

—Original Article—

Expression of matrix metalloproteinases in bovine luteal cells induced by prostaglandin F2 α , interferon γ and tumor necrosis factor α

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Abstract. We recently demonstrated that luteal cells flow out from the ovary via lymphatic vessels during luteolysis. However, the regulatory mechanisms of the outflow of luteal cells are not known. Matrix metalloproteinases (MMPs) can degrade the extracellular matrix and basal membrane, and tissue inhibitors of matrix metalloproteinases (TIMPs) inhibit the activity of MMPs. To test the hypothesis that MMP expression in luteal cells is regulated by luteolytic factors, we investigated the effects of prostaglandin F2 α (PGF), interferon γ (IFNG) and tumor necrosis factor α (TNF) on the mRNA expression of MMPs and TIMPs in cultured luteal cells. Luteal cells obtained from the CL at the mid-luteal stage (days 8–12 after ovulation) were cultured with PGF (0.01, 0.1, 1 μ M), IFNG (0.05, 0.5, 5 nM) and TNF (0.05, 0.5, 0.5 nM) alone or in combination for 24 h. PGF and IFNG significantly increased the expression of *MMP-1* mRNA. In addition, 1 μ M PGF in combination with 5 nM IFNG stimulated *MMP-1* and *MMP-9* mRNA expression significantly more than either treatment alone. In contrast, IFNG significantly decreased the level of *MMP-14* mRNA. The mRNA expression of TIMP-1, which preferentially inhibits MMP-1, was suppressed by 5 nM IFNG. One μ M PGF and 5 nM IFNG suppressed *TIMP-2* mRNA expression. These results suggest a new role of MMPs: luteal MMPs stimulated by PGF and IFNG break down the extracellular matrix surrounding luteal cells, which accelerates detachment from the CL during luteolysis, providing an essential prerequisite for outflow of luteal cells from the CL to lymphatic vessels.

Key words: Luteal cells, Lymphatic vessel, Matrix metalloproteinases, Structural luteolysis
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In mammals, the corpus luteum (CL) is an essential endocrine gland for the establishment and maintenance of pregnancy. If pregnancy is not established, the CL loses its capacity to produce progesterone (functional luteolysis), decreases in volume and disappears from the ovary (structural luteolysis) [1]. During structural luteolysis, luteal cells are eliminated by apoptosis and phagocytosis by macrophages [2–4]. We recently found that during structural luteolysis luteal cells flow out of the CL via lymphatic vessels [5], although the mechanisms regulating the outflow are unclear.

Matrix metalloproteinases (MMPs) are a family of structurally-related proteins that degrade the extracellular matrix (ECM) and basal membrane [6]. The ECM, besides providing structural support between cells, has been implicated in modulating cell processes, such as differentiation, migration, gene expression and apoptosis [6, 7]. In cancer metastasis, MMPs are required for cancer cells to degrade the ECM and intravasation at nearby blood vessels or lymphatic vessels [8–10]. In luteal cells, ECM degradation by MMPs induces changes in the mRNA expression of luteinizing hormone receptor, low density lipoprotein receptor and 3 β -hydroxy steroid dehydrogenase,

which are factors required to produce steroid hormones in luteal cells [11]. Hence, although these changes mediated by MMPs have been suggested to be involved in the loss of capacity of luteal tissue to synthesize progesterone during luteolysis, the roles of MMPs in structural luteolysis are unclear.

Luteal cells that flow out to the lymphatic vessels during structural luteolysis must be detached from the CL tissue. In the bovine CL tissue, MMP-1, 2, 9 and 14 increase drastically during luteolysis induced by prostaglandin F2 α (PGF) injection [12]. The authors implied that the main source of MMP-1 and MMP-9 in the CL tissue is immune cells invading the CL during luteolysis [12]. However, since luteal cells have been immunohistochemically shown to be the source of MMPs in the CL during luteolysis [12], MMPs secreted by luteal cells may break down the ECM surrounding luteal cells in the same way that MMPs secreted by cancer cells break down the ECM in metastasis. The aim of the present study was to validate possibility of the above idea. Since outflow of luteal cells occurs during luteolysis [5], we hypothesized that luteolytic factors increase MMP expression in luteal cells. To test this hypothesis, we investigated the effects of three factors that are known to induce luteolysis PGF, interferon γ (IFNG) and tumor necrosis factor α (TNF) [4] on the mRNA expressions of four MMPs involved in degrading collagen and the basal membrane in cultured luteal cells. Since tissue inhibitors of matrix metalloproteinases (TIMPs) are known to inhibit MMP activity [7], in the present study, we also investigated the effects of PGF, IFNG and TNF on mRNA expression of TIMPs in cultured luteal cells.

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Materials and Methods

Collection of bovine corpora lutea

In this study, we did not perform any animal experiments. The ovaries were collected from nonpregnant Holstein cows at a local abattoir (Tsuyama Meat Center) in accordance with protocols approved by the local institutional animal care committee. All the samples and data analyzed in the present study were obtained with the permission of the above center.

Ovaries with CLs at the mid-luteal stage were collected at a local abattoir within 10–20 min after exsanguination. The luteal stage was classified by macroscopic observation of the ovary and uterus [13, 14]. For cell culture experiments, ovaries with a mid-luteal CL (days 8–12 of the estrous cycle) were submerged in ice-cold physiological saline and transported to the laboratory.

Cell isolation

Luteal cells were obtained as described previously [15]. Briefly, mid-luteal CL tissue from four cows was enzymatically dissociated, and the resulting cell suspensions were centrifuged (5 min at 50 × g) three times to separate the luteal cells (pellet) from endothelial cells and other types of luteal non-steroidogenic cells (supernatant). The dissociated luteal cells were suspended in culture medium (Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham ([D/F], 1:1, v/v; no. D8900; Sigma-Aldrich, St. Louis, MO, USA) containing 5% calf serum (no. 16170-078; Life Technologies, Grand Island, NY, USA) and 20 µg/ml gentamicin (no. 15750-060; Life Technologies) and cultured with 5% CO₂ in air. Cell viability was greater than 80%, as assessed by trypan blue exclusion. The cells in the cell suspension after centrifugation consisted of about 70% small luteal cells, 20% large luteal cells, 10% endothelial cells or fibrocytes, and no erythrocytes.

Cell culture

The dispersed luteal cells were seeded at 2.0×10^5 viable cells per milliliter in 24-well culture dishes (no. 662160; Greiner Bio-One, Frickenhausen, Germany) to evaluate mRNA expression. The cultures were kept in a humidified atmosphere of 5% CO₂ in air at 38 C in an N₂-O₂-CO₂-regulated incubator (no. BNP-110; ESPEC, Osaka, Japan). After 24 h of culture, the culture medium was replaced with fresh medium containing 0.1% BSA (no. 15408; Roche Diagnostics, Mannheim, Germany); 5 ng/ml sodium selenite (no. S5261; Sigma-Aldrich); 5 µg/ml holo-transferrin (no. T3400; Sigma-Aldrich); and 0.01, 0.1 or 1 µM PGF (no. 16010; Cayman chemical, Ann Arbor, MI, USA), 0.05, 0.5 or 5 nM TNF (Dainippon Pharmaceutical, Osaka, Japan) or 0.05, 0.5 or 5 nM IFNG (Kindly donated by Dr S Inumaru, NIAH, Ibaraki, Japan) for 24 h. The concentrations of PGF, IFNG and TNF for combination treatment were determined based on those that had the most effect on the expression of *MMP* mRNA in single treatments with PGF, IFNG and TNF. The luteal cells were also exposed to 1 µM PGF in combination with 5 nM IFNG or 5 nM TNF, 5 nM IFNG in combination with 5 nM TNF, 1 µM PGF in combination with 5 nM IFNG and 5 nM TNF for 24 h.

RNA isolation and cDNA synthesis

Total RNA was extracted from CL tissues and cells using TRIzol reagent (no. 15596-026; Invitrogen, Carlsbad, CA, USA) according to the manufacturer's directions. One microgram of each total RNA was reverse transcribed using a ThermoScript RT-PCR System (no. 11146-016; Invitrogen).

Quantitative PCR (Real-Time PCR)

Ten percent of the reaction mixture was used in each PCR using specific primers for MMPs (Table 1). The expression of mRNA was quantified using iQ SYBR Green Supermix (no. 170-8880;

Table 1. Primers for MMPs and TIMPs used in quantitative RT-PCR

Target	Primer	Sequence (5'–3')	Accession no.	Product (bp)
MMP-1	Forward	AGGTGCAGGTATCGGAGGAG	NM174112	275
	Reverse	CACACACTTCTGGGGTTTGG		
MMP-2	Forward	GGCATCTCTCAGATCCGTGG	NM174745.2	155
	Reverse	TGTGGGTCTTCGTACACAGC		
MMP-9	Forward	CTAGTTGGGATCCGGCAGAC	NM174744	128
	Reverse	CTAGTTGGGATCCGGCAGAC		
MMP-14	Forward	GAGTGACAGGCAAGGCTGAT	NM174390.2	200
	Reverse	AAATGTGGCATACTCGCCCA		
TMP-1	Forward	CATCTACACCCTGCCATG	NM174471.3	231
	Reverse	CAGGGGATGGATGAGCAG		
TMP-2	Forward	GGGTCTCGCTGGACATTG	NM174472.4	256
	Reverse	TTGATGTTCTTCTCCGTGACC		
TMP-3	Forward	ACTTTGGAGACTCGAGCAGC	NM174473.4	231
	Reverse	GGTGTAGACCATCGTGCCAA		
TMP-4	Forward	CAGCCCTTTATCCCTGCCTC	NM001045871.2	220
	Reverse	TACTGTCACCCACGTTCTGC		
ACTB	Forward	CAGCAAGCAGGAGTACGATG	AY141970	137
	Reverse	AGCCATGCCAATCTCATCTC		

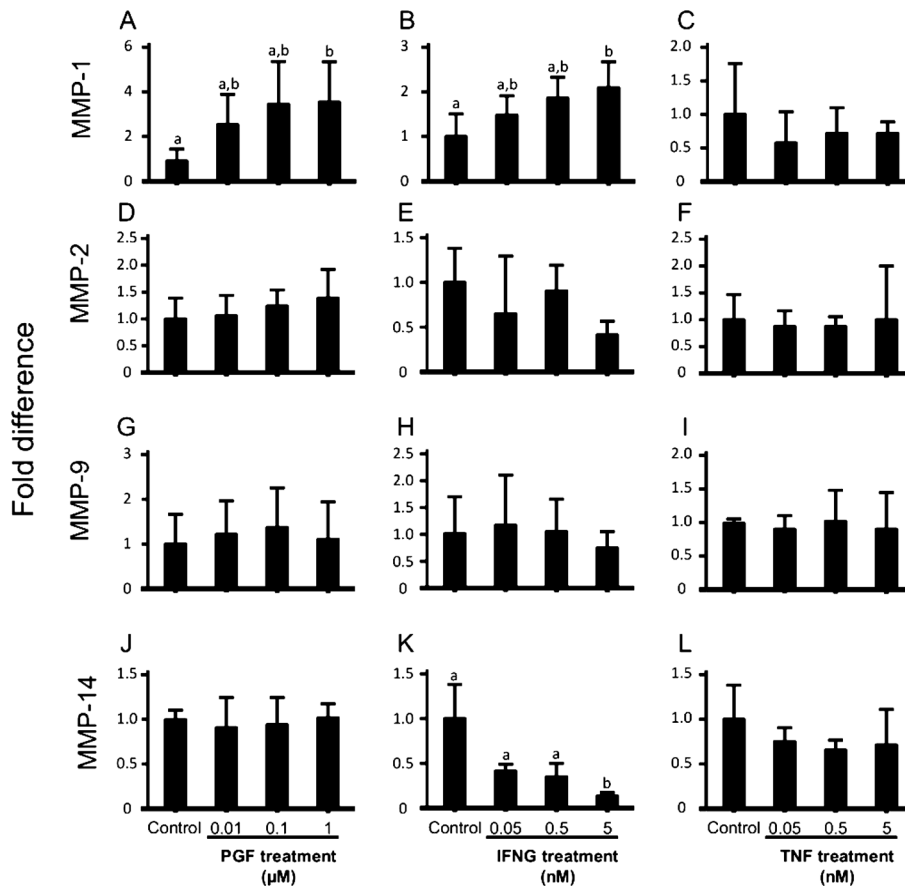


Fig. 1. Regulation of *MMP* mRNA expression in cultured bovine luteal cells following single treatments with different concentrations of PGF, IFNG and TNF for 24 h. (A–C) *MMP-1*. (D–F) *MMP-2*. (G–I) *MMP-9*. (J–L) *MMP-14*. Different superscript letters indicate significant differences ($P < 0.05$) compared with other columns as assessed by the Kruskal-Wallis test followed by Dunn's multiple comparisons test (A and K) or one-way ANOVA followed by Tukey's multiple comparisons test (B–J and L). Data are the mean \pm SEM of 4 experiments.

Bio-Rad Laboratories, Hercules, CA, USA) starting with 2 ng of reverse-transcribed total RNA. The PCR conditions were 95 C for 15 min, followed by 45 cycles of 94 C for 15 sec, 55 C for 30 sec and 72 C for 30 sec. Use of a QuantiTect™ SYBR Green PCR system at elevated temperatures resulted in reliable and sensitive quantification of the RT-PCR products with high linearity. The relative level of expression of each mRNA was analyzed by the $2^{-\Delta\Delta CT}$ method [16, 17].

Statistical analyses

All experimental data are shown as the mean \pm SEM. The statistical significances of differences in the expression of *MMP* mRNA were assessed by one-way ANOVA followed by Tukey's multiple comparisons test or the Kruskal-Wallis test followed by Dunn's multiple comparisons test and by the Student's *t*-test or Mann-Whitney test based on a test for homogeneity of variance. The statistical analyses performed for each experiment are described the figure legends.

Results

Effects of a single treatment with PGF, IFNG or TNF on *MMP* mRNA expression

MMP-1 mRNA expression in cultured luteal cells was stimulated by PGF and IFNG (Fig. 1A and B). The levels of *MMP-2* and *MMP-9* mRNA expression were not affected by PGF, IFNG and TNF (Fig. 1D–I). IFNG suppressed *MMP-14* mRNA expression (Fig. 1K).

Effects of combination treatment with PGF, IFNG and/or TNF on *MMP* and *TIMP* mRNA expression

Based on the above results, 1 μ M PGF, 5 nM IFNG and 5 nM TNF were used in this experiment. *MMP-1* mRNA expression was stimulated more by PGF in combination with IFNG than by each treatment alone (Fig. 2A). IFNG in combination with or without PGF and TNF decreased *MMP-2* mRNA expression (Figs. 3A and C). PGF in combination with IFNG increased *MMP-9* mRNA expression (Fig. 4A), whereas PGF in combination with IFNG and TNF did not affect *MMP-9* mRNA expression (Fig. 4D). IFNG in combination with or without PGF and TNF reduced *MMP-14* mRNA expression

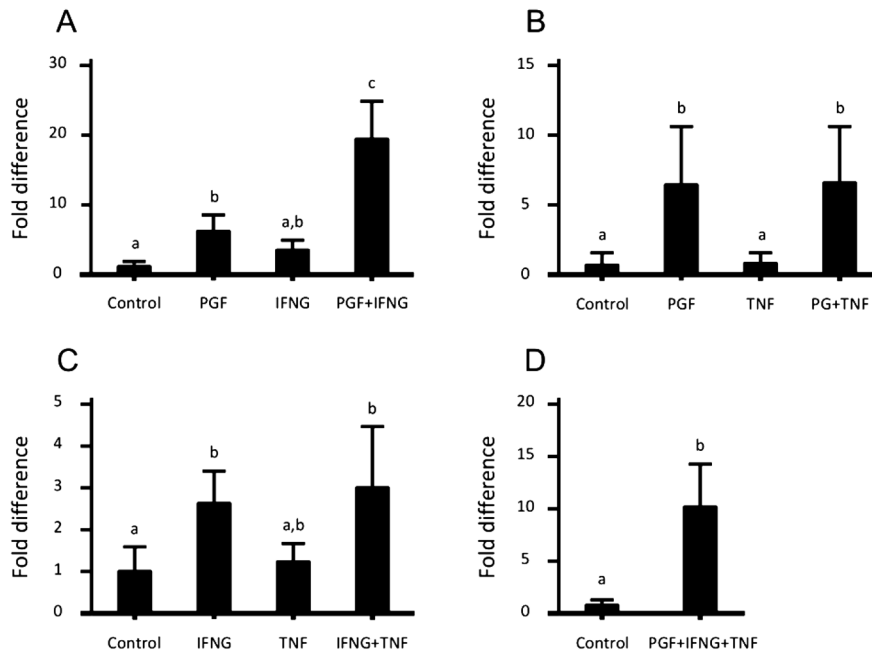
MMP-1 mRNA expression

Fig. 2. Regulation of *MMP-1* mRNA expression in cultured bovine luteal cells following combination treatment with 1 μ M PGF, 5 nM IFNG and 5 nM TNF for 24 h. (A) PGF in combination with IFNG significantly stimulated the expression of *MMP-1* mRNA in luteal cells compared with a single treatment with PGF or IFNG. (B, C, D) The other results were the same as those induced by single-dose treatments. Different superscript letters indicate significant differences ($P < 0.05$) compared with other columns as assessed by the Kruskal-Wallis test followed by Dunn's multiple comparisons test (A, B, C) and the Mann-Whitney test (D). Data are the mean \pm SEM of 4 experiments.

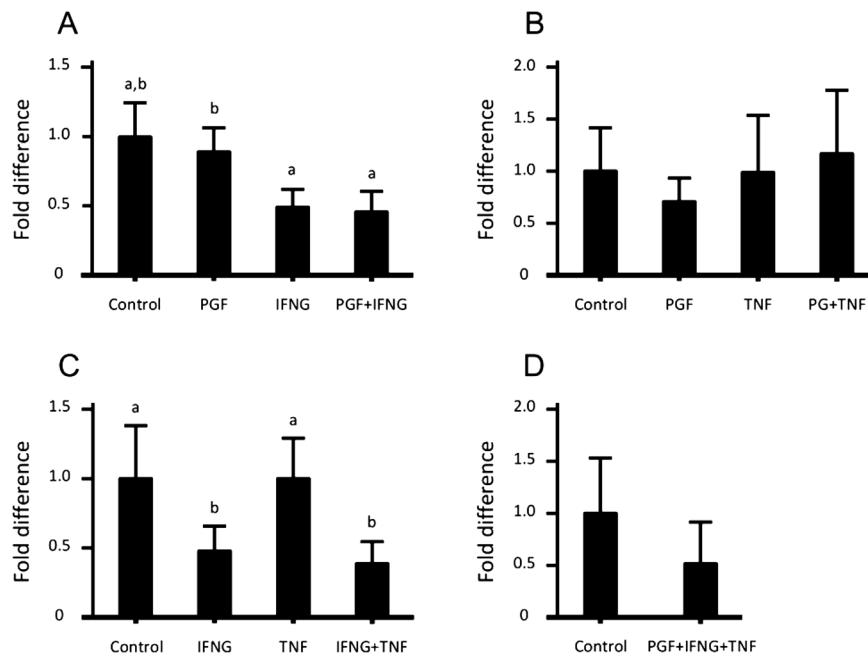
MMP-2 mRNA expression

Fig. 3. Regulation of *MMP-2* mRNA expression in cultured bovine luteal cells following combination treatment with 1 μ M PGF, 5 nM IFNG and 5 nM TNF for 24 h. (A, B, C, D) All changes in *MMP-2* mRNA expression induced by combination treatments were the same as those induced with a single-dose treatment. Different superscript letters indicate significant differences ($P < 0.05$) compared with other columns as assessed by one-way ANOVA followed by Tukey's multiple comparisons test (A, B, C) and the Student's *t*-test (D). Data are the mean \pm SEM of 4 experiments.

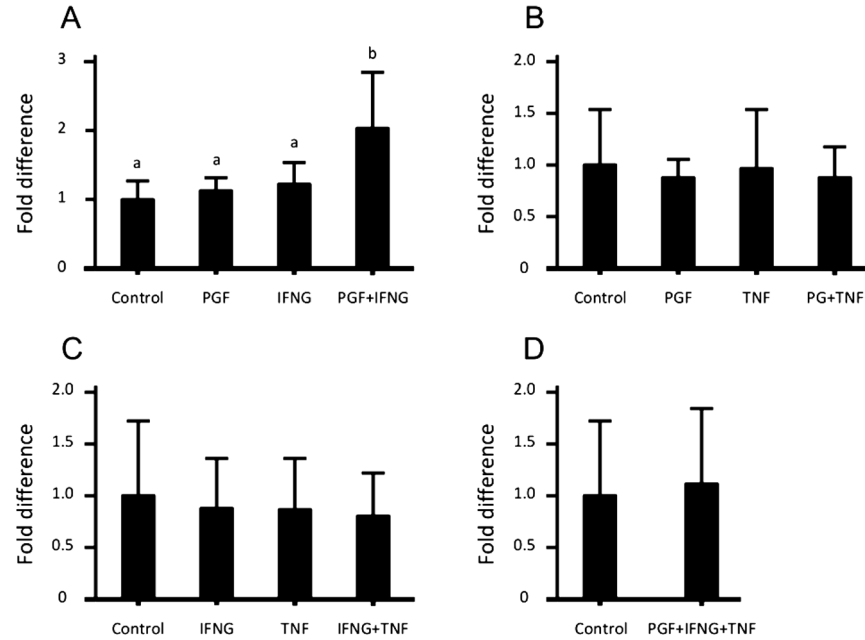
MMP-9 mRNA expression

Fig. 4. Regulation of *MMP-9* mRNA expression in cultured bovine luteal cells following combination treatment with 1 μ M PGF, 5 nM IFNG and 5 nM TNF for 24 h. (A) PGF in combination with IFNG significantly stimulated *MMP-9* mRNA expression in luteal cells compared with that in control and single treatments of PGF or IFNG. (B, C) PGF in combination with TNF and IFNG in combination with TNF did not affect the *MMP-9* mRNA expression. (D) PGF in combination with IFNG and TNF did not stimulate *MMP-9* mRNA expression. Different superscript letters indicate significant differences ($P < 0.05$) compared with other columns as assessed by one-way ANOVA followed by Tukey's multiple comparisons test (A, B, C) and the Student's *t*-test (D). Data are the mean \pm SEM of 4 experiments.

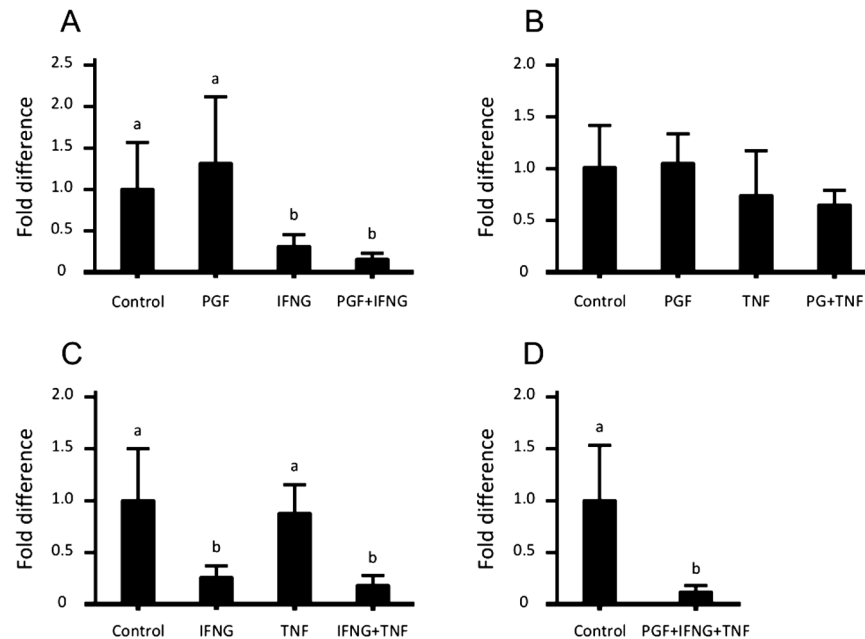
MMP-14 mRNA expression

Fig. 5. Regulation of *MMP-14* mRNA expression in cultured bovine luteal cells following combination treatment with 1 μ M PGF, 5 nM IFNG and 5 nM TNF for 24 h. (A) PGF in combination with IFNG suppressed the expression of *MMP-14* mRNA. (B) PGF in combination TNF did not affect *MMP-14* mRNA expression. (C) IFNG in combination with TNF suppressed the expression of *MMP-14* mRNA. (D) PGF in combination with IFNG and TNF suppressed *MMP-14* mRNA expression. Different superscript letters indicate significant differences ($P < 0.05$) compared with other columns as assessed by the Kruskal-Wallis test followed by Dunn's multiple comparisons test (A, C) or one-way ANOVA followed by Tukey's multiple comparisons test (B) and the Mann-Whitney test (D). Data are the mean \pm SEM of 4 experiments.

(Figs. 5A, C and D).

Since luteolytic factors affected *MMP* mRNA expression, we investigated the effect of luteolytic factors on *TIMP* mRNA expression in luteal cells. IFNG suppressed *TIMP-1* mRNA expression (Figs. 6A and C), while IFNG in combination with PGF and TNF did not affect *TIMP-1* mRNA expression. PGF and IFNG suppressed *TIMP-2* mRNA expression (Figs. 7A, B and C), while PGF in combination with IFNG and TNF did not affect *TIMP-2* mRNA expression (Fig. 7D). PGF, IFNG and TNF did not affect *TIMP-3* or *TIMP-4* mRNA expression (Figs. 8 and 9).

Discussion

The preceding results confirmed that *MMP-1*, *-2*, *-9* and *14* mRNA are expressed in cultured bovine luteal cells. The expression of *MMP-1*, *2*, *9* and *14* mRNA increased during luteolysis [12]. These MMPs were previously found to be involved in functional luteolysis [11, 18, 19]. PGF secreted by the uterus is a trigger that induces luteolysis in the cow. IFNG and TNF, which are secreted by macrophages that invade the CL, are also involved in inducing luteolysis [4, 20–22]. Until now, immune cells infiltrating the regressing CL were considered the main source of *MMPs* during luteolysis [12]. In the present study, PGF and IFNG stimulated the expression of *MMP-1* and *MMP-9* mRNA in cultured bovine luteal cells, which suggests that luteal cells are also important sources of *MMPs*, especially *MMP-1*, during luteolysis.

During structural luteolysis, luteal cells flow out of the CL via lymphatic vessels drained from the CL [5]. Luteal cells have to be detached from the CL tissue to flow out to the lymphatic vessels. The CL tissue is composed of collagens, which are the main components of the ECM, and the predominant luteal collagen is collagen type I [23, 24]. *MMP-1*, secreted from cells, and *MMP-14*, anchored to plasma membrane, have the ability to cleave collagen types I, II and III [25, 26]. In the present study, PGF in combination with IFNG strongly stimulated *MMP-1* mRNA expression in cultured luteal cells, which suggests that *MMP-1* secreted by luteal cells degrades the ECM surrounding the luteal cells and promotes the detachment of luteal cells from the CL tissue during luteolysis.

Since *MMP-14* is expressed on the cell membrane of large luteal cells [12], *MMP-14* may assist the outflow of luteal cells by digesting the pericellular ECM. We expected that luteolytic factors such as PGF, IFNG and TNF would upregulate *MMP-14* mRNA expression in luteal cells. However, *MMP-14* mRNA expression was not changed by any dose of these factors. On the other hand, IFNG strongly suppressed the expression of *MMP-14* mRNA in luteal cells. *MMP-14* mRNA expression in the CL tissue has been reported to increase during luteolysis [12]. Our results suggest that luteal cells are not involved in the increase of *MMP-14* mRNA expression during luteolysis. Since macrophages express *MMP-14* [27, 28], the increase of *MMP-14* mRNA expression in the CL tissue during luteolysis may be due to the increase of macrophages invading the CL [20]. In cancer cell invasion, *MMP-14* is localized at the front of migrating cells and degrades the ECM [29, 30]. Therefore, we expected that luteal cells would be detached by *MMP-14* and enter the luteal lymphatic vessels. However, the present results contradict this hypothesis. Another explanation for the movement of luteal cell

could be that luteal cells detached from the CL tissue by *MMP-1* are transported by interstitial fluids that come from blood plasma and flow into the lymphatic vessels. Further study is needed to clarify how luteal cells move in the CL tissue to the lymphatic vessels.

The basal membrane, which provides structural support to the walls of blood vessels, is degraded by *MMP-2* and *MMP-9* [26]. The basal membrane has been found surrounding the lymphatic vessels that differentiate from veins [31–33]. Immune cells such as lymphocytes and macrophages invading the CL migrate into the lymphatic node from the CL during luteolysis [34]. Therefore, degradation of the basal membrane when immune cells enter the lymphatic vessels is a necessary process. In this study, IFNG suppressed *MMP-2* mRNA expression, while PGF in combination with IFNG induced *MMP-9* mRNA expression. Interestingly, PGF in combination with IFNG and TNF eliminated the effect of PGF in combination with IFNG on the expression of *MMP-9* mRNA. *MMP-2* and *MMP-9* secreted by luteal cells may support immune cells to enter the lymphatic vessels by degrading the basal membrane, and their expressions in luteal cells may be regulated by macrophages, which have the capacity to secrete IFNG and TNF.

The present study showed that PGF and IFNG reduced *TIMP-1* and *TIMP-2* mRNA expression in cultured luteal cells. TIMPs form a 1:1 complex with MMPs and inhibit their proteolytic activity [35]. *TIMP-1* preferentially inhibits *MMP-1*, while *TIMP-2* is a more effective inhibitor of *MMP-2* [36]. *TIMP-1* mRNA expression in CL tissue decreases during luteolysis [12, 18]. In the present study, although PGF in combination with IFNG strongly stimulated *MMP-1* mRNA expression, the same treatment suppressed *TIMP-1* mRNA expression. These findings suggest that *MMP-1* breaks down the ECM in the bovine CL during luteolysis. *TIMP-2* mRNA expression in the bovine and ovine CL tissue decreases during luteolysis [12, 19]. We showed that PGF and IFNG suppressed *TIMP-2* mRNA expression in cultured bovine luteal cells. These results suggest that the decrease of *TIMP-2* mRNA expression during luteolysis is caused by luteolytic factors such as PGF and IFNG. During luteolysis, T lymphocytes invade the CL [20, 37]. T lymphocytes secrete *MMP-2* and *MMP-9*, and pass through the basal membrane into extravascular tissue sites [27]. Since IFNG suppresses *TIMP-2* expression in luteal cells, IFNG secreted by macrophages appears to conveniently enable T lymphocytes to break down basal membrane with *MMP-2* during luteolysis. PGF released by the uterus causes luteolysis in ruminants, and the number of leukocytes in the CL (e.g., T lymphocytes, macrophages) increases at the time of luteolysis [20]. Since leukocytes are known to produce a variety of cytokines including IFNG and TNF [38], the uterus and macrophages seem to be sources of PGF and IFNG. In addition, we described an auto-amplification system for PGF in the bovine CL [39]. Luteal PGF may also play a role in regulating *MMP-1* and *TIMP* expression during luteolysis.

MMPs are known to play roles in cell proliferation, migration, differentiation and apoptosis [6]. In the CL, a reduction of P4 secretion and apoptotic cell death of luteal cells are associated with the loss of ECM integrity [40]. Endothelial cells in the ovine CL detach from the ECM [41] and undergo apoptosis during luteolysis [42]. In cattle, capillaries in the CL have been reported to disappear during luteolysis [43]. It is possible that *MMPs* induce apoptosis of bovine capillary cells by degrading the ECM. During luteolysis,

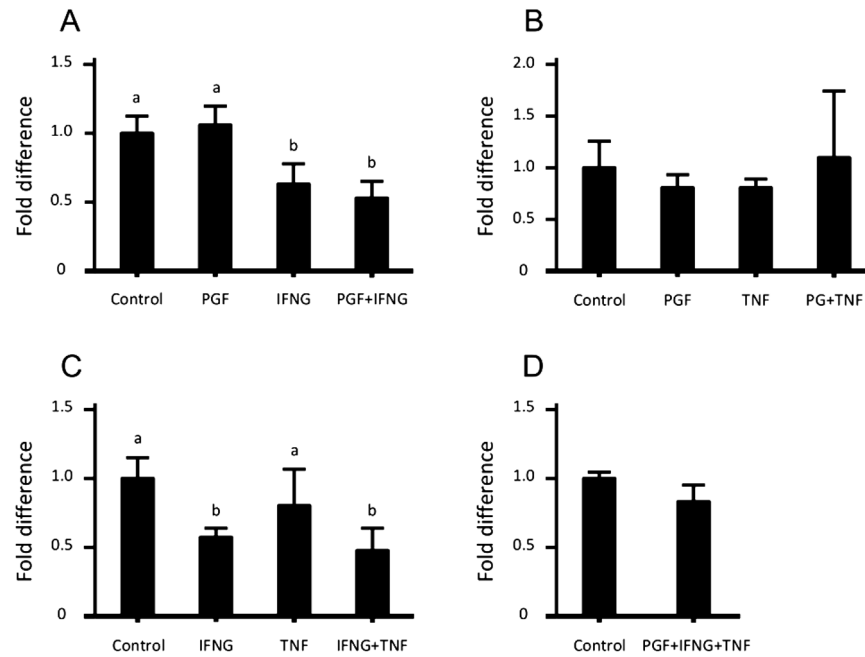
TIMP-1 mRNA expression

Fig. 6. Regulation of *TIMP-1* mRNA expression in cultured bovine luteal cells following combination treatment with 1 μ M PGF, 5 nM IFNG and 5 nM TNF for 24 h. (A, C) IFNG suppressed the expression of *TIMP-1* mRNA. (B, C) PGF and TNF did not affect *TIMP-1* mRNA expression. (D) IFNG in combination with PGF and TNF did not affect the expression of *TIMP-1* mRNA. Different superscript letters indicate significant differences ($P < 0.05$) compared with other columns as assessed by one-way ANOVA followed by Tukey's multiple comparisons test (A, C) or the Kruskal-Wallis test followed by Dunn's multiple comparisons test (B) and the Mann-Whitney test (D). Data are the mean \pm SEM of 4 experiments.

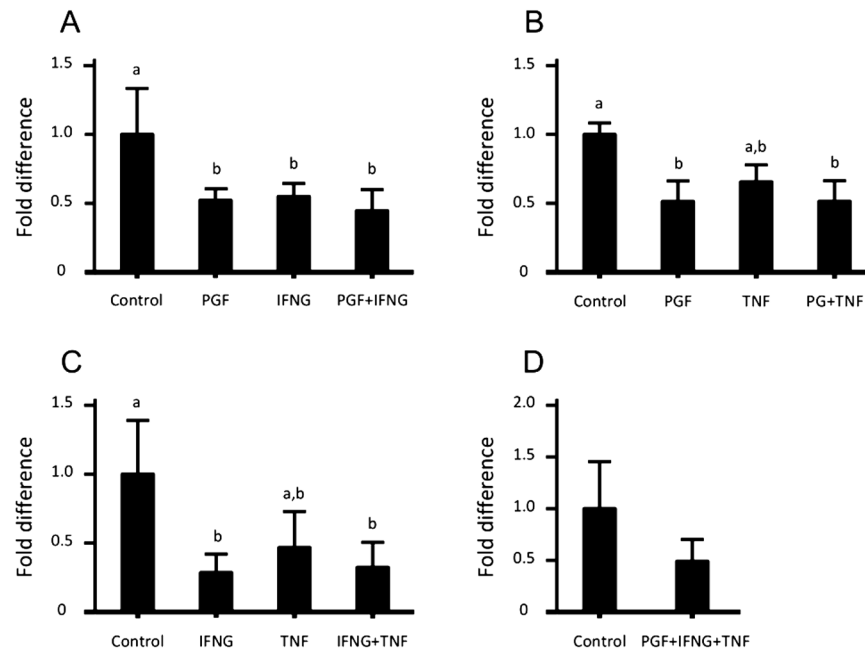
TIMP-2 mRNA expression

Fig. 7. Regulation of *TIMP-2* mRNA expression in cultured bovine luteal cells following combination treatment with 1 μ M PGF, 5 nM IFNG and 5 nM TNF for 24 h. (A, C) PGF and IFNG suppressed the expression of *TIMP-2* mRNA. (B, C) TNF did not affect *TIMP-2* mRNA expression. (D) PGF in combination with IFNG and TNF did not affect the expression of *TIMP-2* mRNA. Different superscript letters indicate significant differences ($P < 0.05$) compared with other columns as assessed by one-way ANOVA followed by Tukey's multiple comparisons test (A, B, C) and the Student's *t*-test (D). Data are the mean \pm SEM of 4 experiments.

TIMP-3 mRNA expression

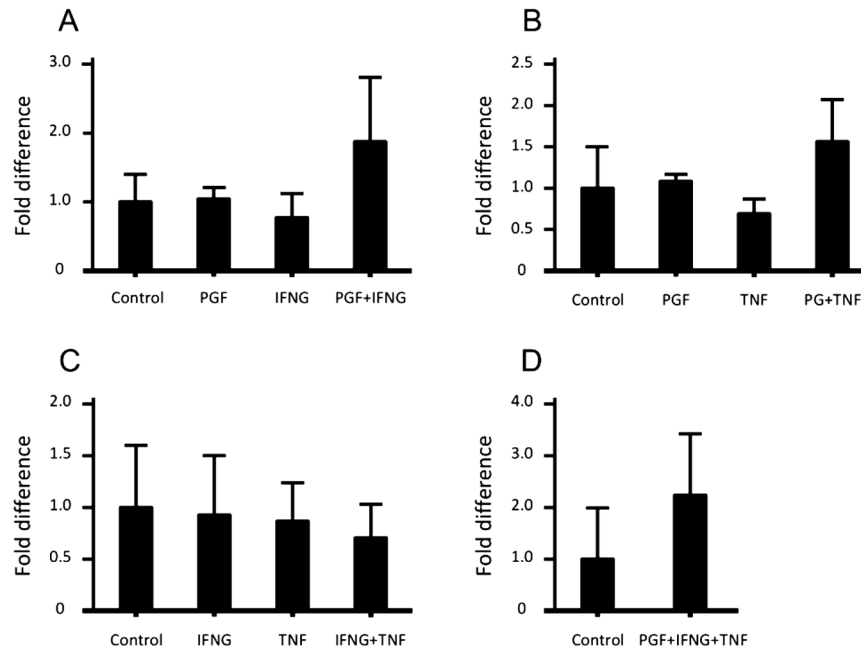


Fig. 8. Regulation of *TIMP-3* mRNA expression in cultured bovine luteal cells following combination treatment with 1 μ M PGF, 5 nM IFNG and 5 nM TNF for 24 h. (A, B, C, D) PGF, IFNG and TNF did not affect the expression of *TIMP-3* mRNA. Data are the mean \pm SEM of 4 experiments.

TIMP-4 mRNA expression

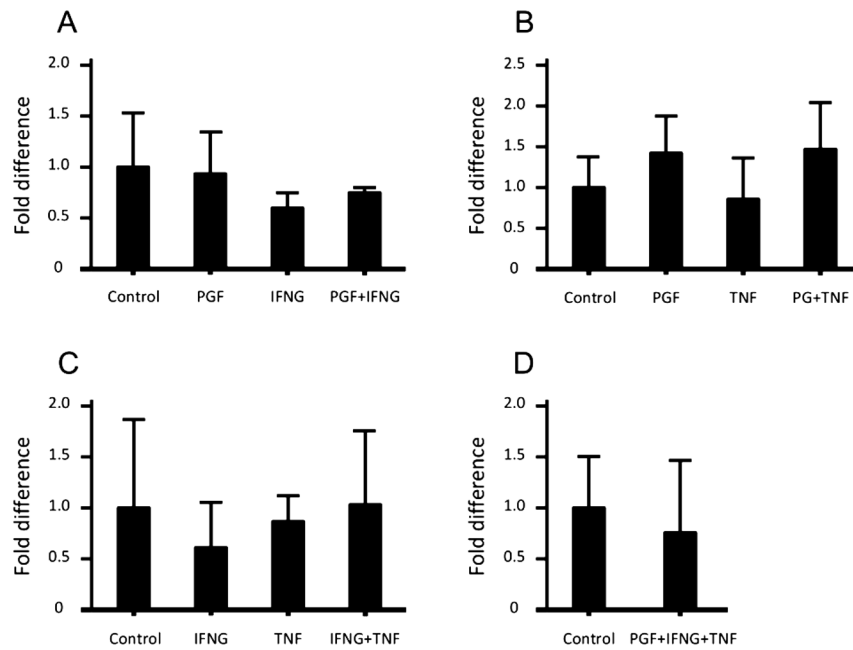


Fig. 9. Regulation of *TIMP-4* mRNA expression in cultured bovine luteal cells following combination treatment with 1 μ M PGF, 5 nM IFNG and 5 nM TNF for 24 h. (A, B, C, D) PGF, IFNG and TNF did not affect the expression of *TIMP-4* mRNA. Data are the mean \pm SEM of 4 experiments.

IFNG and TNF induce apoptosis of luteal cells [4, 44]. MMP-1 and MMP-9 have been implicated in the conversion of TNF and Fas ligand, which is a protein homologous with TNF, to active, soluble forms [45, 46]. Therefore, MMP-1 secreted by luteal cells may locally activate TNF, and regulate apoptosis of luteal cells. Interestingly, most of the luteal cells found in the lymphatic fluid were small luteal cells that were alive [5]. MMP-1 produced by luteal cells may induce apoptosis of large luteal cells by activation of TNF and the outflow of small luteal cells by degradation of the ECM surrounding luteal cells.

During luteolysis induced by PGF administration on day 10 post ovulation *in vivo*, the volume of the CL decreases to less than half of its original size in 24 h [47]. Recently, we demonstrated the involvement of lymphatic vessels in this rapid shrinking of the CL [5], and the present findings seem to support this idea. PGF administration seems to upregulate the synthesis of MMP-1 in luteal cells. MMP-1 may degrade the ECM surrounding luteal cells, resulting in detachment of the luteal cells from the CL tissue and their outflow through the lymphatic vessels.

In summary, our results show that luteolytic factors such as PGF and IFNG regulate the expression of *MMPs* and *TIMPs* mRNA in cultured luteal cells. PGF and IFNG during luteolysis may be involved in inducing detachment of luteal cells from the CL tissue by increasing the production of MMPs in luteal cells, providing an essential prerequisite for outflow of luteal cells from the CL tissue to lymphatic vessels.

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