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.


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$_{2\alpha}$, PG$_{E_2}$, 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 PGF$_{2\alpha}$, 13,14-dihydro-15-keto-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 PGF$_{2\alpha}$ analogue (cloprostenol; Estrumate; Takeda Co., Osaka, Japan); 100 µg gonadotrophin releasing hormone (GnRH) (Conceal; Takeda Co.) were injected i.m. 60 h after the 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 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 ovarioectomized. 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 transrectal 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, 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, 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, PGF$_{2\alpha}$ and PGFM were added to Ringer’s solution, and the obtained values were 65 and 66% respectively. The EIAs for PGF$_{2\alpha}$ (Miyamoto et al. 1995) and PGFM (Meyer et al. 1989)
were described previously. The standard curve for PGF$_{2\alpha}$ ranged from 9.5 to 9500 pg ml$^{-1}$, and the ED$_{50}$ 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 ED$_{50}$ 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$_{2\alpha}$, 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 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$_{2\alpha}$ 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$_{2\alpha}$ 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$_{2\alpha}$ in OVP and PGFM in JVP, and that of PGF$_{2\alpha}$ 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. 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$_{2\alpha}$ in OVP and PGFM in JVP**

In the present study, three kinds of relationship were observed between the PGF$_{2\alpha}$ peaks in OVP and the PGFM peaks in JVP during spontaneous luteolysis. Pattern I was classified as a concomitant appearance of a PGF$_{2\alpha}$ peak in OVP with a PGFM peak in JVP. Pattern II was classified as a PGF$_{2\alpha}$ 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$_{2\alpha}$ in OVP. The changes in PGF$_{2\alpha}$ in OVP and PGFM in JVP in three
individual cows are shown in Fig. 3 with examples of these three patterns. In total, 58 peaks of PGF$_{2\alpha}$ 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$_{2\alpha}$ (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$_{2\alpha}$ or PGFM secretion prior to and after the onset of spontaneous luteolysis (Table 1). In addition, the peak concentrations of PGF$_{2\alpha}$ 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$_{2\alpha}$ between OVP and MDS**

An example of changes in PGF$_{2\alpha}$ 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$_{2\alpha}$ within the CL (PGF$_{2\alpha}$ in MDS) and the release of PGF$_{2\alpha}$ from the CL into the ovarian vein (PGF$_{2\alpha}$ in OVP) during spontaneous luteolysis.

**Discussion**

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. The intra-luteal PGF$_{2\alpha}$ secretion slightly increased after the onset of luteolysis and drastically increased from 24 h. Furthermore, the

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**Table 1** Distribution of the occurrence of types I, II and III pulse patterns of PGF$_{2\alpha}$ (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.

<table>
<thead>
<tr>
<th></th>
<th>Prior to luteolysis (~48 ~ 0 h)</th>
<th>After luteolysis (0 ~ 72 h)</th>
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<tbody>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>1.60 ± 0.25</td>
<td>1.63 ± 0.13</td>
</tr>
<tr>
<td>Pattern I</td>
<td>0.72 ± 0.19</td>
<td>1.02 ± 0.20</td>
</tr>
<tr>
<td>Pattern II</td>
<td>0.88 ± 0.13</td>
<td>0.55 ± 0.20</td>
</tr>
<tr>
<td>Pattern III</td>
<td>0.67 ± 0.21</td>
<td>0.48 ± 0.22</td>
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OVP, ovarian venous plasma; JVP, jugular venous plasma.

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**Table 2** Characteristics of releasing profiles of PGF$_{2\alpha}$ 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.

<table>
<thead>
<tr>
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<th>Prior to luteolysis (~48 ~ 0 h)</th>
<th>After luteolysis (0 ~ 72 h)</th>
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<tbody>
<tr>
<td>Peak concentration Pattern I</td>
<td>379.0 ± 224.7</td>
<td>575.6 ± 161.1</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$ (OVP)</td>
<td>22.3 ± 3.5</td>
<td>28.7 ± 9.9</td>
</tr>
<tr>
<td>Pattern II</td>
<td>145.5 ± 41.9</td>
<td>133.9 ± 33.4</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$ (OVP)</td>
<td>20.1 ± 1.6</td>
<td>20.1 ± 2.9</td>
</tr>
<tr>
<td>Pattern III</td>
<td>56.8 ± 17.1</td>
<td>55.9 ± 9.5</td>
</tr>
<tr>
<td>Basal concentration Pattern I</td>
<td>10.7 ± 0.7</td>
<td>11.8 ± 1.3</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$ (OVP)</td>
<td>10.7 ± 0.7</td>
<td>11.8 ± 1.3</td>
</tr>
</tbody>
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OVP, ovarian venous plasma; JVP, jugular venous plasma.

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**Figure 3** Relationship between PGF$_{2\alpha}$ in OVP ipsilateral to the CL and PGFM in JVP. Pattern I was classified as a concomitant appearance of a PGF$_{2\alpha}$ peak in OVP with a PGFM peak in JVP. Pattern II was classified as a PGF$_{2\alpha}$ 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$_{2\alpha}$ in OVP.
results provide the first direct evidence that PGF$_{2\alpha}$ is released from the CL into the ovarian vein 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.

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$_{2\alpha}$ 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$_{2\alpha}$ 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$_{2\alpha}$ during spontaneous luteolysis.

The bovine CL contains relatively large amounts of arachidonic acid that is comparable to the endometrial cells (Łukaszewska & 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$_{2\alpha}$ 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$_{2\alpha}$ secretion was drastically increased from 24 h after the onset of luteolysis. These findings suggest that the intra-luteal PGF$_{2\alpha}$ may mediate structural rather than functional luteolysis.

In the systemic circulation, PGF$_{2\alpha}$ 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$_{2\alpha}$ secretion. On the other hand, it was reported that PGF$_{2\alpha}$ can be converted to PGFM in ovine CL (Silva et al. 2000). In the present study, the increases in PGF$_{2\alpha}$ and PGFM secretion in CL were positively correlated with each other after the onset of luteolysis. The data support the concept that PGF$_{2\alpha}$ is catabolised to PGFM in the CL, and thus, a PGF$_{2\alpha}$ metabolic pathway exists in the CL of the cow. The fact that PGF$_{2\alpha}$ increased to about 300% while PGFM increased to only about 150% during luteolysis may imply that the active synthesis, but not catabolism, of PGF$_{2\alpha}$ 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$_{2\alpha}$ plays a role as an autocrine/paracrine modulator of CL function (Miyamoto & Schams 1994, Olofsson & Leung 1994). The increased PGF$_{2\alpha}$ within the CL after the onset of luteolysis may act as an amplifier of uterine PGF$_{2\alpha}$ during spontaneous luteolysis.

In the present study, three kinds of relationship were observed between the PGF$_{2\alpha}$ peaks in OVP and the PGFM peaks in JVP during spontaneous luteolysis. Pattern I was classified as a concomitant appearance of a PGF$_{2\alpha}$ peak in OVP with a PGFM peak in JVP (Fig. 5a). Presumably, uterine PGF$_{2\alpha}$ was released into the uterine vein, and then branched into two pathways. In the first pathway PGF$_{2\alpha}$ 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$_{2\alpha}$ moves into the ovarian vein and is detected as a peak in OVP. In the second pathway PGF$_{2\alpha}$ 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$_{2\alpha}$ pulse.

Pattern II was classified as a peak of PGF$_{2\alpha}$ 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$_{2\alpha}$ 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$_{2\alpha}$ into OVP cannot be excluded, the source of this PGF$_{2\alpha}$ can be considered to be the CL. In support of this idea, Griffith et al. (2002) reported that in ewes hysterectomized on day 5 of the oestrous cycle, multiple administrations of PGF$_{2\alpha}$ on day 10 induced clear
pulsatile releases of PGFM in the circulation during luteolysis, which is independent of the administered PGF2\(_{2a}\). The data suggest that the regressing CL may release PGF2\(_{2a}\) 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 PGF2\(_{2a}\) in OVP (Fig. 5c). The observation that PGF2\(_{2a}\) in the ovarian vein does not show a peak suggests that the CL does not release PGF2\(_{2a}\) at that time. Thus, the weak PGFM peak may be due to a small amount of PGF2\(_{2a}\) 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 PGF2\(_{2a}\) 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 PGF2\(_{2a}\) 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 PGF2\(_{2a}\) within the CL (PGF2\(_{2a}\) in MDS) and the PGF2\(_{2a}\) released from the CL into the ovarian vein (PGF2\(_{2a}\) in OVP) during spontaneous luteolysis. Even though PGF2\(_{2a}\) detected in the ovarian vein may contain both uterus- (pattern I) and CL-derived (pattern II) PGF2\(_{2a}\), the changing profiles of PGF2\(_{2a}\) within the CL and in OVP do not coincide. Therefore, it is unlikely that the production of PGF2\(_{2a}\) within CL tissue is reflected in circulating PGF2\(_{2a}\) 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 PGF2\(_{2a}\) during spontaneous luteolysis, and provide the first in vivo evidence that the CL releases PGF2\(_{2a}\) during spontaneous luteolysis in the cow. Although the physiological relevance of CL-derived PGF2\(_{2a}\) 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 PGF2\(_{2a}\) within the regressing CL amplifies the luteolytic action of PGF2\(_{2a}\) from the uterus.

**Acknowledgements**

The authors thank Dr K Okuda, Okayama University, Japan, for progesterone antiserum, Dr S Ito, Kansai University of Medicine, Japan, for PG antiserum, and Fresenius AG, St Wendel, Germany for the microdialysis capillary membranes. This study was supported by the Grant-in-Aid for Scientific Research of the Japan Society for the Promotion of Science (JSPS), the Novartis Foundation (Japan) for the Promotion of Science, and the 21st Century COE Program (A-1), Ministry of Education, Culture, Science and Technology, Japan. T J A and M P B W are postdoctoral fellows supported by Japan Society for the Promotion of Science. M M is supported by the COE Program.

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Received 23 January 2004
First decision 24 March 2004
Revised manuscript received 13 May 2004
Accepted 14 May 2004

www.reproduction-online.org


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