Genomic and non-genomic effects of progesterone on prostaglandin (PG) F2α and PGE2 production in the bovine endometrium

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Progesterone (P4) acts through different actuating pathways called genomic and non-genomic pathways. Here we investigated whether P4 regulates prostaglandin (PG) F2α (PGF) and PGE2 production in bovine endometrium through different pathways. Cultured endometrial cells were exposed to P4 for a short time (5–20 min) or bovine serum albumin (BSA)-conjugated P4 (P4-BSA) for 24 h. Progesterone treatment for 24 h stimulated PGE2 production in epithelial cells, but suppressed both PGF and PGE2 production and the expression of PG-metabolising enzymes including phospholipase A2 (PLA2) and cyclooxygenase-2 (COX2) in stromal cells. Short-term (5–20 min) P4 treatment did not affect *PLA2* or *COX2* transcript levels in either cell type. P4-BSA increased PGF and PGE2 production only in epithelial cells. *Nuclear P4 receptor* mRNA expression in endometrium was higher at the follicular phase than at the early- to mid-luteal stages, whereas *membrane P4 receptor* mRNA expression did not change throughout the oestrous cycle. The overall results suggest that P4 controls PG production by inhibiting enzymes via a genomic pathway and by stimulating signal transduction via a non-genomic pathway. Consequently, P4 may protect the corpus luteum by attenuating PGF production in stromal cells and by increasing PGE2 secretion from epithelial cells.

Additional keywords: Uterus; cows; steroid hormone; luteolysis

Endometrial prostaglandins (PGs) are the most important modulators of the oestrous cycle and are regulated by ovarian hormones. This study aimed to clarify the regulatory mechanisms of progesterone on PG production. Our findings demonstrate that progesterone has at least two actuating pathways and precisely controls PG production by regulating enzymes of the arachidonate cascade via genomic pathways and by controlling signal transduction via non-genomic pathways.

Introduction

Prostaglandin (PG) F2α (PGF) is synthesised and secreted from the endometrium to induce luteolysis and to modulate the oestrous cycle in the cow (McCracken *et al.* 1970; Silvia *et al.* 1991; Mann and Lamming 2006). In contrast, PGE2 has anti-luteolytic activity in the sheep (Pratt *et al.* 1977). In the bovine endometrium, epithelial cells principally produce PGF and stromal cells

produce PGE2 (Kim and Fortier 1995; Asselin *et al.* 1996). The luteolytic pulses of PGF are produced by endometrium in response to oxytocins (OT) binding to OT receptors (OTRs) (Flint and Sheldrick 1986; McCracken *et al.* 1999). Progesterone (P4) acts to block expression of oestrogen receptor and OTR to prevent luteolysis (Spencer and Bazer 2004). However, continuous exposure of the endometrium to P4 downregulates P4 receptor expression in endometrial epithelial cells, which allows the rapid increase of OTR expression (Spencer and Bazer 2004).

The synthesis of PGs is initiated by arachidonate isolation from plasma membrane phospholipids by phospholipase A2 (PLA2) (Flint *et al.* 1986). Cyclooxygenase-2 (COX2) converts arachidonic acid to PGH2 and then two specific downstream enzymes, PGE synthase (PGES) and PGF synthase (PGFS), catalyse the conversion of PGH2 to PGE2 or PGF, respectively (Arosh *et al.* 2002). Thus, the change in expression of these enzymes influences endometrial PG production.

Progesterone is a sex-steroid hormone that is abundantly secreted from the corpus luteum (CL) to establish and maintain pregnancy (Mann and Lamming 2006). Progesterone acts as a direct transcription factor and enhances the transcriptional activity of nuclear P4 receptor (PGR) via the activation of the Ras/Raf/Mek/Erk cascade by binding to the PGR (genomic effect) (Leonhardt and Edwards 2002; Lange 2004). On the other hand, there is some evidence that P4 also has rapid effects (non-genomic effects) due to cell membrane-initiated signalling pathways in several cell types (Losel *et al.* 2003; Verikouki *et al.* 2008). Rapid actions at the membrane level are thought to activate intracellular transduction pathways that induce the synthesis of transcriptional factors required for the genomic effect of P4 (Koulen *et al.* 2008). Progesterone binding to progestin/adipoQ receptor family member VII (PAQR7), also known as membrane P4 receptor α, stimulates Erk activity (Zhu *et al.* 2003; Karteris *et al.* 2006). In addition, P4 receptor membrane component 1 (PGRMC1), when it interacts with serpine 1 mRNA-binding protein (SERBP1), inhibits Erk activity (Peluso *et al.* 2003), while only minimal information is available for PGRMC2 (Gerdes *et al.* 1998). The Ras/Raf/Mek/Erk cascade, which is known as the traditional mitogen-activated protein kinase (MAPK) pathway, phosphorylates PG synthases (Anfuso *et al.* 2007).

In the present study, to clarify whether P4 modulates PG synthesis through genomic or non-genomic pathways in bovine endometrial cells, we investigated: (1) the effect of P4 on PGF and PGE2 production by cultured endometrial cells, (2) time-dependent expression of enzymes associated with the arachidonate cascade after P4 treatment in cultured endometrial cells, (3) the effect of bovine serum albumin (BSA)-conjugated P4 (P4-BSA) on PGF and PGE2 production and (4) the expression of PGR, PAQR7, PGRMC1/2 and SERBP1 in bovine endometrium throughout the oestrous cycle.

Materials and methods

Collection of endometrial tissues

Apparently-healthy uteri of Holstein cows without a visible conceptus were obtained from a local slaughterhouse (Okayama Meat Center) within 10–20 min of exsanguination and were immediately transported to the laboratory. The stages of the oestrous cycle were confirmed by macroscopic observation of the ovaries and uterus as described previously (Okuda *et al.* 1988; Miyamoto *et al.* 2000). For mRNA analysis, endometrial tissues (n = 5 per stage) were collected from cows at six different stages of the oestrous cycle (oestrus, Day 0; early-luteal, Days 2–3; developing, Days 5–6; mid-luteal, Days 8–12; late-luteal, Days 15–17 and follicular stage, Days 19–21). Intercaruncular endometrial tissues from the uterine horn, ipsilateral to the CL or the dominant follicle, were used for experiments. The endometrial tissues were immediately frozen in liquid nitrogen and stored at –80°C until processed for mRNA isolation.

Isolation of endometrial cells

Uteri of the early and developing luteal stages (Days 2–3 and 5–6) were used for isolation. The epithelial and stromal cells from bovine endometrium were enzymatically separated (0.05% collagenase; Worthington Biochemical Co., Lakewood, NJ, USA) using procedures described previously (Murakami *et al.* 2003). A polyvinyl catheter was inserted into the side of the oviduct and the ends of the horn were tied to retain trypsin solution used to detach the epithelial cells as described herein. The uterine lumen was washed three times with 30–50 mL of sterile Ca^{2+}-free and Mg^{2+}-free Hank's balanced salt solution (HBSS) supplemented with 100 IU mL^{-1} penicillin (Meiji Seika Pharma, Tokyo, Japan), 100 µg mL^{-1} streptomycin (Meiji Seika Pharma) and 0.1% (w/v) bovine serum albumin (BSA) (Roche Diagnostics, Manheim, Germany). Thirty to fifty millilitres of sterile HBSS containing 0.3% (w/v) trypsin (Sigma-Aldrich, St. Louis, MO, USA) was then infused into the uterine lumen through the catheter.

After collection of the epithelial cells, the uterine lumen was washed with sterile HBSS supplemented with antibiotics and 0.1% (w/v) BSA. The horn was then cut transversely with scissors into several segments, which were slit to expose the endometrial surface. Intercaruncular endometrial strips were dissected from the myometrial layer with a scalpel and washed once in 50 mL of sterile HBSS containing antibiotics. The endometrial strips were then minced into small pieces (1 mm^3). The minced tissues (~5 g) were digested by stirring for 60 min in 50 mL of sterile HBSS containing 0.05% (w/v) collagenase, 0.005% (w/v) DNase I (BBI Enzymes, Cardiff, UK) and 0.1% (w/v) BSA. The dissociated cells were filtered through metal meshes (100 µm and 80 µm) to remove undissociated tissue fragments. The filtrate was washed three times by centrifugation (4°C, 10 min at 100*g*) with Dulbecco's modified Eagle medium (DMEM) (Sigma-Aldrich) supplemented with antibiotics and 0.1% (w/v) BSA. After the washes, the cells were counted using a haemocytometer. The cell viability was greater than 85% as assessed by 0.5% (w/v) Trypan blue dye exclusion.

Cell culture

The final pellets of the epithelial and stromal cells were separately resuspended in culture medium (DMEM/Ham's F-12, 1 : 1 (v/v); Invitrogen, Carlsbad, CA, USA) supplemented with 10% (v/v) bovine serum (Invitrogen), 20 μg mL^{-1} gentamicin (Sigma-Aldrich) and 2 μg mL^{-1} amphotericin B (Sigma-Aldrich) (Skarzynski *et al.* 2000). Epithelial cells were seeded at a density of 1×10^5 viable cells mL^{-1} in culture flasks (Greiner Bio-One, Frickenhausen, Germany) and stromal cells were seeded at a density of 1×10^5 viable cells mL^{-1} in 4-well cluster dishes (Thermo Fisher Scientific, Yokohama, Japan) and 24-well cluster dishes (Greiner Bio-One) and were cultured at 38.5°C in a humidified atmosphere of 5% CO_2 in air. For the stromal preparation, the medium was changed 2 h after plating, by which time selective attachment of stromal cells had occurred (Fortier *et al.* 1988; Skarzynski *et al.* 2000). Alternatively, since the epithelial cells attached 24–48 h after plating, the medium in the epithelial cell culture was replaced 48 h after plating. The medium was changed every 2 days until the cells reached confluence. When the epithelial cells were confluent, 0.02% trypsin solution was added to the cells to collect the pure epithelial cells. Epithelial cells were seeded at a density of 1×10^5 viable cells mL^{-1} in 4-well cluster dishes and 24-well cluster dishes and were cultured at 38.5°C in a humidified atmosphere of 5% CO_2in air until the cells reached confluence. When these cells were confluent (6–7 days after the start of the culture), the medium was replaced with fresh DMEM/Ham's F-12 supplemented with 0.1% (w/v) BSA, 5 ng mL^{-1} sodium selenite (Sigma-Aldrich), 0.5 mM ascorbic acid (Wako Pure Chemical Industries, Osaka, Japan), 5 mg mL^{-1} transferrin (Sigma-Aldrich), 2 mg mL^{-1} insulin (Sigma- Aldrich) and 20 mg mL^{-1} gentamicin.

Experiment 1: effect of P4 on endometrial PGF and PGE2 production in cultured bovine endometrial cells

To reveal the effect of P4 on PGF and PGE2 production, endometrial epithelial and stromal cells were exposed to P4 (0, 1, 10 or 100 nM) for 24 h. PGF and PGE2 concentrations in the media of both cells were measured by enzyme immunoassay. DNA content was measured by the spectrophotometric method (Labarca and Paigen 1980) and used to standardise the results.

Experiment 2: effect of P4 on PLA2 and COX2 mRNA expression in cultured bovine endometrial cells

To elucidate whether P4 affects *PLA2* and *COX2* mRNA expression in bovine endometrial epithelial and stromal cells, both cell types were cultured with P4 (0, 1, 10 or 100 nM) for 24 h. After incubation, total RNA was extracted for determination, then *PLA2* and *COX2* mRNA expression was examined by quantitative reverse transcription polymerase chain reaction (RT-PCR).

Experiment 3: short-term effect of P4 on PLA2 and COX2 mRNA expression in cultured bovine endometrial cells

To clarify whether *PLA2* and *COX2* mRNA expression was influenced by P4 in a very short time, endometrial epithelial and stromal cells were pre-incubated with fresh medium for 1 h. After

incubation, the medium was replaced with fresh medium and cells were exposed to P4 (0 or 10 nM) for 5, 10, 15 or 20 min. Total RNA was extracted for determination, then *PLA2* and *COX2* mRNA expression was examined by quantitative RT-PCR.

Experiment 4: non-genomic effect of P4 on endometrial PGF and PGE2 production in cultured bovine endometrial cells

To reveal the effect of membrane-mediated P4 on PGF and PGE2 production, endometrial cells were cultured with 0, 1, 10 or 100 nM P4-BSA (Sigma-Aldrich) for 24 h. P4-BSA specifically binds to cell surface membranes. PGF and PGE2 concentrations in the media of both cell types were measured by enzyme immunoassay. DNA content was measured by the spectrophotometric method (Labarca and Paigen 1980) and used to standardise the results.

Experiment 5: PGR, PAQR7, PGRMC1, PGRMC2 and SERBP1 mRNA expression in the bovine endometrium throughout the oestrous cycle

Expression of *PGR*, *PAQR7*, *PGRMC1*, *PGRMC2* and *SERBP1* mRNA in endometrial tissues of each stage (n = 5 per stage) were examined by quantitative RT-PCR.

Enzyme immunoassay

The concentrations of PGF and PGE2 in the culture medium were determined by an enzyme immunoassay as described previously (Uenoyama *et al.* 1997; Tanikawa *et al.* 2005). The PGF standard curve ranged from 0.016 to 4 ng mL^{-1} and the median effective dose (ED_{50}) of the assay was 0.25 ng mL^{-1}. The intra- and inter-assay coefficients of variation were, on average, 3.94 and 13.2%, respectively. The PGE2 standard curve ranged from 0.039 to 10 ng mL^{-1} and the ED_{50} of the assay was 0.625 ng mL^{-1}. The intra- and inter-assay coefficients of variation were, on average, 1.36 and 16.7%, respectively.

Reverse transcription and real-time PCR

Total RNA was extracted from cultured cells using TRIsure (Bioline, London, UK) according to the manufacturer's directions. One microgram of each total RNA was reverse transcribed using a ThermoScript RT-PCR System (Invitrogen) and 10% of the reaction mixture was used in each PCR reaction using specific primers for PGR, PAQR7, PGRMC1, PGRMC2, SERBP1, cytosolic phospholipase A2, group IVA (PLA2G4), COX2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the bovine sequence (Table 1). The primers were chosen using Primer 3, an online software package (http://primer3.sourceforge.net/). Gene expression was measured by real-time PCR using the Mx3000P QPCR System (Stratagene, La Jolla, CA, USA) and the QuantiTect SYBR Green PCR system (Qiagen GmbH, Hilden, Germany) starting with 2 ng of reverse-transcribed total RNA as described previously (Sakumoto *et al.* 2006). GAPDH expression was used as an internal control. For quantification of the mRNA expression levels PCR was performed under the following conditions: 95°C for 15 min followed by 55 cycles of 94°C for 15 s, 55°C for 20 s and 72°C for 15 s.

Use of the QuantiTect SYBR Green PCR system at elevated temperatures resulted in reliable and sensitive quantification of the RT-PCR products with high linearity (Pearson product moment correlation coefficient, $r > 0.99$).

Statistical analysis

All experimental data are shown as the mean ± s.e.m. of values obtained in three to six separate experiments, where each experimental triplicate was performed using stromal cells from a single bovine endometrium. The statistical significance of differences was assessed by analysis of variance (ANOVA) followed by Bonferroni–Dunn test for multiple comparisons using StatView (SAS Institute, Cary, NC, USA).

Results

Experiment 1: effect of P4 on endometrial PGF and PGE2 production in cultured bovine endometrial cells

Progesterone (100 nM) increased only PGE2 production compared with controls in cultured bovine endometrial epithelial cells (Fig. 1*c*; $P < 0.05$). Gradual decreases of PGF and PGE2 production were observed after P4 treatment in the stromal cells (Fig. 1*b*, *d*; $P < 0.05$). The ratio of PGF to PGE2 production was suppressed by P4 (10 and 100 nM) in the stromal cells (Fig. 1*f*; $P < 0.05$) but there was no difference in the epithelial cells (Fig. 1*e*).

Experiment 2: effect of P4 on PLA2 and COX2 mRNA expression in cultured bovine endometrial cells

Progesterone (1–100 nM) decreased both *PLA2* and *COX2* mRNA expression in a dose-dependent manner in cultured bovine endometrial stromal cells (Fig. 2*b*, *d*; $P < 0.05$) but not in epithelial cells (Fig. 2*a*, *c*).

Experiment 3: short-term effect of P4 on PLA2 and COX2 mRNA expression in cultured bovine endometrial cells

There was no effect on *PLA2* and *COX2* mRNA expression by exposing cells to P4 (10 nM) for a short time (5, 10, 15 or 20 min) in either cultured endometrial stromal or epithelial cells (Fig. 3).

Experiment 4: non-genomic effect of P4 on endometrial PGF and PGE2 production in cultured bovine endometrial cells

Progesterone-BSA (100 nM) significantly increased PGF and PGE2 production in cultured bovine endometrial epithelial cells (Fig. 4*a*, *c*; $P < 0.05$) but not in stromal cells (Fig. 4*b*, *d*). There was no difference in the ratio of PGF to PGE2 production in bovine endometrial epithelial or stromal cells (Fig. 4*e*, *f*).

Experiment 5: PGR, PAQR7, PGRMC1, PGRMC2 and SERBP1 mRNA expression in the bovine endometrium throughout the oestrous cycle

Expression of *PGR, PAQR7, PGRMC1, PGRMC2* and *SERBP1* mRNA was detected in the bovine endometrium throughout the oestrous cycle (n = 5 cows per stage). *PGR* mRNA expression was higher at the follicular phase (Days 19–21) than at the early- (Days 2–3) to mid- (Days 8–12) luteal stages (Fig. 5*a*). There were no changes in *PAQR7, PGRMC1*, *PGRMC2* or *SERBP1* mRNA expression during the oestrous cycle (Fig. 5*b–e*).

Discussion

In previous studies, P4 treatment for a long time (72 h) stimulated both PGF and PGE2 production in cultured bovine endometrial epithelial cells, but did not affect stromal cells (Asselin *et al.* 1996; Xiao *et al.* 1998). Furthermore, P4 inhibited OT-stimulated PGF secretion both from endometrial slices and from epithelial cells during 4 h of incubation (Duras *et al.* 2005). Moreover, P4 pre-treatment for 30 min reduced intracellular mobilisation of Ca^{2+} in response to OT (Duras *et al.* 2005). This effect was supposed to occur via activation of a membrane receptor that belongs to the class of membrane receptors coupled to phospholipase C. In the present study, P4 treatment (24 h) significantly stimulated PGE2 production but not PGF production by cultured endometrial epithelial cells, whereas P4 significantly inhibited PGF and PGE2 production and the ratio of PGF to PGE2 production by stromal cells. The findings suggest that the endometrial cellular reaction to P4 changes according to exposure time. Thus, it is important to clarify the extensive mechanisms of intracellular P4 function. Prostaglandin biosynthesis begins with PLA2, which leads to the release of arachidonic acid from plasma membrane phospholipids (Clark *et al.* 1991), and then COX2 converts free arachidonic acid to PGH2 (Wiltbank and Ottobre 2003). Therefore, both enzymes act as important physiological control points of PG production. In the present study, P4 inhibited *PLA2* and *COX2* mRNA expression in cultured endometrial stromal cells but not in epithelial cells. The suppression of PGF and PGE2 production by P4 in Experiment 2 seems to be due to the downregulation of *PLA2* and *COX2* mRNA expression in bovine endometrial stromal cells. In the epithelial cells, P4 did not affect the expression of these enzymes, although P4 stimulated PGE2 production. These findings suggest that P4 stimulates the expression and activation of other factors involved in PGE2 production in bovine endometrial epithelial cells. In stromal cells, short-term (5, 10, 15 or 20 min) P4 treatment did not affect either *PLA2* or *COX2* mRNA expression, although P4 treatment for 24 h suppressed *PLA2* and *COX2* mRNA expression in Experiment 3. On the other hand, short-term and 24-h exposure to P4 did not affect either *PLA2* or *COX2* mRNA expression in epithelial cells. These findings suggest that the effect of P4 on the production of PGs is induced via a PGR-activating pathway in bovine endometrial stromal cells.

A membrane-initiated action of P4 was demonstrated using P4-BSA, because P4 covalently linked to BSA cannot enter the cells and its binding is restricted to cell-surface membrane progesterone

receptors (Peluso *et al.* 2002, 2003). In the present study, P4-BSA stimulated PGF and PGE2 production in epithelial cells but not in stromal cells. The above findings suggest that P4 dominantly binds membrane receptors to stimulate production of PGs in epithelial cells, whereas P4 selectively binds to PGR in stromal cells to suppress production of PGs.

In the present study, PGR expression was higher at the follicular phase than at the early- to mid-luteal stages. The expression patterns of P4 receptors, except SERBP1, throughout the oestrous cycle are consistent with those observed in a previous study (Kowalik *et al.* 2013). Following ovulation, P4 concentrations begin to increase due to the formation of the CL. The granulosa and theca cells of the ovulated dominant follicle luteinise and produce P4 in readiness for the establishment and maintenance of pregnancy or resumption of the oestrous cycle (Forde *et al.* 2011). Endometrial P4 concentration is highest on Days 1–5 of the oestrous cycle and it subsequently decreases and is maintained at a low level during the remainder of the cycle (Kowalik *et al.* 2013). These findings suggest that the genomic effect of P4 on endometrial PGF production may be mediated by interaction with PGR to protect the CL against endometrial PGF production. Rapid effects of P4 through the membrane P4 receptor is thought to continuously induce expression of various genes throughout oestrous cycle.

Conclusions

In conclusion, we revealed the existence of a non-genomic pathway of P4 that acts in parallel with a genomic effect on PGF and PGE2 production in the bovine endometrium.

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Fig. 1. Mean (± s.e.m.) secretion of PGF, PGE2 and the ratio of PGF to PGE2 by endometrial epithelial and stromal cells on Days 2–5 of the bovine oestrous cycle (n = 3–8). Cultured bovine endometrial (*a*, *c*, *e*) epithelial and (*b*, *d*, *f*) stromal cells were incubated for 24 h with P4. Different superscript letters indicate significant difference ($P < 0.05$) as determined by ANOVA followed by a Bonferroni–Dunn as a multiple-comparison test.

Fig. 2. Effects of progesterone (P4) on the level of *PLA2* and *COX2* mRNA expression in cultured bovine (*a*, *c*) epithelial and (*b*, *d*) stromal cells. Endometrial cells were exposed to P4 (1–100 nM) for 24 h. All values (mean ± s.e.m.) are expressed as the relative ratio of *PLA2* and *COX2* mRNA to *GAPDH* mRNA. Different letters indicate significant difference ($P < 0.05$) as determined by ANOVA followed by a Bonferroni–Dunn as a multiple-comparison test.

Fig. 3. Time-dependent effects of progesterone (P4) on *PLA2* and *COX2* mRNA expression in cultured bovine endometrial (*a*, *c*) epithelial and (*b*, *d*) stromal cells. After 1 h of pre-incubation, both endometrial cell types were exposed to 10 nM P4 for 0, 5, 10, 15 or 20 min. All values (mean ± s.e.m.) are expressed as the relative ratio of *PLA2* and *COX2* mRNA to *GAPDH* mRNA. Different letters indicate significant difference ($P < 0.05$) as determined by ANOVA followed by a Bonferroni–Dunn as a multiple-comparison test.

Fig. 4. Mean (± s.e.m.) secretion of PGF, PGE2 and the ratio of PGF to PGE2 by endometrial epithelial and stromal cells on Days 2–5 of the bovine oestrous cycle ($n = 3–8$). Cultured bovine endometrial (*a*, *c*, *e*) epithelial and (*b*, *d*, *f*) stromal cells were incubated for 24 h with P4-BSA. Different superscript letters indicate significant difference ($P < 0.05$) as determined by ANOVA followed by a Bonferroni–Dunn as a multiple-comparison test.

Fig. 5. Expression of (*a*) *PGR*, (*b*) *PAQR7*, (*c*) *PGRMC1*, (*d*) *PGRMC2* and (*e*) *SERBP1* mRNA in bovine endometrium throughout the oestrous cycle (oestrus, Day 0; early-luteal, Days 2–3; developing, Days 5–6; mid-luteal, Days 8–12; late-luteal, Days 15–17; follicular stage, Days 19–21). All values (mean ± s.e.m.) are expressed as the relative ratio of receptor expression to *GAPDH*. Different superscript letters indicate significant difference ($P < 0.05$) as determined by ANOVA followed by a Bonferroni–Dunn as a multiple-comparison test.

Table 1. Primers for real-time PCR

F, forward; R, reverse

Gene	Primer sequence (5′–3′)	Accession number	Product size (bp)
PGR	F: AACGAAAGCCAAGCCCTAAG R: GCTGGAGGTATCAGGTTTGC	AY656812	147
PAQR7	F: CTGGAAGCCGTATATCTACGT R: GCTGTAATGCCAGAACTCGGAC	XM005203177.1	286
PGRMC1	F: TCTTCAGGGGTGTGTGTGAA R: CATTGTCCTGTGCTCTTTGG	NM001075133.1	266
PGRMC2	F: TGCCTCTTTGCCTCGTATGA R: GAGGCATCCCTACCAGCAAAT	NM001099060.1	179
SERBP1	F: AGCTCAGACCAACTCCAATGC R: CGGCTCAGACCTTCTTTCTTCA	NM001046449.1	149
PLA2G4	F: AGGTGCACAACTTCATGCTG R: GGCATCCAATTCGTCTTCAT	BC134610	107
COX2	F: TGTGAAAGGGAGGAAAGAGC R: GGCAAAGAARGCAAACATCA	AF004944	115
GAPDH	F: CACCCTCAAGATTGTCAGCA R: GGTCATAAGTCCCTCCACGA	BC102589	103