

## Genomic and non-genomic effects of progesterone and pregnenolone on the function of bovine endometrial cells

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**ABSTRACT:** Progesterone ( $P_4$ ) decreases oxytocin (OT)-stimulated prostaglandin ( $PG$ ) $F_{2\alpha}$ , but not  $PGE_2$  secretion from bovine endometrial cells and this effect is partly elicited via a non-genomic route. The aim of this study was to determine whether  $P_4$  and pregnenolone ( $P_5$ ), in the presence or absence of OT, influence: (a) the gene expression of enzymes responsible for PG synthesis: cyclooxygenase-2 (COX-2), synthase of  $PGF_{2\alpha}$  (PGFS) and  $PGE_2$  (PGES), (b) protein expression of COX-2, PGFS and PGES, and (c)  $P_4$  receptor membrane component 1 (PGRMC1) gene expression in bovine endometrial cells. The epithelial endometrial cells ( $2.5 \times 10^5/ml$ ) from Days 14–16 of the oestrous cycle were incubated for 72–96 h to attach the cells to the bottom of a well. Next, the cells were preincubated for 30 min with  $P_4$  and  $P_5$  ( $10^{-5}M$  each) and incubated for 4 h and 6 h alone or with OT ( $10^{-7}M$ ). Thereafter, the medium was collected for  $PGE_2$  and PGFM determination, while cells were harvested for gene and protein expression analysis. The used steroids: (a) inhibited OT-stimulated  $PGF_{2\alpha}$ , but not  $PGE_2$  secretion from endometrial cells, (b) did not affect the expression of mRNA for COX-2, PGFS, PGES and PGRMC1 in endometrial cells after 4 and 6 h, (c) they decreased OT-stimulated COX-2 mRNA expression only after 6 h incubation, and (d) did not influence COX-2, PGFS and PGES protein expression after 6 h. These results indicate that  $P_4$  and  $P_5$  inhibit OT-stimulated secretion/production of luteolytic  $PGF_{2\alpha}$  by a transcription-independent mechanism and partly by down-regulation of COX-2 mRNA.

**Keywords:** non-genomic effect; progesterone; progesterone membrane receptor component 1; endometrium; bovine

Prostaglandins (PGs) produced in the uterus play an important role in the regulation of the oestrous cycle and the successful implantation of blastocysts in many species, including cattle (McCracken et al., 1999; Goff, 2004; Fortier et al., 2008). During the bovine oestrous cycle, Days 15–17 are crucial for the initiation of luteolysis or for maternal recognition of pregnancy (McCracken et al., 1999; Niswender et al., 2000). At this time, endometrial  $PGF_{2\alpha}$  is released in a pulsatile manner in the first case (McCracken et al., 1999), while  $PGE_2$  acts as

luteotropic and antiluteolytic factor preventing luteolysis during early pregnancy (Goff, 2004). Both these PGs are synthesized in endometrial cells from arachidonic acid, which is converted into prostaglandin  $H_2$  ( $PGH_2$ ) by cyclooxygenase (COX-1 and COX-2). Next,  $PGH_2$  is metabolized to  $PGF_{\alpha}$  and  $PGE_2$  by PGF synthase (PGFS) and PGE synthase (PGES), respectively (Goff, 2004; Fortier et al., 2008). In the bovine endometrium, a change in COX-1 mRNA levels and protein product has not been detected. However, a significant in-

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crease in COX-2 mRNA and protein expression has been detected on Days 13–21 of the estrous cycle (Arosh et al., 2002). Moreover, it was shown that oxytocin (OT) stimulated COX-2 mRNA and protein expression, but did not affect COX-1 mRNA expression in cows (Asselin et al., 1997; Parent et al., 2003). Thus, it is suggested that COX-2 is essential for OT influence on  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$  synthesis (Asselin et al., 1997). It has been discovered that the PGFS, involved in the production of  $\text{PGF}_{2\alpha}$  in bovine endometrium at the time of luteolysis, is aldoketoreductase 1B5 (AKR1B5) (Madore et al., 2003; Fortier et al., 2008). Moreover, it was found that microsomal PGES-1 (m-PGES1) is highly correlated with COX-2 (Murakami et al., 2002) and that this PGES is responsible for  $\text{PGE}_2$  synthesis in the bovine endometrium (Arosh et al., 2002; Parent et al., 2002).

Progesterone ( $\text{P}_4$ ) and oestrogens may directly affect the basal  $\text{PGF}_{2\alpha}$  secretion by the endometrium (Xiao et al., 1998; Goff, 2004). Moreover, these steroids can regulate endometrial responsiveness to OT and to other regulatory factors, and OT further stimulates  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$  secretion from endometrial cells of cows (Kim and Fortier, 1995). It has been demonstrated that  $\text{P}_4$  inhibits OT-stimulated  $\text{PGF}_{2\alpha}$  secretion from bovine endometrial cells via genomic (Skarzynski et al., 1999) and non-genomic mechanisms (Grazzini et al., 1998; Bogacki et al., 2002; Rekawiecki and Kotwica, 2007; Rekawiecki et al., 2008).

Recently, we showed that  $\text{P}_4$ , its precursor – pregnenolone ( $\text{P}_5$ ) and metabolite –  $17\beta$ -hydroxyprogesterone impaired OT-stimulated  $\text{PGF}_{2\alpha}$ , but not  $\text{PGE}_2$  secretion from endometrial cells on Days 14–16 of the oestrous cycle (Duras et al., 2005; Kowalik and Kotwica, 2007). This inhibitory effect appeared after short-term culture (4 h), so the effect of steroids could not be mediated through genomic mechanisms. It is possible that steroids indirectly suppress the transcription of specific genes, e.g., the OT receptor and/or PG synthase (Salomonsen et al., 1990; Raw et al., 1996). Steroids used in our studies also decrease intracellular  $\text{Ca}^{2+}$  mobilization, which plays an important role in mediating the stimulatory effect of OT on  $\text{PGF}_{2\alpha}$  secretion (Duras et al., 2005). This data suggests that different cellular mechanisms exist for steroids affecting secretion of both prostaglandins from endometrial epithelial cells. There is no clear explanation for this phenomenon, but one possibility is that the reduction in the  $\text{Ca}^{2+}$  response may affect enzymes

that control the ratio of  $\text{PGE}_2$  to  $\text{PGF}_{2\alpha}$  (Duras et al., 2005; Kowalik and Kotwica, 2007). It is also possible that the decrease of  $\text{Ca}^{2+}$  mobilization in endometrial cells is evoked by interaction of  $\text{P}_4$  with its membrane receptor – progesterone membrane receptor component 1 (PGRMC1) (Peluso, 2006).

Although the rapid, non-genomic effect of  $\text{P}_4$  on the function of endometrial cells has been well documented (Bogacki et al., 2002; Dunlap and Stormshak, 2004; Duras et al., 2005; Bishop and Stormshak, 2006; Franczak et al., 2006) the molecular mechanism of its action on the secretion of  $\text{PGE}_2/\text{PGF}_{2\alpha}$  in the bovine endometrial cells is poorly understood. The present study was undertaken to determine whether this inhibitory effect of  $\text{P}_4$  and  $\text{P}_5$  on OT-stimulated secretion  $\text{PGF}_{2\alpha}$  involved genomic or non-genomic mechanisms. Therefore, we studied whether  $\text{P}_4$  and  $\text{P}_5$ , with and without OT, have effects on: (a) mRNA expression of enzymes responsible for PG biosynthesis, COX-2, PGFS and PGES, (b) protein expression of COX-2, PGFS and PGES and (c) PGRMC1 mRNA expression in bovine endometrial cells from Days 14–16 of the estrous cycle.

## MATERIAL AND METHODS

### Tissue collection

Uteri were collected from a commercial slaughterhouse from mature heifers and nonpregnant cows on Days 14–16 of the estrous cycle and transported within 1 h to the laboratory in ice-cold phosphate buffered saline (PBS; 137mM NaCl; 27mM KCl; 10mM  $\text{Na}_2\text{HPO}_4$ ; 2mM  $\text{KH}_2\text{PO}_4$ ; pH 7.4). Days of the oestrous cycle were assessed by morphological observations of the reproductive tract (Fields and Fields, 1996). Uteri from 4 animals were collected for each experiment. All materials used in these studies were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise stated.

### Isolation of endometrial epithelial cells

Endometrial epithelial cells were isolated using a procedure described previously (Duras et al., 2005). The number of cells and cell viability were determined by means of 0.04% trypan blue exclusion, and only the cells with a viability of above 85% were used. The cells were suspended ( $2.5 \times$

$10^5$  cell/ml medium) in DMEM/Ham's-F12 medium with 10% FCS and 20  $\mu\text{g/ml}$  gentamycin added into each of 6-well plates (Nunc GmbH&Co. KG, Wiesbaden, Germany), and incubated (Heraeus BB-6060, Hanau, Germany) at 38°C in a humidified atmosphere of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ . Medium was changed after 48 and 72 h and after 96 h if necessary for the attachment of cells to the bottom of the well. Cells were then incubated in DMEM/Ham's-F12 medium containing 0.1% BSA and 20  $\mu\text{g/ml}$  gentamycin for 24 h.

**Experiment 1.** To study the mRNA expression of COX-2, PGFS and PGES, endometrial epithelial cells were preincubated for 30 min with:  $\text{P}_4$  ( $10^{-5}\text{M}$ ) or  $\text{P}_5$  ( $10^{-5}\text{M}$ ; Serva, Germany), and then the medium was supplemented with OT ( $10^{-7}\text{M}$ ). Cells incubated without hormones served as controls. After a 4 or 6 h incubation the medium was collected into tubes containing 10  $\mu\text{l}$  of solution (30  $\mu\text{M}$  EDTA, 1% acetylosalicylic acid), and stored (at  $-20^\circ\text{C}$ ) until analysis of  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  metabolite – PGFM concentrations. PGFM concentrations reflects 50% of the  $\text{PGF}_{2\alpha}$  secretion of the endometrium as reported earlier (Skarzynski et al., 1999) and both these terms are used in this paper. The cells were stored at  $-80^\circ\text{C}$  until RNA isolation.

**Experiment 2.** To establish the protein level for COX-2, PGFS and PGES in the endometrial epithelial cells, these were preincubated for 30 min with:  $\text{P}_4$  ( $10^{-5}\text{M}$ ; Serva, Germany), and then the medium was supplemented with OT ( $10^{-7}\text{M}$ ) or OT with  $\text{P}_4$ . Cells incubated without hormones served as the control. After 6 h incubation, the medium was collected for determination of PGFM and  $\text{PGE}_2$  concentration. Thereafter, 200  $\mu\text{l}$  of lysis buffer (0.25M sacharose, 10mM Tris, 0.1mM EDTA, pH 7.4) with Protease Inhibitor Cocktail was added into each well. The cells were scraped off, transferred into tubes and homogenized on ice with an ultrasound homogenizer (Sonic&Materials Inc, USA). The concentration of protein was determined by the Bradford method (1976) and the protein was used for Western blot analysis.

**Experiment 3.** To investigate mRNA expression for PGRMC1, the endometrial epithelial cells were incubated in the same way as in Experiment 1, but arachidonic acid (AA) was used as positive control for the cellular response as measured by prostaglandin secretion. After 4 or 6 h the medium was collected for PGFM and  $\text{PGE}_2$  determination, while the cells were stored at  $-80^\circ\text{C}$  for RNA isolation and semi-quantitative PCR.

## EIA of prostaglandins

Concentrations of PGFM were determined directly in medium by enzyme immunoassay (EIA) using horseradish peroxidase-labelled PGFM as a tracer (1 : 40 000; final dilution) and anti-PGFM serum (1 : 80 000) described previously (Homonics and Silvia, 1988). The standard curve ranged from 62.5 to 32 000  $\text{pg/ml}$ . The intra- and inter-assay coefficients of variation were 13.4% and 16.4%, respectively.

$\text{PGE}_2$  concentrations were determined also by EIA using horseradish peroxidase-labelled  $\text{PGE}_2$  (1 : 30 000; final dilution) and anti- $\text{PGE}_2$  serum (diluted 1 : 80 000). The standard curve ranged from 78 to 20 000  $\text{pg/ml}$ . The intra- and inter-assay coefficients of variation were 5.75% and 11.3%, respectively.

## RNA isolation and semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cells with the Total RNA Prep Plus Kit (A&A Biotechnology, Gdansk, Poland) according to the manufacturer instructions. Concentration and quality of RNA were determined spectrophotometrically (BioRad, Hercules, CA, USA). Total RNA (1  $\mu\text{g}$ ) was treated with DNase and reverse transcribed into cDNA in a total reaction volume of 20  $\mu\text{l}$  containing: 50mM Tris-HCl (pH 8.3), 75mM KCl, 3mM  $\text{MgCl}_2$ , 5 mM dithiothreitol, 10 mM dNTP mix, 1  $\mu\text{g}$  oligo(dT)23 primers (Fermentas, Vilnius, Lithuania), 200 IU of reverse transcriptase RevertAid<sup>TM</sup> M-MuLV (Fermentas, Vilnius, Lithuania). RNA was denatured at 70°C for 10 min. The subsequent RT reaction was carried out at 42°C for 60 min and terminated by heating for 10 min at 70°C. Based on the gene sequences available in GenBank, (NCBI), primers were designed using primer 3 software (<http://frodo.wi.mit.edu/primer3/input.htm>); for PGRMC1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Primer Express 3.0 software (Applied Biosystems, Foster City, CA, USA); for: COX-2, PGFS, PGES and 18 sRNA. These primers were synthesized by TIB MolBiol (Poznan, Poland). The sequences of the primers, the expected PCR product length and the number of cycles are shown in Table 1. Complementary DNA was amplified by a PCR reaction carried out in total volume of 25  $\mu\text{l}$  containing 12.5  $\mu\text{l}$  REDTaq ReadyMix

Table 1. Forward and reverse primer sequences, expected amplicon length and number of cycles used in PCR. Every primer set is designed according to the accession number in the The Entrez Nucleotide database

Primer	Sequence (forward/reverse)	Amplicon length (bp)	Number of cycles	Accession number
COX-2	5' GATCCCCAGGGCACAAATCT 3' 5' GGTGAAGTGCTGGGCAAAGA 3'	50	27	NM_174445
mPGES	5' TTCCTGGCAACTGGCTGAG 3' 5' AAACACACACAGGCCCCCT 3'	54	29	NM_174443
PGFS	5' CAATCGACCTTGGGTACCGT 3' 5' TCATTCTGGTACACGTGGGC 3'	51	28	S54973
PGRMC1	5' GCCTTTGCATCTTTCTGCTC 3' 5' TGGCTCCTCCTTGCTGAGT 3'	448	25	NM_001075133
18sRNA	5' GTTCGATTCCGGAGAGGGA 3' 5' CCTTCCTTGATGTGGTAGCC 3'	51	21	AF176811
GAPDH	5' TGTTCAGTATGATTCCACCC 3' 5' TCCACCACCCTGTTGCTGTA 3'	850	21	NM_001034034

PCR Reaction Mix, 2 µl cDNA, and 0.5 µl (50 µM) of both PCR primers for each studied gene. The optimal number of cycles ensuring the termination of amplification for studied genes in the lag phase was determined in a series of preliminary studies. The PCR profiles consisted of initial denaturation at 94°C for 3 min, an appropriate number of cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The PCR reaction products were separated by electrophoresis on a 2% agarose (Eurogentec, Seraing, Belgium) gel (PGRMC1 and GAPGH) or 15% polyacrylamide gel (COX-2, PGFS, PGES and 18sRNA) together with molecular mass standards (DNA, Gdansk, Poland). The intensity of each band was quantified using Kodak EDAS 290 Software (Rochester, New York, USA). The relative amounts of PCR products were estimated by normalizing the signal intensities of the gene products in relation to housekeeping gene products.

### Western Blots

Total cellular protein obtained from the endometrial cells (20 µg) was dissolved in SDS gel-loading buffer (50mM Tris-HCl, pH 6.8; 4% SDS, 20% glycerol, 2% β-mercaptoethanol), heated to 95°C for 5 min and separated on 10% (for COX-2 and PGFS) and 15% (for PGES) SDS-PAGE. Separated proteins were electroblotted onto Immobilon PVDF membrane (Millipore, USA) in transfer buffer (25mM Tris-HCl, pH 8.3; 192mM glycine;

20% methanol; 0.05% SDS). The nonspecific binding sites were blocked with 5% non-fat dry milk in TBS buffer (50mM Tris-HCl; 0.9% NaCl) containing 0.05% Tween-20, for 1.5 h at room temperature. Thereafter, the membrane was incubated overnight at 4°C with the primary polyclonal antibodies, diluted as follows: 1/250 rabbit COX-2, 1/250 rabbit PGES (Cayman Chemicals, Ann Arbor, MI), 1/750 rabbit PGFS (Kindly provided by Professor M.A. Fortier, Ontogeny and Reproduction Unit CHUQ-CHUL, Quebec, Canada). Next, the membrane was washed three times for 10 min in TBS-T buffer and incubated with secondary antibody (alkaline phosphatase-conjugated goat anti-rabbit IgG) for 1.5 h at room temperature at a dilution of 1/30 000. Immunoreactive bands were detected using a mixture of 5-bromo-4-chloro-3-indolyl phosphate and nitroterazolium blue chloride, as visualisation stock solution (NBC/BCIP with alkaline phosphatase assay buffer 0.1 M NaCl, 5 mM MgCl<sub>2</sub>, 100 mM Tris-HCl, pH 9.5). Western blots were quantitated using Kodak EDAS 290 Software (Eastman Kodak, Rochester, New York, USA).

### Statistical analysis

All data were expressed as means ± (S.E.M.). Prostaglandins concentrations were presented as percentages and compared to control values accepted as 100%. Due to the heterogeneity of the data they were transformed into logarithms (ln) before ANOVA analysis for repeated measures and

followed by the Newman-Keuls post test (PRISM, Graph Pad Software, San Diego, CA, USA).

**RESULTS**

**Experiment 1**

Oxytocin increased PGFM and PGE<sub>2</sub> concentration in culture medium after 4 h and 6 h ( $P < 0.05 - P < 0.001$ ) of incubation of endometrial cells (Figure 1). This indicates that the cells responded to physiological stimulation. Any of the used steroids affected the basal secretion of PGF<sub>2α</sub> (Figure 1a, b). However, both P<sub>4</sub> and P<sub>5</sub> decreased ( $P < 0.05$ ) the effect of OT on PGF<sub>2α</sub> secretion after 4 h (Figure 1a) and 6 h (Figure 1b). Only P<sub>5</sub> (Figure 1d) increased ( $P < 0.05$ ) the basal secretion of PGE<sub>2</sub> after 6 h of incubation. There was no observed inhibitory effect of the used steroids on OT-stimulated PGE<sub>2</sub> secretion ( $P > 0.05$ ) after 4 h (Figure 1c) and 6 h of

incubation (Figure 1d). All of the used treatments affected the expression of mRNA for COX-2 (Figure 2a), PGFS and PGES (data not shown) in bovine endometrial cells after 4 h of incubation. After 6 h of incubation OT only increased ( $P < 0.05$ ) the expression of COX-2, while applied steroids decreased ( $P < 0.05$ ) the effect of OT on COX-2 mRNA level in these cells (Figure 2b). All used treatments had an impact on PGFS and PGES expression after 6 h incubation of the cells (data not shown).

**Experiment 2**

Since P<sub>5</sub> impaired the effect of OT on gene expression with an intensity similar to P<sub>4</sub>, only the latter was used in this experiment. Oxytocin increased ( $P < 0.05$ ) PGFM and PGE<sub>2</sub> concentration in medium after 6 h of incubation (Figure 3). Progesterone did not affect basal PGFM and PGE<sub>2</sub> concentrations, but it decreased the effect of OT on the secretion of PGF<sub>2α</sub>

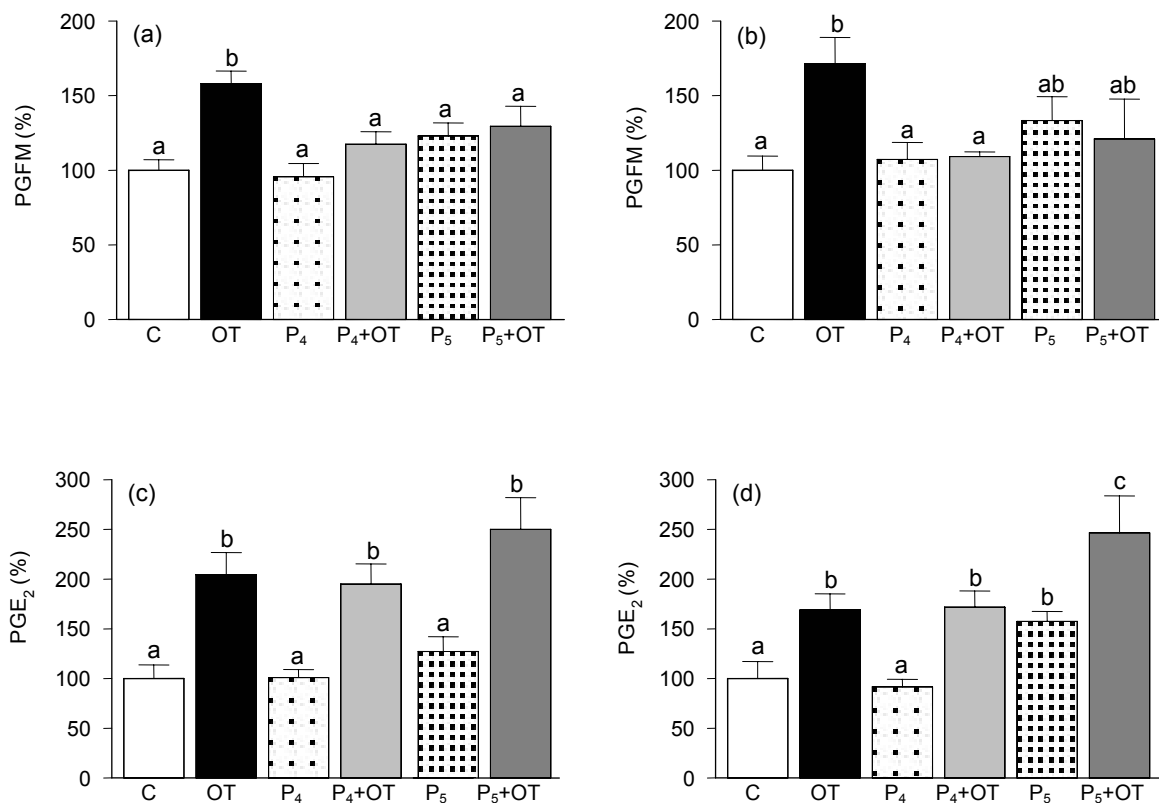


Figure 1. Mean (±SEM) concentration of PGFM (a), (b) and PGE<sub>2</sub> (c), (d) in medium incubated with endometrial cells on Days 14–16 of the oestrous cycle of the cows ( $n = 4$ ). The cells were pre-incubated for 30 min with progesterone (P<sub>4</sub>; 10<sup>-5</sup>M) and pregnenolone (P<sub>5</sub>; 10<sup>-5</sup>M), and next incubated 4 h (a),(c) or 6 h (b), (d) with oxytocin (OT; 10<sup>-7</sup>M), and OT together with each steroid. Values were compared to controls (100%). Bars with different superscripts are significantly different ( $P < 0.05$ )

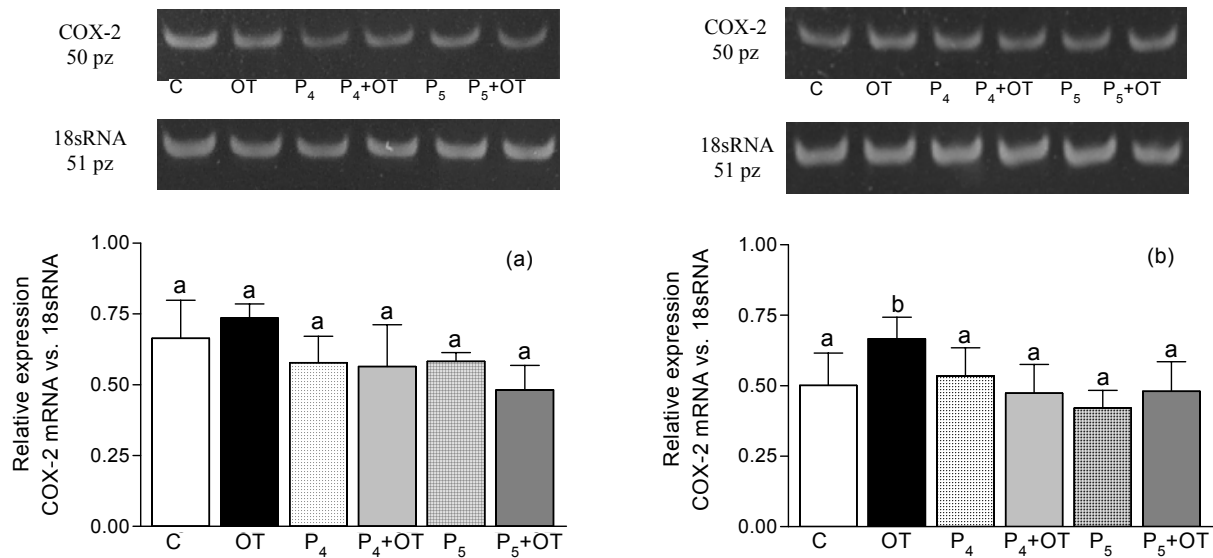


Figure 2. Mean ( $\pm$  SEM) expression of COX-2 mRNA levels in bovine endometrial cells after 4 h (a) and 6 h (b) incubation of cells with control medium (C), oxytocin (OT;  $10^{-7}$ M), progesterone (P<sub>4</sub>;  $10^{-5}$ M), pregnenolone (P<sub>5</sub>;  $10^{-5}$ M) and OT together with each steroids. All values are expressed as a ratio of COX-2 to 18sRNA ( $n = 4$ ). Different superscripts indicate significant differences ( $P < 0.05$ )

(Figure 3). However, none of the used treatments changed the levels of COX-2, PGFS and PGES protein in bovine endometrial cells from Days 14–16 of the estrous cycle after 6 h of incubation (Figure 4).

had an effect on the expression of PGRMC1 mRNA in endometrial cells after 4 h and 6 h incubation of the cells (Figure 5).

### Experiment 3

The expression of PGRMC1 mRNA was observed in the bovine endometrial cells from Days 14–16 of the estrous cycle (Figure 5). However, neither OT nor the used steroids separately or jointly with OT

### DISCUSSION

The treatment of bovine endometrial cells on Days 14–16 of the estrous cycle with OT increased PGF<sub>2 $\alpha$</sub>  and PGE<sub>2</sub> secretion after both 4 h and 6 h stimulation and this was the main criterion used to determine the usefulness of endometrial cells

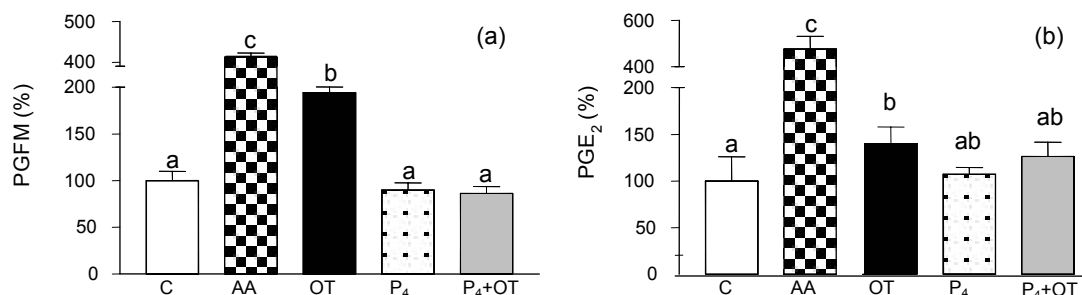


Figure 3. Concentration (mean  $\pm$  SEM) of PGFM (a) and PGE<sub>2</sub> (b) in medium incubated with endometrial cells on Days 14–16 of the oestrous cycle of the cows ( $n = 4$ ). The cells were pre-incubated for 30 min with progesterone (P<sub>4</sub>;  $10^{-5}$ M), and next incubated 6 h with arachidonic acid (AA;  $10^{-5}$ M), oxytocin (OT;  $10^{-7}$ M), and OT together with P<sub>4</sub>. Values were compared to controls (100%). Bars with different superscripts are different ( $P < 0.05$ ). Different superscripts indicate significant differences ( $P < 0.05$ )

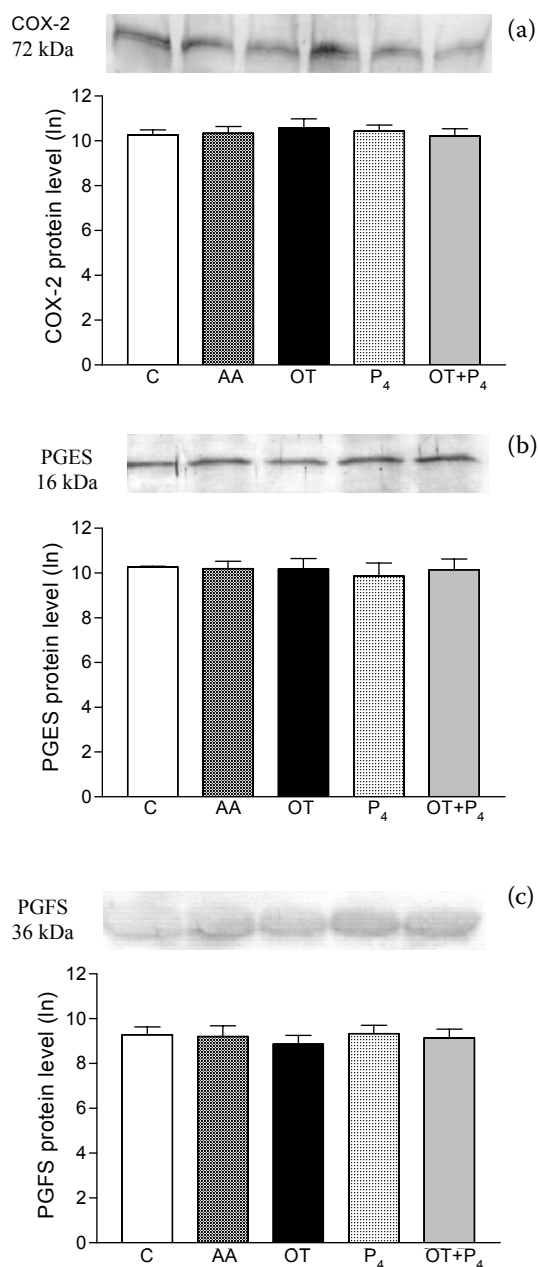


Figure 4. COX-2 (a), PGES (b) and PGFS (c) protein level in bovine endometrial cells ( $n = 4$ ) after 6 h incubation of cells with control medium (C), arachidonic acid (AA;  $10^{-5}$ M), oxytocin (OT;  $10^{-7}$ M), progesterone ( $P_4$ ;  $10^{-5}$ M), and OT together with each steroids. Upper panels: representative blots of COX-2, PGES and PGFS antibodies. Lower panel: densitometric analyses of COX-2, PGES and PGFS

for the further experiments. As shown previously (Bogacki et al., 2002; Duras et al., 2005; Kowalik and Kotwica, 2007),  $P_4$  and  $P_5$  do not affect the basal  $PGF_{2\alpha}$  secretion, but both steroids suppressed OT-stimulated  $PGF_{2\alpha}$  secretion from endometrial

cells. This inhibitory effect of steroids was observed after both 4 h and 6 h of cell culture, therefore it is assumed that both transcription-independent and transcription-dependent mechanisms may be involved in this process. A non-genomic effect of  $P_4$  has been found in a number of tissues from the female reproductive tract (Grazzini et al., 1998; Bogacki et al., 2002; Duras et al., 2005; Bishop and Stormshak, 2006, 2008; Franczak et al., 2006), but the nature of this rapid action has not been fully understood. It was found that  $P_4$  impaired binding of OT to its membrane receptor (Grazzini et al., 1998; Bogacki et al., 2002; Dunlap and Stormshak, 2004; Bishop and Stormshak, 2008). However, this effect was not confirmed by other authors (Burger et al., 1999; Ivell et al., 2000). It was also suggested that  $P_4$  may influence the concentration of cholesterol in the cell membrane causing conformational changes in the OT receptor and in this way to reduce the effect of OT on cells (Gimpl and Fahrenholz, 2001). Moreover,  $P_4$  can influence cell function via its membrane receptors (Bramley, 2003; Peluso, 2006; Bishop and Stormshak, 2008). It was also found that  $P_4$  directly decreases the expression of the OT receptor and prostaglandin synthases (Salomonsen et al., 1990; Raw et al., 1996). Our earlier studies (Duras et al., 2005; Kowalik and Kotwica, 2007) suggested that  $P_4$  and other steroids inhibit PGFS mRNA expression. These steroids did not affect expression of mRNA for PGES but they blocked OT-stimulated  $PGF_{2\alpha}$  secretion. The present study demonstrated that  $P_4$  and  $P_5$  did not affect COX-2, PGFS and PGES mRNA expression in endometrial cells after 4 h and 6 h of incubation. Moreover, we did not observe changes in genes expression after 4 h incubation of cells supplemented with OT, so it can be assumed that the steroids in question act on PG secretion via a transcription-independent mechanism. On the other hand, COX-2 mRNA expression was stimulated by OT after 6 h, but this effect was not observed in cells pretreated with  $P_4$  and  $P_5$ . These results indicate that the used steroids may inhibit OT-stimulated  $PGF_{2\alpha}$  secretion/production by down-regulation of the transcription of the COX-2 gene.

Moreover, we studied the influence of  $P_4$  with and without OT on the expression of COX-2, PGFS and PGES protein after 6 h of incubation with the endometrial cells. None of the treatments influenced the expression of the protein level for the studied enzymes. It should be noted that OT stimulated the expression of both the COX-2 mRNA and protein in

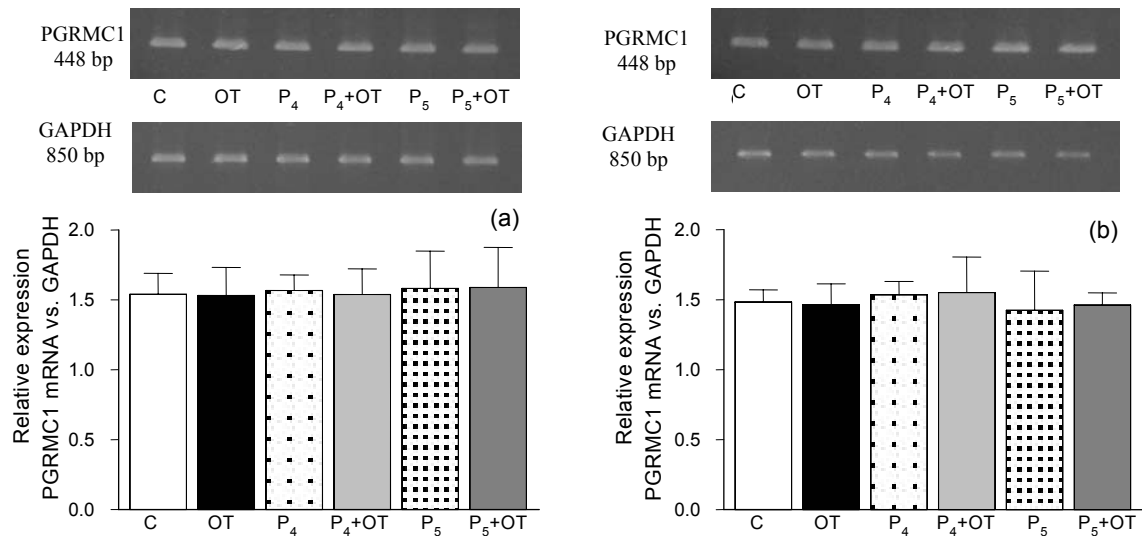


Figure 5. Means ( $\pm$  SEM) expression of PGRMC1 (progesterone receptor membrane component 1) mRNA determined by semi-quantitative PCR in bovine endometrial cells ( $n = 4$ ) after 4 h (a) and 6 h (b) incubation of cells with control medium (C), oxytocin (OT;  $10^{-7}$ M), progesterone ( $P_4$ ;  $10^{-5}$ M), pregnenolone ( $P_5$ ;  $10^{-5}$ M) and OT together with each steroids. All values are expressed as a ratio of PGRMC1 to GAPDH. Upper panel: representative images of agarose gels; lower panel: densitometric analyses of PGRMC1 mRNA relative to GAPDH mRNA

bovine endometrial cells after 9 h and 24 h (Asselin et al., 1997; Parent et al., 2003), while  $P_4$  did not affect COX-2 mRNA expression (Xiao et al., 1998). Moreover, neither OT (Xiao et al., 1998), nor  $P_4$  (Asselin and Fortier, 2000) had an effect on PGFS mRNA in the bovine endometrium. In our study, OT and  $P_4$  did not influence COX-2, PGFS and PGES protein expression after 6 h, however, they affected the secretion of  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$ . These results strengthen the idea that  $P_4$  may affect PG secretion via a non-genomic mechanism.

It is possible that the non-genomic effect of  $P_4$  observed in endometrial cells is evoked partly via progesterone membrane receptor component 1 (PGRMC1) (Peluso, 2006). This protein may be involved in the regulation of cholesterol and steroid synthesis (Losel et al., 2008) and this latter stabilizes the membrane OT receptor and improves its affinity for the ligand (Klein et al., 1995).  $P_4$ , on the other hand, inhibits the signal transduction of G-protein-coupled receptors and the intracellular transport of cholesterol (Gimpl and Fahrenholz, 2001). Therefore, it is possible that  $P_4$  action via PGRMC1 can influence the concentration of cholesterol in the cell membrane and change the effect of OT on the target cell. The results presented in this study show PGRMC1 mRNA expression

in bovine epithelial endometrial cells from Days 14–16 of the estrous cycle. However, no effect of OT and steroids on PGRMC1 gene expression was observed in this study. Nevertheless, the obtained results did not exclude PGRMC1 participation in the non-genomic activity of  $P_4$  in endometrial cells, since expression of PGRMC1 mRNA has been demonstrated during the development of *corpus luteum* in cows (Kowalik and Kotwica, 2008).

In conclusion, this study indicated differential effects of  $P_4$  and  $P_5$  on the regulation of  $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$  secretion stimulated by OT in cultured bovine endometrial cells. The results show that the studied steroids inhibit OT-stimulated secretion/production of  $\text{PGF}_{2\alpha}$  in endometrial cells in a transcription-independent manner and partly by down-regulation of COX-2 mRNA. This inhibitory effect of steroids on  $\text{PGF}_{2\alpha}$  secretion may be crucial for the lifespan of the *corpus luteum* and for the establishment of early pregnancy.

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