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—Original Article—

The prostaglandin E_2 receptor PTGER2 and prostaglandin $F_{2\alpha}$ receptor PTGFR mediate oviductal glycoprotein 1 expression in bovine oviductal epithelial cells

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Abstract. Oviductal glycoprotein 1 (OVGP1), an oviductin, is involved in the maintenance of sperm viability and motility and contributes to sperm capacitation in the oviduct. In this study, the regulatory effects exerted by prostaglandin E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) on OVGP1 expression via their corresponding receptors in bovine oviductal epithelial cells (BOECs) were investigated. BOECs were cultured *in vitro*, and their expression of receptors of PGE_2 (PTGER1, PTGER2, PTGER3, and PTGER4) and $PGF_{2\alpha}$ (PTGFR) was measured using RT-qPCR. Ca^{2+} concentration was determined with a fluorescence-based method and cAMP was quantified by enzyme-linked immunosorbent assays to verify activation of PTGER2 and PTGFR by their corresponding agonists in these cells. OVGP1 mRNA and protein expression was measured using RT-qPCR and western blotting, respectively, following PTGER2 and PTGFR agonist-induced activation. PTGER1, PTGER2, PTGER4, and PTGFR were found to be present in BOECs; however, PTGER3 expression was not detected. OVGP1 expression was significantly promoted by 10^{-6} M butaprost (a PTGER2 agonist) and decreased by 10^{-6} M fluprostenol (a PTGFR agonist). In addition, 3 μM H-89 (a PKA inhibitor) and 3 μM U0126 (an ERK inhibitor) effectively inhibited PGE_2-induced upregulation of OVGP1, and 5 μM chelerythrine chloride (a PKC inhibitor) and 3 μM U0126 negated OVGP1 downregulation by $PGF_{2\alpha}$. In conclusion, this study demonstrates that OVGP1 expression in BOECs is enhanced by PGE_2 via PTGER2-cAMP-PKA signaling, and reduced by $PGF_{2\alpha}$ through the PTGFR-Ca^{2+}-PKC pathway.

Key words: Oviduct, Oviductal glycoprotein 1 (OVGP1), Prostaglandin E_2 (PGE_2), Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), PTGER2, PTGFR

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Prostaglandins (PGs), a family of bioactive lipids including PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 and thromboxane A_2, are produced from arachidonic acid by cyclooxygenase-1 (COX-1), COX-2, and specific PG synthases [1–3]. PGs play important roles in many reproductive processes, such as ovulation, menstruation, fertilization, implantation, and parturition [2, 4]. Wijayagunawardane *et al*. have demonstrated that PGE_2 and $PGF_{2\alpha}$ can be secreted by bovine oviductal epithelial cells (BOECs) [5]. $PGF_{2\alpha}$ is involved in uterine smooth muscle contraction [6] and contributes to the retention of sperm at the junction of the uterus and oviduct, whereas PGE_2 interacts with the hypothalamus and causes the secretion of luteinizing hormone [7]. In early childbirth, the level of $PGF_{2\alpha}$ in the amniotic fluid increases significantly [8], and $PGF_{2\alpha}$ receptor (PTGFR)-knockout mice cannot initiate birth [9]. Studies have shown that binding of $PGF_{2\alpha}$, PGI_2, and PGE_2 to their receptors is important in the regulation of smooth muscle contraction and secretion active substance in human oviducts [10]. In addition, such binding has significant functions in gamete transmission, fertilization, and early embryo development through suitable constriction in the oviduct [11]. Together, PGs are among the most important regulatory factors underlying the activities of the oviduct.

As a reproductive organ, the oviduct is key in the regulation of mammalian reproduction, creating a microenvironment facilitating gamete and early embryo development [12]. A family of molecules known as oviductins, including oviductal glycoprotein 1 (OVGP1), are among the various factors secreted by oviductal epithelial cells [13]. These molecules contribute to maintaining the viability and motility of bovine sperm, and possibly modulate their capacitation [14]. It is unknown whether there exists an association between PGs and OVGP1 secretion by oviductal epithelial cells. Therefore, in this study, the expression of receptors of PGE_2 (PTGERs) and $PGF_{2\alpha}$ in BOECs was measured, and the effects of PTGER2 and PTGFR activation on OVGP1 expression in these cells were investigated *in vitro*.

Materials and Methods

Ethics statement

All of the animal studies in the present work were conducted in

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Table 1 Primers used in this study

Genes		Nucleotide sequence (5'-3')	Length (bp)	GenBank accession number
β-actin	Forward	5'CCAAGGCCAACCGTGAGAAGAT3'	256	NM_173979.3
	Reverse	5'CCACGTTCCGTGAGGATCTTCA3'		
OVGP1	Forward	5' CACCTCCTCAAAGCCTCACAGA 3'	194	NM_001080216.1
	Reverse	5' TCATAGCCAACCCACTCCTTCC 3'		
PTGER1	Forward	5' TGGTGGTGGTGCTGGCTGTC3'	221	NM_001192148
	Reverse	5'GCTGGCCTCCCAAGGTGCTCTTGGTTT3'		
PTGER2	Forward	5'GGAGCGCTACCTAGCCATC3'	229	AF539402.1
	Reverse	5'GATGAGCAACAGCAGCAGAG3'		
PTGER3	Forward	5' CAGTATGGCAAAGGCAGAA3'	288	NM_181032.1
	Reverse	5'CCGCACTGGTACTCAAGC3'		
PTGER4	Forward	5'CGGTGATGTTCATCTTCGG3'	302	NM_174589.2
	Reverse	5'GTAGGCGTGGTTGATGGC3'		
PTGFR	Forward	5'GCAGACCAAGCACAGTGAAA3'	151	NM_181025.3
	Reverse	5'CTGACAGCCAACCACGTATG3'		

accordance with the experimental practices and standards approved by the Animal Welfare and Research Ethics Committee of Inner Mongolia Agricultural University (Approval ID: 2011-1).

Cell culture and treatments

Fresh oviducts were provided by a local abattoir on the day of slaughter, being transported to the laboratory in ice-cold physiological saline within 4 h. The cows were judged to be in proestrus based on previous evidence that PGE_2 and $PGF_{2\alpha}$ secretion levels are lower during this stage than other stages of the estrous cycle [15]. The ampullae of the oviducts were cut open longitudinally and washed once with sterile PBS, before being washed three times with PBS containing penicillin-streptomycin (100 IU/ml). The lumens of 10 oviducts were then gently scraped. In a previous study, we found that the smooth muscle of the ampulla, rather than the isthmus, exhibits stronger contraction and PG secretion [16]. The harvested cells were washed three times with PBS and trypsinized for 3 min, which was terminated with phenol red-free Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Gaithersburg, MD) containing 15% fetal calf serum (FCS). Cells were seeded at 1×10^5 ml^{-1} in DMEM containing 15% FCS. Once an 85% confluent monolayer had formed, the cells were trypsinized and re-plated. To eliminate any effects of endogenous PGs and other hormones, fourth-generation cells were used in the experiments. Cell viability was determined by trypan blue exclusion, and the proportion of viable BOECs in cultures used for experiments was always greater than 90%. Cell type was verified using the epithelial cell-specific marker cytokeratin, for which 90% of the cultured cells were positive.

Fourth-generation BOECs were plated in dishes [12-well dishes for mRNA extraction and enzyme-linked immunosorbent assays (ELISAs), 96-well dishes for Ca^{2+} measurement, and 6-well dishes for protein assays]. When the cell monolayer reached approximately 90% confluency, the culture supernatant was replaced with serum- and phenol red-free medium. After incubating the cells for 12 h, control BOECs were cultured in parallel. Then, after 2, 4, 8, 16, 24, and 48 h, the cultures were tested for OVGP1 expression. Butaprost (a PTGER2 agonist, 10^{-6} M) (Cayman Chemical, Ann Arbor, MI) and fluprostenol (a PTGFR agonist, 10^{-6} M) (Cayman) were added to the experimental BOECs cultures, and the experimental and control BOECs were cultured in separate dishes in parallel. Expression of OVGP1 was subsequently measured after 2, 4, 8, 16, 24, and 48 h and compared to that of the cells cultured in the absence of receptor agonists. H-89 (a PKA inhibitor, 3 μM) (Sigma-Aldrich, St. Louis, MO), chelerythrine chloride (a PKC inhibitor, 5 μM) (Sigma-Aldrich), and U0126 (an ERK inhibitor, 3 μM) (Sigma-Aldrich) were added to the experimental BOECs cultures, and the experimental and control BOECs were then cultured in separate dishes in parallel. OVGP1 expression was determined after 4 h and compared with that of cells cultured in the absence of inhibitors.

RNA extraction, reverse transcription, and qPCR

Total RNA was extracted from cells using RNAiso Plus (Takara, Kusatsu, Japan) according to the manufacturer's instructions at 2, 4, 8, 16, 24, and 48 h post-stimulation, and treated with DNase I to prevent DNA contamination. cDNA was then prepared using a PrimeScript™ RT Reagent Kit (Takara). qPCR was performed using SYBR Premix Ex Taq™ II (Takara), 500 ng cDNA, and 400 nM primers. The composition of the qPCR mixture was based on the SYBR Premix Ex Taq™ II manufacturer's instructions, and was as follows: 12.5 μl SYBR Premix Ex Taq™ II, 1 μl forward primer, 1 μl reverse primer, 8.5 μl ddH_2O, and 2 μl cDNA. qPCR conditions consisted of an initial denaturation at 95°C for 30 sec, followed by 40 amplification cycles (95°C for 5 sec and 60°C for 34 sec) (n = 6). cDNA levels were determined with the $2^{-\Delta Ct}$ method, using β-actin as an internal control. Sequences of the primers employed in these assays, which were designed with Primer5.0 software (Premier company, Canada), are listed in Table 1.

Western blotting

Proteins were extracted from fourth-generation cells for western blotting. In brief, cells were washed in physiological salt, treated with 300 μl cell lysis buffer containing 1% proteinase inhibitors, and

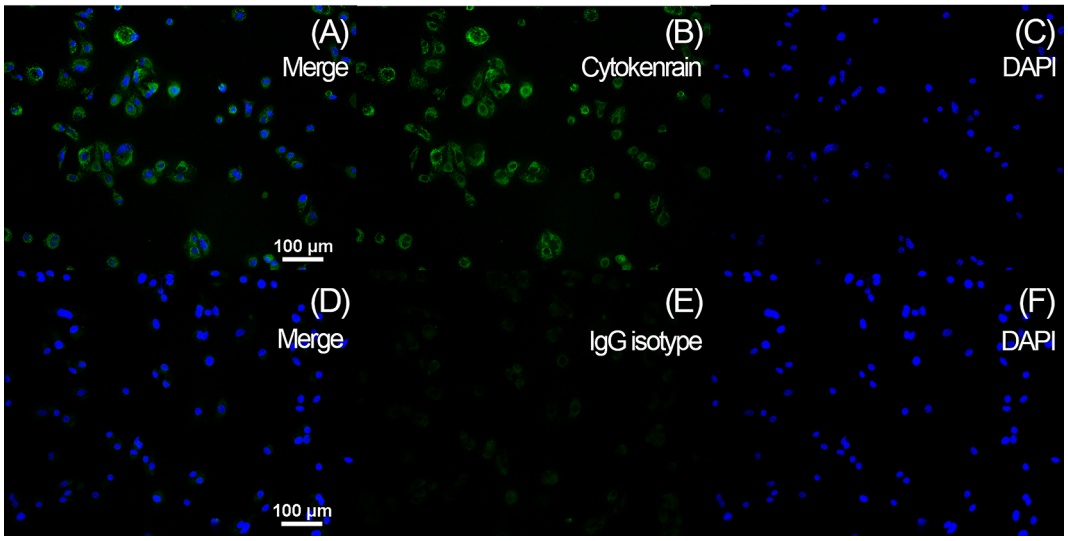

Fig. 1. Identification of cultured bovine oviductal epithelial cells (BOECs) by fluorescence microscopy. Upper panels: (A) merge of cytokeratin (green) and nuclear (blue) staining; (B) cytokeratin (epithelial cell marker, green) staining in BOECs; (C) nuclear staining (DAPI, blue). Lower panels: (D) Merge of IgG isotype control and nuclear staining; (E) IgG isotype control staining; (F) nuclear staining (DAPI, blue).

centrifuged at 14,000 *g* for 10 min at 4°C to obtain the supernatant. Protein concentration was measured with the bicinchoninic acid (BCA) method. The samples (containing 80–100 μg protein) (n = 4) were mixed with sample buffer (5% SDS, 5% dithiothreitol, 50 mM Tris-HCl, and 50% glycerol; pH 6.8) at a ratio of 1:5, and heated at 100°C for 5 min before being subjected to SDS-PAGE on 10% gels for 2 h at 120 V. The proteins in the gels were electroblotted onto PVDF membranes for 90 min at 350 mA. After blocking for 2 h, the membranes were incubated overnight at 4°C with polyclonal primary antibodies against OVGP1 (diluted 1:200; sc-46432; Santa Cruz Biotechnology, Dallas, TX) and β-actin (diluted 1:1,000; ab16039; Abcam, Cambridge, UK). Horseradish peroxidase (HRP)-conjugated donkey anti-goat (Santa Cruz Biotechnology) and HRP-conjugated goat anti-mouse secondary antibodies (Cell Signaling Technology, MA) diluted 1:3,000 and SuperSignal West Femto (Thermo Scientific, Waltham, MA) chemiluminescent substrate were used to detect bound primary antibodies. High-performance chemiluminescence film was subsequently exposed to the membranes, and protein band densities were analyzed using ImageJ software (please see https://imagej.net/Citing).

Immunofluorescence

Fourth-generation BOECs were seeded in 6-well slides. When the cell monolayers reached approximately 90% confluency, they were fixed with cold acetone for 15 min and washed with PBS. The cells were subsequently blocked with 10% goat serum and incubated with a primary antibody against cytokeratin (ab668; Abcam) at 4°C overnight, followed by an Alexa Fluor® 488-conjugated goat anti-mouse secondary antibody (Abcam) for 2 h. A mouse IgG isotype control (R&D Systems, Minneapolis, MN) was used in this study.

Effects of PGE_2 and $PGF_{2\alpha}$ on Ca^{2+} concentration

Ca^{2+} concentration was measured after butaprost and fluprostenol treatment of fourth-generation BOECs. Cells were incubated with the fluorescent indicator fluo3-AM (3 μM; s1056; Beyotime, Shanghai, China) at 37°C for 30 min, and subsequently washed three times with PBS to remove any excess. Next, 100 μl PBS was added, and the cells were examined using a multimode reader at 37°C with excitation at λ = 488 nm and emission at λ = 528 nm. The following formula was then applied: $[Ca^{2+}] = Kd[(F-Fmin)/(Fmax-F)]$, where Kd = 450,(Kd means the dissociation constant and F means fluorescence).

Effects of PGE_2 and $PGF_{2\alpha}$ on cAMP concentration in BOECs

Fourth-generation BOECs were incubated at 37°C for 1 h with medium containing 10^{-3} M IBMX(3-Isobutyl-1-methyxanthine). Following application of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, or 10^{-5} M PGE_2 or butaprost for 5 min (with the exception of the control BOECs, to which no drugs or medium were added), liquid nitrogen was used to terminate the reaction, and the solution was removed by washing three times with PBS. The cells were then exposed to 1 ml 1 M HCl for 20 min, before being transferred to a centrifuge tube and centrifuged at 1,200 *g* for 10 min to obtain the supernatant. cAMP concentration was measured with a cAMP EIA Kit (581001; Cayman Chemical). The concentration of protein in each sample was measured using the BCA method and used as an internal reference.

Statistical analysis

All data are presented as means ± standard errors of the mean. Statistical significance was analyzed using one-way analysis of variance followed by post hoc analysis (Dunnett's test for the data in Fig. 2B, C, and D and Fig. 3A, B, C, and D; Tukey's test for those in Fig. 3E and F and Fig. 4). P-values less than 0.05 were considered to indicate significant differences. GraphPad Prism 5

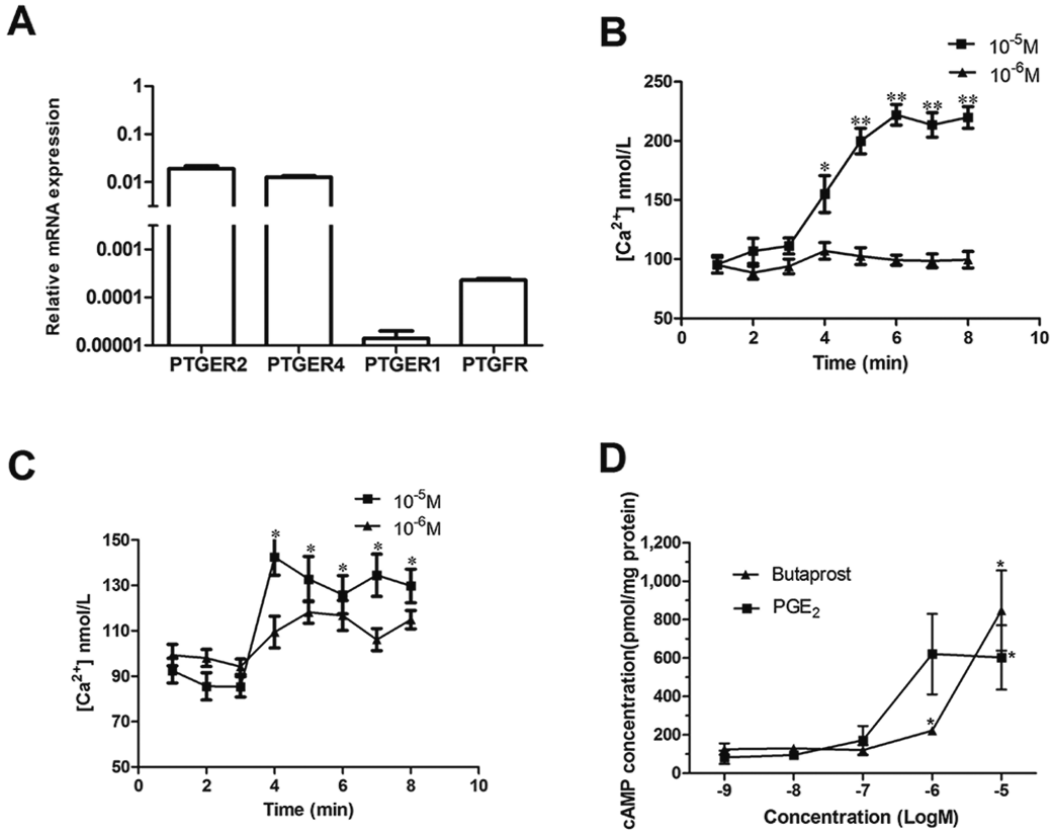

Fig. 2. Expression of prostaglandin (PG) receptors in bovine oviductal epithelial cells (BOECs) was measured using RT-qPCR (A). Effects of PGE_2 (B) and $PGF_{2\alpha}$ (C) on $[Ca^{2+}]$ in BOECs. Effects of PGE_2 and butaprost on cAMP levels in BOECs (D). Data are means ± standard errors of the mean from four independent experiments; * $P < 0.05$, ** $P < 0.01$.

software (GraphPad Software, La Jolla, CA) was used to conduct the statistical analysis.

Results

Cultured BOEC morphology

As shown in Fig. 1, BOECs were found to be polygonal or irregularly shaped, with clear borders and closely packed, granular cytoplasm. Their nuclei were large and circular.

Expression of receptors of PGE_2 and $PGF_{2\alpha}$ in BOECs

PTGER1, PTGER2, PTGER4, and PTGFR were found to be present in BOECs (Fig. 2A); however, PTGER3 expression was not detected (not shown). Whereas 10^{-5} M PGE_2 significantly increased Ca^{2+} concentration in these cells ($P < 0.01$), 10^{-6} M PGE_2 had little to no effect in this regard (Fig. 2B). Furthermore, 10^{-6} M and 10^{-5} M $PGF_{2\alpha}$ significantly raised the concentration of Ca^{2+} in BOECs ($P < 0.05$) (Fig. 2C), suggesting the existence of Gq protein-coupled receptors in these cells, and implying that PGE_2 and $PGF_{2\alpha}$ increased Ca^{2+} concentration through activation of their corresponding receptors. The concentration of cAMP in BOECs significantly increased as the concentration of PGE_2 was raised. Administration of 10^{-5} and 10^{-6} M PGE_2 or 10^{-5} M butaprost resulted in significantly heightened BOEC cAMP concentration (Fig. 2D).

PTGER2 and PTGFR mediate OVGP1 expression in BOECs

To determine whether the receptors PTGER2 and PTGFR mediate regulation of OVGP1 expression in BOECs, we treated these cells with selective receptor agonists and antagonists to evaluate their effects on OVGP1 mRNA and protein expression. No significant difference ($P < 0.05$) in the expression of OVGP1 was noted between the treatment groups at 0 h and the control group at any time point (data not shown). Therefore, the 0 h time point was used as the control. Expression of OVGP1 mRNA and protein was significantly increased 4 h and 8 h following treatment with 10^{-6} M butaprost (a PTGER2 agonist) compared with the control (Fig. 3A and 3B). In addition, 10^{-5} M AH6809 (a PTGER2 antagonist) significantly inhibited the upregulation of OVGP1 mRNA and protein induced by treatment with butaprost for 4 h (Fig. 3E and 3F). These results suggest that PGE_2 promotes OVGP1 expression through activation of PTGER2 in BOECs. In contrast, addition of 10^{-6} M fluprostenol (a PTGFR agonist) led to significant suppression of OVGP1 mRNA and protein expression at all tested time points, most notably so at 2 h and 4 h ($P < 0.01$, Fig. 3C and 3D). Moreover, this fluprostenol-induced downregulation of OVGP1 mRNA and protein was blocked to a significant extent by 10^{-5} M AL8810 (a PTGFR antagonist; Fig. 3E

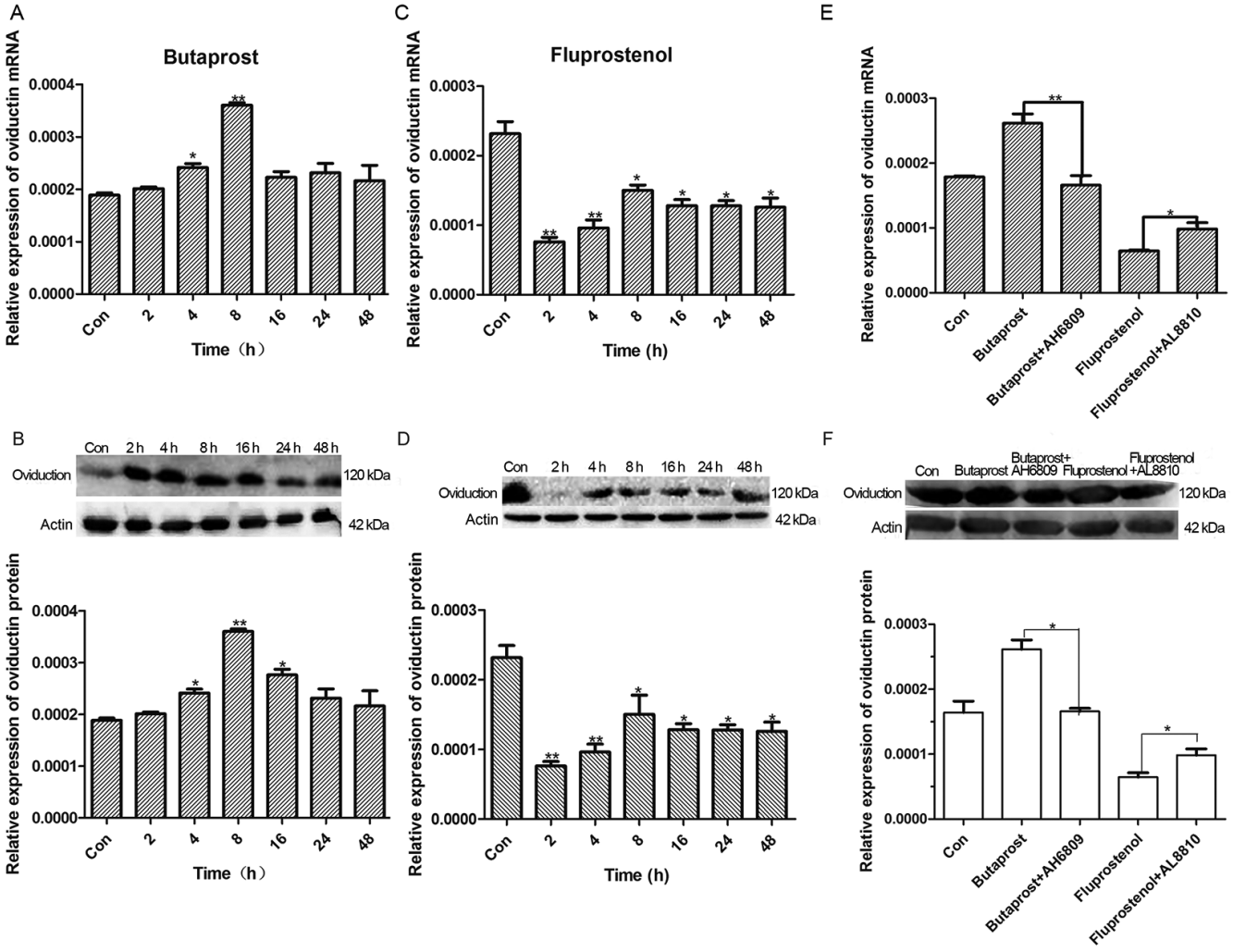

Fig. 3. PTGER2 and PTGFR mediate OVGP1 expression in bovine oviductal epithelial cells. Effects of 10^{-6} M butaprost on OVGP1 mRNA (A) and protein expression (B). Effects of 10^{-6} M fluprostenol on OVGP1 mRNA (C) and protein expression (D). Effects of AH6809 and AL8810 (both 10^{-6} M) on OVGP1 mRNA (E) and protein expression (F). Data are means ± standard errors of the mean from four independent experiments; * $P < 0.05$, ** $P < 0.01$. Con, control.

and 3F). These results suggest that the interaction between $PGF_{2\alpha}$ and PTGFR results in inhibition of OVGP1 expression.

Effects of PKA, PKC, and ERK inhibitors on PGE_2- and $PGF_{2\alpha}$-mediated OVGP1 expression in BOECs

To identify the intracellular signal transduction pathways involved in PGE_2- and $PGF_{2\alpha}$-mediated OVGP1 regulation in BOECs, we examined the effects of PKA, PKC, and ERK inhibitors on PGE_2- and $PGF_{2\alpha}$-induced OVGP1 expression. PGE_2 significantly increased OVGP1 mRNA (4 h) and protein (8 h) expression in BOECs, and addition of 3 μM H-89 (a PKA inhibitor) or 3 μM U0126 (an ERK inhibitor) markedly inhibited this effect (Fig. 4A and 4B). These results suggest that OVGP1 expression was promoted by PGE_2 via the PTGER2-cAMP-PKA-ERK pathway. In contrast, $PGF_{2\alpha}$ significantly decreased expression of OVGP1 mRNA (4 h) and protein (8 h) in BOECs, similar to the effects of fluprostenol, and addition of 5 μM chelerythrine chloride (a PKC inhibitor) or 3 μM U0126 effectively blocked this $PGF_{2\alpha}$-induced suppression of OVGP1 expression (Fig. 4C and 4D). This indicates that expression of OVGP1 was restricted by $PGF_{2\alpha}$ through the PTGFR-Ca^{2+}-PKC-ERK pathway.

Discussion

In the present study, we evaluated the effects of the PTGER2 agonist butaprost and the PTGFR agonist fluprostenol on OVGP1 mRNA and protein levels in BOECs using RT-qPCR and western blotting, respectively. Our results showed that butaprost and fluprostenol significantly promoted and suppressed OVGP1 expression in BOECs, respectively. Moreover, PGE_2 was shown to promote expression of OVGP1 through its receptor PTGER2, and $PGF_{2\alpha}$ to exert a down regulatory effect on OVGP1 via activation of its receptor PTGFR. Furthermore, the possibility that PGE_2 and $PGF_{2\alpha}$ affect OVGP1

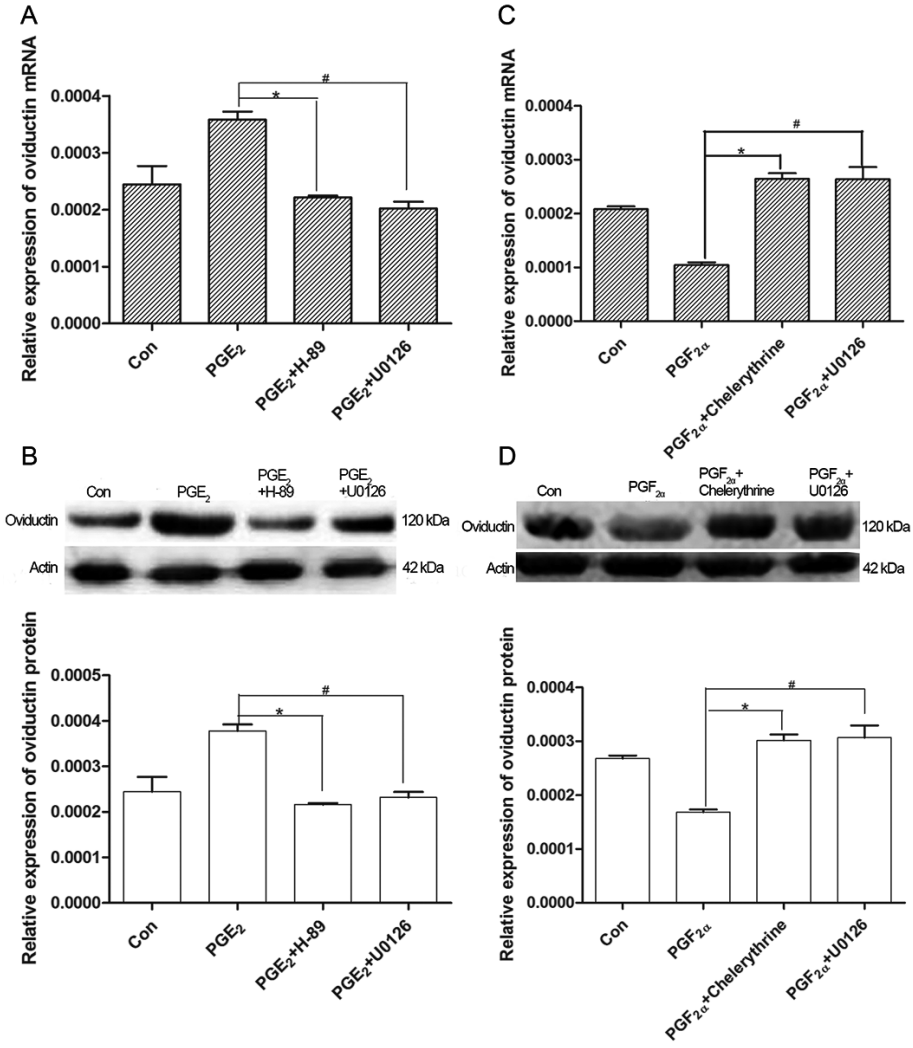

Fig. 4. Effects of PKA, PKC, and ERK inhibitors on PGE_2- and $PGF_{2\alpha}$-mediated changes in OVGP1 expression in bovine oviductal epithelial cells. Effect of PKA and ERK inhibitors on OVGP1 mRNA (A) and protein expression (B). Effect of PKC and ERK inhibitors on OVGP1 mRNA (C) and protein expression (D); * and # $P < 0.05$. Con, control.

through the PTGER2-cAMP-PKA and PTGFR-Ca^{2+}-PKC signaling pathways, respectively, has been strengthened by our data.

OVGP1 is the primary component of oviduct secretions. Mugnier *et al*. have demonstrated that oviductal epithelial cell secretions can increase the rate of *in vitro* fertilization in horses, and have speculated that OVGP1 is involved in the fertilization process [17]. This conclusion has also been reached by Goncalves *et al*. [18], who indicated that OVGP1 also plays an important role in fertilization in the cow oviduct. Transferal of sperm to the oviduct stimulates oviductal epithelial cells to secrete high levels of PGs, which may target either sperm or oviductal epithelial cells themselves [19, 20]. However, no reports indicating the expression of receptors of PGE_2 and $PGF_{2\alpha}$ on sperm cells have been published to our knowledge, suggesting that PGE_2 and $PGF_{2\alpha}$ secreted by oviductal epithelial cells act on these cells themselves, or on targets such as oviductal stromal cells and fertilized eggs. Oviductal epithelial cells play important roles in reproduction, including in fertilization and early embryonic development. They also serve as a model for studies of the oviduct and related areas of research [21]. In the current work, we cultured BOECs *in vitro* (Fig. 1) and used them to analyze the effect of receptors of PGE_2 and $PGF_{2\alpha}$ on OVGP1 expression.

PGE_2 and $PGF_{2\alpha}$ are found in semen, cervical fluid, and the oviduct. PGE_2 receptors are divided into four subtypes: PTGER1, PTGER2, PTGER3, and PTGER4, whereas only one $PGF_{2\alpha}$ receptor has been identified (PTGFR) [22]. Our results indicated that PTGER1, PTGER2, PTGER4, and PTGFR were present in BOECs. Furthermore, PTGER2 and PTGER4 were highly expressed in these cells, whereas PTGER1 and PTGFR expression was low. As mentioned above, $PGF_{2\alpha}$, PGE_2, and butaprost significantly increased intracellular Ca^{2+} and cAMP levels (Fig. 2), suggesting that PTGER2 and PTGFR are functional in BOECs. Although PTGER1 and PTGER4 are expressed in oviductal epithelial cells, knockout studies in mice have shown that

PTGER2, rather than PTGER1, PTGER3, or PTGER4, is associated with reproductive dysfunction [23]. Therefore, in the present work, we primarily focused on the effect of PGE_2 and activation of its receptor PTGER2 on OVGP1 expression in oviductal epithelial cells. In order to clarify the nature of OVGP1 regulation by PGE_2 and $PGF_{2\alpha}$, we determined the effects of PTGER2 and PTGFR on OVGP1 synthesis in BOECs.

PGE_2 is the main hormone regulating early and late egg growth in the uterus [24]. Throughout gestation, regulation by PGE_2 predominates, influencing the effects of OVGP1. OVGP1 expression increases during the estrus cycle, but this increase is transient and $PGF_{2\alpha}$ is required to adjust the balance. PGE_2 possesses luteoprotective properties, whereas $PGF_{2\alpha}$ is a luteolysin in ruminants [25, 26]. Other similar studies have suggested that 24 h after the effects of estrogen are observed, maintenance of PGE_2 levels and a decrease in $PGF_{2\alpha}$ concentration lead to an increased $PGE_2/PGF_{2\alpha}$ ratio [27]. Kaczmarek *et al*. reported that seminal plasma affects PGE_2 and $PGF_{2\alpha}$ synthesis in the porcine oviduct to increase this ratio [20]. PGE_2 and $PGF_{2\alpha}$ have opposing effects, but it has been suggested that their expression is coordinated to achieve a balance *in vivo*. It has also been proposed that PGF2α significantly stimulated intraluteal $PGF_{2\alpha}$ production in all luteal phases, but did not affect PGE2 production [28]. PGE_2 rapidly increased TbetaRIII mRNA and protein expression and enhanced TbetaRIII gene promoter activity ctivity, whereas $PGF_{2\alpha}$ does not [29]. De Moraes *et al*. reported that PTGFR mRNA expression is stable during pregnancy in cows, and lower in rats postpartum [30]. The results of the present study indicate that in BOECs, PGE_2 promotes OVGP1 expression via activation of PTGER2, and $PGF_{2\alpha}$ downregulates OVGP1 via PTGFR activation. The different regulatory effects of PGs on OVGP1 expression might contribute to the maintenance of a balanced state within the oviduct.

The inducible COX-2 enzyme modulates PGE_2-PTGER2-cAMP-PKA singling [31]. In addition, PGE_2 has been reported to induce VEGF expression through activation of the PGE_2-Gas-cAMP-PKA signaling pathway [32]. Via PTGFR, $PGF_{2\alpha}$ upregulates COX-2 expression through PKC activation in human amniotic fibroblasts [33] and activates the PLC system during the mid-luteal phase [34]. The simultaneous activation of PTGER2 and PTGFR amplifies cAMP release through the PTGFR- Gq protein-Ca^{2+} signaling pathway [32]. Therefore, we speculate that PGE_2 promotes OVGP1 expression via PTGER2 activation and the cAMP-PKA signaling pathway, while $PGF_{2\alpha}$ downregulates OVGP1 via PTGFR activation and Ca^{2+}-PKC signaling. Our results showed that PGE_2-induced OVGP1 expression was significantly suppressed by H-89 and U0126 treatment, suggesting that OVGP1 in BOECs is regulated through the PGE_2-PTGER2-cAMP-PKA pathway and resulting activation of ERK signaling. Moreover, reduction of OVGP1 expression by $PGF_{2\alpha}$ in BOECs was found to be negated by administration of chelerythrine chloride and U0126, indicating that the $PGF_{2\alpha}$-PTGFR-Ca^{2+}-PKC pathway and simultaneous regulation of ERK activation are also involved in OVGP1 regulation in these cells. Together, these findings contribute to clarifying the molecular mechanisms underlying the effect of PGE_2 and $PGF_{2\alpha}$ on OVGP1 synthesis.

In conclusion, this study supports the idea that in BOECs, PGE_2 promotes OVGP1 expression via activation of PTGER2, whereas $PGF_{2\alpha}$ downregulates OVGP1 by activating PTGFR. In these cells, the upregulatory effect of PGE_2 on OVGP1 is exerted via PTGER2-cAMP-PKA signaling, and is counterbalanced by $PGF_{2\alpha}$ through the PTGFR-Ca^{2+}-PKC pathway. These results represent an essential next step in understanding the regulatory effects of these PGs on the mammalian oviduct and changes in the biological components of oviduct fluid.

Conflict of Interest: There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Acknowledgements

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