# Expression of mRNA for Cell Adhesion Molecules in the Bovine Corpus Luteum During the Estrous Cycle and PGF2α-Induced Luteolysis


doi: info:doi/10.1262/jrd.19082

*Journal of Reproduction and Development*, Vol. 53, No. 6, 2007

—Research Note—

# Expression of mRNA for Cell Adhesion Molecules in the Bovine Corpus Luteum During the Estrous Cycle and $PGF_{2\alpha}$-Induced Luteolysis

**Koumei SHIRASUNA[1)], Sho WATANABE[1)], Kaya NAGAI[1)], Kiemi SASAHARA[1)], Takashi SHIMIZU[1)], Albert M. RICKEN[2)], Katharina SPANEL-BOROWSKI[2)] and Akio MIYAMOTO[1)]**

*[1)]Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan and [2)]Institute of Anatomy, University of Leipzig, Leipzig, D-04103, Germany*

**Abstract.** Cell-to-cell interaction via cell contact-dependent pathway is essentially important for maintenance and regulation of corpus luteum (CL) integrity and its physiological actions. The objective of the present study was to evaluate the mRNA expression of the cell adhesion molecules (CAMs) that are constituent factors of gap junctions [connexin (Cx) 43] and adherence junctions (VE-, E-, N-cadherin) in two types of endothelial cells from the mid CL and in CL tissue during the estrous cycle and $PGF_{2\alpha}$-induced luteolysis in the cow. Specific mRNA expression for Cx43 and N-cadherin was detected in cytokeratin-positive (CK+) and cytokeratin-negative (CK-) luteal endothelial cells (EC) and fully luteinized granulosa cells (LGC). E-cadherin mRNA was expressed in CK+EC and LGC, but not in CK-EC. VE-cadherin mRNA was expressed in both CK+ and CK-EC. During the estrous cycle, Cx43 mRNA expression was significantly lower in the regressing CL. VE-cadherin expression also tended to increase in the mid CL and increased significantly in the regressing CL. E-cadherin mRNA expression was higher in the early and late CL than in the mid- and regressing CL. N-cadherin mRNA expression gradually increased from the early to late CL followed by a decrease in the regressing CL. During $PGF_{2\alpha}$-induced luteolysis, Cx43 mRNA expression appeared to increase, and VE-cadherin and E-cadherin mRNA significantly increased at 24 h. N-cadherin mRNA expression decreased 2 and 4 h after $PGF_{2\alpha}$ administration. Collectively, expression of the mRNAs for CAMs was different in the two types of luteal endothelial cells and fully luteinized granulosa cells and changed independently in the CL during the estrous cycle and $PGF_{2\alpha}$-induced luteolysis in the cow. The results suggest that CAMs play physiological roles in cell-to-cell communication to regulate both gap and adherence junctions during CL development and regression in the cow.

**Key words:** Cadherin, Connexin, Corpus luteum, Cow, Luteolysis

**(J. Reprod. Dev. 53:** 1319–1328, 2007)

The corpus luteum (CL) is a transient organ that secretes progesterone (P), which is a prerequisite for establishment and maintenance of pregnancy. The CL is formed from the residual cells of a follicle after ovulation, in which cell growth and differentiation occur together with neoangiogenesis, and the CL acquires the capacity to secrete P. If pregnancy does not occur, prostaglandin (PG) $F_{2\alpha}$ is released from the uterus [1, 2], and the CL rapidly looses its P secretory ability (functional luteolysis) [1, 2]. Subsequently, its

Accepted for publication: July 31, 2007
Published online: September 4, 2007
Correspondence: A. Miyamoto (e-mail: akiomiya@obihiro.ac.jp)

structure is disrupted, and where apoptosis of luteal cell and endothelial cells is prominent (structural luteolysis) [3].

It is likely that a cell-to-cell interaction is essentially important for maintenance and regulation of CL integrity and its physiological actions. The bovine CL consists of several cell types, such as luteal cells, endothelial cells, vascular smooth muscle cells, pericytes and fibroblast cells, and it constitutes a heterogeneous structure [4]. Therefore, it has been suggested that there are intercellular communications via a contact-dependent pathway among various cell types in the CL.

The gap junction is formed with a tunnel-like structure and enables regulatory molecules, nutrients and ions of less than about 1 kDa (i. e., calcium ions, cAMP and inositol 1,4,5-triphosphate) to be exchanged between adjacent cells [5]. Gap junctions are formed by connexin (Cx) proteins such as Cx26, Cx32, Cx43 and Cx45 [6, 7], and Cx43 is the main member of the gap junctional protein family. Gap junctions and Cx43 have been identified in the CLs of several species [8–10], and gap junctions formed by Cx43 has been suggested to be predominantly important for regulation of growth, differentiation and regression of the CL [8–10].

On the other hand, the adherence junction is another cell adhesion type, and cadherin, which is a calcium ion-dependent cell adhesion molecule, has a key role in this junction. Cadherin is a single pass transmembrane glycoprotein, and its extracellular domain links to the same cadherin of an adjacent cell homophilically, while its intercellular domain binds to the cytoskeleton [11, 12]. These cadherin families, including vascular endothelial cell cadherin (VE-cadherin), epithelial cadherin (E-cadherin) and neuronal cadherin (N-cadherin), act as major factors in tissue development and differentiation of many organs, including ovarian tissues. The cytoplasmic domain of these cadherins is anchored to the actin cytoskeleton via proteins of the armadillo family, such as α-catenin [13] and β-catenin [14]. The association with actin may be important in modulating paracellular permeability, stabilization of the junctional complex and intracellular signal transduction [15]. These adhesion junctions are required not only for tight junctions but also for the formation of gap junctions [16]. It has been demonstrated that cadherins and catenins are expressed in the CL of humans [10, 17], baboons [10, 18], mice [19] and rats [20–22], but there is currently no information available concerning the bovine CL. Therefore, the aim of the present study was to determine the mRNA expression of these cell adhesion molecules (CAMs) during formation, maturation and regression of the CL in the cow.

## Materials and Methods

All animal experiments were conducted at the Field Center of Animal Science and Agriculture, Obihiro University, and the experimental procedures complied with the Guidelines for the Care and Use of Agricultural Animals of Obihiro University.

*Reagent for cell cultures*

Dulbecco's modified Eagle's medium (DMEM)/nutrient mixture and Ham's F-12 medium (1:1), phosphate buffered saline (PBS), insulin, amphotericin B, and gentamicin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Forskolin was purchased from Wako (Osaka, Japan). Fetal bovine serum (FBS) and TRIzol reagent were purchased from Invitrogen Corporation (CA, USA). Vitrogen 100 was purchased from Angiotech BioMaterials (Palo Alto, CA, USA). Tissue culture plates (24-wells/plate) and flasks were purchased from Nunc (Kampstrup, Denmark).

*Isolation and luteinization of granulosa cells and luteal endothelia cells, and ollection of bovine CLs during the estrous cycle*

Ovaries containing CLs from Holstein cows were collected at a local slaughterhouse. Granulosa cells (GC) were collected and luteinized according to the method of Meidan *et al.* [23]. GCs were collected from follicles with diameters of 5 to 8 mm and were luteinized in DMEM/F-12 medium containing 10% FBS, gentamicin (50 mg/l), insulin (2 mg/l), forskolin (10 mM) and amphotericin B (2.5 mg/l). We regarded GCs obtained by culture for 8 days as luteinized GCs based on the data concerning P secretion [23]. Luteinized GCs (LGC) were collected and stored at –80 C until analysis of mRNA expression.

Cytokeratin-positive (CK+) and cytokeratin-negative (CK-) endothelial cells (EC) isolated from the CLs of the cows during the mid-luteal phase were used as described previously [24]. These cells were grown on plates precoated with 1% Vitrogen in

complete DMEM Ham's F12 containing 5% FBS. The experiments were carried out on cells from passages 3 to 5, and ECs were collected and stored at –80 C until the analysis of mRNA expression.

The luteal stages were classified as early, mid, late or regressed by macroscopic observation of the ovary as described previously [25]. After the stages were determined, the CL were immediately separated from the ovaries. Thereafter, they were minced, placed into a 1.5 ml microcentrifuge tube with 400 $\mu$l of TRIzol reagent, homogenized immediately and stored at –80 C until being analyzed.

*$PGF_{2\alpha}$-induced luteolysis in the cow*

Experimented design: Ten multiparous, nonlactating Holstein cows were used for this study ($PGF_{2\alpha}$-treated group, n=5; control group, n=5). The day of estrus was designated as day 0. On days 10–12 of the estrous cycle, 500 $\mu$g of $PGF_{2\alpha}$ analog (cloprostenol, Estrumate®; Takeda Pharmaceutical, Osaka, Japan) or saline was injected into the cows (injection=0 h). Serial luteal biopsies, as described previously [26], were collected at 0 (pre-injection), 0.25, 2, 4 and 24 h after $PGF_{2\alpha}$ or saline administration to determine the expression of mRNA in the CL. Blood samples were collected by caudal venepuncture. Plasma was obtained by centrifugation at 900 g for 20 min at 4 C and was stored at –30 C until being analyzed.

RNA extraction and RT-PCR: Total RNA was extracted from cells and luteal tissues using TRIzol reagent according to the manufacturer's directions and was treated with DNase using a commercial kit (SV Total RNA Isolation System; Promega, Madison, WI, USA). It was then frozen at –20 C in THE RNA Storage Solution (Ambion, Austin, TX, USA). The mRNA expressions of Cx43, VE-cadherin, E-cadherin, N-cadherin, α-catenin, β-catenin, endothelin-1 (ET-1) and β-actin in CK+EC, CK-EC, LGC and CL tissue (mid-luteal phase) were examined by RT-PCR as previously reported [26]. The primers used for RT-PCR are indicated in Table 1.

Quantitative real-time PCR: The mRNA expressions of Cx43, VE-cadherin, E-cadherin, N-cadherin, α-catenin, β-catenin, steroidogenic acute regulatory protein (StAR), ET-1 and GAPDH were quantified by real-time PCR using a LightCycler (Roche Diagnostics, Mannheim, Germany) as per our previous report [26]. The primers used for real-time PCR are shown in Table 1. The values were normalized using GAPDH as the internal standard.

Hormone determination: The concentration of P in the plasma was determined in duplicate by enzyme immunoassay (EIA) after diethyl ether extraction [27]. The standard curve ranged from 0.05 to 50 ng/ml, and the effective dose 50 ($ED_{50}$) of the assay was 2.4 ng/ml. The intra- and interassay coefficients of variation (CVs) were 6.2 and 9.3%, respectively.

*Statistical analysis*

All data were expressed as the mean ± SEM. The time of $PGF_{2\alpha}$ analogue injection was defined as 0 h. The expression of mRNA was expressed as the percentage of the individual baseline (0 h). The data were examined by ANOVA followed by the Bonferroni multiple comparison test. Probabilities of less than 5% ($P<0.05$) were considered significant.

## Results

*Detection of the mRNA expression of Cx43, VE-cadherin, N-cadherin, E-cadherin, α-catenin, β-catenin and ET-1 in different cell types*

The bovine CLs expressed the mRNA of all CAMs (Fig. 1). Specific transcripts for Cx43, N-cadherin, α-catenin and β-catenin were detected in CK+ECs, CK-ECs and LGCs. The mRNA expression of E-cadherin was detected in CK+ECs and LGCs, but not in CK-ECs. The mRNAs of VE-cadherin and ET-1, as markers of ECs, were mainly expressed in CK-ECs, and were weekly expressed in CK+ECs, but not in LGCs.

*Relative mRNA levels of ET-1, Cx43, VE-cadherin, E-cadherin, N-cadherin, α-catenin and β-catenin in luteal endothelial cells*

The mRNA levels of ET-1 (A), Cx43 (B), VE-cadherin (C), E-cadherin (D), N-cadherin (E), α-catenin (F) and β-catenin (G) were determined for CK+ECs and CK-ECs using real-time PCR (Fig. 2). Expression of ET-1, Cx43, VE-cadherin, N-cadherin, α-catenin and β-catenin mRNA was detected in both CK+ECs and CK-ECs. The expression levels of ET-1, VE-cadherin and N-cadherin were significantly higher in CK-ECs compared with those in CK+ECs. However, mRNA expression of E-cadherin was only detected in CK+ECs.

**Table 1.** Primer sequence and resulting fragment sizes of Cx43, VE-cadherin, N-cadherin, E-cadherin, α-catenin, β-catenin, ET-1, StAR, β-actin and GAPDH, which was used as the internal standard

| | Primer sequence | Product size |
|---|---|---|
| RT-PCR | | |
| *Cx 43* | Fwd 5′-TTATTGGCCCAGTTTTGCTC-3′<br>Rev 5′-GGACATGCACTTGAAGCAGA-3′ | 570 bp |
| *N-Cadherin* | Fwd 5′-CGAGCCAGCAGATTTTAAGG-′<br>Rev 5′-TGACAGCTGACCTGAGATGG-3′ | 439 bp |
| *E-Cadherin* | Fwd 5′-TGTGACTGTGATGGGATCGT-3′<br>Rev 5′-TAGCAGCTTCGGAACCACTT-3′ | 475 bp |
| *VE-Cadherin* | Fwd 5′-GATGCACACGACGAAGAGA-3′<br>Rev 5′-GCTGGTACACGACAGAAGCA-3′ | 419 bp |
| *α-catenin* | Fwd 5′-TAAGAAGGCCCATGTTTTGG-3′<br>Rev 5′-TTTGGCTGCCATAATGTTCA-3′ | 415 bp |
| *β-catenin* | Fwd 5′-GGTTCGTGCACATCAGGATA-3′<br>Rev 5′-GGGCTCCAGTACAACCTTCA-3′ | 422 bp |
| *ET-1* | Fwd 5′-CGTGGCCGGAAGAAAAACAA-3′<br>Rev 5′-GGCGCCTGAGTGAGACACAA-3′ | 512 bp |
| *β-actin* | Fwd 5′-CAAAGGCCAACCGTGAGAAGAT-3′<br>Rev 5′-CCACGTTCCGTGAGGATCTTCA-3′s | 458 bp |
| Real-time PCR | | |
| *Cx 43* | Fwd 5′-TGAGTGCCGTTTACACTTGC-3′<br>Rev 5′-GGCAAGAGACACCAATGACA-3′ | 125 bp |
| *N-Cadherin* | Fwd 5′-TCCCCCTCTCATCTGAACAC-3′<br>Rev 5′-ATCCTCAGGTAGGGCTGGTT-3′′ | 113 bp |
| *E-Cadherin* | Fwd 5′-CCTGCCAATCCTGATGAAAT-3′<br>Rev 5′-TAGCAGCTTCGGAACCACTT-3′ | 130 bp |
| *VE-Cadherin* | Fwd 5′-TGGGCAAGATTAAATCGAGTG-3′<br>Rev 5′-TCCCTGTCCAGCCTCTCTAA-3′ | 130 bp |
| *α-catenin* | Fwd 5′-TTACCCGGCTGCTTATTTTG-3′<br>Rev 5′-AGTGCCAGCATTCCTCAGTT-3′ | 104 bp |
| *β-catenin* | Fwd 5′-GGTTCGTGCACATCAGGATA-3′<br>Rev 5′-GGGCTCCAGTACAACCTTCA-3′ | 110 bp |
| *StAR Fwd* | 5′-GTGGATTTTGCCAATCACCT-3′<br>Rev 5′-TTATTGAAAACGTGCCACCA-3′ | 203 bp |
| *ET-1* | Fwd 5′-CAAATGCATCCTGCCTGGTC-3′<br>Rev 5′-ATTGCCACCCCCATAGAGGA-3′ | 160 bp |
| *GAPDH* | Fwd 5′-CTCTCAAGGGCATTCTAGGC-3′<br>Rev 5′-TGACAAAGTGGTCGTTGAGG-3′ | 120 bp |

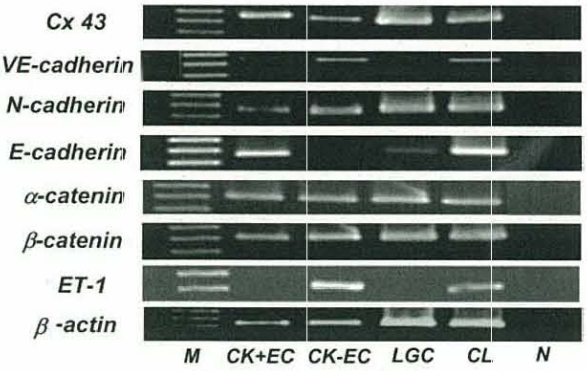


**Fig. 1.** Representative photos of specific RT-PCR products for Cx43, VE-cadherin, N-cadherin, E-cadherin, α-catenin, β-catenin, ET-1 and β-actin. Lane M indicates the DNA ladder, lane CK+EC indicates luteal endothelial cells having cytokeratin, lane CK-EC indicates luteal endothelial cells not having cytokeratin, Lane LGC indicates luteinized granulosa cells, lane CL indicates bovine luteal tissue (Days 8–12) and lane N indicates the negative control. The products were separated by agarose gel electrophoresis.

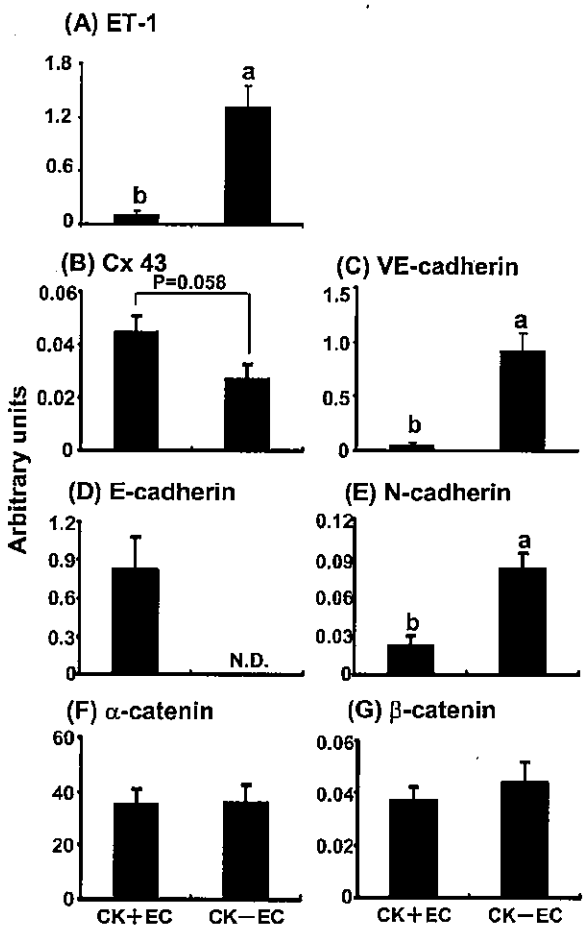


Fig. 2. Relative mRNA levels of ET-1 (A), Cx43 (B), VE-cadherin (C), E-cadherin (D), N-cadherin (E), α-catenin (F) and β-catenin (G) in CK+ECs and CK-ECs (n=8, respectively). All values are shown as the mean ± SEM (relative to β-actin mRNA levels). Different superscript letters indicate significant differences (P<0.05) as determined by ANOVA followed by the Bonferroni multiple comparison test.

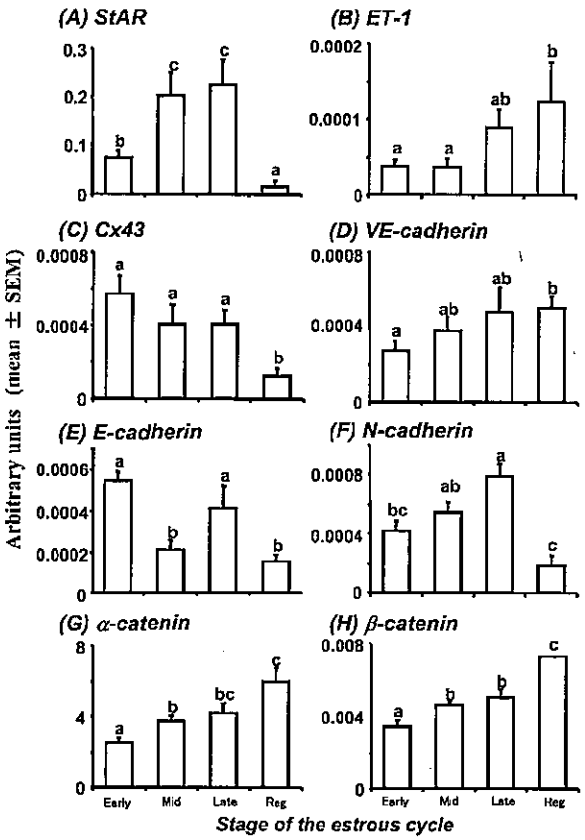


Fig. 3. Relative mRNA levels of StAR (A), ET-1 (B), Cx43 (C), VE-cadherin (D), E-cadherin (E), N-cadherin (F), α-catenin (G) and β-catenin (H) in bovine CLs throughout the estrous cycle (n=4, respectively). All values are shown as the mean ± SEM (relative to β-actin mRNA levels). Different superscript letters indicate significant differences (P<0.05) as determined by ANOVA followed by the Bonferroni multiple comparison test.

*Expression of StAR, ET-1, Cx43, VE-cadherin, E-cadherin, N-cadherin, α-catenin and β-catenin mRNA throughout the estrous cycle*

The mRNA expression in the CL tissue during the estrous cycle is shown Fig. 3. Expression of StAR mRNA, as a marker of luteal function, was higher in the mid- and late luteal stage (Fig. 3A). ET-1 is a luteolytic mediator of $PGF_{2\alpha}$ action, and ET-1 mRNA expression increased as the luteal phase progressed (Fig. 3B). Cx43 mRNA expression was significantly lower in the regressed luteal stage (Fig. 3C). VE-cadherin expression also tended to increase beginning in the mid CL and significantly increased in the regressed CL compared with the early CL (Fig. 3D). E-cadherin mRNA expression was higher in the early and late luteal stages than in the mid- and regressed luteal stages (Fig. 3E). The mRNA expression of N-cadherin gradually increased from the early to late luteal stage and then decreased in the regressed luteal stage (Fig. 3F). The mRNA expressions of α-catenin and β-catenin gradually increased throughout the

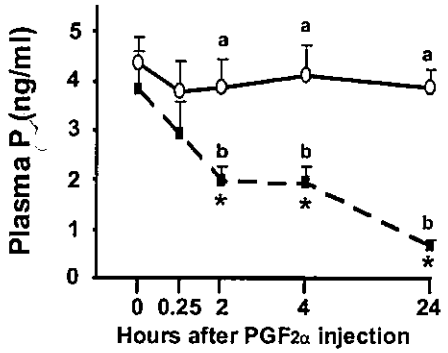


Fig. 4. Changes in the plasma P concentrations during $PGF_{2\alpha}$-induced luteolysis (n=5, respectively). All values are shown as the mean ± SEM. The white circles indicate the control group injected with saline, and the black squares indicate the $PGF_{2\alpha}$-treated group. Different superscript letters indicate significant differences (P<0.05) between the control and $PGF_{2\alpha}$-treated groups as determined by ANOVA followed by the Bonferroni multiple comparison test.

estrous cycle (Fig. 3G and H).

*Changes in the plasma P concentration and mRNA expressions of StAR, ET-1, Cx43, VE-cadherin, E-cadherin, N-cadherin, α-catenin and β-catenin during $PGF_{2\alpha}$-induced luteolysis in the cow*

The changes in the plasma P concentration after $PGF_{2\alpha}$ or saline administration during the mid-luteal phase are shown in Fig. 4. The plasma P concentration began to decrease significantly 2 h after $PGF_{2\alpha}$ administration, but was unchanged in the control group.

The changes in the mRNA expressions of StAR, ET-1, Cx43, VE-cadherin, E-cadherin, N-cadherin, α-catenin and β-catenin are shown in Fig. 5. StAR mRNA expression began to decrease significantly at 2 h in the $PGF_{2\alpha}$-treated group (Fig. 5A). The Cx43 mRNA expression of the $PGF_{2\alpha}$-treated group appeared to be higher than that of the control at 24 h (P=0.053; Fig. 5C). The mRNA expressions of ET-1, VE-cadherin and E-cadherin increased at 24 h during $PGF_{2\alpha}$-induced luteolysis (Fig. 5B, D and E). The mRNA expression of N-cadherin decreased at 2 h and 4 h in the $PGF_{2\alpha}$-treated group (Fig. 5F). The mRNA expressions of both α- and β-catenin did not change after $PGF_{2\alpha}$ administration (Fig. 5G and H).

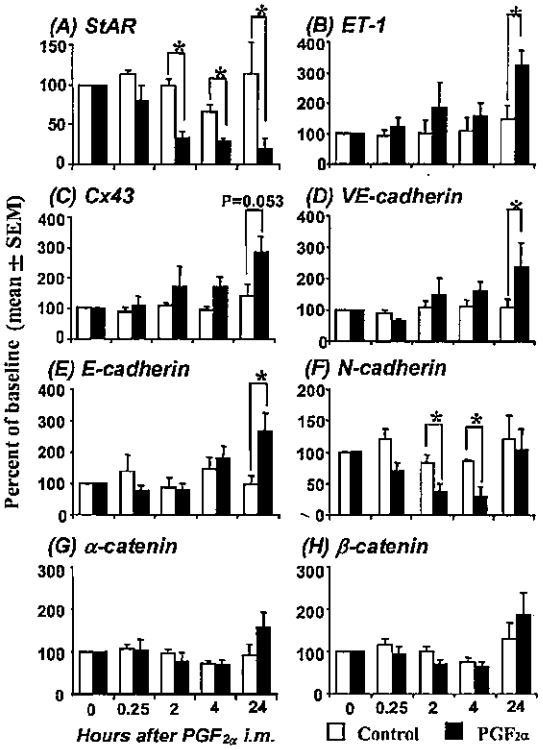


Fig. 5. Relative mRNA levels of StAR (A), ET-1 (B), Cx43 (C), VE-cadherin (D), E-cadherin (E), N-cadherin (F), α-catenin (G) and β-catenin (H) in bovine CLs during $PGF_{2\alpha}$-induced luteolysis (n=5, respectively). All values are shown as the mean ± SEM (relative to β-actin mRNA levels). The white bars indicate the control group injected with saline, and the black bars indicate the $PGF_{2\alpha}$-treated group. The symbols indicate significant differences (P<0.05) between the control and $PGF_{2\alpha}$-treated groups as determined by ANOVA followed by the Bonferroni multiple comparison test.

## Discussion

In the present study, we evaluated the mRNA expression of CAMs in two types of endothelial cells from CLs and the relative changes in the mRNA expression of CAMs in CL tissue during the estrous cycle and $PGF_{2\alpha}$-induced luteolysis in the cow. The profiles of the mRNA expressions of the CAMs that contribute to formation of gap and adherence junctions were different. Thus, this suggests that the gap and adherence junctions constituted by CAMs are regulated by related factors depending on the type of endothelial cell, luteal phase and action of $PGF_{2\alpha}$ in the cow.

Gap junctions are implicated in the regulation and coordination of cellular metabolism and function during growth and differentiation of organs and tissues [5]. Recent studies have shown the presence of Cx43 in the luteal tissues and cultured luteal cells of rats [28], humans [10], baboons [10], sheep [29, 30] and cows [8]. In the present study, Cx43 mRNA expression was detected in luteal endothelial cells (CK+ECs and CK-ECs) and in luteinized GCs. Indeed, Cx43 is detected on the borders of luteal cells and also between luteal cells and endothelial cells [9]. Grazul-Bilska *et al.* [8] demonstrated that the expression of Cx43 in luteal tissues is greatest during the early and mid-luteal phases and decreases during the late luteal phase of the estrous cycle in the cow, and this was fully supported by our present result. Moreover, inhibition of Cx43 mRNA expression by siRNA results in decreased P production by ovine luteal cells *in vitro* [31]. These findings suggest that luteal cells and luteal endothelial cells have a gap junction and are associated with P production during development and maintenance of the CL [9, 10].

During CL regression, expression of Cx43 is low in baboons [9], sheep [29] and cows [8]. In the present study, Cx43 mRNA expression was significantly low during the regressing luteal stage and tended to increase, but not decrease, 24 h after $PGF_{2\alpha}$ administration. Expression of Cx43 mRNA remains at a relatively high level during $PGF_{2\alpha}$-induced luteolysis in sheep [30], and $PGF_{2\alpha}$ enhances gap junctional intercellular communication of bovine luteal cells from the late luteal phase [9]. In addition, the mRNA expressions of VE-cadherin and E-cadherin increased at 24 h during $PGF_{2\alpha}$-induced luteolysis. Cell adhesion is essential for formation of gap junction and cell-to-cell communication. These observations suggest that maintenance of Cx43, VE-cadherin and E-cadherin mRNA expression during regression may contribute to ensure the cell adhesion and gap junction necessary for transferring the luteolytic signal between luteal cells and endothelial cells [9, 30].

As its name suggests, VE-cadherin is a specific factor of endothelial cells. VE-cadherin mRNA was expressed in CK-ECs and CK+ECs but not in LGCs, and CK-ECs expressed VE-cadherin mRNA more strongly than in CK+ECs in the present study. A previous report showed that luteal endothelial cells, both CK+ECs and CK-ECs, have endothelial cells markers, such as Tie2 and CD31; however, only CK-ECs expressed endothelial nitric oxide synthase [32]. Moreover, the mRNA expression of ET-1, another endothelial cell marker, was only evident in CK-ECs [32], as was the case in the present study. Thus, CK-ECs may be a dominant cell type in the CL. During the estrous cycle, VE-cadherin mRNA expression also tended to increase in the mid CL. VE-cadherin has been shown to be involved in endothelial cells survival by shedding molecules promoting apoptosis from the cell surface [33]. A recent study indicated that VE-cadherin antibody blocks neovascularization during the luteal phases, resulting in inhibition of CL development, a decrease in the vascular area and a reduction of P production in mice [19]. Therefore, VE-cadherin appears to be required for full development of a functional CL.

We found that E-cadherin mRNA is expressed in CK+ECs and LGCs, but not in CK-ECs. It has been reported that rat granulosa cells do not have E-cadherin but rather that luteal cells express E-cadherin *in vivo* [21, 22]. During the estrous cycle, E-cadherin mRNA expression was at its highest level during the early luteal phase in the bovine CL, and this was similar to previous studies in humans [17], baboons [18] and rats [22], suggesting that expression of E-cadherin may be related to luteinization [21, 22]. Also, addition of E-cadherin antibody induces cell disruption within 30 min in baboon luteal cells *in vitro* [18], indicating that cell-to-cell adhesion by E-cadherin may be necessary for maximal intercellular communication between luteal cells and luteal endothelial cells as synthesis of P progresses.

N-cadherin-neutralizing antibody inhibits granulosa cell aggregation and increases apoptosis in these cells in the rat [20, 34]; this indicates that there are direct relationships between N-cadherin-mediated granulosa cell adhesion and survival [35]. N-cadherin has been demonstrated in granulosa cells, LGCs and luteal cells in humans [35] and rats [20, 22]. In the present study, all examined cell types (CK+ECs, CK-ECs and LGCs) expressed N-cadherin mRNA, suggesting that luteal cell-luteal cell and/or luteal cell-endothelial cell communications are formed by an adherence junction of N-cadherin within the bovine CL. Luteal cells are strongly positive for N-cadherin in the early and mid-luteal phases, whereas there is only weak N-cadherin staining during the late luteal phase [35]. Our present results support the above finding that the

mRNA expression of N-cadherin in the bovine CL gradually increased from the early to late luteal stage and then decreased in the regressed luteal stage. Therefore, N-cadherin expression is an integral component of the remodeling processes, including development and maintenance of the bovine CL.

On the other hand, N-cadherin mRNA expression temporarily declined 2–4 h after $PGF_{2\alpha}$ injection in the present study. At this time, production of P from the CL drastically decreases, whereas basic fibroblast growth factor (bFGF) is upregulated by $PGF_{2\alpha}$ administration in the CL [36]. Indeed, bFGF can suppress granulosa cell apoptosis, and this bFGF effect is prevented by exposure to the N-cadherin antibody [36]. Moreover, exposure to the N-cadherin antibody reduces the level of tyrosine-phosphorylated FGF receptor [20]. Therefore, this demonstrates that interaction between N-cadherin and bFGF via the FGF receptor may be required to prevent apoptosis. Taking these findings into consideration, bFGF may not be capable of acting as an anti-apoptotic through the FGF receptor since downregulation of N-cadherin at the initial step of $PGF_{2\alpha}$-induced luteolysis resulted in weakening of the blockade of apoptosis at an early stage of luteolysis in the bovine CL.

All examined cell types (CK+ECs, CK-ECs and LGCs) expressed both α- and β-catenins, suggesting that signal transduction via binding to cadherins may occur in luteal cell-luteal cell and/or luteal cell-endothelial cell communication in the bovine CL. At the time of initiation of full structural luteolysis (around 24 h after $PGF_{2\alpha}$ injection), expression of CAMs within the CL was upregulated and/or maintained, whereas the CL volume was gradually decreased by cell apoptosis [3]. Indeed, the mRNA expression of both α- and β-catenin were also maintained during $PGF_{2\alpha}$-induced luteolysis. Expression of E-cadherin is essential for Cx43 phosporylation and communication competence [37]. Therefore, the cadherin-catenin complex might have a role in maintaining the gap junction for communication of luteolytic signals. Thereafter, gap and adherence junctions may disappear due to progress of the luteolytic cascade.

In summary, we have shown that the mRNAs of CAMs were expressed differently in two types of luteal endothelial cell and in fully luteinized granulosa cells and that they changed independently in the CL during the estrous cycle and $PGF_{2\alpha}$-induced luteolysis in the cow. The results suggest that CAMs play physiological roles in the cell-to-cell communication of the cow in order to regulate both gap and adherence junctions during CL development and regression.

## Acknowledgments

The authors thank Dr. K. Okuda (Okayama University, Okayama, Japan) for P antiserum and Takeda Pharmaceutical (Osaka, Japan) for Estrumate® and Conceral®. This study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) and the 21st Century COE Program (A-1) of the Ministry of Education, Culture, Sports, Science and Technology of Japan. K.S. was supported by a JSPS Research Fellowship for Young Scientists.

## References

1. **McCracken JA, Carlson JC, Glew ME, Goding JR, Baird DT, Green K, Samuelsson B.** Prostaglandin $F_{2\alpha}$ identified as a luteolytic hormone in sheep. *Nat New Biol* 1972; 238: 129–134.
2. **Niswender GD, Reimers TJ, Diekman MA, Nett TM.** Blood flow: a mediator of ovarian function. *Biol Reprod* 1976; 14: 64–81.
3. **Juengel JL, Garverick HA, Johnson AL, Youngquist RS, Smith MF.** Apoptosis during luteal regression in cattle. *Endocrinology* 1993; 132: 249–254.
4. **Farin CE, Moeller CL, Sawyer HR, Gamboni F, Niswender GD.** Morphometric analysis of cell types in the ovine corpus luteum throughout the estrous cycle. *Biol Reprod* 1986; 35: 1299–1308.
5. **Yamasaki H, Naus CC.** Role of connexin genes in growth control. *Carcinogenesis* 1996; 17: 1199–1213.
6. **Beyer EC, Paul DL, Goodenough DA.** Connexin family of gap junction proteins. *J Membr Biol* 1990; 116: 187–194.
7. **Dermietzel R, Hwang TK, Spray DS.** The gap junction family: structure, function and chemistry. *Anat Embryol (Berl)* 1990; 182: 517–528.
8. **Grazul-Bilska AT, Redmer DA, Johnson ML, Jablonka-Shariff A, Bilski JJ, Reynolds LP.** Gap

junctional protein connexin 43 in bovine corpora lutea throughout the estrous cycle. *Biol Reprod* 1996; 54: 1279–1287.
9. **Grazul-Bilska AT, Reynolds LP, Redmer DA.** Gap junctions in the ovaries. *Biol Reprod* 1997; 57: 947–957.
10. **Khan-Dawood FS, Yang J, Dawood MY.** Expression of gap junction protein connexin-43 in the human and baboon (*Papio anubis*) corpus luteum. *J Clin Endocrinol Metab* 1996; 81: 835–842.
11. **Nose A, Nagafuchi A, Takeichi M.** Expressed recombinant cadherins mediate cell sorting in model systems. *Cell* 1988; 54: 993–1001.
12. **Takeichi M.** Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* 1991; 251: 1451–1455.
13. **Geiger B, Ayalon O.** Cadherins. *Annu Rev Cell Biol* 1992; 8: 307–332.
14. **Molenaar M, van de Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, Korinek V, Roose J, Destree O, Clevers H.** XTcf-3 transcription factor mediates $\beta$-catenin-induced axis formation in Xenopus embryos. *Cell* 1996; 86: 391–399.
15. **Navarro P, Caveda L, Breviario F, Mandoteanu I, Lampugnani MG, Dejana E.** Catenin-dependent and -independent functions of vascular endothelial cadherin. *J Biol Chem* 1995; 270: 30965–30972.
16. **Meyer RA, Laird DW, Revel JP, Johnson RG.** Inhibition of gap junction and adherens junction assembly by connexin and A-CAM antibodies. *J Cell Biol* 1992; 119: 179–189.
17. **Khan-Dawood FS, Yang J, Dawood MY.** Immunocytochemical localization and expression of E-cadherin and $\beta$-catenin in the human corpus luteum. *Mol Hum Reprod* 1996; 2: 753–757.
18. **Khan-Dawood FS, Yang J, Ozigi AA, Dawood MY.** Immunocytochemical localization and expression of E-cadherin, $\beta$-catenin, and plakoglobin in the baboon (*Papio anubis*) corpus luteum. *Biol Reprod* 1996; 55: 246–253.
19. **Nakhuda GS, Zimmermann RC, Bohlen P, Liao F, Sauer MV, Kitajewski J.** Inhibition of the vascular endothelial cell (VE)-specific adhesion molecule VE-cadherin blocks gonadotropin-dependent folliculogenesis and corpus luteum formation and angiogenesis. *Endocrinology* 2005; 146: 1053–1059.
20. **Trolice MP, Pappalardo A, Peluso JJ.** Basic fibroblast growth factor and N-cadherin maintain rat granulosa cell and ovarian surface epithelial cell viability by stimulating the tyrosine phosphorylation of the fibroblast growth factor receptors. *Endocrinology* 1997; 138: 107–113.
21. **Sundfeldt K, Piontkewitz Y, Billig H, Hedin L.** E-cadherin-catenin complex in the rat ovary: cell-specific expression during folliculogenesis and luteal formation. *J Reprod Fertil* 2000; 118: 375–385.
22. **Machell NH, Farookhi R.** E- and N-cadherin expression and distribution during luteinization in the rat ovary. *Reproduction* 2003; 125: 791–800.
23. **Meidan R, Girsh E, Blum O, Aberdam E.** *In vitro* differentiation of bovine theca and granulosa cells into small and large luteal-like cells: morphological and functional characteristics. *Biol Reprod* 1990; 43: 913–921.
24. **Spanel-Borowski K.** Diversity of ultrastructure in different phenotypes of cultured microvessel endothelial cells isolated from bovine corpus luteum. *Cell Tissue Res* 1991; 266: 37–49.
25. **Miyamoto Y, Skarzynski DJ, Okuda K.** Is tumor necrosis factor alpha a trigger for the initiation of endometrial prostaglandin $F_{2\alpha}$ release at luteolysis in cattle? *Biol Reprod* 2000; 62: 1109–1115.
26. **Watanabe S, Shirasuna K, Matsui M, Yamamoto D, Berisha B, Schams D, Miyamoto A.** Effect of intraluteal injection of endothelin type A receptor antagonist on $PGF_{2\alpha}$-induced luteolysis in the cow. *J Reprod Dev* 2006; 52: 551–559.
27. **Miyamoto A, Okuda K, Schweigert FJ, Schams D.** Effects of basic fibroblast growth factor, transforming growth factor-$\beta$ and nerve growth factor on the secretory function of the bovine corpus luteum *in vitro*. *J Endocrinol* 1992; 135: 103–114.
28. **Risek B, Guthrie S, Kumar N, Gilula NB.** Modulation of gap junction transcript and protein expression during pregnancy in the rat. *J Cell Biol* 1990; 110: 269–282.
29. **Grazul-Bilska AT, Redmer DA, Bilski JJ, Jablonka-Shariff A, Doraiswamy V, Reynolds LP.** Gap junctional proteins, connexin 26, 32, and 43 in sheep ovaries throughout the estrous cycle. *Endocrine* 1998; 8: 269–279.
30. **Borowczyk E, Johnson ML, Bilski JJ, Borowicz PP, Redmer DA, Reynolds LP, Grazul-Bilska AT.** Expression of gap junctional connexins 26, 32, and 43 mRNA in ovarian preovulatory follicles and corpora lutea in sheep. *Can J Physiol Pharmacol* 2006; 84: 1011–1020.
31. **Borowczyk E, Johnson ML, Bilski JJ, Bilska MA, Redmer DA, Reynolds LP, Grazul-Bilska AT.** Role of gap junctions in regulation of progesterone secretion by ovine luteal cells *in vitro*. *Reproduction* 2007; 133: 641–651.
32. **Klipper E, Gilboa T, Levy N, Kisliouk T, Spanel-Borowski K, Meidan R.** Characterization of endothelin-1 and nitric oxide generating systems in corpus luteum-derived endothelial cells. *Reproduction* 2004; 128: 463–473.
33. **Herren B, Levkau B, Raines EW, Ross R.** Cleavage of $\beta$-catenin and plakoglobin and shedding of VE-cadherin during endothelial apoptosis: evidence for a role for caspases and metalloproteinases. *Mol Biol Cell* 1998; 9: 1589–1601.
34. **Makrigiannakis A, Coukos G, Blaschuk O, Coutifaris C.** Follicular atresia and luteolysis: evi-

dence of a role for N-cadherin. *Ann NY Acad Sci* 2000; 900: 46–55.

35. **Makrigiannakis A, Coukos G, Christofidou-Solomidou M, Gour BJ, Radice GL, Blaschuk O, Coutifaris C.** N-cadherin-mediated human granulosa cell adhesion prevents apoptosis: a role in follicular atresia and luteolysis? *Am J Pathol* 1999; 154: 1391–1406.
36. **Neuvians TP, Berisha B, Schams D.** Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) expression during induced luteolysis in the bovine corpus luteum. *Mol Reprod Dev* 2004; 67: 389–395.
37. **Musil LS, Cunningham BA, Edelman GM, Goodenough DA.** Differential phosphorylation of the gap junction protein connexin 43 in junctional communication-competent and -deficient cell lines. *J Cell Biol* 1990; 111: 2077–2088.