side-chain cleavage enzyme (P450scc), 3$\beta$-HSD and $\beta$-actin. The levels of mRNA expressions were quantified by real-time PCR with a LightCycler (Roche Diagnostics, Indianapolis, IN, USA) using a commercial kit (LightCycler FastStart DNA Master SYBR Green I, Roche Diagnostics). The primers were designed using Primer-3 based on bovine sequences. The amplification program consisted of 15 min activation at 95 C followed by 40 cycles of PCR steps (15 sec denaturation at 94 C, 30 sec annealing at 58 C and a 20 sec extension at 72 C). For quantification of the target genes, a series of standards was constructed by amplifying a fragment of DNA (150–250 bp) that contains the target sequence for real-time PCR. The primers used for real-time PCR are listed in Table 1. The PCR products were subjected to electrophoresis, and the target band was cut out and purified using a DNA purification kit (SUPREC™-01, TaKaRa Bio. Otsu, Japan). Three to five stepwise-diluted DNA standards were included in every PCR run. The quantification of mRNA expression was performed using Light Cycler Software (Version 3.5; Roche). Primer sets were tested in luteal tissue samples to confirm amplification of single bands, and amplified products were cloned and sequenced to confirm their identify, prior to use of primers in analysis of samples. The values were normalized using $\beta$-actin as the internal standard.

### *Statistical analysis*

All mRNA expression data are presented as means ± SEM. All data were analyzed by Student's *t*-test following by the *F*-test. Probabilities less than 5% ($P<0.05$) were considered to be significant.

## Results

### *The mRNA expression of angiogenic-related factors in the early and mid CL after $PGF_{2\alpha}$ administration*

The results of quantitative analysis of the mRNA expressions of VEGF120 (A), VEGF164 (B), Vasohibin (C), FGF2 (D), ANPT1 (E), ANPT2 (F), ANPT2/ANPT1 ratio (G), Tie2 (H), IGF-I (I) and

**Table 1.** Primers used in realtime PCR

| Gene | | Sequence of nucleotide (5'-3') [a] | Accession No. |
|---|---|---|---|
| $VEGF_{120}$ | FWD | CCCAGATGAGATTGAGTTCATTTT | M32976 |
| | REV | GCCTCGGCTTGTCACATTTT | |
| $VEGF_{164}$ | FWD | CCCAGATGAGATTGAGTTCATTTT | M32976 |
| | REV | AGCAAGGCCCACAGGGATTT | |
| Vasohibin | FWD | CAGTCAAGGATTTGGGGATG | BC111257 |
| | REV | ACCCCGGTTAACAAAGAAGG | |
| FGF2 | FWD | GAACGGGGGCTTCTTCCT | M13440 |
| | REV | CCCAGTTCGTTTCAGTGCC | |
| ANPT1 | FWD | TGCCAGAACCCAAAAAGGTA | AF093573 |
| | REV | CCCCAACCAATATTCACCAG | |
| ANPT2 | FWD | ACCCTTCAGGTGAACACTGG | AF094699 |
| | REV | CGTGAGGCCTTTAAGGTGAA | |
| Tie2 | FWD | GAATGCCCCAAAGGTGATCG | X71424 |
| | REV | TCCGTCCGAGGCTCCAAATA | |
| IGF-I | FWD | TGCAAAAATGACCCTGGAGT | MN001077828 |
| | REV | ACAAGCCTGCTGAATGAATGT | |
| IGF-II | FWD | GACCGCGGCTTCTACTTCAG | MN174087 |
| | REV | AAGAACTTGCCCACGGGGTAT | |
| EDN1 | FWD | CAAATGCATCCTGCCTGGTC | X52740 |
| | REV | ATTGCCACCCCCATAGAGGA | |
| EDNAR | FWD | GCATCCAGTGGAAGAACCAT | X57765 |
| | REV | AACCAGTCAACCCTTCAACG | |
| EDNBR | FWD | GCTCCATCCCACTCAGAAAA | D90456 |
| | REV | GCCAACACAGAGCAAAGACA | |
| ACE | FWD | GGTCCATCCCCTACTTC | AJ309016 |
| | REV | GCGTGCAGGTTCAGGTAGA | |
| AT1R | FWD | GGAAACAGCTTGGTGGTGAT | MN174233 |
| | REV | AAACACGCTGGCATAGAGGT | |
| AT2R | FWD | AGCTGGGATTGCCTTAATGA | XM589248 |
| | REV | GAAGGGAAGCCAACAAATGA | |
| eNOS | FWD | GGAAATCGGGGGTCTGGAGT | M89952 |
| | REV | TTGGCGAGCTGAAAGCTGTG | |
| iNOS | FWD | TCATCTTCGCCACCAAGCAG | U14640 |
| | REV | CAGTGATGGCCGACCTGATG | |
| FPr | FWD | GGCCAGCATTGGAATGAATA | MN181025 |
| | REV | CTCCACAACAGCGTCTGGTA | |
| COX-2 | FWD | TCCTGAAACCCACTCCCAACA | AF031698 |
| | REV | TGGGCAGTCATCAGGCACAG | |
| PGFS | FWD | GATGGCCACTTCATTCCTGT | J03570 |
| | REV | CACAGTGCCATCTGCAATCT | |
| PGES | FWD | CGCTGCTGGTCATCAAAAT | AY032727 |
| | REV | GGAAGGGGTAGATGGTCTCC | |
| PGIS | FWD | AGGATGAAGGAGAAGCATGG | MN174444 |
| | REV | TGTGGAGGAGAGTCGGTTTC | |
| StAR | FWD | GTGGATTTTGCCAATCACCT | MN174189 |
| | REV | TTATTGAAAACGTGCCACCA | |
| P450scc | FWD | CTGCAAATGGTCCCACTTCT | K02130 |
| | REV | CACCTGGTTGGGTCAAACTT | |
| 3$\beta$-HSD | FWD | TCCACACCAGCACCATAGAA | X17614 |
| | REV | AAGGTGCCACCATTTTTCAG | |
| $\beta$-actin | FWD | CCAAGGCCAACCGTGAGAAAAT | K00622 |
| | REV | CCACATTCCGTGAGGATCTTCA | |

[a] FWD, forward; REV, reverse.

IGF-II (J) are shown in Fig. 1. $PGF_{2\alpha}$ increased the mRNA expressions of VEGF120, VEGF164 and Vasohibin in the periphery area of the early CL, but decreased these expressions in both areas of the mid CL after $PGF_{2\alpha}$ injection (Fig. 1A–C). $PGF_{2\alpha}$ increased FGF2 mRNA expression in the periphery and center area of the early CL, but decreased the expression in the center area of the mid CL after $PGF_{2\alpha}$ injection (Fig. 1D). Although $PGF_{2\alpha}$ did not change ANPT1 mRNA expression in the early and mid CL (Fig. 1E), it did increase

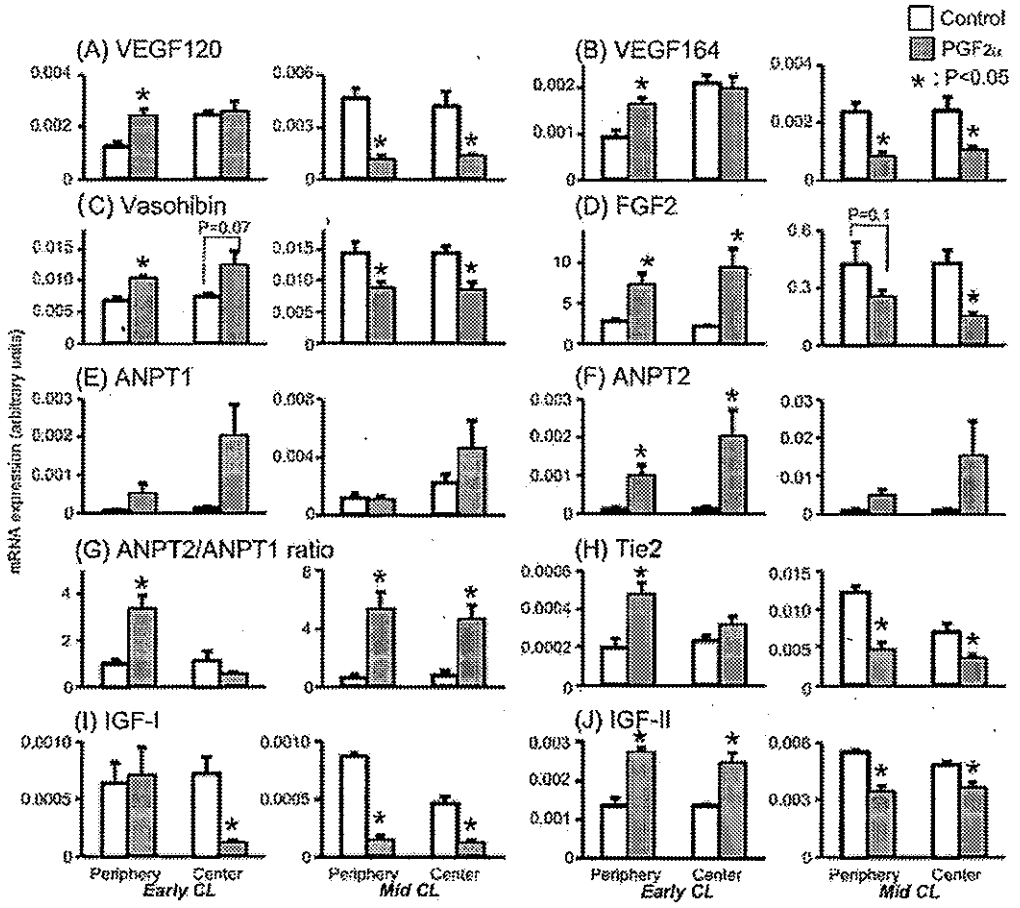


**Fig. 1.** Changes of mRNA expressions of angiogenic-related factors in the early and mid CL after $PGF_{2\alpha}$ administration. A: VEGF120. B: VEGF164. C: Vasohibin. D: FGF2. E: ANPT1. F: ANPT2. G: ANPT2/ANPT1 ratio. H: Tie2. I: IGF-I. J: IGF-II. In each figure, the left panel indicates the early CL, and the right panel indicates the mid CL. White bars indicate the control treatment, and black bars indicate the $PGF_{2\alpha}$ treatment. Means ± SEM are presented. Different superscripts indicate statistically different values ($P<0.05$).

ANPT2 mRNA expression in both areas of the early CL, but not in the mid CL (Fig. 1F). In terms of the expression ratio of ANPT2/ANPT1, $PGF_{2\alpha}$ increased the ratios in the periphery area of the early CL and in both areas of the mid CL (Fig. 1G). Although $PGF_{2\alpha}$ stimulated Tie2 mRNA expression in the periphery area of the early CL, it decreased the expression in both areas of the mid CL (Fig. 1H). IGF-I mRNA expression was decreased by $PGF_{2\alpha}$ injection in the center area of the early CL and in both areas of the mid CL (Fig. 1I). IGF-II mRNA expression was increased by $PGF_{2\alpha}$ injection in the early CL but decreased in the mid CL (Fig. 1J).

*The mRNA expression of vasoactive-related factors in the early and mid CL after $PGF_{2\alpha}$ administration*

The results of quantitative analysis of the mRNA expressions of EDN1 (A), EDNAR (B), EDNBR (C), ACE (D), AT1R (E), AT2R (F), eNOS (G) and iNOS (H) are shown in Fig. 2. Although $PGF_{2\alpha}$ inhibited EDN1 mRNA expression in both areas of the early CL, it stimulated EDN1 mRNA expression in the periphery of the mid CL (Fig. 2A). $PGF_{2\alpha}$ did not change the mRNA expressions of EDNAR and EDNBR in the early CL, but it did decrease EDNAR mRNA expression (in both areas) and increase EDNBR mRNA expression (in the periphery area) in the mid CL (Fig. 2B and C). ACE mRNA expression was only increased by $PGF_{2\alpha}$ injection in the periphery area of the mid CL (Fig. 2D). Although AT2R mRNA expression did not change (Fig. 2F), $PGF_{2\alpha}$ decreased AT1R mRNA expression in both areas of the early CL and in the periphery area of the mid CL (Fig. 2E). In the mid CL, the mRNA expressions of eNOS and iNOS were stimulated by $PGF_{2\alpha}$ injection in the periphery area, and $PGF_{2\alpha}$ decreased eNOS mRNA expression but not iNOS mRNA expression in the early CL (Fig. 2G and H).

*The mRNA expression of prostaglandin-related factors in the early and mid CL after $PGF_{2\alpha}$ administration*

The results of quantitative analysis of the mRNA expressions of

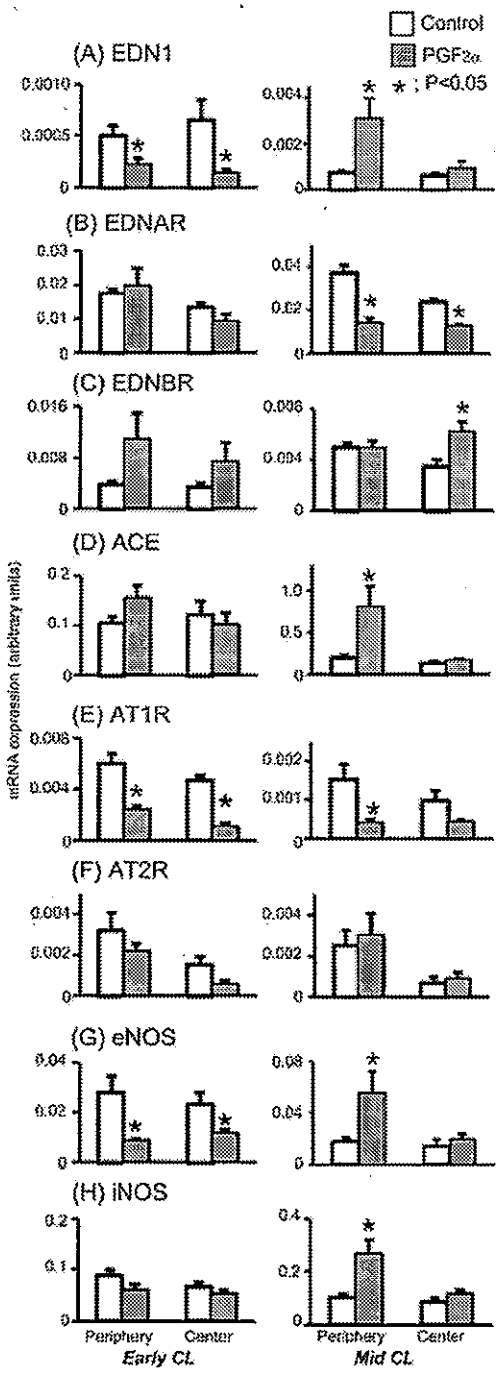


**Fig. 2.** Changes of mRNA expressions of vasoactive-related factors in the early and mid CL after $PGF_{2\alpha}$ administration. A: EDN1. B: EDNAR. C: EDNBR. D: ACE. E: AT1R. F: AT2R. G: eNOS. H: iNOS. In each figure, the left panel indicates the early CL, and the right panel indicates the mid CL. White bars indicate the control treatment, and the black bars indicate the $PGF_{2\alpha}$ treatment. Means ± SEM are presented. Different superscripts indicate statistically different values ($P<0.05$).

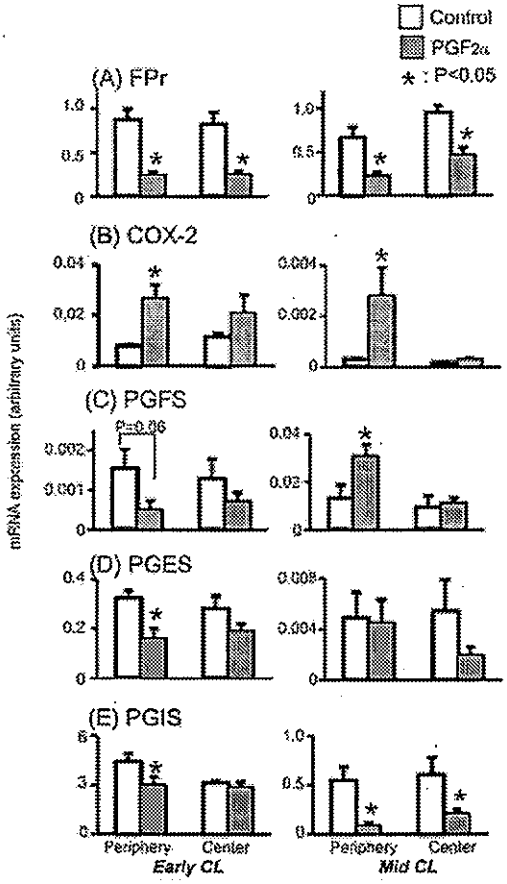


**Fig. 3.** Changes of mRNA expressions of prostaglandin-related factors in the early and mid CL after $PGF_{2\alpha}$ administration. A: FPr. B: COX-2. C: PGFS. D: PGES. E: PGIS. In each figure, the left panel indicates the early CL, and the right panel indicates the mid CL. White bars indicate the control treatment, and black bars indicate the $PGF_{2\alpha}$ treatment. Means ± SEM are presented. Different superscripts indicate statistically different values ($P<0.05$).

FPr (A), COX-2 (B), PGFS (C), PGES (D) and PGIS (E) are shown in Fig. 3. In all experimental groups, $PGF_{2\alpha}$ decreased FPr mRNA expression (Fig. 3A). $PGF_{2\alpha}$ increased COX-2 mRNA expression in the periphery but not in the center area of both the early and mid CL (Fig. 3B). $PGF_{2\alpha}$ tended to decrease the mRNA expression of PGFS in the periphery of the early CL and increased PGFS mRNA expression in the periphery area of the mid CL (Fig. 3C). Although PGES mRNA expression did not change in the mid CL, it was decreased by $PGF_{2\alpha}$ injection in the periphery area of the early CL (Fig. 3D). $PGF_{2\alpha}$ decreased PGIS mRNA expression in the periphery area of the early CL and in both areas of the mid CL (Fig. 3E).

### *The mRNA expression of steroidogenic-related factors in the early and mid CL after $PGF_{2\alpha}$ administration*

The results of quantitative analysis of the mRNA expressions of StAR (A), P450scc (B) and 3$\beta$-HSD (C) are shown in Fig. 4.

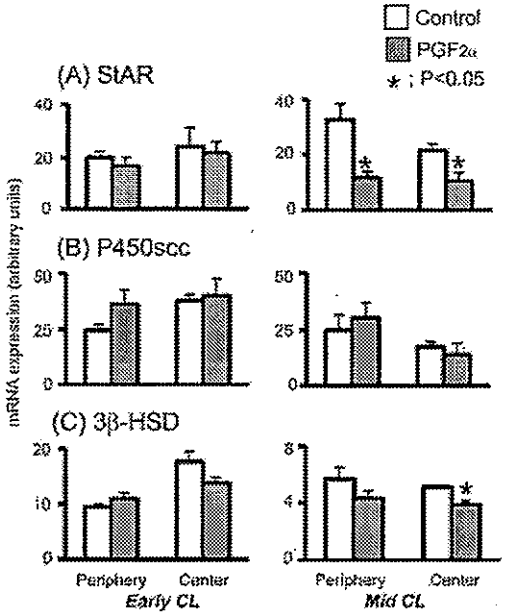


**Fig. 4.** Changes of mRNA expressions of steroidogenic-related factors in the early and mid CL after $PGF_{2\alpha}$ administration. A: StAR. B: P450scc. C: 3$\beta$-HSD. In each figure, the left panel indicates the early CL, and the right panel indicates the mid CL. White bars indicate the control treatment, and black bars indicate the $PGF_{2\alpha}$ treatment. Means ± SEM are presented. Different superscripts indicate statistically different values ($P<0.05$).

$PGF_{2\alpha}$ decreased StAR mRNA expression in the mid CL, but not in the early CL (Fig. 4A). $PGF_{2\alpha}$ did not change P450scc mRNA expression in both the early and mid CL (Fig. 4B). Although 3$\beta$-HSD mRNA expression did not change in the early CL, it was decreased by $PGF_{2\alpha}$ injection in the center area of the mid CL (Fig. 4C).

## Discussion

The results of the present study showed distinctly different responses in mRNA expression in the early and mid CL at 30 min after $PGF_{2\alpha}$ injection in the cow (Table 2). It was very clear that mRNA expressions relating to angiogenesis such as VEGFs (in the periphery area), FGF2, vasohibin (in the periphery area) and IGF-II were up-regulated after $PGF_{2\alpha}$ injection in the early CL, whereas they were down-regulated in the mid CL in the cow. Moreover, in the vasoactive-related factors, $PGF_{2\alpha}$ stimulated mRNA expressions of EDN1, ACE, eNOS and iNOS in the mid CL, but not in the early CL. However, $PGF_{2\alpha}$ similarly inhibited FPr and AT1R mRNA expression in both the early and mid CL. These data suggest that $PGF_{2\alpha}$ acutely, but differentially, regulates gene transcription between the early and mid CL in the cow.

The bovine CL produces many angiogenic factors including VEGF [14] and FGF2 [17]. These two angiogenic factors are

**Table 2.** Summary of mRNA expression changes in the early CL and mid CL after $PGF_{2\alpha}$ administration

| Angiogenic-related factors | Early CL | | Mid CL | |
|---|---|---|---|---|
| mRNA | Periphery | Center | Periphery | Center |
| VEGF120, 164 | ⇧ | → | ⇩ | ⇩ |
| FGF2 | ⇧ | ⇧ | ↓ | ⇩ |
| Vasohibin | ⇧ | ↑ | ⇩ | ⇩ |
| ANPT2/ANPT1 | ⇧ | → | ⇧ | ⇧ |
| Tie2 | ⇧ | → | ⇩ | ⇩ |
| IGF-I | → | ⇩ | ⇩ | ⇩ |
| IGF-II | ⇧ | ⇧ | ⇩ | ⇩ |

| Vasoactive-related factors | Early CL | | Mid CL | |
|---|---|---|---|---|
| mRNA | Periphery | Center | Periphery | Center |
| EDN1 | ⇩ | ⇩ | ⇧ | → |
| EDNAR | → | → | ⇩ | ⇩ |
| EDNBR | → | → | → | ⇧ |
| ACE | → | → | ⇧ | → |
| AT1R | ⇩ | ⇩ | ⇩ | → |
| AT2R | → | → | → | → |
| eNOS | ⇩ | ⇩ | ⇧ | → |
| iNOS | → | → | ⇧ | → |

| Prostaglandin-related factors | Early CL | | Mid CL | |
|---|---|---|---|---|
| mRNA | Periphery | Center | Periphery | Center |
| FPr | ⇩ | ⇩ | ⇩ | ⇩ |
| COX-2 | ⇧ | → | ⇧ | → |
| PGFS | ↓ | → | ⇧ | → |
| PGES | ⇩ | → | → | → |
| PGIS | ⇩ | → | ⇩ | ⇩ |

| Steroidogenic-related factors | Early CL | | Mid CL | |
|---|---|---|---|---|
| mRNA | Periphery | Center | Periphery | Center |
| StAR | → | → | ⇩ | ⇩ |
| P450scc | → | → | → | → |
| 3β-HSD | → | → | → | ⇩ |

⇧ : Significant increase ↑ : Tend to increase
⇩ : Significant decrease ↓ : Tend to decrease
→ : No change

potent mitogens for endothelial cells [18], stimulators of vascular permeability [19] and strong luteotropic factors in the CL [20–22]. Indeed, VEGF and FGF2 can stimulate P secretion from the bovine CL *in vitro* [23]. These findings suggest that VEGF and FGF2 have a stimulatory role in both angiogenesis and P regulation during the early luteal phase in the cow. Interestingly, in the early CL, $PGF_{2\alpha}$ clearly stimulated the mRNA expressions of VEGFs and FGF2. It has been reported that $PGF_{2\alpha}$ up-regulates VEGF transcription in human cancer cells *in vitro* [24]. Moreover, the production of $PGF_{2\alpha}$ and the mRNA expression of COX-2 are higher in the early CL than in other luteal phases [23, 25]. Importantly, in the early bovine CL *in vitro*, $PGF_{2\alpha}$ clearly stimulates P secretion [23, 26], and VEGF and FGF2 stimulate $PGF_{2\alpha}$ and P secretion [23]. Consequently, it is proposed that $PGF_{2\alpha}$ in the early CL acts as a local regulator to stimulate VEGF and FGF2, and this is how the CL acquires the ability to resist the effects of $PGF_{2\alpha}$.

Although $PGF_{2\alpha}$ did not change mRNA expressions of steroidogenic factors in the early CL, $PGF_{2\alpha}$ injection decreased the mRNA expressions of StAR and 3$\beta$-HSD in the mid CL. Indeed, $PGF_{2\alpha}$ decreases StAR mRNA expression in the mid CL but not in the early CL in the cow and porcine [4, 27], indicating the key enzyme to reduce steroidogenesis by $PGF_{2\alpha}$ administration. Additionally, in the present study, $PGF_{2\alpha}$ injection down-regulated the mRNA expressions of VEGFs and FGF2 in the mid CL, suggesting the rational phenomenon to inhibit angiogenesis for progressing luteolysis. It has been shown that $PGF_{2\alpha}$ administration drastically decreases VEGF protein expression after 30 min in the mid bovine CL *in vivo* [28]. These data suggest that $PGF_{2\alpha}$ differentially regulates mRNA expressions of steroidogenic factors as well as VEGFs and FGF2 between the early and mid CL in the cow.

ANPT1 is necessary for maintenance and stabilization of blood vessels [29], while ANPT2, acting as a natural antagonist for ANPT1, has a role in causing endothelial cells to undergo active remodeling, thereby destabilizing the vascular structure [29]. ANPT1 and ANPT2 bind to the same receptor, Tie2, and the balance of ANPT2 and ANPT1 binding to Tie2 appears to play a crucial role in vascular stability. The presence of VEGF may also determine the fate of destabilized blood vessels [30]. When the expression of VEGF is high, destabilization of blood vessels results in the formation of a new vascular network, whereas a lack of support by VEGF results in regression of blood vessels [30]. In the present study, $PGF_{2\alpha}$ stimulated the ANPT2/ANPT1 ratio in both the early and mid CL. In this situation, VEGF mRNA was stimulated by $PGF_{2\alpha}$ in the early CL but was inhibited in the mid CL. In addition, Tie2 mRNA expression was also synchronized with the ANPT2/ANPT1 ratio in the early CL but not in the mid CL. These data suggest that after $PGF_{2\alpha}$ injection, the early CL response favors angiogenic destabilization and that the mid CL response favors disruptive destabilization of blood vessels.

Vasohibin is a novel VEGF-inducible angiogenesis inhibitor predominantly expressed in endothelial cells [31]. VEGF up-regulates vasohibin expression to inhibit excessive angiogenesis [31, 32]. Indeed, vasohibin inhibits VEGF-induced angiogenesis *in vivo* and *in vitro* [31–33]. In the present study, we first determined the mRNA expression of vasohibin in the bovine CL, and $PGF_{2\alpha}$ regulated vasohibin mRNA expression as well as those of the VEGFs, suggesting that inhibition of hyper-angiogenesis in the early CL and a drastic down-regulation in the mid CL occur to induce angiolysis after $PGF_{2\alpha}$ injection. However, the detailed function of vasohibin for angiogenesis and endothelial cell proliferation and migration, should be investigated in the bovine CL in a future study.

The IGF system is essential for support of P secretion. The bovine CL has been identified as a site of IGF-I and IGF-II mRNA expressions as well as peptide production and action throughout the luteal phase [34, 35]. In the present study, although $PGF_{2\alpha}$ significantly inhibited mRNA expression of both IGF-I and IGF-II in the mid CL, IGF-II mRNA expression was stimulated by $PGF_{2\alpha}$ in the early CL. Interestingly, the stimulatory effect on P secretion of IGF-II is greater than that of IGF-I in bovine CL *in vitro* tissue culture [36]. These data suggest that $PGF_{2\alpha}$ may have potential as an anti-luteolytic agent in the early CL that is different from that of the mid CL.

Recent studies have clearly indicated that EDN1, angiotensin II (Ang II) and NO are involved in the process of luteal regression [37–46]. In fact, EDN1 and NO can inhibit P secretion in the bovine [38, 39], ovine [41] and human CL [47] *in vitro*. Angiotensin II can also suppress P secretion stimulated by LH in bovine luteal cells [48]. Moreover, $PGF_{2\alpha}$ stimulates biosynthesis of EDN1 (and EDN1 mRNA expression), Ang II (and ACE mRNA expression) and NO (and eNOS/iNOS mRNA expression) *in vivo* and *in vitro* [37, 39–41, 43, 46]. In support of the above findings, the present results indicated that $PGF_{2\alpha}$ stimulated mRNA expressions of EDN1, ACE, eNOS and iNOS in the periphery of the mid CL. These findings suggest that $PGF_{2\alpha}$, EDN1, Ang II and NO may act cooperatively to initiate functional luteolysis in the cow. Contrary to the mid CL, $PGF_{2\alpha}$ significantly decreased EDN1 and eNOS mRNA expression in the early CL. Therefore, we suggest that $PGF_{2\alpha}$ has a dual function depending on the luteal phase, acting as an anti-luteolytic factor (in the early CL) and a luteolytic factor (in the mid CL) in the cow.

Previous reports have shown that FPr mRNA expression is down-regulated by $PGF_{2\alpha}$ administration in both the early and mid-luteal phases *in vivo* [4, 8]. Additionally, administration of $PGF_{2\alpha}$ drastically inhibited FPr mRNA expression after 30 min in both the early and mid-cycle CL in the present study. Thus, this indicates that the refractoriness of the early-cycle CL to the luteolytic effect of $PGF_{2\alpha}$ is not caused by a lack of the FPr system. On the other hand, administration of $PGF_{2\alpha}$ elevates COX-2 expression after 4 h in the mid-cycle CL but not in the early-cycle CL in the cow [4, 49]. In the present study, however, $PGF_{2\alpha}$ clearly stimulated COX-2 mRNA expression in the periphery of both the early and mid CL at 30 min after $PGF_{2\alpha}$ injection. This discrepancy may be due to the different time point for the CL collection after $PGF_{2\alpha}$ injection. Interestingly, although the mRNA expression of PGFS tended to decrease in the periphery of the early CL, administration of $PGF_{2\alpha}$ stimulated PGFS mRNA expression in the periphery of the mid CL in the cow. This finding suggests that luteal $PGF_{2\alpha}$ production is stimulated by the COX-2/PGFS system due to exogenous $PGF_{2\alpha}$ in the mature CL and not in the developing CL. In addition, a drastic decrease of PGIS mRNA expression in the mid CL likely correlates to a luteolytic response of $PGF_{2\alpha}$, since $PGI_2$ produced from PGIS

has strong potential to stimulate P secretion in bovine luteal cells *in vitro* [26, 50]. We have no clear explanation for the small changes in PGES mRNA in the early and mid CL, although $PGE_2$ is well known as a potent luteotropic PG in the CL [26].

In the present study, $PGF_{2\alpha}$ action was different not only in the luteal phases (the early and mid CL) but also in the luteal regions (periphery and center region of the CL). For example, in the mid CL, $PGF_{2\alpha}$ stimulated mRNA expressions of vasoactive related-factors such as EDN1, ACE, eNOS, iNOS and PG-related factors such as COX-2 and PGFS in the periphery but not in the center area. We have previously shown that there are many arteriolovenous vessels in the periphery area of the mid CL compared with the center area, and distribution of arteriolovenous vessels is related to the increase of luteal blood flow caused by $PGF_{2\alpha}$ administration [13]. Therefore, the difference of vascular structure may be one of the reasons why $PGF_{2\alpha}$ affects the mRNA expressions of vasoactive and PG related-factors in the periphery area of the mid CL. On the other hand, $PGF_{2\alpha}$ also regulated mRNA expressions in the early CL without steroidogenic-related factors. However, there are similar distributions of capillaries and the arteriolovenous vessels in the periphery and center of the early CL [13]. It is possible that the distribution of luteal cells, these derivations (small luteal cells derived from theca cells and large luteal cells derived from granulosa cells) and the degree of differentiation are different between the periphery and center of the early CL.

In conclusion, $PGF_{2\alpha}$ has a stage-specific action depending on the luteal phase (early *vs.* mid) during the estrous cycle in the cow. Taken together, $PGF_{2\alpha}$ may have a dual function, acting as an anti-luteolytic factor due to stimulation of angiogenic factors in the early CL and acting as a luteolytic factor due to increase of vasoactive- and PG-related factors, especially COX-2 and PGFS, in the mid CL in the cow.

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