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Peroxisome proliferator activated receptor ligands affect porcine endometrial steroids production during the estrous cycle and early pregnancy: an *in vitro* study

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ABSTRACT: In the present study, we investigated the effect of PPAR ligands on progesterone (P_4) and 17β-estradiol (E_2) secretion, as well as 3β-hydroxysteroid dehydrogenase/Δ(5)-Δ(4) isomerase (*3β-HSD*) mRNA expression, in porcine endometrial slices collected on days 10–12 and 14–16 of the estrous cycle or early pregnancy. The explants were incubated *in vitro* for 6 h in the presence of PPARα ligands – WY-14643 (agonist) and MK 886 (antagonist); PPARβ ligands – L-165041 (agonist) and GW 9662 (antagonist); PPARγ ligands – 15d-prostaglandin J_2 and rosiglitazone (agonists) and T0070907 (antagonist). During the estrous cycle, all PPAR ligands inhibited P_4 secretion during the mid-luteal phase (days 10–12). During early pregnancy, a stimulatory effect of PPARα agonist was observed during maternal recognition of pregnancy (days 10–12), while an inhibitory effect was observed at the beginning of implantation (days 14–16). PPAR ligands inhibited the expression of *3β-HSD* mRNA on days 14–16 of the estrous cycle (β and γ isoforms) or pregnancy (α, β, γ isoforms) but did not affect gene expression on days 10–12 of the estrous cycle or early pregnancy. An inhibitory effect of PPARα, PPARγ, and PPARβ on E_2 secretion was observed during maternal recognition of pregnancy, but a stimulatory effect was observed during mid- (γ isoform) or late-luteal (β isoform) phases of the estrous cycle. Our study indicates, for the first time, that PPARs are engaged in P_4 and E_2 production in porcine endometrium. It is possible that the diverse receptivity of endometrial tissue to the PPAR ligands can be associated with the reproductive status of gilts.

Keywords: PPAR; progesterone; estradiol; luteal phase; periimplantation

INTRODUCTION

Peroxisome proliferator activated receptors (PPARs), -α, -β/δ, and -γ, are members of the nuclear receptor family. As transcriptional factors, they modulate the expression of genes controlling many physiological functions. Besides the role of PPAR in the regulation of lipid and glucose metabolism, they are also involved in the modulation of female reproductive functions. Synthetic ligands of PPARα (fibrates) and PPARγ (thiazolidinediones) are used as therapeutic agents in metabolic disorders including insulin resistance, type 2 diabetes, and dislipidemia. In reproductive disorders the treatment with thiazolidinediones induces ovulation in women with polycystic ovary syndrome (PCOS) and improves fertility (Du et al. 2012).

Deletion of PPARγ gene in mice leads to embryo death due to disorders in placental vascularization (Barak et al. 1999). Also in PPARβ-null mice abnormalities in placenta development were observed (Barak et al. 2002). All PPAR isoforms are expressed

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in tissues of the hypothalamic–pituitary–ovarian axis and placenta of different species (Michalik et al. 2002; Bogacka et al. 2015). As reported, PPARs regulate ovarian steroidogenesis, angiogenesis, and tissue remodelling, as well as placental development and maternal-fetal nutrient transport (Velez et al. 2013). Their presence was also observed in gametes and embryos (Huang 2008). A limited amount of data indicates that PPARs also play an important role in the regulation of uterine functions. This can be supported by the fact that all PPAR isoforms are expressed in the uterus of different species, including human, bovine, ovine, porcine, and murine (Bogacka and Bogacki 2011; Nishimura et al. 2011). In addition, the diversified PPAR expression profile in the endometrium, which depends on the physiological status of animals, underlines their significance in this tissue. Our previous results demonstrated a higher level of PPARγ1 mRNA in porcine endometrial tissue on days 13–15 of the estrous cycle (during luteolysis) and decreased levels of PPARα and PPARβ on days 11–12 of pregnancy (during maternal recognition of pregnancy) (Bogacka and Bogacki 2011). We have also shown that PPAR activators stimulated luteolytic $PGF_2\alpha$ release by porcine endometrium during the estrous cycle, while during pregnancy, they were less effective (Bogacka et al. 2013a). Moreover, we found that PPAR activation during the luteal phase of the estrous cycle and the time of peri-implantation promoted luteotropic PGE_2 production by porcine endometrial tissue (Bogacka et al. 2013b).

Recently, it has been demonstrated that the uterus is able to produce steroid hormones *de novo* in both early pregnant and cyclic pigs (Franczak and Kotwica 2008; Franczak et al. 2013). Interestingly, their endometrial expression varied, depending on physiological status of animals. A greater level of progesterone (P_4) secretion was observed on days 10–16 of pregnancy compared with corresponding days of the estrous cycle (Wojciechowicz et al. 2013). In addition, changes in the expression of 3β-hydroxysteroid dehydrogenase/Δ(5)-Δ(4) isomerase (*3β-HSD*), the gene involved in the P_4 production, were demonstrated mostly during the estrous cycle (Wojciechowicz et al. 2013). Enhanced amounts of estradiol (E_2) released by the endometrial explants were noted during early pregnancy compared with luteolysis period (Franczak and Kotwica 2008). The above results might suggest that the porcine endometrium is a significant source of steroids and may supplement steroidogenesis by the conceptus during maternal recognition of pregnancy (Franczak and Kotwica 2008).

In the present study we examined the *in vitro* effect of PPAR ligands on P_4 and E_2 secretion by porcine endometrium on days 10–12 and 14–16 of the estrous cycle and the corresponding days of pregnancy. Additionally, the expression of the gene coding 3β-HSD, an enzyme that catalyzes the synthesis of P_4, was also determined.

MATERIAL AND METHODS

Experimental animals. The study was conducted on crossbred gilts (100 kg, 7 months old) from a commercial farm. The animals were treated hormonally as described previously (Bogacka et al. 2013a, b). Firstly, a single intramuscular injection of 750 IU PMSG (Folligon; Intervet International B.V., Boxmeer, the Netherlands) was followed by 500 IU hCG (Chorulon; Intervet International B.V.) administered 72 h later. The animals were randomly assigned to four experimental groups: cyclic – days 10–12 (n = 4) and 14–16 (n = 4) of the estrous cycle, pregnant – days 10–12 (n = 4) and 14–16 (n = 4) of pregnancy. Two groups of cyclic gilts were infused with saline, and two groups of pregnant gilts were inseminated twice, 24 h and 36 h after the hCG treatment. Two stages of the cyclic gilts represent, respectively, mid- and late-luteal phases of the estrous cycle, while the analyzed periods of pregnancy reflected maternal recognition and the beginning of implantation, respectively. During slaughter, porcine uteri were dissected and transported to *in vitro* laboratory on ice in sterile PBS with antibiotics (penicillin and streptomycin) (Polfa Tarchomin S.A., Warsaw, Poland). All procedures relative to the care and use of animals were approved by the Local Animal Ethics Committee of the University of Warmia and Mazury in Olsztyn (Poland), and the study was conducted in accordance with the national guidelines for animal care.

In vitro culture of endometrial explants. The procedure for the collection and incubation of endometrial tissue was described previously (Bogacka et al. 2013b). The endometrium was separated from the myometrium, cut into small pieces (200–210 mg), and washed twice with PBS. Each piece of tissue

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was placed in a sterile culture vial with 2 ml of medium 199 (Sigma-Aldrich, St. Louis, USA) supplemented with 0.1% BSA, gentamycin (40 mg/ml), and nystatin (120 IU/ml). The endometrial explants were pre-incubated in a water bath for 18 h in an atmosphere of 95% O_2 and 5% CO_2 and then treated for 6 h with PPAR ligands with the following reagents: PPARα ligands – WY-14643 (agonist; 1 and 10μM; Cayman Chemical Company, Ann Arbor, USA) and MK 886 (antagonist; 10μM; Enzo Life Sciences International, Farmingdale, USA); PPARβ ligands – L-165041 (agonist; 1 and 10μM; TOCRIS Bioscience, Bristol, UK) and GW 9662 (antagonist; 10μM; Cayman Chemical Company); PPARγ ligands – 15d-prostaglandin J_2 (agonist; 10μM), rosiglitazone (agonist; 1 and 10μM; Cayman Chemical Company), and T0070907 (antagonist; 1μM; Cayman Chemical Company). The PPAR ligand concentrations and incubation times were selected according to our preliminary study and previous reports (Lovekamp et al. 2001; Schoppee et al. 2002; Seto-Young et al. 2007). The tested compounds were dissolved in a total volume of 20 μl dimethyl sulfoxide (DMSO) (Sigma-Aldrich). Controls (without the ligands) contained culture media or medium with DMSO. After incubation, the endometrial slices were washed with PBS and frozen at –80°C for total RNA isolation and real time PCR quantification. Incubation media were collected for radioimmunoassay and frozen at –20°C.

Steroid hormones assay. Concentrations of P_4 and E_2 in media collected after 6 h of incubation of endometrial explants with the tested factors were determined by 3H-radioimmunoassay (RIA), which is routinely used in our laboratory. The specificity of the anti-progesterone (SO/91/4) and anti-estradiol (BS/88/754) antibodies has been reported previously (Ciereszko et al. 2001; Szafranska et al. 2002). Standard curves following a log/logit transformation were established using: 0.5–200 pg/ml standards for E_2 and 2–1500 pg/ml standards for P_4. P_4 was extracted using ether, and the efficiency was 85.3%. E_2 was not extracted. The validity of the assay was confirmed by parallelism between undiluted and serially diluted samples. The sensitivity of the assays for E_2 was 0.5 pg/ml, and for P_4 – 2 pg/ml. Inter- and intra-assay coefficients were less than 10.0%.

Genes expression analysis. Total RNA was isolated with the Total RNA kit (A&A Biotechnology, Gdynia, Poland), quantified spectrophotometrically, and the integrity of the product was confirmed on 1.5% agarose gel. The concentrations of the PCR primers were 300nM and 200nM of the TaqMan fluorogenic probes labelled with FAM (6-FAM, 6-carboxyfluorescein) dye. The sequences of primers and TaqMan probes for *3β-HSD* (GenBank No. AF232699) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*; GenBank No. U48832) were designed using Primer Express Software Version 3 (Applied Biosystems Inc.) and were synthesized by Applied Biosystems Inc. The following primer and probe sequences were used: *3β-HSD* forward ACCGTCATGAAGGTCAATGTGA, *3β-HSD* reverse GATGAAGACCGGCACGCT, *3β-HSD* probe CAGCTCCTGCTGGAGGCCTGTGTC; *GAPDH* forward CATCAATGGAAAGGCCATCAC, *GAPDH* reverse CAGCATCGCCCCATTTG, and *GAPDH* probe CTTCCAGGAGCGAGATCCCGCC. Real time RT-PCR assay was run in duplicates and the expressions of mRNA encoding *3β-HSD* and *GAPDH* were determined using the TaqMan®RNA-to-CT™ 1-Step Kit (Applied Biosystems Inc.). Standard curves for the quantifications of *3β-HSD* and *GAPDH* were run by serial dilution of a known amount of total RNA. The tested agents did not affect *GAPDH* mRNA levels. All expression data was normalized to the amount of *GAPDH* mRNA and presented as arbitrary units as previously described (Bogacka et al. 2013b).

Statistical analysis. Results were analyzed by STATISTICA software (Version 10.0, 2010). The effect of the treatment as independent variable was performed by one-way analysis of variance for repeated measurements followed by Duncan's post-hoc test. Statistical significances were assigned at $P \leq 0.05$ while not significant differences indicated $P > 0.05$. The data were presented as means ± SEM.

RESULTS

Impact of PPAR ligands on P_4 secretion. The concentration of P_4 in the culture media collected after incubation of porcine endometrial explants with PPAR ligands is presented in Table 1. A treatment of the tissue with PPARα agonist – WY-14643 – inhibited (1μM) P_4 secretion on days 10–12 of the estrous cycle and on days 14–16 of pregnancy, 1 and 10μM). In contrast, a stimulatory effect of the ligand (1μM) on P_4 release was noted on days 10–12 of pregnancy.

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Table 1. Effect of PPAR ligands on progesterone release by the endometrium of gilts on days 10–12 (n = 4) and 14–16 (n = 4) of the estrous cycle and days 10–12 (n = 4) and 14–16 (n = 4) of pregnancy

Progesterone (ng/ml)	Estrous cycle		Pregnancy	
	days 10–12	days 14–16	days 10–12	days 14–16
PPARα ligands				
Control	0.26 ± 0.05^{a}	0.32 ± 0.08^{a}	0.13 ± 0.06^{a}	0.24 ± 0.02^{a}
WY 1μM	0.12 ± 0.04^{b}	0.30 ± 0.08^{a}	0.20 ± 0.04^{b}	0.15 ± 0.04^{b}
WY 10μM	0.20 ± 0.03^{ab}	0.42 ± 0.13^{a}	0.18 ± 0.07^{ab}	0.15 ± 0.04^{b}
MK 10μM	0.25 ± 0.04^{a}	0.31 ± 0.05^{a}	0.20 ± 0.07^{ab}	0.17 ± 0.05^{ab}
MK + WY (10 + 10μM)	0.19 ± 0.04^{ab}	0.38 ± 0.08^{a}	0.15 ± 0.06^{ab}	0.18 ± 0.06^{ab}
PPARβ ligands				
Control	0.26 ± 0.05^{a}	0.32 ± 0.08^{a}	0.13 ± 0.06^{a}	0.24 ± 0.02^{a}
L 1μM	0.15 ± 0.03^{b}	0.34 ± 0.07^{a}	0.12 ± 0.06^{a}	0.21 ± 0.04^{a}
L 10μM	0.16 ± 0.04^{b}	0.44 ± 0.11^{a}	0.17 ± 0.07^{a}	0.18 ± 0.04^{a}
GW 10μM	0.19 ± 0.02^{ab}	0.35 ± 0.07^{a}	0.18 ± 0.09^{a}	0.19 ± 0.02^{a}
GW + L (10 + 10μM)	0.17 ± 0.02^{b}	0.40 ± 0.13^{a}	0.20 ± 0.06^{a}	0.21 ± 0.07^{a}
PPARγ ligands				
Control	0.26 ± 0.05^{a}	0.32 ± 0.08^{a}	0.13 ± 0.06^{a}	0.24 ± 0.02^{a}
PGJ_2 10μM	0.25 ± 0.04^{ab}	0.32 ± 0.06^{a}	0.17 ± 0.09^{a}	0.18 ± 0.04^{a}
RO 1μM	0.17 ± 0.03^{b}	0.35 ± 0.06^{a}	0.21 ± 0.10^{a}	0.20 ± 0.05^{a}
RO 10μM	0.16 ± 0.02^{b}	0.26 ± 0.07^{a}	0.16 ± 0.09^{a}	0.22 ± 0.06^{a}
T 1μM	0.19 ± 0.03^{ab}	0.30 ± 0.08^{a}	0.11 ± 0.06^{a}	0.21 ± 0.06^{a}
T + RO (1 + 1μM)	0.22 ± 0.01^{b}	0.31 ± 0.08^{a}	0.15 ± 0.06^{a}	0.19 ± 0.04^{a}

^{a,b}different letters indicate significant differences ($P \leq 0.05$) in steroid level between treatments (control and different agonist and/or antagonist within each status (the estrous cycle or pregnancy) and time point (days 10–12 or 14–16)); the same letters indicate no differences between treatments

PPARβ activation by 1 and 10μM of L-165045 diminished P_4 release by endometrial explants on days 10–12 of the estrous cycle, but did not change the secretion on days 14–16 of the estrous cycle and during both stages of pregnancy. Surprisingly, a simultaneous addition of the agonist and antagonist to the medium also abolished P_4 secretion by the tissue collected on days 10–12 of the estrous cycle.

A synthetic PPARγ agonist – rosiglitazone at doses 1 and 10μM – reduced P_4 release by endometrial explants on days 10–12 of the estrous cycle. The addition of the agonist together with the antagonist also inhibited P_4 release by the tissue in this group. The tested factors did not affect P_4 secretion by the tissue collected from the remaining experimental groups of gilts.

Impact of PPAR ligands on E_2 secretion. The concentration of E_2 in culture media collected after incubation of porcine endometrial explants with PPAR ligands is presented in Table 2. An inhibitory effect of PPARα agonist – WY-14643 (1 and 10μM) as well as combination of agonist with antagonist MK 886 on E_2 release by endometrial explants was observed on days 10–12 of pregnancy. Unexpectedly, a simultaneous treatment of the explants with the agonist and antagonist markedly increased steroid release on days 10–12 of the estrous cycle. PPARα ligands did not affect E_2 secretion by the tissue collected on days 14–16 of the estrous cycle and pregnancy.

The treatment of endometrial slices with 10μM of L-165045 (PPARβ agonist) increased E_2 secretion by the tissue on days 14–16 of the estrous cycle. Moreover, on days 10–12 of pregnancy, a combined addition of PPARβ agonist with antagonist (GW9662, 10μM) inhibited E_2 secretion. It should be emphasized that some doses of this antagonist may also affect the activity of PPARα and PPARγ.

Activation of PPARγ by a natural (PGJ_2, 10μM) as well as synthetic (rosiglitazone, 10μM) ligand increased E_2 release by the explants collected on days 10–12 of the estrous cycle. In contrast, 1μM of rosiglitazone inhibited steroid secretion on days

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Table 2. Effect of PPAR ligands on 17-β estradiol release by the endometrium of gilts on days 10–12 (n = 4) and 14–16 (n = 4) of the estrous cycle and days 10–12 (n = 4) and 14–16 (n = 4) of pregnancy

Estradiol (pg/ml)	Estrous cycle		Pregnancy	
	days 10–12	days 14–16	days 10–12	days 14–16
PPARα ligands				
Control	51.64 ± 12.54^{a}	56.10 ± 17.22^{a}	41.59 ± 9.39^{a}	69.41 ± 30.33^{a}
WY 1μM	58.60 ± 11.27^{a}	28.36 ± 4.95^{a}	23.48 ± 5.55^{b}	72.91 ± 33.04^{a}
WY 10μM	60.78 ± 9.13^{a}	30.65 ± 8.52^{a}	25.18 ± 1.16^{b}	58.03 ± 19.56^{a}
MK 10μM	53.06 ± 12.34^{a}	39.71 ± 4.54^{a}	31.96 ± 0.99^{ab}	43.40 ± 6.92^{a}
MK + WY (10 + 10μM)	140.90 ± 18.23^{b}	65.09 ± 23.39^{a}	26.03 ± 3.03^{b}	43.38 ± 11.48^{a}
PPARβ ligands				
Control	51.64 ± 12.54^{a}	56.10 ± 17.22^{a}	41.59 ± 9.39^{a}	69.41 ± 30.33^{a}
L 1μM	64.72 ± 28.72^{a}	85.94 ± 21.14^{ab}	29.36 ± 7.75^{ab}	57.19 ± 2.75^{a}
L 10μM	67.83 ± 11.91^{a}	115.4 ± 24.43^{b}	22.52 ± 11.70^{ab}	49.62 ± 0.87^{a}
GW 10μM	42.97 ± 15.45^{a}	53.39 ± 11.26^{a}	28.94 ± 3.02^{ab}	55.83 ± 3.17^{a}
GW + L (10 + 10μM)	79.64 ± 27.66^{a}	62.82 ± 10.75^{a}	18.98 ± 5.34^{b}	44.14 ± 2.12^{a}
PPARγ ligands				
Control	51.64 ± 12.54^{a}	56.10 ± 17.22^{a}	41.59 ± 9.39^{a}	69.41 ± 30.33^{a}
PGJ_2 10μM	104.0 ± 26.97^{b}	30.98 ± 8.29^{a}	39.98 ± 6.77^{a}	56.90 ± 3.65^{a}
RO 1μM	71.80 ± 18.74^{ab}	28.38 ± 2.25^{a}	17.80 ± 0.90^{bc}	53.97 ± 11.11^{a}
RO 10μM	107.1 ± 4.66^{b}	41.29 ± 6.19^{a}	33.66 ± 6.47^{ab}	78.12 ± 4.05^{a}
T 1μM	54.51 ± 10.45^{a}	69.18 ± 27.07^{a}	26.06 ± 7.47^{ab}	66.10 ± 11.28^{a}
T + RO (1 + 1μM)	63.89 ± 30.00^{ab}	63.47 ± 30.90^{b}	5.82 ± 0.58^{c}	70.40 ± 11.86^{a}

$^{a–c}$different letters indicate significant differences ($P \leq 0.05$) in steroid level between treatments (control and different agonist and/or antagonist within each status (estrous cycle or pregnancy) and time point (days 10–12 or 14–16)); the same letters indicate no differences between treatments

10–12 of pregnancy. Moreover, PPARγ antagonist (1μM) in combination with the agonist inhibited E_2 secretion by endometrial explants collected on days 10–12 of pregnancy.

Impact of PPAR ligands on 3β-HSD mRNA level. The expression of *3β-HSD* in porcine endometrial explants is presented in Table 3. We observed an inhibitory effect of PPARβ and PPARγ ligands on the *3β-HSD* mRNA level in the tissue collected on days 14–16 of the estrous cycle and days 14–16 of pregnancy. PPARα ligands decreased *3β-HSD* gene expression in the tissue on days 14–16 of pregnancy, but they were ineffective during the estrous cycle. The tested compounds did not affect *3β-HSD* gene expression in the endometrium on days 10–12 of the estrous cycle and pregnancy.

DISCUSSION

The current study, for the first time, indicates that PPARs are involved in P_4 and E_2 release by porcine endometrial tissue during the estrous cycle and early pregnancy. An inhibitory effect of all PPAR (α, β, γ) agonists on P_4 secretion was noted during the mid-luteal phase of the estrous cycle. During early pregnancy, a stimulatory effect of the PPARα agonist was observed during maternal recognition of pregnancy, while with an inhibitory effect, it was observed at the beginning of implantation. The present results showed an inhibitory effect of PPARα and PPARγ agonists on E_2 secretion by porcine endometrium on days 10–12 of pregnancy. An opposite – stimulatory – effect on E_2 secretion was observed after PPARβ and PPARγ activation on days 10–12 and 14–16 of the estrous cycle, respectively.

There is a lack of data describing the link between PPARs and steroid production in uterine tissue. Such a relationship has been observed in ovarian cells in different species, but the results are contradictory. For instance, PPARγ ligands enhanced P_4 release by porcine theca cells (Schoppee et al. 2002) and mixed human ovarian (stroma, theca, and granulosa) cells (Seto-Young et al. 2007), but inhibited hormone secretion by porcine and hu-

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Table 3. Expression of *3β-HSD* mRNA in the porcine endometrium

3β-HSD (arbitrary units)	Estrous cycle		Pregnancy	
	days 10–12	days 14–16	days 10–12	days 14–16
PPARα ligands				
Control	0.21 ± 0.06[a]	0.27 ± 0.06[a]	0.06 ± 0.01[a]	0.19 ± 0.04[a]
WY 1μM	0.31 ± 0.08[a]	0.24 ± 0.08[a]	0.05 ± 0.02[a]	0.05 ± 0.01[b]
WY 10μM	0.26 ± 0.08[a]	0.28 ± 0.05[a]	0.09 ± 0.05[a]	0.07 ± 0.04[b]
MK 10μM	0.28 ± 0.07[a]	0.17 ± 0.04[a]	0.06 ± 0.02[a]	0.06 ± 0.02[b]
MK + WY (10 + 10μM)	0.38 ± 0.16[a]	0.19 ± 0.05[a]	0.10 ± 0.04[a]	0.04 ± 0.02[b]
PPARβ ligands				
Control	0.21 ± 0.06[a]	0.27 ± 0.06[a]	0.06 ± 0.01[a]	0.19 ± 0.04[a]
L 1μM	0.17 ± 0.03[a]	0.13 ± 0.02[b]	0.04 ± 0.01[ab]	0.05 ± 0.02[b]
L 10μM	0.21 ± 0.09[a]	0.13 ± 0.01[b]	0.04 ± 0.01[a]	0.05 ± 0.01[b]
GW 10μM	0.15 ± 0.04[a]	0.11 ± 0.03[b]	0.08 ± 0.02[ac]	0.06 ± 0.04[b]
GW + L (10 + 10μM)	0.22 ± 0.02[a]	0.09 ± 0.03[b]	0.05 ± 0.01[a]	0.04 ± 0.02[b]
PPARγ ligands				
Control	0.21 ± 0.06[a]	0.27 ± 0.06[a]	0.06 ± 0.01[a]	0.19 ± 0.04[a]
PGJ_2 10μM	0.20 ± 0.05[a]	0.15 ± 0.04[b]	0.07 ± 0.01[a]	0.04 ± 0.01[b]
RO 1μM	0.21 ± 0.09[a]	0.16 ± 0.04[ab]	0.08 ± 0.03[ab]	0.04 ± 0.02[b]
RO 10μM	0.22 ± 0.04[a]	0.10 ± 0.03[b]	0.06 ± 0.02[a]	0.06 ± 0.01[b]
T 1μM	0.16 ± 0.01[a]	0.27 ± 0.05[a]	0.05 ± 0.02[a]	0.04 ± 0.01[b]
T + RO (1 + 1μM)	0.22 ± 0.03[a]	0.16 ± 0.03[ab]	0.09 ± 0.01[b]	0.03 ± 0.01[b]

[a,b]different letters indicate significant differences ($P \leq 0.05$) in gene expression between treatments (control and different agonist and/or antagonist within each status (estrous cycle or pregnancy) and time point (days 10–12 or 14–16)); the same letters indicate no differences within columns

man granulosa cells (Gasic et al. 1998). In turn, a lack of changes in P_4 production has been demonstrated in human granulosa-lutein cells (Chen et al. 2009). Our previous results have shown an inhibitory effect of PPAR (α, β, γ) agonists on P_4 release by porcine corpora lutea (CLs) during the peri-implantation stage, while the ligands were ineffective during mid- and late-luteal phases of the estrous cycle (Kurzynska et al. 2014). It seems that porcine ovarian CLs were more sensitive to the ligands during early pregnancy, whereas endometrial tissue was more receptive during the mid-luteal phase of the estrous cycle. This also suggests that PPAR-dependent P_4 production within these two tissues (uterus and ovary) is differentially regulated during the analyzed stages of the estrous cycle and pregnancy.

It should be highlighted that changes in P_4 secretion, observed in the current study, are not followed by changes in *3β-HSD* gene expression (Table 4). The ligands of PPARs inhibited the expression on days 14–16 of the estrous cycle (β, γ isoforms) or pregnancy (α, β, γ isoforms), while they were ineffective on days 10–12 of the estrous cycle or pregnancy. This phenomenon has also been observed by other researchers. Alterations in P_4 secretion after PPAR activation were not reflected in differences in the expression and/or activity of enzymes regulating P_4 synthesis (e.g. *CYP11A1* or *3β-HSD*) in porcine theca cells or ovine granulosa cells (Froment et al. 2003). Therefore, studies investigating molecular mechanisms of PPAR action in the endometrium are justified.

It has been recently reported that the uterus, along with the ovary, can be a source of estrogens (Mann et al. 2007). The impact of the reproductive status of animals on endometrial E_2 production was reflected by markedly greater amounts of E_2 released during early pregnancy compared with luteolysis (Franczak and Kotwica 2008). It is obvious that the sustaining of proper embryo development and pregnancy depends on a complex cross-talk between the embryo and mother. In pigs, such communication begins on day 12 of pregnancy, when the fetus secretes an increased amount of estrogens (Geisert et al. 1990). It is pos-

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Table 4. Effect of PPAR ligands on progesterone/*3β-HSD* and estradiol level

	Progesterone concentration/*3β-HSD* mRNA				Estradiol concentration			
	estrous cycle		pregnancy		estrous cycle		pregnancy	
	days 10–12	days 14–16	days 10–12	days 14–16	days 10–12	days 14–16	days 10–12	days 14–16
PPARα ligands								
WY 1μM	↓/↔	↔/↔	↑/↔	↓/↓	↔	↔	↓	↔
WY 10μM	↔/↔	↔/↔	↔/↔	↓/↓	↔	↔	↓	↔
MK 10μM	↔/↔	↔/↔	↔/↔	↔/↓	↔	↔	↔	↔
MK + WY (10 + 10μM)	↔/↔	↔/↔	↔/↔	↔/↓	↑	↔	↓	↔
PPARβ ligands								
L 1μM	↓/↔	↔/↓	↔/↔	↔/↓	↔	↔	↔	↔
L 10μM	↓/↔	↔/↓	↔/↔	↔/↓	↔	↑	↔	↔
GW 10μM	↔/↔	↔/↓	↔/↔	↔/↓	↔	↔	↔	↔
GW + L (10 + 10μM)	↓/↔	↔/↓	↔/↔	↔/↓	↔	↔	↓	↔
PPARγ ligands								
PGJ_2 10μM	↔/↔	↔/↓	↔/↔	↔/↓	↑	↔	↔	↔
RO 1μM	↓/↔	↔/↔	↔/↔	↔/↓	↔	↔	↓	↔
RO 10μM	↓/↔	↔/↓	↔/↔	↔/↓	↑	↔	↔	↔
T 1μM	↔/↔	↔/↔	↔/↔	↔/↓	↔	↔	↔	↔
T + RO (1 + 1μM)	↓/↔	↔/↔	↔/↔	↔/↓	↔	↔	↓	↔

↑↓values within a row with different signs show stimulatory or inhibitory effects that differ significantly at $P \leq 0.05$
↔ values showing no effect

sible that the cooperative action of embryonic and uterine E_2 stimulates endometrial PGE_2 synthesis (Waclawik 2011). Consequently, an increased ratio of $PGE_2/PGF_2\alpha$ protects the corpus luteum against luteolysis. Our previous data indicates that PPARs are engaged in PGE_2 and $PGF_2\alpha$ production in endometrial tissue, depending on the physiological status of pigs (Bogacka et al. 2013a, b). The present study suggests that PPARs are also involved in E_2 release by porcine endometrium. An inhibitory effect of PPARα and PPARγ on E_2 secretion was observed during maternal recognition of pregnancy, but a stimulatory effect was observed during mid- (γ isoform) or late-luteal (β isoform) phases. This differential response of endometrial tissue could play a role in the regulation of the uterine prostaglandins (PGE_2 and $PGF_2\alpha$) ratio and, in consequence, may cause the sustaining of CL during pregnancy or luteolysis during the estrous cycle. It should be underlined that in some cases, simultaneous treatment of endometrial explants with agonist and antagonist, surprisingly, affected E_2 secretion. This paradoxical additive response might arise from possible additional intracellular pathways which are activated in the presence of both ligands. It also cannot be excluded that ligands applied under specific condition exhibited various specificity and/or affinity to PPARs present in porcine endometrium.

A direct interaction between PPARs and endometrial E_2 synthesis has not been investigated so far, but available data has noted such a relationship in the ovary. Available inconsistent results indicate that the diverse impact of PPAR ligands on E_2 production depends on cell or ligand types, time of incubation with PPAR ligands and various species. For example, PPARγ activation inhibited E_2 production in human ovarian (mixture of stroma, theca, and granulosa) cells (Seto-Young et al. 2007) but enhanced the secretion in rat granulosa cells (Zhang et al. 2007). There are also reports showing a lack of PPARγ activation in E_2 production in porcine ovarian follicles (Waclawik 2011). Our previous studies demonstrated a differential (inhibitory or lack of effect) influence of PPAR ligands on E_2 release by the CL depending on the stage of the estrous cycle or pregnancy (Kurzynska et al. 2014).

CONCLUSION

This is the first report indicating that PPARs participate in the regulation of P_4 and E_2 produc-

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tion by porcine endometrium. Our study demonstrates the diverse receptivity of endometrial tissue to PPAR ligands, which can be associated with the reproductive status of gilts. Generally, the agonists of PPAR isoforms (α, β, γ) markedly inhibited P_4 production in the endometrium during mid-luteal phase (days 10–12) of the estrous cycle, whereas E_2 production was inhibited by the ligands (β and γ isoforms) during maternal recognition of pregnancy (days 10–12). However, additional experiments are needed to clarify the mechanisms of PPAR action in female uterine tissue.

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REFERENCES

Barak Y., Nelson M.C., Ong E.S, Jones Y.Z., Ruiz-Lozano P., Chien K.R., Koder A., Evans R.M. (1999): PPAR gamma is required for placental, cardiac, and adipose tissue development. Molecular Cell, 4, 585–595.

Barak Y., Liao D., He W., Ong E.S., Nelson M.C., Olefsky J.M., Boland R., Evans R.M. (2002): Effects of peroxisome proliferator-activated receptor delta on placentation, adiposity, and colorectal cancer. Proceedings of the National Academy of Sciences of the United States of America, 99, 303–308.

Bogacka I., Bogacki M. (2011): The quantitative expression of peroxisome proliferator activated receptor (PPAR) genes in porcine endometrium through the estrous cycle and early pregnancy. Journal of Physiology and Pharmacology, 62, 559–565.

Bogacka I., Bogacki M., Kurzynska A., Chojnowska K. (2013a): The involvement of peroxisome proliferator activated receptors (PPARs) in prostaglandin F2α production by porcine endometrium. Reproductive Biology, 13, 309–316.

Bogacka I., Bogacki M., Gaglewska M., Kurzynska A., Wasielak M. (2013b): In vitro effect of peroxisome proliferator activated receptor (PPAR) ligands on prostaglandin E_2 synthesis and secretion by porcine endometrium during the estrous cycle and early pregnancy. Journal of Physiology and Pharmacology, 64, 47–54.

Bogacka I., Kurzynska A., Bogacki M., Chojnowska K. (2015): Peroxisome proliferator-activated receptors in the regulation of female reproductive functions. Folia Histochemica et Cytobiologica, 53, 189–200.

Chen Q., Sun X., Chen J., Cheng L., Wang J., Wang Y., Sun Z. (2009): Direct rosiglitazone action on steroidogenesis and proinflammatory factor production in human granulosa-lutein cells. Reproductive Biology and Endocrinology, 7, 147.

Ciereszko R., Opalka M., Kaminska B., Wojtczak M., Okrasa S., Dusza L. (2001): Luteotrophic action of prolactin during the early luteal phase in pigs: the involvement of protein kinases and phosphatases. Reproductive Biology, 1, 62–83.

Du Q., Yang S., Wang Y.J., Wu B., Zhao Y.Y., Fan B. (2012): Effects of thiazolidinediones on polycystic ovary syndrome: a metaanalysis of randomized placebo-controlled trials. Advances in Therapy, 29, 763–774.

Franczak A., Kotwica G. (2008): Secretion of estradiol-17beta by porcine endometrium and myometrium during early pregnancy and luteolysis. Theriogenology, 69, 283–289.

Franczak A., Wojciechowicz B., Kotwica G. (2013): Novel aspects of cytokine action in porcine uterus – endometrial and myometrial production of estrone (E_1) in the presence of interleukin 1beta (IL1beta), interleukin 6 (IL6) and tumor necrosis factor (TNFalpha) – in vitro study. Folia Biologica (Krakow), 61, 53–61.

Froment P., Fabre S., Dupont J., Pisselet C., Chesneau D., Staels B., Monget P. (2003): Expression and functional role of peroxisome proliferator-activated receptor-gamma in ovarian folliculogenesis in the sheep. Biology of Reproduction, 69, 1665–1674.

Gasic S., Bodenburg Y., Nagamani M., Green A., Urban R.J. (1998): Troglitazone inhibits progesterone production in porcine granulosa cells. Endocrinology, 139, 4962–4966.

Geisert R.D., Zavy M.T., Moffatt R.J., Blair R.M., Yellin T. (1990): Embryonic steroids and the establishment of pregnancy in pigs. Journal of Reproduction and Fertility, 40 (Suppl.), 293–305.

Huang J.C. (2008): The role of peroxisome proliferator-activated receptors in the development and physiology of gametes and preimplantation embryos. PPAR Research, 2008, 732303.

Kurzynska A., Bogacki M., Chojnowska K., Bogacka I. (2014): Peroxisome proliferator activated receptor ligands affect progesterone and 17β-estradiol secretion by porcine corpus luteum during early pregnancy. Journal of Physiology and Pharmacology, 65, 709–717.

Lovekamp T.N., Davis B.J. (2001): Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. Toxicology and Applied Pharmacology, 172, 217–224.

Mann G.E., Scholey D.V., Robinson R.S. (2007): Identification of elevated concentrations of estradiol in bovine uterine endometrium. Domestic Animal Endocrinology, 33, 437–441.

Michalik L., Desvergne B., Dreyer C., Gavillet M., Laurini R.N., Wahli W. (2002): PPAR expression and function

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during vertebrate development. The International Journal of Developmental Biology, 46, 105–114.

Nishimura K., Yamauchi N., Chowdhury V.S., Torii M., Hattori M.A., Kaneto M. (2011): Expression of peroxisome proliferator-activated receptor isoforms in the rat uterus during early pregnancy. Cell and Tissue Research, 345, 275–284.

Schoppee P.D., Garmey J.C., Veldhuis J.D. (2002): Putative activation of the peroxisome proliferator-activated receptor gamma impairs androgen and enhances progesterone biosynthesis in primary cultures of porcine theca cells. Biology of Reproduction, 66, 190–198.

Seto-Young D., Avtanski D., Strizhevsky M., Parikh G., Patel P., Kaplun J., Holcomb K., Rosenwaks Z., Poretsky L. (2007): Interactions among peroxisome proliferator activated receptor-gamma, insulin signaling pathways, and steroidogenic acute regulatory protein in human ovarian cells. The Journal of Clinical Endocrinology & Metabolism, 92, 2232–2239.

Szafranska B., Ziecik A., Okrasa S. (2002): Primary antisera against selected steroids or proteins and secondary antisera against γ-globulins – as available tool for studies of reproductive processes. Reproductive Biology, 2, 187–204.

Velez L.M., Abruzzese G.A., Motta A.B. (2013): The biology of the peroxisome proliferator-activated receptor system in the female reproductive tract. Current Pharmaceutical Design, 19, 4641–4646.

Waclawik A. (2011): Novel insights into the mechanisms of pregnancy establishment: regulation of prostaglandin synthesis and signaling in the pig. Reproduction, 142, 389–399.

Wojciechowicz B., Kotwica G., Kolakowska J., Franczak A. (2013): The activity and localization of 3β-hydroxysteroid dehydrogenase/Δ(5)-Δ(4) isomerase and release of androstenedione and progesterone by uterine tissues during early pregnancy and the estrous cycle in pigs. Journal of Reproduction and Development, 59, 49–58.

Zhang H., Li Q., Lin H., Yang Q., Wang H., Zhu C. (2007): Role of PPAR gamma and its gonadotrophic regulation in rat ovarian granulosa cells in vitro. Neuroendocrinology Letters, 28, 289–294.

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