Ovarian steroids regulate prostaglandin secretion in the feline endometrium

Marta J. Siemieniuch a,∗, Anom Bowolaksonob, Dariusz J. Skarzynski a, Kiyoshi Okuda b

a Department of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima-St. 10, Olsztyn 10-747, Poland
b Graduate School of Natural Science and Technology, Okayama University, Tsushima Naka 700-8530, Japan

A B S T R A C T

Sex steroids, i.e. progesterone (P4) and 17β-estradiol (E2), fluctuate during the feline estrous cycle and their alterations correspond to many events in cat reproduction. In order to investigate possible effects of sex steroids on prostaglandin (PG) secretion in the cultured endometrial cells, mRNA expressions coding for PG-endoperoxide synthase (PTGS2) in the epithelial and stromal cells harvested with sex steroids were studied by RT-PCR. The effects of ovarian steroids on PG secretion in the epithelial and stromal cells were also investigated. E2 at a dose 10⁻⁷ M significantly increased prostaglandin F2α (PGF2α) secretion in the epithelial cells (P < 0.01). PGF2α production was intensified by the treatment in combination with both steroids (P < 0.001). P4 at any dose alone had no effect on PGF2α secretion in the epithelial cells, whereas at a dose 10⁻⁵ M enhanced prostaglandin E2 (PGE2) production (P < 0.05). The ovarian steroids stimulated both PGF2α and PGE2 in the epithelial cells of the feline endometrium via an E2 receptor (ESR1)- and P4 receptor (PGR)-dependent genomic-pathway. In contrast to the epithelial cells, neither P4 nor E2 affected PG secretion in the stromal cells. PTGS2 mRNA expression was not affected by ovarian steroids in either cell types. The overall results suggest that PG secretion is regulated by P4 and E2 and this effect is not due to changes in PTGS2 mRNA expression.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Controlling the estrous cycle of cats could be important in developing methods of control for feral cats (Olson and Moulton, 1993). A deeper knowledge of the regulation of the estrous cycle may also help to improve techniques used in wild or endangered cat populations. Prostaglandins (PGs) have been demonstrated to participate in majority of events in the reproductive tract and constitute potent factors controlling the endometrial function in several species (Weems et al., 2006). PGs are metabolites of arachidonic acid (AA) liberated from membrane phospholipids by phospholipase A2 (PLA2). The rate-limiting step during PG formation is catalysed by PG-endoperoxide synthase (PTGS) which converts the PG precursor to the unstable form – PGG2 (Salamonsen and Findlay, 1990). Data collected in knockout mice revealed that PTGS2 plays a markedly greater role in reproductive events and is responsive to cytokines and growth factors (Lim et al., 1997; Lim et al., 1999). The terminated products of AA pathway constitute a wide variety of PGs, i.e. prostaglandin F2α (PGF2α) and prostaglandin E2 (PGE2), produced by specific PG synthases (Salamonsen and Findlay, 1990; Wiltbank and Ottobre, 2003). PGF2α and PGE2 are believed to play opposing roles in reproduction with PGF2α released by the endometrium at the end of the estrous cycle acting as a potent luteolytic factor in cattle (Poyser, 1995; McCracken et al., 1999; Okuda et al., 2002). In contrast PGE2 produced by the blastocyst or secreted by the endometrial stroma after conception is thought to support the embry-
maternal dialogue at the initial steps of pregnancy and induce vascularisation of the endometrium, thereby playing a significant role as a luteotrophic factor (Pratt et al., 1977; Magness et al., 1981; Kennedy, 1983).

Since the activity of PTGS2 has been identified in the endometrium of humans (Jones et al., 1997) and several species (Arosh et al., 2002; Boerboom et al., 2004; Ashworth et al., 2006), and PG secretion fluctuate during the estrous cycle (Poyser, 1995; Okuda et al., 2002; Stout and Allen, 2002), it is assumed that PGs are involved in majority of physiological processes in the endometrium. However, modulators of PG secretion have not been completely clarified.

Cyclical alterations of ovarian steroids seem to be responsible for fluctuations of PG levels throughout the menstrual and estrous cycle (Salamonsen and Findlay, 1990). Sex steroids may directly affect spontaneous PGF2α and PGE2 production by the endometrium of the cow menstrual and the estrous cycle (Salamonsen and Findlay, 2002), it is assumed that PGs are involved in majority of physiology processes in the endometrium. However, modulators of PG secretion have not been completely clarified.

2. Materials and methods

2.1. The animals and collection of the uterine tissue

The uteri together with the ovaries and oviducts were collected from an adult domestic cat (Felis catus domestica, L. 1758) (n = 11) at the mid-luteal stage (Days 15–20 after the monitored estrus onset) after a routine ovariohysterectomy at the owner’s request and with the owner’s consent (Okayama, Japan). The reproductive tracts were transported to the laboratory on ice within 1.5 h. The phase of the estrous cycle was confirmed by the macroscopic observation of the ovaries and uterus as described previously (Karja et al., 2002). The ovaries, oviducts and connected tissue were dissected from the uteri horns.

2.1.1. Isolation of the endometrial cells

Both the epithelial and stromal cells from the feline endometrium were enzymatically separated. The epithelial cells were isolated using a peristaltic pump system. The uterine horns were separated and both were used for cells isolation. One polyvinyl catheter was inserted into the side of the oviductal horn and the other – into the side of the uterine body. The end of the first catheter was placed in a container with an enzymatic solution. The digestive solution was pumped into the uterine horn and channeled to a container with an enzymatic solution by the other catheter. Before the experiment started, the uterine lumen had been washed three times with 15–20 ml sterile Ca2+- and Mg2+- free and Hanks Balanced Salt Solution (HBSS) supplemented with 100 U/ml of penicillin, 100 µg/ml of streptomycin and 0.1% bovine serum albumin (BSA, Roche Diagnostics, Indianapolis, IN). Subsequently, the enzymatic solution (collagenase [2 mg/ml, Sigma Aldrich, St. Louis, MO]; DNase I [200 µg/ml, Sigma Aldrich] and Dispase [1.2 U/ml, Sigma Aldrich]) was circulated by using a peristaltic pump. The total amount of the enzyme solution was 50 ml. The enzyme solution was circulated with the constant velocity of 3.5 ml/min. After 60 min the equipment was disconnected and the uteri horns were longitudinally dissected. The rest of the epithelial cells were detached by gentle scratching with a scalpel blade.

After collection of the epithelial cells the uterine lumen was washed with sterile HBSS supplemented with antibiotics and 0.1% (w/v) BSA. Each longitudinally cut uterine horn was slit to expose the endometrial surface. The endometrial strips were dissected from the myometrial layer by a scalpel blade and washed once in sterile HBSS containing antibiotics. The endometrial fragments were minced into small pieces (approximately 1 mm2) and digested by stirring for 60 min in 50 ml of sterile HBSS containing 2 mg/ml (w/v) collagenase and 200 µg/ml (w/v) DNase I. The dissociated cells were then filtered through a metal mesh (100 µm and 80 µm) to remove undissociated tissue fragments. Both the epithelial and stromal cells suspensions were washed three times by centrifugation (10 min at 100 × g) with Dulbecco modified Eagle medium (DMEM, Sigma–Aldrich) and suspended in 10 ml fresh medium. The cells concentration was counted using a hemocytometer. The cells viability exceeded 90% as assessed by 0.05% (w/v) trypan blue dye exclusion.

2.1.2. Culture of the endometrial epithelial and stromal cells

The final pellet of both the isolated epithelial and stromal cells was resuspended in a culture medium (DMEM/Ham F-12 [D/F], 1:1 (v/v), Sigma–Aldrich) supplemented with 10% (v/v) calf serum (Sigma–Aldrich), 20 µg/ml of gentamycin (Invitrogen, San Diego, CA), and 2 µg/ml of amphotericin B (Sigma Aldrich) (Skarzynski et al., 2000). The cells were seeded at a density of 2 × 105 viable cells/ml in 75-cm2 culture flasks (Greiner Bio-One, Monroe, NC) and were cultured at 37.5 °C in a humidified atmosphere of 5% CO2 in air. The epithelial cells were seeded on a collagen matrix (Type I collagen, from calf skin, 10 mg/cm2; Sigma) to facilitate cell attachment. For the stromal preparation the medium was changed 4 h after plating, by which time selective attachment of stromal cells had occurred. For the epithelial preparation the medium was changed 48 h after plating.

After reaching confluence (6–7 days after the start of the culture) the cells were rinsed twice with sterile Ca2+- and Mg2+-free phosphate-buffered saline (PBS). In order to
collect the stromal cells, the cells were incubated in 0.02% trypsin and 0.008% ethylene diamide tetraacetate (EDTA) for 4–5 min at room temperature (RT) while to collect the epithelial cells, the cells were incubated with 0.008% EDTA for 2 min at RT. The cell culture was then rinsed by PBS to remove the contaminated stromal cells or fibroblasts. The cells were then incubated with 0.02% trypsin for 8–10 min at RT and at the end of the incubation period 25 ml of D/F supplemented with 10% CS was added to stop the enzymatic reaction. Both types of cells were washed once by centrifugation (10 min at 100 × g). The pellet of both types of cells was resuspended in 10 ml of fresh D/F medium and the cells concentration was counted using a hemocytometer. The cells viability exceeded 90% as assessed by 0.05% (w/v) trypan blue dye exclusion. The cells were seeded at a density of 2 × 10^5 viable cells/ml in a 48-well cluster dish (Greiner-Bio One, Monroe, NC), a 96-well cluster dish (Greiner-Bio One, Monroe, NC) or a 6-well cluster dish (Greiner-Bio One, Monroe, NC) and harvested as described for the primary cells culture.

The homogeneity of each cell culture and contamination of the epithelial and stromal cultures (with the stromal and epithelial cells, respectively) was assessed by immunofluorescent staining for specific markers of the epithelial cells (cytokeratin) and stromal cells (vimentin) as described previously (Malayer and Woods, 1998) with own modification. Briefly, the epithelial- or stromal-derived cells were seeded at 2 × 10^5 cells/ml in special slide flasks (Slide Flasks No. 170920, Nunc, Denmark, DK-4000 Roskilde) and cultured as described above. After 48 h of culture the slides were washed three times in PBS, fixed in methanol for 10 min and air-dried. Slides were then washed three times in PBS. Triton 0.01% × 100 diluted in PBS was added to the cell cultures for 10 min at RT. Then slides were again washed three times in PBS and incubated for 12 h at +4 °C with the primary antibody against either cytokeratin (mouse monoclonal anti-human cytokeratin peptide 18; Sigma; 1:100 dilution) or vimentin (mouse monoclonal anti-pig eye lens vimentin; Sigma; 1:200 dilution) in PBS. Subsequently, the slides were washed three times in PBS and then incubated with the second antibody (anti-mouse IgG conjugated to alkaline phosphatase; Sigma; 1:200 dilution) for 1 h at RT and light protected. The controls were prepared as described above in the absence of the primary antibody.

2.2. Experimental design

2.2.1. Experiment 1 – optimization of the culture conditions: spontaneous PGF_2α and PGE_2 secretion from the cultured endometrial epithelial and stromal cells; the dose-dependent effects of ovarian steroids on PGF_2α and PGE_2 secretion; effects of ovarian steroids on cell viability

All experiments were conducted after the first cell passage in order to minimize heterogeneity of the cells and in order to optimize culture conditions, i.e. the time of incubation and an effective dose of E_2 and P_4, several preliminary experiments were done. AA (precursor of PGs) at a dose 10^{-6} M was used to check the possibility of PG synthesis by both types of cells.

To examine the pattern of spontaneous PG secretion, 500 µl of the conditioned media was collected after incubation for 0, 1, 2, 4, 12, 18 and 24 h and frozen at –30 °C until PGF_2α and PGE_2 measurements (n=3).

The cultured epithelial and stromal cells were treated with E_2 (E4389, Sigma–Aldrich) or P_4 (P7556, Sigma–Aldrich) in a dose-dependent manner (0, 10^{-9}–10^{-5} M) (n=4). After 24 h cell viability was determined by WST-1 assay. PGF_2α and PGE_2 concentrations of the epithelial and stromal cells harvested with E_2 or P_4 were measured by enzyme-immunoassay (EIA). The DNA content, estimated by a spectrophotometric method described by Labarca and Paigen (1980), was used to standardize the results.

2.2.2. Experiment 2 – estrogen and progesterone receptors in the cultured endometrial epithelial and stromal cells

In order to determine the expression of mRNA coding for estrogen receptor (ESR1) and progesterone receptor (PGR), the endometrial epithelial and stromal cells cultures were subjected to the semi-quantitative RT-PCR (n=3).

2.2.3. Experiment 3 – effects of 17β-estradiol and/or progesterone on PGF_2α and PGE_2 secretion from the cultured endometrial epithelial and stromal cells

In order to determine the effects of sex steroids on PG secretion, the endometrial epithelial and stromal cells were treated with E_2 and/or P_4 at the dose identified in experiment 1 (E_2 at 10^{-7} M, P_4 at 10^{-5} M) (n=4). The intracellular mechanism of E_2 and P_4 action on the feline endometrial epithelial cell was examined by cells pre-incubated for 0.5 h with a specific ESR1 blocker (ICI-7a,17β-[9,(4,4,5,5,5pentfluoropentyl)sulfanyl]nonyl)estr-1,3,5(10)-triene-3,17-diol; #1047, Tocris Cookson Inc., Ellisville, MO; 10^{-3} M) or specific PGR blocker (mifepristone, RU486, 11β-(4-dimethylamino)phenyl-17β-hydroxy-17-(1-propynyl)estradiol-4,9-dien-3-one; #M8046, Sigma–Aldrich; 10^{-5} M). After preincubation the cells were treated with E_2 or P_4, respectively. After 24 h of incubation, conditioned media were collected and frozen at –30 °C until PGF_2α and PGE_2 measurements.

2.2.4. Experiment 4 – effects of 17β-estradiol and/or progesterone on prostaglandin-endoperoxide synthase mRNA expression

In order to determine the effects of E_2 and/or P_4 on mRNA expression coding for prostaglandin-endoperoxide synthase (PTGS2), the endometrial epithelial and stromal cells after 18 h of incubation with or without E_2 and/or P_4 were subjected to the semi-quantitative PCR (n=4). β-Actin (ACTB) was used as a reference gene.

2.3. Reverse transcription and mRNA expression

For the studies on mRNA expression coding for ESR1, PGR and PTGS2 the harvested cells were treated with the sex steroids for 18 h and then collected. Total RNA was prepared from the uterine cell cultures using TRIzol Reagent (Life Technologies, Gibco BRL) according to the manufacturer's instructions. One µg of each sample of total RNA
was reverse transcribed using a PrimeScript™ First Strand cDNA Synthesis Kit for RT-PCR (6110 A, TaKaRa, Kyoto, Japan) according to the manufacturer’s instruction, and the reaction mixture was used in each PCR together with appropriate oligonucleotide primer pairs. The PCR amplification was calibrated in order to determine the optimal number of cycles that would allow detection of appropriate mRNA transcripts while still keeping amplification of these genes in the log phase. The semi-quantitative RT-PCR was carried out using the housekeeping gene, ß-actin, as an internal control. ß-Actin primer was added at the appropriate cycle number by the “primer-dropping method” according to Wong et al. (1994). The conditions for the PCRs were as follows: 22 (ACTB), 35 (ESR1), 35 (PGR), 30 (PTGS2) cycles of reactions including denaturation for 30 s at 95 °C, annealing for 1 min at 60 °C, and extension for 1 min at 72 °C, followed by an additional extension for 5 min at 72 °C. The free software Primer 3 (http://www.genome.wi.mit.edu/genome_software/other/primer3.html) was used to design primers. Aliquots of the PCR reaction products were electrophoresed on a 1.5% agarose gel containing ethidium bromide with a known standard (100 bp Ladder; D-1030; BIONEER Korea), and photographed under ultraviolet illumination. The band intensities were analyzed by using NIH Image computerized densitometry (National Institute of Health, Bethesda, MD, USA). Bands of the appropriate size were cut out of the gel and sent to the sequencing service (GENOMED, Warsaw, Poland). The sequence obtained was compared with databases using BLAST analysis. The details of the PCR reaction: primers used and length (bp) are given in Table 1.

### Table 1: Primers used in the study.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5′→3′</th>
<th>Length (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTB</td>
<td>For gatcttgccacacaccttcttagagggtgctgggat</td>
<td>588</td>
<td>AB051104</td>
</tr>
<tr>
<td></td>
<td>Rev</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR1</td>
<td>For cgctctaccgcacctgtaagtt</td>
<td>368</td>
<td>AY605260</td>
</tr>
<tr>
<td></td>
<td>Rev accgatgctgcaatacaagt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGR</td>
<td>For accgccccctcttctacac</td>
<td>110</td>
<td>AY462088</td>
</tr>
<tr>
<td></td>
<td>Rev ttgcctatccccacagatg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTGS2</td>
<td>For tcgacccagagcacaagagtt</td>
<td>341</td>
<td>EF036473</td>
</tr>
<tr>
<td></td>
<td>Rev ctagtcagggagcctgttgga</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2.4. PGF2α and PGE2 determination

The concentrations of PGF2α and PGE2 in conditioned media were determined with an enzyme-immunoassay (EIA). The PGF2α standard curve ranged from 0.156 to 4 ng/ml, and the median effective dose (ED50) of the assay was 0.25 ng/ml. The intra- and inter-assay coefficients of variation were 5.3% and 8.9%, respectively. The PGE2 standard curve ranged from 0.039 ng/ml to 10 ng/ml and the ED50 of the assay was 0.625 ng/ml. The intra- and inter-assay coefficients of variation were on average 6.1% and 8.7%, respectively. The cross-reactivities of the anti-PGE2 serum, validated by comparing the inhibition of peroxidase-labeled PGE2 binding with that of antiserum, were as follows: 100% for PGE2, 15% for PGE1, 10% for PGA1, 6.7% for PGB2, 4.6% for PGA2, 2.8% for PG2α, 0.13% for PGD2, and 0.05% for 15-keto-PGE2.

### 2.5. WST-1 assay

A derivative of MTT [3-(4,5-dimethyl-2 thiazolyl)-2,5-diphenyl-2H-tetrazolium/Br], WST-1 is a yellow tetrazolium salt that is converted to formazan by active mitochondria of live cells. For the viability assay the culture medium was replaced with 100 µl of D/F medium without phenol red, and 10 µl of assay solution (0.3% WST-1 and 0.2 mM 1-methoxy-5-methylphenazinium methylsulfate in PBS; pH 7.4) were added to each well. The cells were then incubated for 4 h at 37.5 °C. The absorbance (A) was measured at 450 nm using a microplate reader (Model 450; Bio Rad). Cell viability (%) was calculated as:

$$\text{Cell viability } (\%) = 100 \times \frac{A_{\text{test}}}{A_{\text{control}}},$$

where $A_{\text{control}}$ is the mean A of non-treated wells and $A_{\text{test}}$ is the mean A of all the experimental wells.

### 2.6. Statistical analysis

All experimental data are presented as a mean±SEM of values obtained in 3–4 separate experiments in which replications were performed using the epithelial or stromal cells from a single feline endometrium. Amounts of DNA were used to standardize the results obtained from hormone measurements. The statistical differences between the controls and the treated groups were assessed by one-way analysis of variance (ANOVA) followed by Newman–Kuels Multiple Comparison Test (GraphPad PRISM version 4; GraphPad Software Inc., San Diego, CA, USA).

### 3. Results

In the primary cultures, the feline endometrial epithelial cells were spherical or cylindrical in shape with a distinct round nucleus (Fig. 1a), and the stromal cells were spindle-shaped and elongated with a slightly visible nucleus (Fig. 1b). Morphological differences were not observed between the primary and passaged cells. By evaluation of the stained cells, the stromal cell contamination of the stromal cells did not exceed 5% (Fig. 1c and d) and the epithelial cell contamination of the stromal cells did not exceed 1% in the primary cell cultures (Fig. 1d and f).

In experiment 1 the accumulated PGF2α and PGE2 concentrations in conditioned media collected from the endometrial epithelial and stromal cells increased at 12, 18 and 24 h, but reached the maximum at 24 h of incubation ($P<0.001$; data not shown) for both PGs and in both cell types. AA increased PG production in both types of cells compared with the control ($P<0.001$). The AA-stimulated PGF2α secretion increased fivefold in the epithelial and three- and-half-fold in the stromal cells. AA augmented PGE2 secretion twofold compared with the control in both the epithelial and stromal cells. The most effective dose
for PG secretion during 24 h incubation was \(10^{-7}\) M of E2 \((P<0.01)\) and \(10^{-5}\) M of P4 \((P<0.05)\) (data not shown). The examined factors did not affect cell viability assessed by using the WST-1 assay (data not shown).

In experiment 2 ESR1 and PGR mRNA expressions were demonstrated in both epithelial and stromal cells (data not shown).

The effects of E2 and P4 treatment during the culture period of 24 h in experiment 3 on subsequent PG production in the endometrial cells are illustrated in Fig. 2a and b (epithelial) and Fig. 3a and b (stromal). In the epithelial cells, E2 at a dose \(10^{-7}\) M augmented PGF2α secretion compared with the control \((P<0.01)\). P4 alone did not affect PGF2α production in this cell type, whereas P4 in combination with E2 increased PGF2α production significantly \((P<0.001)\). In the case of PGE2 in the epithelial cells only P4 at a dose of \(10^{-5}\) M augmented its production \((P<0.05)\). ICI or RU486 alone did not affect PG secretion \((P>0.05)\). ICI in combination with E2 abolished the stimulating effect of E2 on PGF2α secretion in the epithelial cells. The treatment with RU486 abrogated the positive effect of P4 on PGE2 production in the epithelial cells. In contrast to the epithelial cells, neither E2 nor P4 affected PG secretion in the stromal cells \((P>0.05)\).

PTGS2 mRNA expression was not affected by the treatments in the endometrial epithelial cells \((Fig. 4a, P>0.05)\). In the endometrial stromal cells P4 and E2 in combination with P4 slightly decreased PTGS2 expression \((P=0.064\) and 0.069, respectively) \((Fig. 4b)\); experiment 4.

4. Discussion

In the present study the feline endometrial epithelial and stromal cells were isolated and harvested, and evidence that ovarian steroids regulate PG secretion in the cat endometrium was provided. Separation and culture of the endometrial epithelial and stromal cells have been described for several species including humans \((Liu and Tseng, 1979)\), rats \((McCormack and Classer, 1980)\), rabbits \((Gerschenson et al., 1981; Fortier et al., 1987)\), cattle \((Fortier et al., 1987)\), and...
et al., 1988) and pigs (Zhang et al., 1991a). No distinctive cross-species variations have been reported in the culture of the endometrial cells.

Feline endometrial epithelial cells have been demonstrated to be the main source of PGF$_{2\alpha}$, and the concomitant spontaneous PGE$_2$ secretion was fivefold lower than PGF$_{2\alpha}$ in the epithelial cells. PG production in the stromal cells was definitely lower than in the epithelial cells; however, the former seems to produce slightly more PGE$_2$ than PGF$_{2\alpha}$. The present results suggest that the spontaneous secretory pattern of both endometrial cell types does not differ significantly from that of other species. Indeed, in the cow (Fortier et al., 1988; Asselin et al., 1996), ewe (Cherny and Findlay, 1990), pig (Zhang and Davis, 1991), rabbit (Fortier et al., 1987) and in humans (Smith and Kelly, 1987), the epithelial cells were responsible for majority of PGF$_{2\alpha}$ production, while the stromal cells were the main source of PGE$_2$.

E$_2$-amplified PGF$_{2\alpha}$ secretion, as observed in the present study, was clearly demonstrated in the porcine endometrial epithelial cells (Hu et al., 2003). Estrogens, produce by the porcine embryos, which serve as an early pregnancy recognition factor in pigs are believed to redirect PGF$_{2\alpha}$ secretion from luteolytic endocrine direction (towards the uterine vasculature) to a non-luteolytic exocrine direction (into the uterine lumen) (Bazer and Thatcher, 1977; Mirando et al., 1996; Hu et al., 2003), although, another study favored a retrograde transfer in the area of mesometrium, thus diminishing PGF$_{2\alpha}$ level in the uterine venous blood (Krzymowski and Stefanczyk-Krzymowska, 2008). The capabilities of the epithelial endometrium to transport PG in either apical or basal direction may account for the lack of luteolytic activity of the over-produced PGF$_{2\alpha}$ during early pregnancy in the pig (Ziecik et al., 2006). In the present study E$_2$ significantly augmented PGF$_{2\alpha}$ secretion from the feline endometrium, which is in agree-
ment with the data collected in pigs. By contrast to pigs, the factors involved in the embryo-maternal cross-talk in felines remain unknown, but up-regulation of the PGF<sub>2α</sub> secretion and its accumulation in the area of broad ligament vasculature cannot be ruled out in the cat.

E<sub>2</sub>-stimulated PGF<sub>2α</sub> secretion in the feline epithelium are in contrast with E<sub>2</sub> action reported in the cow, even though E<sub>2</sub> action on PG secretion by the bovine endometrium does not seem to be very consistent. E<sub>2</sub> reduced both PGF<sub>2α</sub> and PGE<sub>2</sub> production in the epithelial cells of the endometrium in cattle (Asselin et al., 1996; Xiao et al., 1998), but removal of E<sub>2</sub> also prolongs the estrous cycle (Villa-Goday et al., 1985; Zhang et al., 1991b), and administration of E<sub>2</sub> at the mid-luteal stage causes luteolysis in cattle (Thatcher et al., 1986). In the endometrium of ruminants, E<sub>2</sub>-upregulated PGF<sub>2α</sub> secretion seems to be dependent on P<sub>4</sub>, since it was noted only after priming with P<sub>4</sub> (Raw and Silvia, 1991; Laming and Mann, 1995; Skarzynski et al., 1999). The presence of a low P<sub>4</sub> concentration (1–2 ng/ml) resulted in maintenance of pulsatile PGF<sub>2α</sub> secretion in the bovine endometrium (Kindahl et al., 1981). It is thought, that in ruminants, P<sub>4</sub> plays a mandatory role in PG synthesis in the endometrium, but E<sub>2</sub> may only facilitate this process.

Orchestrated action of P<sub>4</sub> together with E<sub>2</sub> is required for endometrial PGF<sub>2α</sub> secretion which is involved in luteolysis in the cow (Goff, 2004). By contrast to the bovine endometrium, PGF<sub>2α</sub> secretion in the present study was obligatorily augmented by E<sub>2</sub> in the feline epithelial cells. However, this effect was enhanced in combination with P<sub>4</sub>. In the cat, elevated E<sub>2</sub> levels were occasionally observed throughout diestrus and this increment was particularly seen at the termination of the luteal phase when the low concentration of P<sub>4</sub> is maintained (Wildt et al., 1981). Therefore, we postulate that in the endometrium of the cycling queens E<sub>2</sub>-stimulated PGF<sub>2α</sub> output, primed by a period of P<sub>4</sub> dominance, is involved in the process of luteolysis, which is consistent with the data collected in cattle. Further studies are needed to clarify mechanisms of luteolysis in the cat.

An existing potent antiluteolytic factor which may counteract luteolytic activity of PGF<sub>2α</sub> in felines is also possible as PGF<sub>2α</sub> does not initiate luteolysis when administered at the early- and mid-luteal stage in this species (Shille and Stabenfeld, 1975; Wildt et al., 1979). An evident candidate for this antiluteolytic and luteotropic agent is PGE<sub>2</sub>, as it was demonstrated in other species. In the present study, the sex steroid-stimulated PGE<sub>2</sub> secretion was restricted to the epithelial cells in the cat. This observation is in agreement with previous findings in ruminants, i.e. the enhancement of PGE<sub>2</sub> release after P<sub>4</sub> treatment was limited to the epithelial cells in cows and ewes (Asselin et al., 1996; Raw and Silvia, 1991). It is possible that P<sub>4</sub>-increased PGE<sub>2</sub> secretion advocates PGE<sub>2</sub> as a luteotropic factor in the endometrium of the cat, however, this point deserves closer evaluation. Since PG output in the endometrial stromal cells was not affected by the sex steroids even if both mRNA coding for steroid receptors, i.e. ESR1 and PGR, were present, we assumed that the ovarian steroid-amplified PG secretion is limited to the epithelial cells in the feline endometrium. The sex steroid upregulated PG secretion was confirmed to be dependent on genomic-pathway, since the specific ESR1 and PGR blockers inhibited the stimulating effects of both hormones.

The presence of PTGS2 mRNA expression in both types of the endometrial cells indicates that PGs are locally produced in the feline endometrium. Interestingly, PTGS2 mRNA expression was not affected by the ovarian steroid treatment in cat. The results obtained in this matter in the previous studies are quite ambiguous. An elevated E<sub>2</sub> level together with decreased P<sub>4</sub> concentration have been demonstrated to be the factors determining an increase in PTGS2 mRNA expression in the endometrium and myometrium of the pregnant ewe, just before parturition (Zhang et al., 1996). However, in the endometrium of cycling and early pregnant ewes, E<sub>2</sub> either alone or in combination with P<sub>4</sub> had no effect on PTGS2 mRNA (Charpigny et al., 1997). By contrast to E<sub>2</sub>, prolonged exposure to P<sub>4</sub>-increased AA accumulation and PTGS2 in the endometrium of ewes and cows (Charpigny et al., 1997; Goff, 2004). On the contrary, neither E<sub>2</sub> nor P<sub>4</sub> affected PTGS2 mRNA expression in human amnion cells (Zakar et al., 1995). The data collected in the feline endometrium are in concurrence with the results collected in human amnion cells (Zakar et al., 1995). Interestingly, several data provided evidence, that the ovarian steroids may regulate the local production of PG by the effects on PLA<sub>2</sub> activity in the oviductal epithelium of the rabbit (Morishita et al., 1993), as well as in the rat endometrium (Pakrasi et al., 1983). The sex steroids involvement in the PG synthesis by the feline endometrium needs further studies, but the relationship between uterine PLA<sub>2</sub> activity and increase of PG secretion cannot be ruled out in this species.

In conclusion the method for the endometrial epithelial and stromal cells separation and culture system seems to be valuable as a research technique for further studies into the function of feline endometrium. The overall results suggest that E<sub>2</sub> and P<sub>4</sub> are directly involved in PG secretion only in the endometrial epithelial cells in the cat but these effects are not accompanied by changing PTGS2 mRNA expression.

Acknowledgements

Researches were supported by Grant-in-Aid for Scientific Research from the Polish Ministry of Scientific Research and High Education (MNISW N 308 031 934). MJS was supported by Domestic Grants for Young Scientists of the Foundation for Polish Science (FNP START, 2008, 2009) and Post Doctoral Fellowship Program of the Japan Society for the Promotion of Science (No. P 08733).

Authors thank to Sanyo and Yamagata Veterinary Clinics, Okayama, Japan, for kindly provided material after surgery; Dr. Seiji Ito of Kansai Medical University, Osaka, Japan, for antisera of PGF<sub>2α</sub> and PGE<sub>2</sub>; students of Graduate School of Natural Science and Technology Okayama University, Japan, for help in material collection and Mrs. Urszula Kozak for assistance in language correction.

References


On a note, the text seems to be a mixture of scientific literature references and some sentences that are not clearly categorized. It could be a mistake in the text extraction process.


