Abstract. Prostaglandin F$_2\alpha$ (PGF$_2\alpha$) induces luteolysis in cows and causes infiltration of immune cells, which resembles inflammatory immune response. Since the general immune response is mediated by the lymphatic system, we hypothesized that luteolysis is associated with generation of an immune response that involves lymphatic vessels in the bovine corpus luteum (CL). The CL was obtained from Holstein cows at the mid-luteal phase (days 10–12, ovulation = day 0) by ovariectomy that luteolysis is associated with generation of an immune response that involves lymphatic vessels in the bovine corpus luteum (CL). The CL was obtained from Holstein cows at the mid-luteal phase (days 10–12, ovulation = day 0) by ovariectomy that luteolysis is associated with generation of an immune response that involves lymphatic vessels in the bovine corpus luteum (CL). The CL was obtained from Holstein cows at the mid-luteal phase (days 10–12, ovulation = day 0) by ovariectomy that luteolysis is associated with generation of an immune response that involves lymphatic vessels in the bovine corpus luteum (CL). The CL was obtained from Holstein cows at the mid-luteal phase (days 10–12, ovulation = day 0) by ovariectomy.
lymph nodes drastically increases [34–36]. We recently reported the expression of lymphatic vessel markers, such as lymphatic endothelial hyaluronan receptor 1 (LYVE1) and podoplanin, in the bovine CL [37], suggesting that luteolysis involves the immune response by increasing the number of immune cells that traverse the lymphatic vessels into the CL.

This study aimed to evaluate the lymphatic system, including lymphatic vessel-related factors and cytokines in the CL, at different time points following PGF$_{2\alpha}$-induced luteolysis.

**Materials and Methods**

CL collection was conducted at the Clinic for Cattle of the University of Veterinary Medicine Hannover, Germany. The experimental procedures complied with the guidelines of the Ethics Committee on Animal Rights Protection of Oldenburg, Germany, in accordance with the German legislation on animal rights and welfare. The protocol was approved by the committee on the Ethics of Animal Experiments of the University of Veterinary Medicine Hannover (permit number: 33.9-42502-04/07/1275).

**PGF$_{2\alpha}$-induced luteolysis**

For collecting CLs during luteolysis, 29 normal cyclic German Holstein cows were used in this study. The day of estrus was designated Day 0. Cows (n = 4–5 for each time point) at the mid-luteal phase (days, 10–12) were injected with PGF$_{2\alpha}$ via the intramuscular route (0 min; 0.5 mg of cloprostenol, 2.0 ml EstrumateTM, Essex Tierarznei, Munich, Germany), and ovaries were collected by ovariectomy [3] through the vagina before PGF$_{2\alpha}$ injection (0 min), and at 15 min, 30 min, 2 h and 12 h after injection.

**Processing of the corpus luteum**

The CL was enucleated from the ovary and dissected, free of connective tissues, as described previously [38]. The CL tissue samples were then minced, immediately placed into a 1.5-ml microcentrifuge tube with or without 0.4 ml of TRIzol reagent (Invitrogen, Karlsruhe, Germany) and stored at −80°C until analysis.

**RNA extraction, cDNA synthesis and reverse-transcription quantitative PCR**

Total RNA was extracted from the CL following the protocol of Chomczynski and Sacchi [39] using TRIzol reagent, treated with DNase using a commercial kit (Promega, Madison, WI, USA) and frozen at −20°C in THE RNA Storage Solution (Ambion, Austin, TX, USA). The cDNA was synthesized as previously described [40]. The levels of mRNA expression of LYVE1, podoplanin, VEGFR3, VEGFC, VEGFD, TNFα, CXCL1, CCL21 and β-actin were quantified by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) as previously described [40]. RT-qPCR reactions were performed in duplicate in a final volume of 10 μl containing 5 μl of QuantiTectTM SYBR Green PCR Buffer (QIAGEN GmbH, Hilden, Germany), 2.8 μl of H$_2$O (Sigma, St. Louis, MO, USA), 0.1 μl of 50 μM forward and reverse primers (Table 1 lists primer sequences and accession numbers) and 2 μl of cDNA template or water (as a non-template negative control). RT-qPCR conditions were 10 min at 95°C, followed by 40 cycles of 95°C for 15 sec; 56°C for 30 sec and 72°C for 30 sec using a LightCycler (Roche Diagnostics, Mannheim, Germany). The PCR products were resolved by electrophoresis, and the target bands were cut out and purified using a DNA purification kit (SUPRECTM-01, Takara Bio, Otsu, Japan). The mRNA expression levels in the CL were normalized using β-actin as an internal standard. Each PCR amplification product was sequenced using an Applied Biosystems 3730 × 1 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

**Western blotting**

The CL tissue samples were homogenized in lysis buffer containing 25 mM Tris-HCl (pH 7.4), 0.3 M sucrose, 2 mM Na$_2$EDTA and Complete Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany), and then filtered with a 70-μm filter (Cell Strainer, REF 352350, BD Falcon, Franklin Lakes, NJ, USA). The proteins were dissolved in sample buffer (0.5 M Tris-HCl [pH 6.8], glycerol, 10% SDS and 0.5% bromophenol blue) and steamed for 5 min. The entire samples were subjected to electrophoresis on 10% SDS-PAGE gels for 50 min at 200 V. The proteins were transferred to PVDF membranes (Bio-Rad Laboratories, Hercules, CA, USA) for 2 h at 60 V. The membranes were blocked with 4% Block Ace Powder (DS Pharma Biomedical, Osaka, Japan) in TBS with 0.5% Tween-20 (Sigma) for 1 h at room temperature. The membranes were next incubated with a rabbit anti-mouse-LYVE1 polyclonal antibody (1:500 dilution, Abcam, Cambridge, UK) and a mouse anti-β-actin monoclonal clone AC-15 antibody (1:10,000 dilution, Sigma) at 4°C overnight. The membranes were then washed 3 times in TBS with 0.5% Tween-20, incubated with HRP-conjugated anti-rabbit (1:10,000 dilution, Rockland Immunocore, Gilbertsville, PA, USA) or anti-mouse (1:10,000 dilution, GE Healthcare, Buckinghamshire, UK) IgG antibodies for 1 h at room temperature, and washed 3 times with TBS with 0.5% Tween-20. The signals were detected using an ECL Western Blotting Detection System (GE Healthcare). The optical densities of the immunospecific bands were quantified using an NIH Image computer-assisted analysis system.

**Statistical analysis**

All data are presented as means ± standard error of the mean (SEM). The statistical significance of differences was assessed by one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison. A P value less than 0.05 was considered significant.

**Results**

mRNA expression of LYVE1 and podoplanin and LYVE1 protein expression in the bovine CL during PGF$_{2\alpha}$-induced luteolysis

Figure 1 shows the mRNA expression of LYVE1 and podoplanin (markers of LyECs) and LYVE1 protein expression in the bovine CL during PGF$_{2\alpha}$-induced luteolysis (15 min, 30 min, 2 h and 12 h after PGF$_{2\alpha}$ injection). The level of LYVE1 mRNA expression did not change from 0 min to 2 h, but decreased at 12 h compared with those at 15 min and 2 h after PGF$_{2\alpha}$ injection (Fig. 1A; P<0.05). Lowered expression of LYVE1 protein was found for the first 2 h (P<0.1), and the expression was decreased 12 h after PGF$_{2\alpha}$ injection (Fig. 1B; P<0.05). Lowered Podoplanin mRNA expression was
Table 1. Primer sequences for the investigated genes

| Gene       | Primer sequence     | Accession No. | Product size (bp) |
|------------|---------------------|---------------|------------------|
| LYVE1      | FWD AGG TTG AAG AAG CAC GGA AA | NM_205815     | 231              |
|            | REV AGG GAT CAT CGG TGG TGA TA |               |                  |
| Podoplanin | FWD TGG CTA CGG AGC TTT TTC AT | ENSBTAT       | 291              |
|            | REV CAC ACC CAG GTT TGT TTT CT | 0000002341    |                  |
| VEGFR3     | FWD TGA GGA TAA AGG CAG CAT GGA | AF030379      | 66               |
|            | REV CCC AGA AAA AGA CAG CGA TGA |               |                  |
| VEGFC      | FWD CTC GGA GCC CCA AAC CAG T | NM_174488     | 71               |
|            | REV CAT CCA GCT TAG ACA TGC ATC G |               |                  |
| VEGFD      | FWD GGA GAA TGG CTT TGG AAC CA | NM_001101043  | 272              |
|            | REV CCA GTC CTC GAA GTG TGT GA | XM_590821     |                  |
| TNFα       | FWD TAA CAA GCC GGT AGC CCA CG | K_00622       | 221              |
|            | REV GCA AGG GCT CTT GAT GGC AGA |               |                  |
| CXCL1      | FWD CTA TTT TGG GGA GGG TAT TCC | U66096        | 94               |
|            | REV CGT GAC ATA CTC GTT TGG TGT A |               |                  |
| CCL21      | FWD AGT TGC GCT ATG CCA GCT AT | NM_001038076.2| 184              |
|            | REV TTC CCT TCT TGC CAG ACT TG |               |                  |
| β-actin    | FWD CCA AGG CCA ACC GTG AGA AGA T | K00622       | 256              |
|            | REV CCA GTG TCC GTG AGG ATC TTC A |               |                  |

Fig. 1. mRNA expression of LYVE1 and podoplanin and LYVE1 protein in the bovine CL during PGF2α-induced luteolysis. LYVE1 mRNA expression decreased in the CL at 12 h as compared with 15 min and 2 h after PGF2α injection (A). LYVE1 protein expression also decreased at 12 h compared with that at 0 min after PGF2α injection (B). Podoplanin mRNA expression was decreased in the CL at all points after PGF2α injection (C). All values are shown as means ± standard error of the mean (SEM; n = 4–5 in each time). ** Significant difference (P<0.05 or P<0.01 compared with control) as determined by Bonferroni’s multiple comparison test. * Tendency for a difference (P<0.1) as determined by Bonferroni’s multiple comparison test.
found at 15 min, but the expression tended to recover after that up until 12 h after PGF2α injection (Fig. 1C; P<0.01, P<0.05 or P<0.1).

mRNA expression of lymphangiogenic factors in the bovine CL during PGF2α-induced luteolysis
Changes in the transcripts of lymphangiogenic factors (VEGFC, VEGFD and VEGFR3) in the CL during PGF2α-induced luteolysis are shown in Fig. 2. The levels of VEGFC and VEGFR3 mRNA expression were continuously suppressed after PGF2α injection (Figs. 2A and C; P<0.01 or 0.05). VEGFD mRNA expression decreased at 15 min to 2 h as compared with 0 min after PGF2α injection (P<0.1) and decreased significantly 12 h after PGF2α injection (Fig. 2B; P<0.05).

mRNA expression of TNFα, CXCL1 and CCL21 in the bovine CL during PGF2α-induced luteolysis
TNFα mRNA expression increased significantly in the CL at 15 and 30 min and 2 h as compared with 0 min after PGF2α injection (Fig. 3A; P<0.01 or 0.05). Chemokine (C-X-C motif) ligand 1 (CXCL1) enhances the recruitment of neutrophils and acts as a mediator of inflammation during the early wound healing process [41, 42]. CXCL1 mRNA expression also increased at 15 min (P<0.1) and was higher at 30 min and 2 h (P<0.01 or 0.05) as compared with 0 min after PGF2α injection (Fig. 3B).

Chemokine (C-C motif) ligand 21 (CCL21) is involved in modulation of inflammatory responses and may play a role in the migration of leukocytes from peripheral tissues through afferent lymphatic vessels. CCL21 mRNA expression decreased at 15 min, 2 h and 12 h after PGF2α injection compared with the expression level at 0 min (Fig. 3C; P<0.1 or P<0.05).

Discussion
It is well known that cell death of luteal endothelial cells is induced during luteolysis, which is called structural luteolysis. The treatment with PGF2α resulted in a downregulation of fibroblast growth factor (FGF)-2 mRNA expression and protein expression of VEGFA, which are potent angiogenic factors in the CL [3, 4]. In the CL, PGF2α decreased angiopoietin (ANPT) 1 mRNA expression [43] and stimulated a high level of angiopoietin ANPT 2 in relation to ANPT1, inducing the destabilization of blood vessels [3]. The presence of VEGFA may also define the fate of destabilized blood vessels [44], and thus a deficiency in VEGFA may result in the disruptive destabilization of blood vessels after PGF2α injection. The lymphatic vascular system has a role in the body’s circulation system together with blood vessels, but there have been no studies about the changes of the lymphatic network in the CL during luteolysis. This study showed for the first time that lymphatic vessel markers, such as LYVE1 and podoplanin, and lymphangiogenic factors, such as VEGFC, VEGFD and VEGFR3, were downregulated in luteolysis. Interestingly, VEGFA and FGF2 have potent lymphangiogenic activity [45, 46]. Additionally, ANPT-1 resulted in lymphatic endothelial cell proliferation, lymphatic vessel enlargement, sprouting and branching in vivo [47] and promoted survival and proliferation of LyECs in vitro [48]. Thus, these findings suggest that luteolytic PGF2α downregulates the production of vascular-related factors, resulting in destruction of the vascular system through angiolysis and lymphangiolysis in the CL during luteolysis. Berisha et al. [49] showed that the number of VEGFR-3-immunostained large luteal cells significantly decreased in the bovine CL during regression (day>18). Immunohistochemical observation of lymphatic vessels markers in the CL after luteolysis is required to clarify how the luteal lymphatic structures disintegrate during PGF2α-induced luteolysis.

During luteolysis, leukocytes, especially neutrophils, macrophages and T lymphocytes, significantly increase in number in the CL [10, 14, 21, 22, 50]. Pro-inflammatory cytokines such as TNFα, IL-1β...
mRNA expression of TNFα, CXCL1 and CCL21 in the bovine CL during PGF2α-induced luteolysis. mRNA expression of TNFα (A) and CXCL1 (B) increased in the CL after PGF2α injection, whereas CCL21 mRNA expression decreased (C, mean ± standard error of the mean [SEM; n = 4–5 in each time]). ** Significant difference (P<0.01 or P<0.001 compared with control) as determined by Bonferroni’s multiple comparison test.

**Fig. 3.**

and IFNγ and chemokines such as MCP1 and IL-8 are associated with luteal regression [3, 13, 14, 51, 52]. These findings suggest that the luteolytic phenomenon is an inflammatory-like immune response. Accordingly, we hypothesized that various immune cells promote an immune response involving the lymphatic vessels during luteolysis. The immune cells that enter sites of inflammation, such as neutrophils, dendritic cells and macrophages, migrate from tissues and travel to lymph nodes through peripheral afferent lymphatic vessels [33, 34, 36, 53–55]. In the lymph nodes, dendritic cells present antigens to T cells, and in the case of immune response, this leads to the clonal expansion and differentiation of antigen-specific T cells. These T cells recirculate from the lymph nodes to inflammatory peripheral tissues through the blood vessels, resulting in an effective immune response through the lymphatic vessels and lymph nodes, which is called lymphocyte homing. In this process, the homeostatic chemokine CCL21 plays an important role of regulating outflow of immune cells from tissue. With regard to the exit of leukocytes from peripheral tissues through afferent lymphatic vessels, the expression of homeostatic chemokine CCL21 by dural afferent lymphatic vessels is essential in guiding naïve T cells, dendritic cells and neutrophils [32, 56, 57]. In the CL during luteolysis, CCL21 mRNA expression was decreased. Additionally, the decrease in the expression of lymphatic vessel-related factors suggests the loss of lymphatic vessels in the CL during luteolysis. Thus, luteolysis may be a local, not systemic, inflammatory-immune response that does not utilize lymphatic vessels and lymph nodes.

In summary, expression of lymphatic cell markers and lymphangiogenic factors dramatically decreased in the CL during luteolysis, suggesting that the lymphatic network is disrupted in the CL during luteolysis, as well as the vascular structure.

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**References**

1. Juengel JL, Garverick HA, Johnson AL, Youngquist RS, Smith MF. Apoptosis during luteal regression in cattle. Endocrinology 1993; 132: 249–254. [Medline] [CrossRef]
2. Pate JL. Cellular components involved in luteolysis. J Anim Sci 1994; 72: 1884–1890. [Medline]
3. Arvisais E, Hou X, Wyatt TS, Shirasuna K, Bolwein H, Miyamoto A, Hansen TR, Rueda RR, Davis JS. Prostaglandin F2alpha represses IGF-I-stimulated IRS1/phosphatidylinositol-3-kinase-AKT signaling in the corpus luteum: role of ERK and P70 ribosomal S6 kinase. Mol Endocrinol 2010; 24: 632–643. [Medline] [CrossRef]
4. Berisha B, Meyer HH, Schams D. Effect of progestagen F2 alpha on local luteotropic and angiogenic factors during induced functional luteolysis in the bovine corpus luteum. Biol Reprod 2010; 82: 940–947. [Medline] [CrossRef]
5. Girsh E, Milvae RA, Wang W, Meidan R. Effect of endothelin-1 on bovine luteal cell function: role in progestagen F2alpha-induced antisteroidogenic action. Endocrinology 1996; 137: 1306–1312. [Medline] [CrossRef]
6. Hückley ST, Milvae RA. Endothelin-1 mediates progestagen F2alpha-induced luteal regression in the ewe. Biol Reprod 2001; 64: 1619–1623. [Medline] [CrossRef]
7. Hayashi K, Miyamoto A. Angiotensin II interacts with progestagen F2alpha and endothelin-1 as a local luteolytic factor in the bovine corpus luteum in vitro. Biol Reprod 1999; 60: 1104–1109. [Medline] [CrossRef]
8. Miyamoto A, Kobayashi S, Arata S, Ohtani M, Fukui Y, Schams D. Prostaglandin F2alpha promotes the inhibitory action of endothelin-1 on the bovine luteal function in vitro. J Endocrinol 1997; 152: R7–R11. [Medline] [CrossRef]
9. Cannon MJ, Pate JL. The role of major histocompatibility complex molecules in luteal function. Reprod Biol Endocrinol 2003; 1: 93. [Medline] [CrossRef]
10. Pate JL, Landis Keyes P. Immune cells in the corpus luteum: friends or foes? Reproduction 2001; 122: 665–676. [Medline] [CrossRef]
11. Friedman A, Weiss S, Levy N, Meidan R. Role of tumor necrosis factor alpha and its type I receptor in luteal regression: induction of programmed cell death in bovine corpus luteum-derived endothelial cells. Biol Reprod 2000; 63: 1905–1912. [Medline] [CrossRef]
12. Pate JL. Involvement of immune cells in regulation of ovarian function. J Reprod Fertil Suppl 1995; 49: 365–377. [Medline]
13. Brännström M, Friden B. Immune regulation of corpus luteum function. Semin Reprod
LYMPHATIC VESSELS IN THE BOVINE CORPUS LUTEUM DURING LUTEOLYSIS

Shirasuna K, Watanabe S, Asahi T, Wijayagunawardene MP, Sasahara K, Jiang C, Matsu M, Sasaki M, Shimizu T, Davis JS, Miyamoto A. Prostaglandin F2alpha increases endothelial nitric oxide synthase in the capillaries of the bovine corpus luteum: the possible regulation of blood flow at an early stage of luteolysis. Reproduction 2008; 135: 527–539. [Medline] [CrossRef]

Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156–159. [Medline] [CrossRef]

Watanabe S, Shirasuna K, Matsu M, Yamamoto D, Berisha B, Schams D, Miyamoto A. Effect of intratubal injection of endothelin type A receptor antagonist on PGF2alpha-induced luteolysis in the cow. J Reprod Dev 2006; 52: 551–559. [Medline] [CrossRef]

Engelhardt E, Toksy A, Goebeler M, Debus S, Brocker EB, Gillitzer R. Chemokines IL-8, GRDalpha, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. Am J Pathol 1998; 153: 1849–1860. [Medline] [CrossRef]

Su Y, Raghavanshi SK, Yu Y, Nanney LB, Richardson RM, Richmond A. Altered CXCR2 signaling in beta-aretein 2-deficient mouse models. J Immunol 2005; 175: 5396–5402. [Medline] [CrossRef]

Tanaka J, Acosta TJ, Berisha B, Tetsuka M, Matsu M, Kobayashi S, Schams D, Miyamoto A. Relative changes in mRNA expression of angiopoietins and receptors in bovine corpus luteum during estrous cycle and prostaglandin F2alpha-induced luteolysis: a possible mechanism for the initiation of luteal regression. J Reprod Dev 2004; 50: 619–626. [Medline] [CrossRef]

Hanahan D. Signaling vascular morphogenesis and maintenance. Science 1997; 277: 48–50. [Medline] [CrossRef]

Cursiefen C, Chen L, Borges LF, Jackson D, Cao J, Radziejewski C, D’Amore PA, Dana MR, Wiegand SJ, Streliein JH. VEGF-A stimulates lymphangiogenesis and angiogenesis in inflammatory neovascularization via macrophage recruitment. J Clin Invest 2004; 113: 1040–1050. [Medline] [CrossRef]

Kubo H, Cao R, Brakenhielm E, Makien S, Cao Y, Altalito K. Blockade of vascular endothelial growth factor receptor-3 signaling inhibits fibroblast growth factor-2-induced lymphangiogenesis in mouse cornea. JVASC 2002; 99: 8868–8873. [Medline] [CrossRef]

Tammela T, Saaristo A, Lohela M, Morisada T, Turbon J, Norrmen C, Okke Y, Pajusola K, Thurston G, Suda T, Yla-Herttuala S, Altalito K. Angiopoietin-1 promotes lymphatic sprouting and hyperplasia. Blood 2005; 105: 4642–4648. [Medline] [CrossRef]

Nguyen VPKH, Chen SH, Trinh J, Kim H, Coomber BL, Dumont DJ. Differential response of lymphatic, venous and arterial endothelial cells to angiopoietin-1 and angiopoietin-2. BMC Cell Biol 2007; 8: 10. [Medline] [CrossRef]

Berisha B, Schiffarth S, Kennngott R, Sinowitz F, Meyer HHD, Shams D. Expression of lymphangiogenic vascular endothelial growth factor family members in bovine corpus luteum. Anat Histol Embryol 2012 (In press). doi: 10.1111/ah.e12016. [Medline] [CrossRef]

Murdoch WJ, Steadman LE, Belden EL. Immunoregulation of luteolysis. Med Hypothes 1988; 27: 197–199. [Medline] [CrossRef]

Bukovsky A, Caudle MR, Keenan JA, Wimalasena J, Upadhyaya NB, Van Meter SE. Is corpus luteum regression an immune-mediated event? Localization of immune system components and maturing hormone receptor in human corpora lutea. Biol Reprod 1995; 53: 1373–1384. [Medline] [CrossRef]

Malleto BA, Ropale AS, Ignagni DO, Lisovcyno MV, Rancoco RP, Moron VG, Pistorio-Palencia MC. Presence of neutrophil-bearing antigen in lymphoid organs of immune mice. Blood 2006; 108: 3094–3102. [Medline] [CrossRef]

Abadie V, Badell E, Douillard P, Enserguer D, Leenenn PD, Tanguy M, Fliette L, Saeland S, Gicquel B, Winter N. Neutrophils rapidly migrate via lymphatics after Mycobacterium bovis BCG intradermal vaccination and shuttle live bacilli to the draining lymph nodes. Blood 2005; 106: 1843–1850. [Medline] [CrossRef]

Harmsen AG, Muggenburg BA, Snibbe MR, Bice DE. The role of macrophages in particle translocation from lungs to lymph nodes. Science 1985; 230: 1277–1280. [Medline] [CrossRef]

Beauvillain C, Cunin P, Doni A, Scoet M, Jaillon S, Loiry M, Magistrelli G, Mesternak K, Chevalier A, Delneste Y, Jeanpin P. CCR7 is involved in the migration of neutrophils to lymph nodes. Blood 2011; 117: 1196–1204. [Medline] [CrossRef]

Sacki H, Moore AM, Brown MJ, Hwang ST. Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. J Immunol 1999; 162: 2472–2475. [Medline] [CrossRef]