Real-time dynamics of prostaglandin $F_{2\alpha}$ release from uterus and corpus luteum during spontaneous luteolysis in the cow

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Abstract

Prostaglandin (PG) $F_{2\alpha}$ released from the uterus in a pulsatile fashion is essential to induce regression of the corpus luteum (CL) in the cow. In addition to the uterus, the CL has also been recognized as a site of $PGF_{2\alpha}$ production. Therefore, this study aimed to determine the detailed dynamics of the releasing profile of CL-derived $PGF_{2\alpha}$ together with uterus-derived $PGF_{2\alpha}$ during spontaneous luteolysis in the cow. Non-lactating Holstein cows ($n = 6$) were surgically implanted with a microdialysis system (MDS) on day 15 (oestrus = day 0) of the oestrous cycle. Simultaneously, catheters were implanted to collect ovarian venous plasma ipsilateral to the CL as well as jugular venous plasma. The concentrations of $PGF_{2\alpha}$, 13,14-dihydro-15-keto- $PGF_{2\alpha}$ (PGFM) and progesterone in the MDS and plasma samples were determined by enzyme immunoassays. The intra-luteal $PGF_{2\alpha}$ secretion slightly increased after the onset of luteolysis (0 h) and drastically increased from 24 h, and was maintained at high levels towards the following oestrus. Furthermore, $PGF_{2\alpha}$ was released from the CL into the ovarian vein in a pulsatile manner during spontaneous luteolysis. Also, the fact that intra-luteal secretion of $PGF_{2\alpha}$ and PGFM showed a positive correlation indicates the existence of a local metabolic pathway for $PGF_{2\alpha}$ in the CL. In conclusion, the present study clarified the real-time dynamics of uterus-derived $PGF_{2\alpha}$ and CL-derived $PGF_{2\alpha}$ during spontaneous luteolysis in the cow, and gives the first *in vivo* evidence that the CL releases $PGF_{2\alpha}$ during spontaneous luteolysis in the cow. Although the physiological relevance of CL-derived $PGF_{2\alpha}$ appears to be restricted to a local role as an autocrine/paracrine factor in the CL, overall results support the concept that the local release of $PGF_{2\alpha}$ within the regressing CL amplifies the luteolytic action of $PGF_{2\alpha}$ from the uterus.

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Introduction

Release of prostaglandin (PG) $F_{2\alpha}$ from the uterus in a pulsatile fashion on days 17–18 of the oestrous cycle is essential to induce regression of the corpus luteum (CL) in ruminants (McCracken *et al.* 1984, Wolfenson *et al.* 1985). In addition to uterus-derived $PGF_{2\alpha}$, the functional CL of the cow produces and secretes at least three kinds of PGs, such as $PGF_{2\alpha}$, PGE_2 , and 6-keto- $PGF_{1\alpha}$, the stable inactive metabolite of prostacyclin (PGI_2) (Shemesh & Hansel 1975, Milvae & Hansel 1983, Rodgers *et al.* 1988, Blair *et al.* 1997). Also, the receptors for $PGF_{2\alpha}$ are fully expressed during the lifespan of bovine CL (Rao *et al.* 1979, Sakamoto *et al.* 1995, Mamluk *et al.* 1998). Recently, we and others observed that a luteolytic injection of $PGF_{2\alpha}$ induces a rapid and transient increase of intra-luteal $PGF_{2\alpha}$ during the first 4 h, but it increases again from 24 h (Hayashi *et al.* 2003), and these changes

are well supported by the mRNA expression levels of cyclooxygenase 2 (COX-2) (Tsai & Wiltbank, 1998, Levy *et al.* 2000, Hayashi *et al.* 2003). Moreover, the addition of $PGF_{2\alpha}$ to ovine luteal cells in culture increased the expression of COX-2 protein at 4–12 h, and PGF synthase mRNA concentration increased at 24 h after $PGF_{2\alpha}$ treatment (Tsai & Wiltbank 1997). Thus, $PGF_{2\alpha}$ synthesis is induced in the CL especially at later stages during $PGF_{2\alpha}$ -induced luteolysis. However, the detailed information of $PGF_{2\alpha}$ secretion within the CL during spontaneous luteolysis in the cow has not been well clarified.

The findings above imply that $PGF_{2\alpha}$ secreted in the CL may amplify the luteolytic effect of exogenous $PGF_{2\alpha}$ or the pulsatile release of $PGF_{2\alpha}$ from the uterus. It is therefore important to determine the detailed dynamics of the releasing profile of CL-derived $PGF_{2\alpha}$ together with uterus-derived $PGF_{2\alpha}$ during spontaneous luteolysis in the cow.

For this purpose, we utilized an *in vivo* microdialysis system (MDS) implanted in the CL to observe the real-time changes in $\text{PGF}_{2\alpha}$, 13,14-dihydro-15-keto- $\text{PGF}_{2\alpha}$ (PGFM), and progesterone concentrations within the regressing CL, along with the changes in the concentration of these substances in ovarian venous plasma (OVP) ipsilateral to the CL as well as in jugular venous plasma (JVP).

Materials and Methods

Animals and experimental design

Six multiparous, non-lactating Holstein cows were used for this study. They had had at least two oestrous cycles of normal length (21–22 days) before being used. Luteolysis was induced by intramuscular (i.m.) injection of 500 μg of the $\text{PGF}_{2\alpha}$ analogue (cloprostenol; Estrumate; Takeda Co., Osaka, Japan); 100 μg gonadotrophin releasing hormone (GnRH) (Conceral; Takeda Co.) were injected i.m. 60 h after the $\text{PGF}_{2\alpha}$ injection to ensure ovulation. The day of oestrus was designated as day 0. The cows received surgical implants of MDS membranes into the CL, and the ovarian vein and jugular vein were also catheterized simultaneously on day 15 of the oestrous cycle. After surgery, cows were moved to individual stanchions, and were fed with hay and water available *ad libitum*. Sample collection was started 24 h after surgery and continued until the next oestrus. After the experimental period, the MDS was surgically removed and the cow was ovariectomized. The occurrence of luteolysis was confirmed by macroscopic observation of the dissected CL (Ireland *et al.* 1980). The time schedule of the present study is shown in Fig. 1.

Surgical implantation of the MDS into the CL

The MDS was surgically implanted into the CL on day 15 of the oestrous cycle, via lateral laparotomy under epidural anaesthesia as described previously (Ohtani *et al.* 1998). Before surgery, ovaries were monitored by trans-rectal ultrasonography to determine that the CL was normal and had no cystic cavity. Basically, two to five dialysis capillary membranes (Fresenius SPS 900 Hollow Fibers; cutoff = 01000 kDa, 0.2 mm diameter, 10 mm long; Fresenius AG, St Wendel, Germany) were implanted into the CL. Both ends of the capillary membranes were glued to 25 cm-long pieces of silicone elastomer tubing (inner diameter 0.3 mm) and connected to the MDS. The tubing was fixed on the surface of the CL by Histoacryl blau

(B. Braun-Dexon GmbH, Spangenberg, Germany), and the dialysis pieces with silicone tubing were connected to Teflon tubing that led to the outside of the abdomen. The exteriorized bundle of afferent and efferent Teflon tubing was fixed to the back of the cow. One end of the MDS was connected to a multiple-line peristaltic pump, and the other was connected to a multiple-line fraction collector. The MDS was continuously perfused with Ringer's solution at a flow rate of 2.5 ml h^{-1} throughout the experiment, and fractions of perfusate were collected at 4 h intervals. Sample collection started 24 h after surgery, and all MDS samples were frozen at -30°C immediately after collection until further analysis.

Venous catheterization and collection of OVP and JVP

At surgery, a catheter was placed into the ovarian vein ipsilateral to the CL. The catheter was inserted into the vein about 5 cm away from the ovary, and propelled about 8–10 cm. Catheterization of the jugular vein was also conducted. Blood samples were collected from MDS-implanted cows into sterile 10 ml glass tubes containing 200 μl of a stabilizer solution (0.3 M EDTA, 1% acetyl salicylic acid, pH 7.4) at 4 h intervals until the end of the experiment. All blood samples were immediately chilled in ice water for 10 min, centrifuged at 2000 *g* for 15 min at 4°C , and the plasma was frozen at -30°C until further analysis.

Hormone determination

The concentrations of progesterone, $\text{PGF}_{2\alpha}$ and PGFM in the perfusate fractions of the MDS and in plasma were determined in duplicate by second antibody enzyme immunoassays (EIAs) after extraction using 96-well ELISA plates (NUNC-Immuno Plate, NUNC Brand Products, Roskilde, Denmark).

The progesterone concentrations in the perfusate fractions of the MDS were assayed directly (Miyamoto *et al.* 1992). The standard curve ranged from 0.05 to 50 ng ml^{-1} , and the ED_{50} of the assay was 2.4 ng ml^{-1} . The intra- and interassay coefficients of variation averaged 6.2% and 9.3% respectively.

To extract PGs, the plasma samples (OVP and JVP: 2 ml) and the MDS perfusates (6 ml) were adjusted to pH 3.5 using HCl and extracted using diethyl ether as described previously (Acosta *et al.* 1999). The residue was dissolved in 2 ml and 200 μl assay buffer (40 mM PBS, 0.1% BSA, pH 7.2) for plasma and MDS samples respectively. Samples were thus concentrated 30-fold for the MDS perfusate. To estimate the recovery rate in the plasma, $\text{PGF}_{2\alpha}$ and PGFM were added to plasma, and the obtained values were 60 and 70% respectively. Likewise, to estimate the recovery rate in the MDS perfusate, $\text{PGF}_{2\alpha}$ and PGFM were added to Ringer's solution, and the obtained values were 65 and 66% respectively. The EIAs for $\text{PGF}_{2\alpha}$ (Miyamoto *et al.* 1995) and PGFM (Meyer *et al.* 1989)

Figure 1 Time schedule of the treatment with the MDS *in vivo*.

were described previously. The standard curve for PGF_{2α} ranged from 9.5 to 9500 pg ml⁻¹, and the ED₅₀ of the assay was 145 pg ml⁻¹. The intra- and interassay coefficients of variation were 7.7 and 9.7% respectively. The standard curve for PGFM ranged from 2.5 to 2500 pg ml⁻¹, and the ED₅₀ of the assay was 78 pg ml⁻¹. The intra- and interassay coefficients of variation were 7.7 and 12.5% respectively.

Statistical analysis

For analysis of changes in the concentrations of progesterone, PGF_{2α}, and PGFM in the MDS fractions, the mean concentrations of the first six fractions (24 h) were used for the calculation of an individual proportion of baseline, because of the large variation in the absolute amount of hormones released into each of the microdialysis capillary membranes implanted in different cows. All hormone concentrations in the fractions collected were then expressed as a proportion of this individual baseline. This treatment enables an evaluation of the relative changes of hormonal values between the CL of different animals. The time point when progesterone concentrations in the MDS fractions started to decrease was considered as the onset of spontaneous luteolysis (0 h). Changes in hormonal release after the onset of luteolysis were tested on the basis of individual time points throughout the experiment as compared with the baseline. They were analysed by repeated measures ANOVA followed by *t*-test with the Bonferroni method. Differences were considered significant at a probability less than 5% ($P < 0.05$).

Pulsatile releases of PGF_{2α} in OVP and MDS as well as PGFM in JVP during spontaneous luteolysis were examined. The occurrence of peaks was identified when the proportional changes of PGF_{2α} or PGFM increased from basal values to at least threefold over that of the intra-assay CV of EIAs. The relationship between peaks of PGF_{2α} in OVP and PGFM in JVP, and that of PGF_{2α} peaks between OVP and MDS, were analysed using the Chi-square test of independence for contingency. Probabilities less than 5% ($P < 0.05$) were considered significant.

Results

Oestrous signs were observed in all cows between days 21–23 from the last oestrus, and CLs implanted with MDS were collected by ovariectomy after the following oestrus. The regression of CLs was confirmed by macroscopic observation.

Intra-luteal changes in progesterone, PGF_{2α} and PGFM concentrations during spontaneous luteolysis

The basal levels of release (100%) of progesterone, PGF_{2α} and PGFM into the MDS were 1546 ± 276 pg ml⁻¹, 18.52 ± 1.52 pg ml⁻¹ and 6.61 ± 0.71 pg ml⁻¹ respectively. Intra-luteal progesterone secretion started to decrease ($P < 0.01$) immediately after the onset of luteolysis, and

declined further to about 20% of the baseline at the end of the experiment. Intra-luteal PGF_{2α} secretion slightly increased ($P < 0.05$ to 0.01) after the onset of luteolysis, drastically increased from 24 h to about 300% ($P < 0.01$), and was maintained at high levels towards the following oestrus. Also, a significant increase ($P < 0.05$ to 0.01) in intra-luteal PGFM secretion (150% of baseline) was observed from 20 h after the onset of luteolysis (Fig. 2).

Relationship between PGF_{2α} in OVP and PGFM in JVP

In the present study, three kinds of relationship were observed between the PGF_{2α} peaks in OVP and the PGFM peaks in JVP during spontaneous luteolysis. Pattern I was classified as a concomitant appearance of a PGF_{2α} peak in OVP with a PGFM peak in JVP. Pattern II was classified as a PGF_{2α} peak in OVP and basal release of PGFM in JVP. Pattern III was classified as the appearance of a weak PGFM peak in JVP with basal release of PGF_{2α} in OVP. The changes in PGF_{2α} in OVP and PGFM in JVP in three

Figure 2 Local release of (A) progesterone (P), (B) PGF_{2α} and (C) PGFM into the MDS (bars; 18 lines from 6 cows) during spontaneous luteolysis in the cows ($n = 6$; means $\pm$ S.E.M.). The MDS data are expressed as a percentage of basal release (baseline) for the first 24 h (100% = 1546 ± 276 pg ml⁻¹ for progesterone, 18.52 ± 1.52 pg ml⁻¹ for PGF_{2α} and 6.61 ± 0.71 pg ml⁻¹ for PGFM). Black bars indicate the onset of luteolysis (0h). * and •, higher or lower than baseline ($P < 0.05$ and $P < 0.01$ respectively).

individual cows are shown in Fig. 3 with examples of these three patterns. In total, 58 peaks of PGF_{2α} in OVP (9.67 ± 0.67 peaks/cow) and 56 peaks of PGFM in JVP (9.33 ± 0.56 peaks/cow) were observed in the six cows. The number of cases classified as pattern I, II and III were 32 (5.33 ± 0.99 peaks/cow), 26 (4.33 ± 0.80 peaks/cow) and 24 (4.00 ± 1.03 peaks/cow) respectively.

Characteristics of releasing profiles of PGF_{2α} (OVP) and PGFM (JVP) prior to and after the onset of spontaneous luteolysis

There were no significant differences in the distribution of pulse patterns I, II and III of PGF_{2α} or PGFM secretion

prior to and after the onset of spontaneous luteolysis (Table 1). In addition, the peak concentrations of PGF_{2α} or PGFM in pulse patterns I, II and III were not significantly different prior to and after the onset of spontaneous luteolysis (Table 2).

Relationship of PGF_{2α} between OVP and MDS

An example of changes in PGF_{2α} concentrations in OVP and MDS fractions in an individual cow is shown in Fig. 4. There was no relationship between profiles of local secretion of PGF_{2α} within the CL (PGF_{2α} in MDS) and the release of PGF_{2α} from the CL into the ovarian vein (PGF_{2α} in OVP) during spontaneous luteolysis.

Discussion

The present study clarified the real-time dynamics of uterus-derived PGF_{2α} and CL-derived PGF_{2α} during spontaneous luteolysis in the cow. The intra-luteal PGF_{2α} secretion slightly increased after the onset of luteolysis and drastically increased from 24 h. Furthermore, the

Table 1 Distribution of the occurrence of types I, II and III pulse patterns of PGF_{2α} (OVP) and PGFM (JVP) release prior to (–48 ~ 0 h) and after (0 ~ 72 h) the onset of spontaneous luteolysis. Results are expressed as peak occurrence/cow/day; means ± S.E.M., *n* = 6 cows.

	Prior to luteolysis (–48 ~ 0 h)	After luteolysis (0 ~ 72 h)
PGF _{2α} peak (OVP)	1.60 ± 0.25	1.63 ± 0.13
PGFM peak (JVP)	1.38 ± 0.20	1.03 ± 0.30
Pattern I	0.72 ± 0.19	1.02 ± 0.20
Pattern II	0.88 ± 0.13	0.55 ± 0.20
Pattern III	0.67 ± 0.21	0.48 ± 0.22

OVP, ovarian venous plasma; JVP, jugular venous plasma. There were no significant differences in the distribution of types I, II, and III pulse patterns of PGF_{2α} or PGFM secretion between and within prior to and after the onset of spontaneous luteolysis.

Table 2 Characteristics of releasing profiles of PGF_{2α} and PGFM in types I, II and III pulse patterns prior to (–48 ~ 0 h) and after (0 ~ 72 h) the onset of spontaneous luteolysis. The peaks involve all identified values that increased over threefold from basal values of the intra-assay CV of EIAs. Results are expressed as pg ml^{–1}; means ± S.E.M., *n* = 6 cows.

	Prior to luteolysis (–48 ~ 0 h)	After luteolysis (0 ~ 72 h)
Peak concentration		
Pattern I		
PGF _{2α} (OVP)	379.0 ± 224.7	575.6 ± 161.1
PGFM (JVP)	22.3 ± 3.5	28.7 ± 9.9
Pattern II		
PGF _{2α} (OVP)	145.5 ± 41.9	133.9 ± 33.4
Pattern III		
PGFM (JVP)	20.1 ± 1.6	20.1 ± 2.9
Basal concentration		
PGF _{2α} (OVP)	56.8 ± 17.1	55.9 ± 9.5
PGFM (JVP)	10.7 ± 0.7	11.8 ± 1.3

OVP, ovarian venous plasma; JVP, jugular venous plasma.

Figure 3 Relationship between PGF_{2α} in OVP ipsilateral to the CL and PGFM in JVP. Pattern I was classified as a concomitant appearance of a PGF_{2α} peak in OVP with a PGFM peak in JVP. Pattern II was classified as a PGF_{2α} peak in OVP with basal release of PGFM in JVP. Pattern III was classified as the appearance of a weak PGFM peak in JVP with basal release of PGF_{2α} in OVP.

Figure 4 An example of changes in PGF_{2α} concentrations in OVP and MDS fractions in an individual cow. Luteolysis is represented by the black bars (0 h).

results provide the first direct evidence that PGF_{2α} is released from the CL into the ovarian vein during spontaneous luteolysis. Also, the fact that intra-luteal secretion of PGF_{2α} and PGFM showed a positive correlation indicates the existence of a local metabolic pathway for PGF_{2α} in the CL.

It is well known that the CL during the oestrous cycle is identified as a site of PG production (Shemesh & Hansel 1975, Milvae & Hansel 1983, Rodgers *et al.* 1988) and it expresses mRNA for COX-2 (Tsai & Wiltbank 1998, Levy *et al.* 2000, Silva *et al.* 2000, Kobayashi *et al.* 2002) and PGF synthase (Tsai & Wiltbank 1997), as well as PGF receptors (Rao *et al.* 1979, Wiepz *et al.* 1992, Sakamoto *et al.* 1995). In the present study, the basal release of PGF_{2α} into MDS was about 20 pg ml⁻¹, which increased to about 60 pg ml⁻¹ during the later period of luteolysis. The transfer capacity of the MDS capillary membrane was previously examined to be 1% for PG (Miyamoto *et al.* 1997). Thus, the absolute concentration of PGF_{2α} in the inter-cellular fluid of the CL could be expected to be 100-fold higher than the substance diffused into MDS, which is calculated as around 2000 to 6000 pg ml⁻¹. Thus, this observation provides strong evidence that CL produces high amounts of PGF_{2α} during spontaneous luteolysis.

The bovine CL contains relatively large amounts of arachidonic acid that is comparable to the endometrial cells (Lukaszewska & Hansel 1980), and a functional arachidonic acid-PG metabolic pathway is identified in the bovine CL (Shemesh & Hansel 1975, Milvae & Hansel 1983). In fact, intra-luteal implants of indomethacin, a potent PG synthase inhibitor, on day 11 of the oestrous cycle in ewes resulted in heavier CL on day 18 than that in untreated control ewes (Griffeth *et al.* 2002), suggesting that intra-luteal production of PGF_{2α} is required for structural luteolysis. Furthermore, the systemic administration of PG synthesis inhibitors delayed the structural luteolysis in rats (Kurusu *et al.* 2001). In the present study,

the intra-luteal PGF_{2α} secretion was drastically increased from 24 h after the onset of luteolysis. These findings suggest that the intra-luteal PGF_{2α} may mediate structural rather than functional luteolysis.

In the systemic circulation, PGF_{2α} is inactivated by metabolizing into PGFM during the first passage through the lungs (Piper *et al.* 1970). Hence, the changes in PGFM in peripheral plasma can be considered as an accurate reflection of changes in the uterine PGF_{2α} secretion. On the other hand, it was reported that PGF_{2α} can be converted to PGFM in ovine CL (Silva *et al.* 2000). In the present study, the increases in PGF_{2α} and PGFM secretion in CL were positively correlated with each other after the onset of luteolysis. The data support the concept that PGF_{2α} is catabolised to PGFM in the CL, and thus, a PGF_{2α} metabolic pathway exists in the CL of the cow. The fact that PGF_{2α} increased to about 300% while PGFM increased to only about 150% during luteolysis may imply that the active synthesis, but not catabolism, of PGF_{2α} accelerates the luteolytic cascade by interactions with other local regulators such as endothelin-1 (Girsh *et al.* 1996a,b, Miyamoto *et al.* 1997, Ohtani *et al.* 1998) and angiotensin II (Hayashi & Miyamoto 1999) within the bovine CL. Therefore, it is most likely that luteal PGF_{2α} plays a role as an autocrine/paracrine modulator of CL function (Miyamoto & Schams 1994, Olofsson & Leung 1994). The increased PGF_{2α} within the CL after the onset of luteolysis may act as an amplifier of uterine PGF_{2α} during spontaneous luteolysis.

In the present study, three kinds of relationship were observed between the PGF_{2α} peaks in OVP and the PGFM peaks in JVP during spontaneous luteolysis. Pattern I was classified as a concomitant appearance of a PGF_{2α} peak in OVP with a PGFM peak in JVP (Fig. 5a). Presumably, uterine PGF_{2α} was released into the uterine vein, and then branched into two pathways. In the first pathway PGF_{2α} is transferred to the ovary by utero-ovarian local counter-current transfer mechanisms, and reaches the CL (Barrett *et al.* 1971, Ginther *et al.* 1973, Kawakami *et al.* 1955). After circulating the ovary, the transferred PGF_{2α} moves into the ovarian vein and is detected as a peak in OVP. In the second pathway PGF_{2α} flows to the systemic circulation, and is inactivated by metabolizing into PGFM during the first passage through the lungs, so that it is detected as a peak in JVP. Thus, pattern I is interpreted as the uterus-derived PGF_{2α} pulse.

Pattern II was classified as a peak of PGF_{2α} in OVP together with basal release of PGFM in JVP (Fig. 5b). The observation that PGFM in the systemic circulation does not show a peak at that time strongly suggests that the PGF_{2α} peak in OVP is not derived from the uterus. Although the possibility that the other parts of the ovary such as follicles and stroma release PGF_{2α} into OVP cannot be excluded, the source of this PGF_{2α} can be considered to be the CL. In support of this idea, Griffeth *et al.* (2002) reported that in ewes hysterectomized on day 5 of the oestrous cycle, multiple administrations of PGF_{2α} on day 10 induced clear

Figure 5 Diagrammatic illustration of the relationship between PGF_{2α} in OVP ipsilateral to the CL and PGFM in JVP. (a) Pattern I was classified as a concomitant appearance of a PGF_{2α} peak in OVP with a PGFM peak in JVP. Presumably, uterine PGF_{2α} was released into the uterine vein, and then branched into two pathways. In the first pathway PGF_{2α} transfers to the ovary via the local countercurrent transfer mechanism, and then appears in OVP. In the second pathway PGF_{2α} flows to the systemic circulation, and is inactivated by metabolizing into PGFM, so that it is detected as a peak in JVP. (b) Pattern II was classified as a peak of PGF_{2α} in OVP together with basal release of PGFM in JVP. The observation that PGFM in the systemic circulation does not show a peak at that time strongly suggests that the PGF_{2α} peak in OVP is not derived from the uterus. (c) Pattern III was classified as the appearance of a weak PGFM peak in JVP with basal release of PGF_{2α} in OVP. The weak PGFM peak may be due to a small amount of PGF_{2α} released from the uterus, which is an insufficient amount to be reflected in the OVP via local countercurrent transfer.

pulsatile releases of PGFM in the circulation during luteolysis, which is independent of the administered PGF_{2α}. The data suggest that the regressing CL may release PGF_{2α} into the circulation in a pulsatile manner.

Pattern III was classified as the appearance of a weak PGFM peak in JVP with basal release of PGF_{2α} in OVP (Fig. 5c). The observation that PGF_{2α} in the ovarian vein does not show a peak suggests that the CL does not release PGF_{2α} at that time. Thus, the weak PGFM peak

may be due to a small amount of PGF_{2α} released from the uterus, which is an insufficient amount to be reflected in the OVP via local countercurrent transfer. The observations noted above suggest that the source of PGF_{2α} during spontaneous luteolysis is not only the uterus but also the CL. In the present study, it was observed that the distribution of pulse patterns I, II and III, or peak concentrations of PGF_{2α} and PGFM in pulse patterns I, II and III were constant prior to and after initiation of spontaneous luteolysis.

In the present study, there was no relationship between profiles of local secretion of PGF_{2α} within the CL (PGF_{2α} in MDS) and the PGF_{2α} released from the CL into the ovarian vein (PGF_{2α} in OVP) during spontaneous luteolysis. Even though PGF_{2α} detected in the ovarian vein may contain both uterus- (pattern I) and CL-derived (pattern II) PGF_{2α}, the changing profiles of PGF_{2α} within the CL and in OVP do not coincide. Therefore, it is unlikely that the production of PGF_{2α} within CL tissue is reflected in circulating PGF_{2α} in the whole body, and hence different mechanisms may regulate these two phenomena.

Taken together, the results of the present study show the real-time dynamics of uterine- and CL-derived PGF_{2α} during spontaneous luteolysis, and provide the first *in vivo* evidence that the CL releases PGF_{2α} during spontaneous luteolysis in the cow. Although the physiological relevance of CL-derived PGF_{2α} appears to be restricted to a local role as an autocrine/paracrine factor in the CL, overall results support the concept that the local release of PGF_{2α} within the regressing CL amplifies the luteolytic action of PGF_{2α} from the uterus.

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