

—Original Article—

Expression of C–C motif chemokines and their receptors in bovine placentomes at spontaneous and induced parturition

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Abstract. In bovine placentomes, the inflammatory response is considered important for the detachment of the fetal membrane from the caruncle after parturition. Glucocorticoids, a trigger of the onset of parturition, facilitate functional maturation of placentomes via prostaglandin (PG) and estrogen production in cattle. This study investigated how exogenous glucocorticoids, which exert immunosuppressive effects, affect placental inflammation at parturition. Placentomes were collected immediately after spontaneous or induced parturition. Parturition was conventionally induced using PGF₂ α or dexamethasone or with a combination of triamcinolone acetonide and high-dose betamethasone (TABET treatment). Polymerase chain reaction (PCR) array analysis indicated that 9/13 C–C motif chemokine ligands (*CCLs*) were upregulated > two-fold in spontaneous parturition, with *CCL2* and *CCL8* being highly expressed. The expressions of *CCL2*, *CCL8*, C–C motif chemokine receptor 1 (*CCR1*), and *CCR5* in caruncles were significantly higher in spontaneous parturition than in induced parturition. Although the clinical dose of dexamethasone did not influence the expression of these *CCLs* and *CCRs*, TABET treatment increased *CCR1* expression. *CCL8*, *CCR1*, *CCR2*, and *CCR5* were localized in the caruncular epithelial cells. *CCR2* was also localized in the epithelial cells of the cotyledonary villi. This study is the first report to reveal the disruption in *CCL* and *CCR* expression in bovine placentomes at induced parturition. Enhanced glucocorticoid exposure for the induction of parturition may upregulate *CCR1* expression in placentomes, but the treatment does not adequately promote *CCL* expression. Additionally, immunohistochemistry suggested that the CCL–CCR system is involved in the functional regulation of maternal and fetal epithelial cells in placentomes at parturition.

Key words: Cattle, Chemokine, Parturition, Placenta, Retained fetal membrane

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Retained fetal membrane (RFM) in cows, defined as the fetal membrane still present at least 12 h postpartum, occurs in approximately 5–15% of the pregnant cows and is associated with reduced fertility and milk production [1]. Degradation of the extracellular matrix (ECM) is involved in tissue remodeling during placental separation in the postpartum uterus [2]. Several studies have suggested that matrix metalloproteinase (MMP) 2 and MMP9 possess collagenase activity and play essential roles in the detachment of the fetal membrane from the caruncle [3, 4]. It is thought that

leukocytes infiltrate into placentomes at parturition and participate in tissue remodeling with the release of MMPs [5, 6]. Additionally, a transcriptome study of peripartum placentomes revealed that the physiological processes relating to ECM degradation and the innate immune response are upregulated at parturition [7].

The activity of acid phosphatase, a predominant lysosomal enzyme of the macrophages in placentomes, is higher in cases of non-RFM than in RFM [8]. Several studies have suggested that weak chemotactic activity of cotyledons and caruncles causes RFM [9, 10]. Benedictus *et al.* [11] studied the chemotactic activity of cotyledons for mononuclear leukocytes. The study revealed that cotyledons obtained from cows with RFM in which parturition was induced with dexamethasone possessed lower chemotactic activity. Therefore, studies of the regulation of chemotaxis in term placentomes are essential to reduce the incidence of RFM.

Induction of parturition in cattle is a useful management tool to avoid dystocia by controlling gestation length and fetal size. The

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injection of prostaglandin (PG) F₂ α and/or dexamethasone is commonly performed to induce parturition in pregnant cows. However, parturition induction has a negative effect; i.e. a high rate of occurrence of RFM. Numerous studies have attempted to improve the methods of hormonal induction to decrease the incidence of RFM. Recently, we reported that enhanced glucocorticoid exposure with a combination of the long-acting glucocorticoid triamcinolone acetonide and high-dose betamethasone (TABET treatment) increased expression of PG-endoperoxide synthase 2 (*PTGS2*) and cytochrome P450, family 17, subfamily A, polypeptide 1 (*CYP17A1*) in placentomes at parturition [12]. *PTGS2* is a key enzyme in the production of PGF₂ α , which induces luteolysis and myometrium contraction. *CYP17A1* converts pregnenolone to androstenedione, which is a precursor of estrogens. These findings suggest that exogenous glucocorticoids might induce placental maturation through increased PG or estrogen synthesis. However, it is unclear how the exogenous glucocorticoids, which exert anti-inflammatory and immunosuppressive effects, affect placental inflammatory responses in placentomes at parturition.

Inflammatory chemokines that are a family of small chemotactic proteins are responsible for the recruitment of leukocytes to the site of inflammation. Chemokines are classified into subfamilies according to the arrangement of the N-terminal cysteine residues. In the two major subfamilies, the first two cysteines are adjacent (C–C motif) or separated by one amino acid residue (C–X–C motif). In this study, we conducted placental gene expression profiling of inflammatory cytokines and their receptors at parturition in cows. Since the expression of C–C motif chemokine ligand 2 (*CCL2*) and *CCL8* drastically increased in the caruncles at spontaneous parturition, the effects of the TABET treatment on the expression of *CCLs* and C–C motif chemokine receptors (*CCRs*) were analyzed by a comparison of induced and spontaneous parturition. Furthermore, immunolocalization of these *CCLs* and *CCRs* in placentomes was analyzed.

Materials and Methods

Animals and sample collection

Pregnancy was induced in 24 beef cows (Japanese Black, Angus, and crossbred) by the transfer of *in vivo*-fertilized Japanese Black embryos. The parity of the cows ranged from 1 to 8 (mean \pm standard deviation, 4.0 \pm 2.9). The cows were divided into four groups according to the delivery: spontaneous (SP group; n = 5), after administration of PGF₂ α (PG group; n = 7), after administration of dexamethasone followed by PGF₂ α and estriol (DEX group; n = 6), and after administration of triamcinolone acetonide followed by PGF₂ α and betamethasone sodium phosphate (TABET group; n = 6). The PG group received 25 mg i.m. dinoprost tromethamine (Pronalgon-F® injection; Pfizer Japan, Tokyo, Japan) [13]. The DEX group received dexamethasone 20 mg i.m. (Kyoritsu Seiyaku, Tokyo, Japan) followed 24 h later by PGF₂ α and estriol 20 mg i.m. (Holin®; ASKA Pharmaceutical, Tokyo, Japan) [14]. The TABET group received triamcinolone acetonide 0.017 mg i.m./kg body weight (Kenacort-A®; Bristol-Myers Squibb, Tokyo, Japan) followed 5 days later by PGF₂ α and betamethasone sodium phosphate 0.5 mg i.m./kg body weight (Rinderon®; Shionogi & Co., Ltd., Osaka, Japan) [15].

Immediately after parturition, placental tissues were manually

collected through the birth canal. The placentomes were fixed in 10% neutral buffered formalin solution (pH 7.4) and embedded in paraffin wax blocks for sectioning. The cotyledons and caruncles of additional placentomes were manually separated, and the collected samples were stored at -80°C prior to RNA extraction. The data on parturition and the tissue samples used for gene expression analysis and immunohistochemistry were the same as those used in a previous study [15]. The gestation lengths were 292 \pm 3, 284 \pm 3, 281 \pm 1, and 280 \pm 3 days, in the SP, PG, DEX, and TABET groups, respectively. The incidences of RFM were 0, 71, 50, and 33% in the SP, PG, DEX, and TABET groups, respectively.

All procedures for animal experiments were carried out in accordance with the guidelines and ethics approved by the Animal Experiment Committee of the Animal Research Center.

Polymerase chain reaction (PCR) array analysis

The RT² Profiler PCR Array (PABT-011ZD, Qiagen, Hilden, Germany) was used to profile the expressions of 84 genes encoding bovine inflammatory cytokines and receptors. Total RNA was isolated from the caruncles of two animals from each of the SP and DEX groups using ISOGEN (Nippon Gene, Tokyo, Japan) and reverse transcribed using the RT² First Strand Kit (Qiagen). Real-time PCR was performed according to the manufacturer's instructions using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Richmond, CA). The data were analyzed by a data analysis center on the web (<https://www.qiagen.com/>) and normalized to hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) mRNA. Fold changes in the SP group compared to that of the DEX group were calculated from average of mRNA levels in the two samples.

mRNA expression analysis by real-time PCR

Total RNA of all individuals was isolated from the cotyledons and caruncles using ISOGEN and reverse transcribed using the iScript gDNA Clear cDNA Synthesis Kit (Bio-Rad). Real-time PCR was performed (in duplicate) using the GeneAmp SYBR® qPCR Mix α No ROX (Nippon Gene) for *CCL2*, *CCL5*, *CCL8*, *CCR1*, *CCR5*, *CD11B*, and actin beta (*ACTB*). Details of the oligonucleotide primers are shown in Table 1. The PCR conditions were as follows: 10 min at 95°C followed by 40 cycles of 95°C for 30 sec and 60°C for 60 sec. The real-time PCR was performed using a CFX Connect Real-Time PCR Detection System (Bio-Rad). The data were normalized to *ACTB* mRNA.

Immunohistochemistry

Immunohistochemistry for *CCL8*, *CCR1*, *CCR2*, and *CCR5* in the bovine placental tissue at parturition was performed using the automated Ventana HX System Discovery with a DabMapKit (Roche Diagnostics, Basel, Switzerland), as described previously [16]. The 5 μm -thick sections from formalin-fixed and paraffin-embedded tissue were incubated at room temperature with rabbit polyclonal anti-bovine *CCL8* antibody (MBS2026335; 1:400; MyBioSource, San Diego, CA), rabbit polyclonal anti-human *CCR1* antibody (ab140756; 1:200; Abcam PLC, Cambridge, UK), rabbit polyclonal anti-human *CCR2* antibody (NBP1-48337; 1:100; Novus Biologicals LLC, Littleton, CO), or rabbit polyclonal anti-human *CCR5* antibody (NBP2-31374SS; 1:100; Novus Biologicals LLC) for 12 h. The signals

Table 1. Primer sequences for PCR

Gene	Primer sequence	Fragment size (bp)	Ref Seq
<i>CCL2</i>	F: 5'-TCGCTGCAACATGAAGGTCT-3' R: 5'-TGCTGCTGGTGACTCTTCTG-3'	182	NM_174006.2
<i>CCL5</i>	F: 5'-ACCCACGTCCAGGAGTATTC-3' R: 5'-GATGTACTCTCGCACCCAATT-3'	123	NM_175827.2
<i>CCL8</i>	F: 5'-GGGATTCTGTGTCTGCTGCT-3' R: 5'-TTGGGGTCAGCACAGACATC-3'	221	NM_174006.2
<i>CCR1</i>	F: 5'-AACTGACAGCTTCCGACTCAC-3' R: 5'-TCCCACGGGTCAAGGGAAAT-3'	159	NM_001077839.1
<i>CCR5</i>	F: 5'-AGAGGAGCTGAGACATCCGT-3' R: 5'-GCTGCAATTTGCCTCACGTT-3'	142	NM_001011672.2
<i>CD11B</i>	F: 5'-AAGTCTGCCTCCAAGTTCGC-3' R: 5'-GAGCTTCAGGGTCTCGCATT-3'	194	NM_001039957.1
<i>ACTB</i>	F: 5'-AAAGAGATCACTGCCCTGGC-3' R: 5'-CGGACTCATCGTACTCTGC-3'	154	NM_173979.3

were detected using anti-rabbit IgG-biotin conjugate (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:100 for 1 h and then counterstained with hematoxylin. Negative controls were performed using normal rabbit IgG (NBP2-24891; Novus Biologicals LLC) diluted at concentrations equivalent to the primary antibodies.

Statistical analysis

The results are presented as the mean \pm standard error. Differences between the groups for SP, PG, DEX, and TABET were estimated using a Tukey-Kramer test. Cotyledons and caruncle data were analyzed separately. A P-value < 0.05 was considered significant. We performed all analyses using the R statistical package version 3.3.3 for OS X.

Results

Profiling of inflammatory cytokine and receptor expression

We compared the expression levels of inflammatory cytokine and receptor genes in the caruncles at parturition. As shown in Table 2, 34/84 genes exhibited two-fold higher mRNA expression in the SP group than in the DEX group. In contrast, the expression levels of three genes were two-fold lower in the SP group. Among the differentially expressed genes, *CCL2* and *CCL8* exhibited high normalized expression levels and the highest fold change between the SP and DEX groups (Fig. 1). *CCR1*, *CCR2*, and *CCR5*, which are cell surface receptors for *CCL2* and *CCL8*, exhibited two-fold higher mRNA expression in the SP group (Table 2).

mRNA expression of CCLs and receptors in the placentome

The expression of *CCL2* and *CCL8* mRNA in the caruncles was significantly higher in the SP than that in the PG, DEX, and TABET groups (Fig. 2A and C). *CCL5* mRNA expression in the cotyledon was significantly higher in the PG than that in the SP and DEX groups (Fig. 2B). Although there was no significant difference, *CCL5* mRNA expression in the caruncle was lower in the SP and TABET than that in the PG and DEX groups. *CCR1* mRNA expression in

the caruncle was significantly higher in the SP and TABET than that in the PG and DEX groups (Fig. 2D). *CCR1* mRNA expression in the cotyledon was significantly higher in the TABET than that in the SP group. *CCR5* expression in the caruncle was significantly higher in the SP than that in the DEX and TABET groups (Fig. 2E). Although the difference was not statistically significant ($P = 0.094$), *CCR5* mRNA expression in the caruncle of the PG group was lower than that in the SP group. The expression of *CCR2* mRNA in the placentome was confirmed using reverse transcription (RT)-PCR (data not shown). However, *CCR2* was excluded from the quantitative real-time RT-PCR (qRT-PCR) analysis because of insufficient transcripts. The expression of *CD11B* mRNA was analyzed as a marker of macrophages (Fig. 2F). *CD11B* mRNA expression in the caruncle was higher in the SP group compared with that of the induced parturition groups, but a significant difference was only observed between the SP and DEX groups.

Localization of CCL8 and CCRs

Representative results of immunostaining in the TABET group are shown in Fig. 3. The staining characteristics were similar in all the experimental groups. Immunostaining of *CCL8*, *CCR1*, *CCR2*, and *CCR5* was observed in the caruncular epithelial cells adjacent to the chorionic plate, and in the interdigitation area of the cotyledonary villi and caruncle (Fig. 3). *CCR2* was also observed in the cotyledonary epithelial cells. The localization of *CCL2* in the placentome remains uncertain because a specific antibody for immunohistochemistry was unavailable.

Discussion

This study revealed that many inflammatory cytokines and receptors were upregulated in the caruncle tissue at spontaneous parturition compared with parturition induced with dexamethasone. Gene expression levels that were measured using PCR array analysis indicated that 9/13 *CCLs* were upregulated $>$ two-fold in spontaneous parturition, with *CCL2* and *CCL8* being highly expressed. Streyl *et*

Table 2. Transcripts upregulated and downregulated in caruncle tissue at spontaneous parturition compared with induced (DEX group) parturition

Description	Symbol	Ref seq	Fold change
Chemokine (C–C motif) ligand 8	CCL8	NM_174007	20.22
Chemokine (C–C motif) ligand 2	CCL2	NM_174006	16.64
Chemokine (C–X–C motif) ligand 8/Interleukin 8	CXCL8/IL8	NM_173925	12.65
Interleukin 1, alpha	IL1A	NM_174092	10.53
Chemokine (C–X–C motif) ligand 1	CXCL1/GRO1	NM_175700	8.30
Oncostatin M	OSM	NM_175713	8.30
Chemokine (C–C motif) ligand 24	CCL24	NM_001046596	8.03
Interleukin 1, beta	IL1B	NM_174093	7.70
Chemokine (C–C motif) ligand 4	CCL4	NM_001075147	5.66
Chemokine (C–C motif) ligand 17	CCL17	XM_001788943	5.02
Interleukin 27	IL27	NM_001164653	4.85
Chemokine (C–X–C motif) ligand 5	CXCL5	NM_174300	4.78
Chemokine (C–C motif) ligand 3	CCL3	NM_174511	4.20
Chemokine (C–C motif) receptor 8	CCR8	NM_001194962	3.75
Tumor necrosis factor receptor superfamily, member 11b	TNFRSF11B	NM_001098056	3.72
Interleukin 8 receptor, beta	CXCR2	NM_174360	3.64
Chemokine (C–C motif) ligand 22	CCL22	NM_001099162	3.60
Chemokine (C–X–C motif) ligand 10	CXCL10	NM_001046551	3.56
Interleukin 1 receptor antagonist	IL1RN	NM_174357	3.32
Chemokine (C–C motif) receptor 5	CCR5	NM_001011672	3.32
Chemokine (C–C motif) receptor 2	CCR2	NM_001194959	2.87
Colony stimulating factor 2 (granulocyte-macrophage)	CSF2	NM_174027	2.60
Tumor necrosis factor (ligand) superfamily, member 4	TNFSF4	NM_001205715	2.57
Chemokine (C–C motif) ligand 1	CCL1	XM_001253011	2.50
Tumor necrosis factor	TNF	NM_173966	2.47
Tumor necrosis factor (ligand) superfamily, member 11	TNFSF11	NM_001205770	2.42
Secreted phosphoprotein 1	SPP1	NM_174187	2.38
Chemokine (C–C motif) receptor 4	CCR4	NM_001100293	2.31
Tumor necrosis factor (ligand) superfamily, member 10	TNFSF10	XM_002684917	2.14
Chemokine (C–X3–C motif) receptor 1	CX3CR1	NM_001102558	2.13
Chemokine (C–C motif) receptor 1	CCR1	NM_001077839	2.12
Interleukin 2 receptor, gamma	IL2RG	NM_174359	2.10
Chemokine (C–C motif) receptor 3	CCR3	NM_001194960	2.06
Chemokine (C–C motif) ligand 26	CCL26	NM_001205635	2.05
Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1	AIMP1	NM_001035018	–2.08
Interleukin 17B	IL17B	NM_001192045	–3.35
Chemokine (C–C motif) ligand 5	CCL5	NM_175827	–3.46

al. [7] compared placental gene expressions between preterm cesarean section and spontaneous parturition using microarray analysis. Although their study revealed that the expressions of C–X–C motif chemokine ligand 2 (*CXCL2*) and *CXCL5* were significantly upregulated at spontaneous parturition, changes in the expressions of *CCLs* were not reported. Upregulation of the expression of *CXCL5* in spontaneous parturition was consistently detected in this study, but *CXCL2* was not included in the PCR array. Further research concerning the differential expression of inflammatory genes among induced parturition, preterm cesarean section, and spontaneous parturition will lead to a better understanding of the functional maturation of placentomes at parturition.

Because PCR array analysis was performed using only two caruncle

samples from the SP and DEX groups, the expression level of genes was verified using qRT-PCR. In spontaneous parturition, the expressions of *CCL2* and *CCL8* in the caruncles were significantly higher than those in induced parturition. The clinical dose of dexamethasone and TAbET treatment had no influence on the expression of these chemokines. *CCL2* and *CCL8* are key chemokines that regulate the migration and infiltration of monocytes/macrophages. Therefore, it seems likely that the disruption of *CCL2* and *CCL8* expression in induced parturition leads to reduced infiltration of macrophages into the caruncles.

The chemoattractant action of *CCLs* is induced through seven transmembrane-spanning receptors in target cells [17]. *CCL2* binds specifically to *CCR2*. *CCR2*⁺ monocytes are recruited to the site of

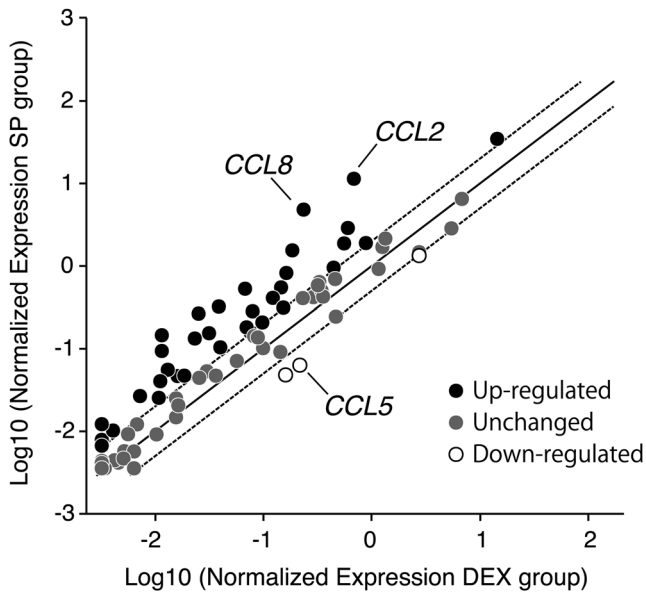


Fig. 1. Comparison of mRNA expression of inflammatory cytokine and receptor genes in caruncle tissue between the SP (spontaneous) and the DEX (induced) groups. Genes that are > 2-fold up- or downregulated in the SP group fall outside the dotted lines. Detailed values for each gene are included in Table 2.

acute inflammation by CCL2 gradients in peripheral tissues. CCL8 is an agonist of multiple CCRs to attract leukocytes. CCL8 has high-affinity to CCR5 and also possesses the capacity to interact with CCR1 and CCR2 [18, 19]. Upregulation of the expression of *CCR1* and *CCR5* in the caruncles at spontaneous parturition was observed in the PCR array and qRT-PCR analyses. Although upregulation of the expression of *CCR2* in spontaneous parturition was detected by PCR array analysis, no verification by qRT-PCR was performed because of quantification limits. These results suggest that the chemotactic activity by the CCL-CCR system is upregulated in placentomes at spontaneous parturition. Consistently, in the caruncles, the expression of *CD11B* that is a surface antigen of monocyte/macrophage, neutrophils, and NK cells was the highest in spontaneous parturition. Among the induced parturition groups, TABET treatment induced an increase in *CCR1* expression in the caruncles compared with the conventional methods for the induction of parturition using PGF2 α or dexamethasone. However, there was no significant difference in *CCR5* expression among the induced groups. The increase of *CCR1* expression in the cotyledon of the TABET group may be caused by contamination with maternal tissue. Placentomes from induced parturition are hard and the separation of firmly attached cotyledons and caruncles is likely to result in cross-contamination. The increase of *CCR1* expression by TABET treatment suggests that the glucocorticoids contribute to the activation of the CCL-CCR system in placentomes at partition.

Although we detected CCL8, CCR1, CCR2, and CCR5 in the caruncular epithelial cells adjacent to the chorionic plate and in the interdigitation area of the cotyledonary villi and caruncle, im-

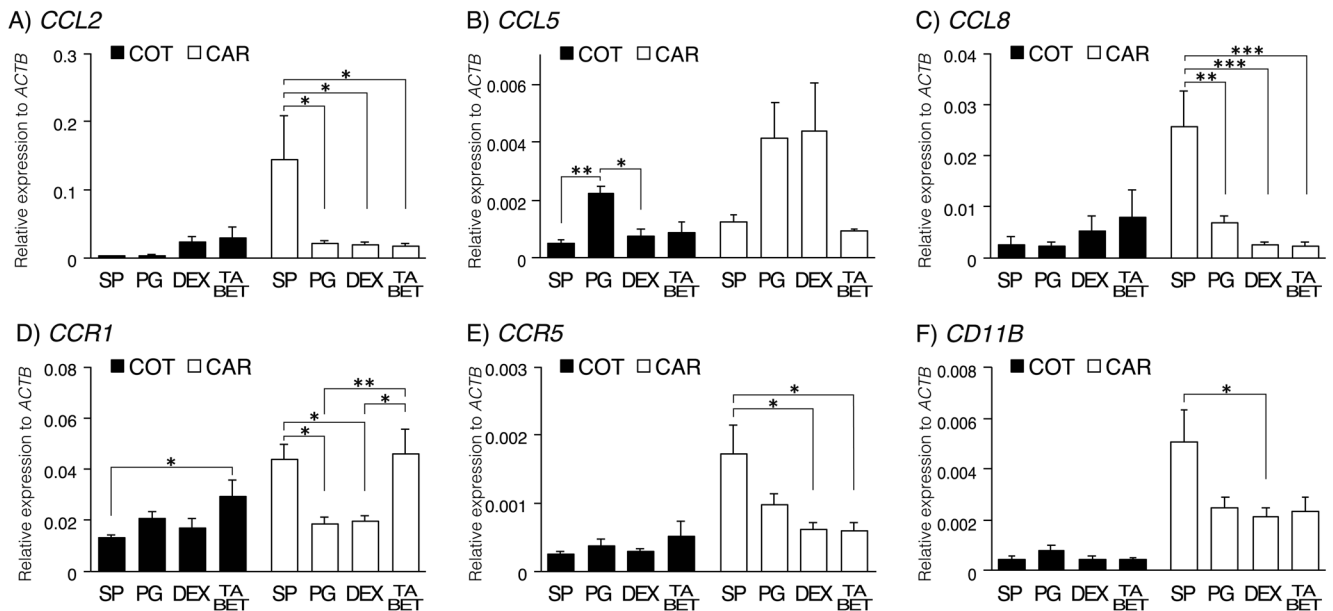


Fig. 2. mRNA expression levels of CCLs and their receptors in the placentome at spontaneous (SP group) and induced (PG, DEX, and TABET groups) parturition. A, *CCL2*; B, *CCL8*; C, *CCR1*; and D, *CCR5* mRNA expression. Statistically significant differences in the relative abundance of *CCL2*, *CCL8*, *CCR1*, and *CCR5* mRNA were analyzed in cotyledon (COT) and caruncle (CAR) tissue. Data are presented as the mean \pm standard error. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

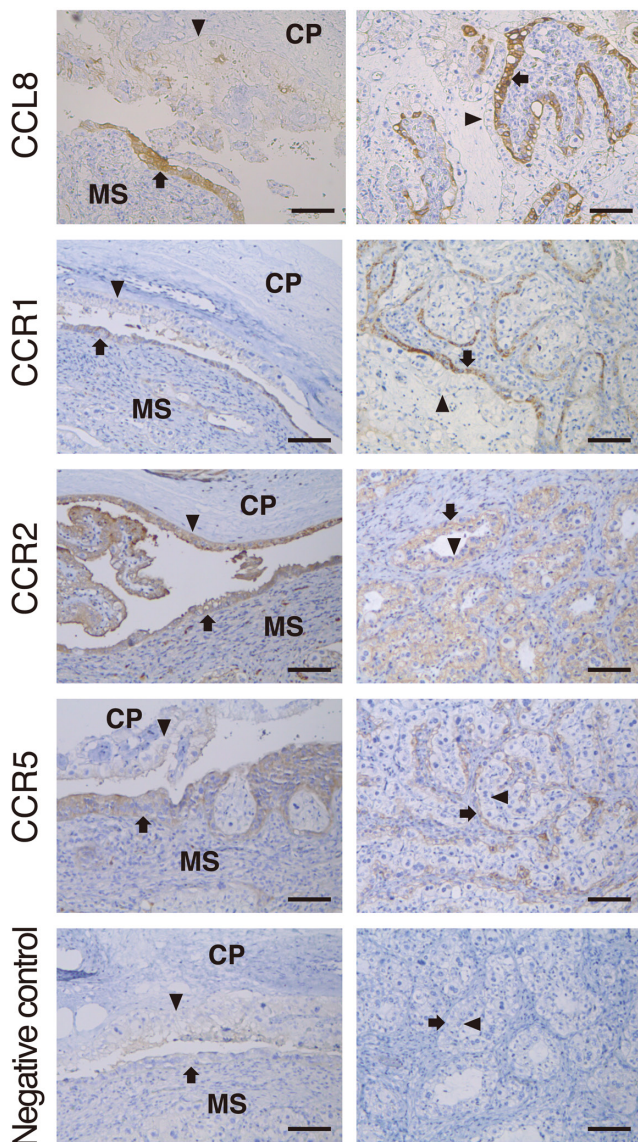


Fig. 3. Localization of CCLs and their receptors in placentomes at parturition. Representative results of the TABET group are shown. Arrows indicate the caruncular epithelial cells, and arrowheads indicate the cotyledonary epithelial cells. The left-hand panels indicate the adjacent area of the chorionic plate and caruncle. The right-hand panels indicate the interdigitation area of the cotyledonary villi and caruncle. CP, chorionic plate; MS, maternal septum. Scale bar = 100 μ m.

munolocalization of CCL2 was not confirmed. Additionally, we observed the localization of CCR2 in the cotyledonary epithelial cells. These results suggest that the increase of CCR expression in spontaneous parturition and TABET treatment may be derived from the caruncular epithelial cells. No immunohistochemical signals of CCLs or CCRs in immune cells were detected in this study. Although the reason is uncertain, this might be attributed to differences in protein expression level or cellular characteristics for tissue fixation by formalin between epithelial cells and immune cells. The current

results suggest that CCLs contribute to functional regulation of epithelial cells of the caruncle and cotyledon.

The role of the CCL–CCR system in epithelial cells in the bovine placentome at parturition is poorly understood. CCR1, CCR2, CCR3, and CXCR3 were found to be expressed in uterine luminal epithelial cells, glandular epithelial cells, or fetal trophoblasts on day 18 of pregnancy [20]. In the study, stimulation with CCL8 in an endometrial tissue culture system decreased *PTGS2* expression. Similarly, CCL2 and CCL8 decreased oxytocin receptor expression. These findings suggest that CCLs contribute to decreasing PG production and preventing luteolysis during early pregnancy in cows. However, luteolysis by PGF2 α is regarded as a prerequisite for the onset of parturition in cows [21]. Additionally, placental PG production and conversion from progesterone to estrogen are augmented as parturition progresses [22, 23]. Therefore, the roles of CCLs in the placentome at parturition may differ from those in the endometrium at early pregnancy, which is a progesterone-dominant environment. Further studies are necessary to investigate the roles of CCLs in placentomes, taking into consideration the estrogen-progesterone ratio.

In spontaneous parturition, there is a gradual rise in plasma cortisol concentrations in the bovine fetus during the week prior to parturition, and the maximum concentration in fetal and maternal plasma is observed immediately before parturition [24]. We conducted TABET treatment to mimic this physiological change in fetal cortisol secretion during spontaneous parturition to improve methods for the induction of parturition. However, a prepartum increase of glucocorticoids that have broad-ranging anti-inflammatory actions is unlikely to be an activating factor of the CCL–CCR system. Although Hamilton *et al.* [25] showed the increase of *CCL2* and *CCL8* expression in human term decidua, *in vitro* betamethasone treatment downregulated chemokine expression in choriodecidual explants. Thus, it appears that glucocorticoids are indirectly involved in the increase in *CCR1* expression in bovine placentomes at parturition. In spontaneous parturition, increased cortisol release from the fetal adrenals stimulates PGE2 and estrogen synthesis in the cotyledons [21, 26]. The inflammatory activities of PGE2 and estrogen might be involved in regulation of the CCL–CCR system in placentomes.

We observed a decrease of *CCL5*, interleukin 17B, and aminoacyl tRNA synthetase complex-interacting multifunctional protein 1 expression in the caruncle of the SP group using the PCR array analysis. Consistently, *CCL5* expression levels using qRT-PCR were lower in the caruncles of the SP and TABET groups, but the differences were not statistically significant. In addition, *CCL5* expression in the cotyledons was significantly lower in the SP and DEX than the PG group. These results suggest that the expression of *CCL5* in placentomes is not upregulated at normal parturition. Although *CCL5* is an agonist of CCR1 [27] that was upregulated in the caruncle of the SP and TABET groups, it might not be a major activating factor of the CCL–CCR system in placentomes at parturition. *CCL5* is chemotactic for a variety of cell types, including T cells, monocytes, dendritic cells, and mast cells [28]. We speculate that the differential expression among CCLs participates in the specificity of leukocyte subsets, which migrate to the placentomes.

In conclusion, this study is the first report to reveal the disruption in CCL and CCR expression in bovine placentomes at induced parturition. Enhanced glucocorticoid exposure for the induction of parturition may

upregulate *CCR1* expression in placentomes, but the treatment does not adequately promote *CCL* expression. However, further studies are needed to elucidate the details of how glucocorticoids facilitate *CCR1* expression. Additionally, immunohistochemistry suggested that the *CCL*–*CCR* system is involved in the functional regulation of maternal and fetal epithelial cells in placentomes at parturition.

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