

Expressions of lipoprotein receptors and cholesterol efflux regulatory proteins during luteolysis in bovine corpus luteum

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The corpus luteum (CL) synthesises and secretes progesterone (P4), which is essential for the establishment and maintenance of pregnancy in mammals. P4 is synthesised from cholesterol. Cholesterol is internalised by low-density lipoprotein receptor (LDLR) and/or scavenger receptor B1 (SR-BI), and is effluxed by ATP-binding cassette (ABC) transporter A1 (ABCA1) and G1 (ABCG1). To test the hypothesis that lipoprotein receptors and ABC transporters are involved in functional luteolysis, we examined the expression of LDLR, SR-BI, ABCA1 and ABCG1 in bovine CL during the luteal stages and after injection of prostaglandin (PG) F_{2α} on Day 10 after ovulation. Expression of LDLR and SR-BI mRNA and protein was lower in the regressed luteal than late luteal stage. Injection of cows with a PGF_{2α} did not affect LDLR mRNA and protein levels in the CL. Although expression of *SR-BI* mRNA did not change, SR-BI protein expression decreased 12 and 24 h after PGF_{2α} injection. The overall findings of the present study suggest that the decreased expression of SR-BI induced by PGF_{2α} is one of the factors responsible for the continuous decrease in P4 production during functional luteolysis.

Additional keywords: luteal phase, ovary, progesterone, prostaglandin, reproduction.

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Scavenger receptor B1 during luteolysis

Progesterone, which is essential for pregnancy in mammals, is synthesised from cholesterol in the corpus luteum (CL). We investigated the involvement of cholesterol uptake receptors and efflux transporters in bovine CL regression (luteolysis), and found that the expression of scavenger receptor B1, a cholesterol uptake receptor, decreased during luteolysis. The decline in cholesterol uptake may reduce progesterone production in the CL during luteolysis.

Introduction

The corpus luteum (CL) is a transient endocrine gland that forms from the Graafian follicle following ovulation. The CL synthesises and secretes progesterone (P4), which is an essential hormone for the establishment and maintenance of pregnancy. If pregnancy is not established, the CL regresses rapidly in order to initiate the next oestrous cycle. Luteolysis consists of two phases: (1) functional luteolysis, which is defined as a decline in P4 production; and (2) structural luteolysis, which is defined as luteal cell death (Sugino and Okuda 2007). In cattle, the pulsatile release of endometrial prostaglandin (PG) F_{2α} is an initiator of functional luteolysis (McCracken *et al.* 1970, 1972; Silvia *et al.* 1991).

P4 is a steroid hormone that is synthesised from cholesterol (Azhar *et al.* 1981; Gwynne and Strauss 1982). Because the CL produces large amounts of P4, the CL needs a large amount of cholesterol (Ferreri and Menon 1992). Because cholesterol is not water soluble, it circulates in the blood as a lipoprotein composed of multiple protein and lipid subunits (Gwynne and Strauss 1982). Therefore, P4 synthesis in luteal cells depends primarily on the presence of lipoproteins such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL; Azhar *et al.* 1981). LDL and HDL bind to LDL receptor (LDLR) and scavenger receptor B1 (SR-BI), respectively, which are located in the cellular membrane (Connelly and Williams 2003; Jeon and Blacklow 2005). The LDLR mediates the cellular uptake of LDL by endocytosis, and SR-BI mediates the selective uptake

of cholesterol ester from HDL (Connelly and Williams 2003; Jeon and Blacklow 2005). HDL also removes excess cholesterol from cells in a process called reverse cholesterol transport (Mahley *et al.* 2006). The excess cholesterol, which can be toxic to cells, is transported to the liver, where it is metabolised. ATP-binding cassette transporter A1 (ABCA1) and ABC transporter G1 (ABCG1) have roles in reverse cholesterol transport (Gelissen *et al.* 2006; Mahley *et al.* 2006).

In primates, the expression of LDLR and SR-BI proteins in the CL is decreased, whereas the expression of ABCA1 protein is increased, during functional luteolysis (Bogan and Hennebold 2010). In the present study, we tested the hypothesis that these changes have roles in functional luteolysis in cattle. To this end, we examined the expression of lipoprotein receptors (LDLR and SR-BI) and ABC transporters (ABCA1 and ABCG1) at the mRNA and protein levels in bovine CL during the luteal stages, both with and without administration of a luteolytic dose of PGF_{2α}.

Materials and methods

Collection of CL throughout the luteal stages

Ovaries were collected from cows at a local slaughterhouse within 30 min after exsanguination and were submerged in ice-cold physiological saline before being transported to the laboratory. The stages of the oestrous cycle were identified by macroscopic observation of the ovary and uterus, as described previously (Miyamoto *et al.* 2000), and ovaries with CLs were classified into the early (Days 2–3 after ovulation), developing (Days 5–6), mid (Days 8–12), late (Days 15–17) and regressed (Days 19–21) luteal stages. After determination of these stages, the CL tissues were immediately separated from the ovaries and stored at –80°C until processing for mRNA and protein analysis.

Collection of CL during PGF_{2α}-induced luteolysis

The collection procedures were approved by the local institutional Animal Care and Use Committee of the Polish Academy of Sciences in Olsztyn, Poland (Agreement No. 5/2007, 6/2007 and 88/2007). Twenty-five healthy, normally cycling Polish Holstein black and white cows (control,

$n = 5$; 2 h, $n = 5$; 4 h, $n = 4$; 12 h, $n = 6$; 24 h, $n = 4$) were used for collection of CL. These cows were multiparous and non-lactating. Oestrus was synchronised by two injections of 25 mg PGF_{2α} (Dinoprost, Dinolytic; Pharmacia and Upjohn, Puurs, Belgium) at 11-day intervals according to the manufacturer's instructions. Ovulation was determined by a veterinarian via transrectal ultrasonography before the second injection. Cows were sedated by injection of xylazine hydrochloride (2% Selactar; Bayer, Leverkusen, Germany), followed by injection of a local anaesthetic (lidocaine hydrochloride; 2% Xylocaine; AstraZeneca, London, UK). Then, ovaries were collected by colpotomy using Hauptner's effeminator (Hauptner and Herberholz; Solingen, Germany; <http://www.hauptner-herberholz.de>, accessed 23rd April 2016) on Day 10 after ovulation before and after injection of a luteolytic dose (25 mg) of a PGF_{2α}. CL tissues were dissected from the ovaries and then immediately stored at -80°C until processing for mRNA and protein analysis.

Quantitative reverse transcription–polymerase chain reaction

Total RNA was extracted from CL tissues using TRIZOL reagent (15596-026; Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA concentrations were measured using the NanoDrop Lite (Thermo Scientific, Waltham, MA, USA). Absorbance was measured at 260 and 280 nm (A260 and A280, respectively) and samples with an A260/A280 purity ratio between 1.8 and 2.0 were used. A 1- μg aliquot of each total RNA sample was reverse transcribed to cDNA using an iScript Reverse Transcription Supermix for quantitative reverse transcription–polymerase chain reaction (qRT-PCR; 170-8841; Bio-Rad Laboratories, Hercules, CA, USA). Gene-specific primers for *LDLR*, *SR-BI*, *ABCA1* and *ABCG1* from the bovine sequence were used for quantitative polymerase chain reaction (qPCR; Table 1). Quantification of mRNA expression was determined by qRT-PCR using the MyiQ Single-Color Real-Time PCR Detection System (170-9770; Bio-Rad Laboratories) and SsoAdvanced Universal SYBR Green Supermix (172-5271; Bio-Rad Laboratories, Hercules, CA, USA) starting with 2 ng reverse-transcribed total RNA. For quantification of *LDLR*, *SR-BI* and *ABCA1* mRNA expression, a PCR was performed

under the following conditions: 95°C for 30 s, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. For quantification of *ABCG1* mRNA expression, a PCR was performed under the following conditions: 95°C for 30 s, followed by 40 cycles of 95°C for 10 s and 69.3°C for 30 s. Use of the MyiQ Single-Color Real-Time PCR Detection System (170-9770; Bio-Rad Laboratories) at elevated temperatures resulted in reliable and sensitive quantification of the RT-PCR products with high linearity (Coefficient of determination $R^2 > 0.98$). To determine the most stable transcribed housekeeping gene, Normfinder software (<http://moma.dk/normfinder-software>, accessed 26th December 2014) was used. β -actin (*ACTB*) mRNA expression was used as an internal control and the expression of each gene was evaluated on the basis of *ACTB* mRNA expression in the individual samples.

Western blotting

CL tissues were homogenised on ice in homogenisation buffer (300 mM sucrose, 32.5 mM Tris-HCl, 2 mM EDTA, pH 7.4) with a tissue homogeniser (Physcotron; NS-50; NITI-ON, Chiba, Japan), and filtered through a metal wire mesh (150 μ m). Part of the filtrate was fragmented ultrasonically with a Vibra-Cell ultrasonic processor (Sonics and Materials, Newtown, CT, USA), and total protein concentrations in the lysates were used for detection of LDLR and SR-BI proteins by western blotting. For protein analysis, the rest of the filtrate was centrifuged at low speed (700g, 5 min, 4°C) to remove nuclei. The supernatant was then centrifuged at 100 000g for 1 h at 4°C to separate the membrane fraction (in the pellet) and the cytoplasmic fraction (in the supernatant). The expression of LDLR and SR-BI proteins in the membrane fraction and total lysate was measured by western blotting. The cell fraction was validated by reprobng with anti-tumour necrosis factor receptor 1 (TNFR1) antibody (ab19139; Abcam, Cambridge, UK) as a plasma membrane marker and anti- β -actin antibody (A2228; Sigma-Aldrich, St Louis, MO, USA) as a cytoplasmic marker (Green *et al.* 2006). Because TNFR1 is expressed in the bovine CL (Sakumoto *et al.* 2011) and generally known as a membrane receptor (Vandenabeele *et al.* 1995), an anti-TNFR1 antibody was used as a

plasma membrane marker (see Fig. S1, available as Supplementary Material to this paper).

Membrane and total protein concentrations in the lysates were determined using a bicinchoninic acid (BCA) protein assay (Pierce BCA Protein Assay Kit (23225; Thermo Scientific)) according to the manufacturer's instructions. The proteins were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using a constant current of 25 mA for 70 min. Gels were stained and destained with Coomassie Brilliant Blue (CBB) to show a similar distribution and intensity of bands for the membrane fraction (Fig. S2). Precision Plus Protein Dual Color Standards (161-0374; Bio-Rad Laboratories) were used as a protein ladder. The proteins were then solubilised in SDS gel loading buffer (50 mM Tris-HCl, 2% SDS (31607-94; Nacalai Tesque, Tokyo, Japan), 10% glycerol, 1% β -mercaptoethanol (137-06862; Wako Pure Chemical Industries, Osaka, Japan), pH 6.8), and heated at 95°C for 5 min. Then, 25 μ g membrane protein of CL tissue throughout the oestrous cycle and 10 μ g total lysate of CL tissue after PGF_{2 α} injection were subjected to SDS-PAGE. Samples were then transferred to polyvinylidene difluoride (PVDF) membranes (RPN303F; GE Healthcare, Buckinghamshire, UK) using a Trans-Blot SD Semi-Dry Transfer Cell (Bio-Rad Laboratories). The membranes were washed in TBST (0.1% Tween 20 in Tris-buffered saline (25 mM Tris-HCl, 137 mM NaCl, pH 7.5]), incubated in blocking buffer (5% non-fat dry milk in TBST or PVDF Blocking Reagent for Can Get Signal (NYPBR01; TOYOBO, Osaka, Japan]) for 30 min at room temperature. After washing with TBST, membranes were incubated with anti-LDLR antibody (LP02; EMD Millipore, Billerica, MA, USA; 1 : 100 dilution), anti-SR-BI antibody (PA5-19538; Thermo Scientific; 1 : 2000 dilution) or anti-ACTB antibody (A2228; Sigma-Aldrich; 1 : 10 000 dilution) overnight at 4°C. After washing with TBST, membranes were incubated with the appropriate secondary antibodies (horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG (NA934; Amersham Biosciences, San Francisco, CA, USA; 1:10 000 dilution] for SR-BI; HRP-conjugated sheep anti-mouse IgG (NA931; Amersham Biosciences) for LDLR (1 : 2000 dilution) and ACTB (1 : 80 000 dilution)) for 30 min at room temperature, and then washed with TBST. Signals were detected by chemiluminescence using Immobilon Western Chemiluminescent HRP Substrate

(P36599; Millipore). ACTB protein expression was used as an internal control in the total lysate. Band intensities were estimated by densitometry using Image Lab Software version 4.0 (Bio-Rad Laboratories).

Statistical analysis

All experimental data are shown as the mean \pm s.e.m. The statistical significance of differences in the expression of *LDLR*, *SR-BI*, *ABCA1* and *ABCG1* mRNA and LDLR and SR-BI protein was assessed by one-way analysis of variance (ANOVA) followed by a Tukey–Kramer multiple comparison test. Two-tailed $P < 0.05$ was considered significant.

Results

LDLR and SR-BI mRNA and protein expression

Specific transcripts for lipoprotein receptors *LDLR* and *SR-BI* were detected in bovine CL throughout the oestrous cycle. The mRNA levels for *LDLR* (Fig. 1a) and *SR-BI* (Fig. 1b) were greater in the mid and late luteal stages than in the early luteal stage, and were lower in the regressed luteal stage than in the late luteal stage ($P < 0.05$). The expression of LDLR protein was lower in the regressed luteal stage than in the late luteal stage (Fig. 1c; $P < 0.05$). The expression of SR-BI protein increased from the early to the mid luteal stage, and decreased thereafter from the late to the regressed luteal stage (Fig. 1d; $P < 0.05$).

ABCA1 and ABCG1 mRNA expression

ABC transporter *ABCA1* and *ABCG1* transcripts were detected throughout the oestrous cycle. *ABCA1* mRNA expression was significantly greater in the developing luteal stage than in the early and mid luteal stages (Fig. 2; $P < 0.05$), whereas *ABCG1* mRNA levels did not change throughout the oestrous cycle (Fig. S3a; $P > 0.05$). *ABCA1* and *ABCG1* protein levels were not examined because *ABCA1* and *ABCG1* antibodies for cattle are not commercially available.

Effects of PGF_{2α}-induced luteolysis on LDLR and SR-BI expression

PGF_{2α}-induced luteolysis significantly decreased *LDLR* mRNA expression at 12 h compared with 2 h after PGF_{2α} injection, but there was no significant difference in *LDLR* mRNA levels between PGF_{2α}-treated and untreated control cows (Fig. 3a; $P > 0.05$). PGF_{2α}-induced luteolysis did not significantly affect *SR-BI* mRNA levels (Fig. 3b; $P > 0.05$) or LDLR protein levels (Fig. 3c; $P > 0.05$) in CL tissues. However, it did reduce the expression of SR-BI protein at 12 and 24 h after PGF_{2α} injection compared with PGF_{2α}-untreated control cows (Fig. 3d; $P < 0.05$).

Effects of PGF_{2α}-induced luteolysis on ABCA1 and ABCG1 expression

PGF_{2α}-induced luteolysis significantly decreased *ABCA1* mRNA levels in bovine CL tissues until 12 h after injection (Fig. 4; $P < 0.05$). There was a significant increase in *ABCG1* mRNA at 12 h compared with 2 h after PGF_{2α} injection, but there was no significant difference in *ABCG1* mRNA levels between PGF_{2α}-treated and untreated control cows (Fig. S3b; $P > 0.05$).

Discussion

The results of the present study suggest that a decline in SR-BI expression is involved in the decrease in P4 production during functional luteolysis in cattle.

Most of the cholesterol used for steroid hormone synthesis in bovine CL is imported to cells from circulating LDL via the LDLR and from circulating HDL via SR-BI (Miranda-Jiménez and Murphy 2007). Thus, LDLR and SR-BI are thought to have roles in steroid hormone synthesis in bovine CL. In the present study, the expression of LDLR mRNA and protein in bovine CL decreased from the late luteal stage (Days 15–17 after ovulation) to the regressed luteal stage (Days 19–21; Fig. 1a, c). The expression of SR-BI mRNA and protein in bovine CL also decreased from the late to the regressed luteal stage (Fig. 1b, d). P4 concentrations in peripheral plasma of cows decrease around Day 17 after ovulation (McCracken *et al.* 1999). Based on the above findings, the decrease in P4 production from around Day 17 after ovulation in bovine CL may be caused by a decline in cholesterol uptake because of decreased LDLR and SR-BI expression.

HDL is involved not only in cholesterol uptake in cells, but also cholesterol efflux (Mahley *et al.* 2006). ABCA1 and ABCG1 remove excess cholesterol from cells through the formation of nascent HDL before cells suffer the toxic effects of excess cholesterol (Mahley *et al.* 2006). The increased levels of ABCA1 and ABCG1 that are involved in reverse cholesterol transport seem to help initiate luteolysis by preventing the accumulation of intracellular cholesterol, which is required for steroidogenesis in the primate CL (Bogan and Hennebold 2010). In the present study, the expression of *ABCA1* mRNA did not change between the late and regressed luteal stages (Fig. 2), and *ABCG1* mRNA did not change across the luteal stages (Fig. S3a). On the basis of these findings, we conclude that ABCA1 and ABCG1 are not involved in functional luteolysis in cattle, at least not at the mRNA level. Further studies are needed to determine whether other ABC transporters are involved in cholesterol efflux. One such transporter (ABCG4) is known to mediate cellular cholesterol efflux (Wang *et al.* 2004).

In cows, PGF_{2α}-injection caused luteal P4 release to decrease rapidly within 4 h and to gradually decrease to 20% of baseline after 24 h (Ohtani *et al.* 1998). In the present study, PGF_{2α} treatment did not significantly affect the expression of *LDLR* mRNA (Fig. 3a) or LDLR protein (Fig. 3c) in CL tissues compared with expression in untreated control cows, suggesting that the LDLR is not involved in functional luteolysis. PGF_{2α} injection did not affect the time course of *SR-BI* mRNA in CL (Fig. 3b), whereas it did decrease the expression of SR-BI protein at 12 and 24 h (Fig. 3d). The PGF_{2α}-induced decrease in SR-BI protein may be responsible for the continuous decline in P4 production. PGF_{2α} injection transiently increases nitric oxide levels in ovarian blood circulation in cows (Acosta *et al.* 2009). Nitric oxide and PGF_{2α} are also involved in the regulation of lipid peroxidation (Motta *et al.* 2001). In human skin fibroblasts, SR-BI binds to 4-hydroxy-2-nonenal (4-HNE), a product of lipid peroxidation. When 4-HNE binds to SR-BI, it increases the ubiquitination of SR-BI, leading to its degradation by proteasomes (Sticozzi *et al.* 2013). These findings suggest that PGF_{2α} and the PGF_{2α}-induced increase in nitric oxide levels increase the

ubiquitination of SR-BI after its binding to 4-HNE, leading to the degradation of SR-BI. The decrease in SR-BI expression may promote functional luteolysis by suppressing the ability of luteal cells to take up cholesterol and produce P4.

Liver X receptors (LXRs) are ligand-activated transcription factors that have roles in the control of cholesterol homeostasis (Janowski *et al.* 1996; Tontonoz and Mangelsdorf 2003). PGF_{2α} has been reported to antagonise an LXR agonist and inhibit ABCA1 promoter activity, as well as *ABCA1* transcription (Zhuang *et al.* 2013), which may be the reason why the expression of *ABCA1* mRNA in CL tissues decreased 12 h after PGF_{2α} injection in cows in the present study (Fig. 4). *ABCA1* expression, which is involved in cholesterol efflux, decreased after PGF_{2α} injection, suggesting that it is not a factor in functional luteolysis in the cow. However, *ABCG1* mRNA levels increased significantly 12 h after PGF_{2α} injection compared with 2 h after injection. It is unclear whether expression of *ABCG1* mRNA at 12 h after PGF_{2α} injection is involved in functional luteolysis because ABCG1 protein levels were not examined. Conversely, *ABCG1* mRNA expression did not change significantly in PGF_{2α}-treated compared with untreated control cows (Fig. S3b; *P* > 0.05). Therefore, ABCG1 may not be directly involved in functional luteolysis.

In conclusion, the overall findings of the present study suggest that the PGF_{2α}-induced decrease in the expression of SR-BI is involved in the continuous decrease in P4 production in bovine CL during functional luteolysis. The decrease in P4 production may be caused by the decline in cholesterol uptake due to the decreased expression of SR-BI. However, LDLR, ABCA1 and ABCG1 may not be directly involved in the continuous decrease in P4 production in cows.

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Fig. 1. Changes in the relative amounts of (a) low-density lipoprotein receptor (*LDLR*) and (b) scavenger receptor B1 (*SR-BI*) mRNA, as determined by quantitative reverse transcription–polymerase chain reaction in bovine CL tissue throughout the oestrous cycle (early, 2–3 days after ovulation; developing, 5–6 days after ovulation; mid, 8–12 days after ovulation; late, 15–17 days after ovulation; regress, regressed luteal stages 19–21 days after ovulation). Data are the mean ± s.e.m. ($n = 7$) and are expressed as the relative ratio of *LDLR* and *SR-BI* mRNA to β-actin (*ACTB*) mRNA. Representative western blot bands and results of densitometric analysis of *LDLR* (c) and *SR-BI* (d) protein expression in the membrane fraction of bovine CL tissue during different luteal phases. Data are the mean ± s.e.m. (*LDLR*, $n = 6$; *SR-BI*, $n = 5$) and show *LDLR* or *SR-BI* protein levels as a percentage of that in the early stage. Different letters above columns indicate significant differences ($P < 0.05$), as determined by ANOVA followed by the Tukey–Kramer multiple comparison test.

Fig. 2. Changes in the relative amount of ATP-binding cassette (ABC) transporter A1 (*ABCA1*) mRNA, as determined by quantitative reverse transcription–polymerase chain reaction in bovine CL tissue throughout the oestrous cycle (early, 2–3 days after ovulation; developing, 5–6 days after ovulation; mid, 8–12 days after ovulation; late, 15–17 days after ovulation; regress, regressed luteal stages 19–21 days after ovulation). Data are the mean ± s.e.m. ($n = 7$) and show the relative ratio of *ABCA1* mRNA to β-actin (*ACTB*) mRNA. Different

letters above columns indicate significant differences ($P < 0.05$), as determined by ANOVA followed by the Tukey–Kramer multiple comparison test.

Fig. 3. Effects of prostaglandin (PG) $F_{2\alpha}$ on the expression of (a) low-density lipoprotein receptor (*LDLR*) and (b) scavenger receptor B1 (*SR-BI*) mRNA, as determined by quantitative reverse transcription–polymerase chain reaction in bovine CL tissue. Holstein cows were injected intramuscularly with 25 mg $PGF_{2\alpha}$ ($n = 4–6$ per time point) or were not injected (Control; $n = 5$) on Day 10 of the oestrous cycle. Data are the mean \pm s.e.m. and show the relative ratio of *LDLR* and *SR-BI* mRNA to β -actin (*ACTB*) mRNA. Representative western blots and densitometric analysis for *LDLR* (c) and *SR-BI* (d) protein in the total lysate of bovine CL tissue after $PGF_{2\alpha}$ injection. Data are the mean \pm s.e.m. (*LDLR*, $n = 3$; *SR-BI*, $n = 4$) and are expressed as the relative ratio of *LDLR* or *SR-BI* protein to β -actin protein. Different letters above columns indicate significant differences ($P < 0.05$) between time points in the $PGF_{2\alpha}$ -injected and control groups as determined by ANOVA followed by the Tukey–Kramer multiple comparison test.

Fig. 4. Effects of prostaglandin (PG) $F_{2\alpha}$ on the expression of ATP-binding cassette (ABC) transporter A1 (*ABCA1*) mRNA, as determined by quantitative reverse transcription–polymerase chain reaction in bovine CL tissue. Holstein cows were injected intramuscularly with 25mg $PGF_{2\alpha}$ ($n = 4–6$ per time point) or were not injected (Control; $n = 5$) on Day 10 of the oestrous cycle. Data are the mean \pm s.e.m. and show the relative ratio of *ABCA1* mRNA to β -actin (*ACTB*) mRNA. Different letters above columns indicate significant differences ($P < 0.05$) between time points in the $PGF_{2\alpha}$ -injected and control groups as determined by ANOVA followed by the Tukey–Kramer multiple comparison test.

Table 1. Primers for quantitative polymerase chain reaction

ACTB, β -actin; *LDLR*, low-density lipoprotein receptor; *SR-BI*, scavenger receptor B1; *ABCA1*, ATP-binding cassette (ABC) transporter A1; *ABCG1*, ABC transporter G1

Gene	Primer (5'–3')	Accession no.	Product (bp)
<i>ACTB</i>	Forward: CAGCAAGCAGGAGTACGATG Reverse: AGCCATGCCAATCTCATCTC	AY141970	137

<i>LDLR</i>	Forward: GCAGCTCCCACCTCTAACTG Reverse: TCGACTTTACGCAGTGTTTCG	NM_001166530.1	107
<i>SR-BI</i>	Forward: TCAGGCACAAGAGCAACATC Reverse: TATCCGGCTGGAACTGGTAG	NM_174597.2	84
<i>ABCA1</i>	Forward: AGCTGTGTTTGTCTGCATCG Reverse: ATCCTCAGGCGCATCATTAC	NM_001024693.1	133
<i>ABCG1</i>	Forward: GACTCGGTCCTCACGCAC Reverse: CGGAGAAACACGCTCATCTC	NM_001205528.1	203
