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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

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Peroxisome proliferator activated receptors (PPARs) are ligand-dependent transcriptional factors which are expressed in distinct tissues of the female reproductive system, including the ovary, uterus and placenta. An important role of PPARs in the regulation of reproductive processes has been previously highlighted in rodents. In the present study we investigated the *in vitro* effect of PPAR ligands on prostaglandin E_2 (PGE_2) release and prostaglandin E synthase (PGES) gene expression in the endometrial explants collected from cyclic (days 10–12 and 14–16 of the estrous cycle) or pregnant (days 10–12 and 14–16) pigs. A stimulatory ($p<0.05$) effect of rosiglitazone (PPARγ agonist) on PGE_2 accumulation was noted during both stages of the estrous cycle and both stages of pregnancy, whereas a higher ($p<0.05$) PGES mRNA level was observed on days 10–12 of the estrous cycle and on days 14–16 of gestation when compared to the controls. The activation of PPARβ by L-165,041 augmented ($p<0.05$) PGE_2 release by the endometrium on days 14–16 of the estrous cycle and on days 14–16 of pregnancy, but the increase ($p<0.05$) in PGES mRNA abundance was noted on days 10–12 of the estrous cycle and during both stages of pregnancy. A stimulatory ($p<0.05$) effect of WY-14643 (agonist) and MK 886 (antagonist) on PGE_2 release was noted on days 10–12 of the estrous cycle, and days 14–16 of pregnancy, respectively. There was a lack of change in PGES mRNA abundance in the endometrium exposed to PPARα ligands. We conclude that PPARs are mediators of prostaglandin E_2 synthesis/accumulation in porcine endometrium during the luteal phase of the estrous cycle and the time of periimplantation.

Key words: *peroxisome proliferator activated receptor alpha,* pregnancy, *peroxisome proliferator activated receptor beta, peroxisome proliferator activated receptor gamma, prostaglandin E synthase, reproduction, implantation*

INTRODUCTION

Peroxisome proliferator activated receptors (PPARs) are transcriptional factors which are strongly involved in carbohydrate and lipid metabolism (1-5). To date, three isotypes of PPARs designated as PPARα, PPARβ (known also as PPARδ) and PPARγ were discovered. A number of natural and synthetic ligands bind PPAR/retinoid X receptor (RXR) heterodimer in distinct tissues (liver, kidney, heart, adipose tissue or skeletal muscle) to control the expression of many target genes (6). Several ligands of PPARs have been applied in clinical practice (7). Synthetic activators of PPARα (fibrates) and PPARγ (thiazolidinediones) are used as therapeutic agents in metabolic disorders including dislipidemia, insulin resistance and type 2 diabetes. Moreover, the treatment with thiazolidinediones improve fertility by the induction of ovulation in women with polycystic ovary syndrome (PCOS) (8).

It has been recently emphasized that PPARs play an important role in the regulation of female reproduction (9, 10). PPARs expression has been reported in many reproductive tissues including the ovary, uterus and placenta (10, 11). They are also present in gametes and embryos (12). PPARγ-knockout mice die due to disorders in placental vascularization (13). PPARβ-null mice also show abnormalities in placenta development (14), whereas PPARα-null rodents display higher risk of maternal abortion and neonatal mortality (15). It has been reported that PPARs regulate placenta development and maternal-fetal nutrient transport as well as ovarian steroidogenesis, angiogenesis and tissue remodeling (for review see 16, 17). Our unpublished data suggest the involvement of PPARs in prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$ possesses a luteolytic activity) production by porcine endometrium during the estrous cycle and the time of implantation (Bogacka *et al*, submitted). A higher release of $PGF_{2\alpha}$ by the endometrial tissue in response to PPAR agonists during the luteal phase of the estrous cycle and lack of changes in $PGF_{2\alpha}$ secretion during maternal recognition of pregnancy might regulate the regression and sustaining of the corpus luteum, respectively. There are limited data regarding the role of PPARs in the regulation of prostaglandin E_2 (PGE_2) production/secretion, a luteotrophic agent in pigs (18). In the present study we investigated the *in vitro* effect of PPAR ligands on PGE_2 release and mRNA expression of prostaglandin E synthase (PGES, the terminal enzyme of

PGE_2 synthesis) in porcine endometrial tissue collected during the estrous cycle (days 10–12 and 14–16) or pregnancy (days 10–12 and 14–16).

MATERIALS AND METHODS

Animals

All procedures relative to the care and use of animals were approved by the Local Animal Ethics Committee and the study was conducted in accordance with the national guidelines for animal care.

The study was performed on crossbred pigs of Polish Landrace and Pietrain (100 kg, 7 month-old) from a commercial farm. Animals were synchronized and superovulated by a single intramuscular (i.m.) injection of 750 I.U. PMSG (Folligon, Intervet, Netherlands) followed by 500 I.U. hCG (im; Chorulon, Intervet, Netherlands) administered 72 hours later as described previously (19-21). The animals were divided into cyclic and pregnant groups. Each group was represented by two different stages of the estrous cycle/pregnancy (days 10–12 and 14–16). Days 10–12 of pregnancy refer to the time of maternal recognition of pregnancy and days 14–16 are associated with major remodeling of the uterus initiating process of the implantation. Days 10–12 and 14–16 of the estrous cycle reflect mid- and late-luteal phases, respectively. The gilts from the pregnant group were inseminated twice: 24 and 36 hours after hCG treatment. Pregnancy was confirmed by the embryo presence in the uterine flushing. After slaughter the uterine horns were dissected and transported to the laboratory in PBS with antibiotics on ice.

Incubation of the endometrial explants

The procedure for the collection and incubation of endometrial tissue was described previously (20, 22). The endometrium was separated from the myometrium by scraping with scalpel blade, minced into small pieces (200–210 mg) and washed twice with PBS. Each tissue piece was placed in a sterile culture vial with 2 ml of medium 199 supplemented with 0.1% BSA, gentamycin (40 mg/ml) and nystatin (120 IU/ml). The endometrial pieces were pre-incubated in a water bath for 18 hours in an atmosphere of 95% O_2 and 5% CO_2 and then treated for 6 hours with the following reagents: PPARγ ligands – 15d-prostaglandin J_2 (agonist; 10 μM), rosiglitazone (agonist; 1 and 10 μM; Cayman Chemical Company, USA) and T0070907 (antagonist; 1 μM, Cayman Chemical Company, USA); PPARβ ligands – L-165,041 (agonist; 1 and 10 μM, TOCRIS Bioscience, USA) and GW 9662 (antagonist; 10 μM; Cayman Chemical Company, USA); PPARα ligands – WY-14643 (agonist; 1 and 10 μM; Cayman Chemical Company, USA) and MK 886 (antagonist; 10 μM; Enzo Life Sciences International, USA). The PPAR ligand concentrations and incubation times were selected according to our preliminary study and previous reports (23-25). Tested compounds were added to culture media in dimethyl sulfoxide (DMSO). Controls (without the treatments) contained culture media or DMSO. After incubation, the endometrial slices were washed with PBS and snap frozen at –80°C for total RNA isolation and real-time PCR quantification. Incubation media were collected for radioimmunology assay and frozen at –20°C.

Determination of prostaglandin E_2 accumulation in culture media

Concentrations of PGE_2 in culture media collected after incubation of endometrial slices for 6 hours with the tested factors were determined by RIA using 'Prostaglandin E_2 RIA kit' according to the protocol provided by Institute of Isotopes Co., Ltd. Budapest, Hungary. The standard curve for PGE_2 ranged from 1.25 pg/tube to 1600 pg/tube. The sensitivity of the PGE_2 was 1.25 pg/tube. The inter- and intra-assay coefficients were less than 7%.

RNA extraction and real-time RT-PCR

Total RNA was isolated with the 'Total RNA' kit (A&A Biotechnology, Poland), quantified spectrophotometrically and the integrity of the product was confirmed on 1.5% agarose gel. The expression of mRNA encoding prostaglandin E synthase (PGES) was determined using TaqMan®RNA-to-CTTM 1-Step Kit (Applied Biosystems, CA, USA). The sequences of primers and Taqman probe for PGES (GenBank No AY857634) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; GenBank No U48832) were designed using Primer Express Software 3 (Applied Biosystems, CA, USA) and were synthesized by Applied Biosystems. The following primer and probe sequences were used: PGES forward TTTGCCAACCCCGAGGAT, PGES reverse TCATTCCGATGGGCCCTAA, PGES probe CCAGTACTGCCGGAGCGACCCAG; GAPDH forward CATCAATGGAAAGGCCATCAC, GAPDH reverse CAGCATCGCCCCATTTG and GAPDH probe CTTCCAGGAGCGAGATCCCGCC. The concentrations of the PCR primers were 300 nM and 200 nM of the TaqMan fluorogenic probes labeled with FAM (6-FAM,6-carboxyfluorescein) dye. Real-time RT-PCR was carried out in an ABI PRSISM 7300 sequence detector (Applied Biosystems, CA, USA) using the following parameters: one cycle at 48°C for 30 min, then one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and one cycle at 60°C for 1 min. All expression data were normalized to the amount of GAPDH mRNA and presented as arbitrary units (19, 20, 26).

Statistical analysis

Results were analyzed using Statistica (version 8.0, StatSoft Inc, USA). Significant differences were determined by Anova for repeated measurements followed by least significant differences (LSD) post-hoc test. Statistical significances were assigned with different letters at $p<0.05$. The data are presented as means ±S.E.M.

RESULTS

Prostaglandin E_2 release

Treatment of the endometrial tissue with 10 μM of rosiglitazone (PPARγ agonist) enhanced ($p<0.05$) PGE_2 release during both stages of the estrous cycle and pregnancy (*Fig. 1A-1D*, *Table 1*). The presence of 1 μM of rosiglitazone stimulated ($p<0.05$) PGE_2 secretion on days 10–12 of the estrous cycle (*Fig. 1A*) and tended to increase ($p=0.07$) PGE_2 secretion on days 14–16 of pregnancy (*Fig. 1D*) when compared to controls. The addition of PGJ_2 tended to increase ($p=0.07$) PGE_2 secretion on days 10–12 of the estrous cycle (*Fig. 1A*) and days 14–16 of pregnancy (*Fig. 1D*) whereas the PPARγ antagonist (T0070907) given alone or in combination with rosiglitazone did not change PGE_2 accumulation in the culture media (*Fig. 1A-1D*).

The addition of 1 or 10 μM of L-165,041 (PPARβ agonist) increased ($p<0.05$) PGE_2 secretion by the endometrium collected on days 14–16 of the estrous cycle (*Fig. 2B*) and days 14–16 of pregnancy (*Fig. 2D*). The treatment with GW9662 (PPARβ antagonist) alone or in combination with L-

Table 1. The effect of PPAR ligands on PGE_2 concentration in the culture media/PGES mRNA abundance in porcine endometrium collected during the estrous cycle or pregnancy (days 10-12 and 14-16).

	Estrous cycle (PGE_2 concentration/PGES mRNA)		**Pregnancy** (PGE_2 concentration/PGES mRNA)	
	Days 10–12	Days 14–16	Days 10–12	Days 14–16
PPARγ ligands				
PGJ_2 10 μM	↔ / ↔	↔ / ↔	↔ / ↔	↔ / ↔
ROSI 1 μM	↑ / ↑	↔ / ↔	↔ / ↔	↔ / ↔
ROSI 10 μM	↑ / ↑	↑ / ↔	↑ / ↔	↑ / ↑
T 1 μM	↔ / ↔	↔ /↔	↔ /↔	↔ / ↔
T+ROSI (1+1 μM)	↔ / ↔	↔ /↔	↔ / ↔	↔ / ↔
PPARβ ligands				
L 1 μM	↔ / ↑	↑ / ↔	↔ / ↑	↑ / ↔
L 10 μM	↔ / ↑	↑ / ↔	↔ / ↔	↑ / ↑
GW 10 μM	↔ / ↔	↔ / ↔	↔ / ↔	↔ / ↔
GW+L (10+10 μM)	↔ / ↔	↔ / ↔	↔ / ↔	↔ / ↔
PPARα ligands				
WY 1 μM	↔ / ↔	↔/ ↔	↔ / ↔	↔ / ↔
WY 10 μM	↑ / ↔	↔ / ↔	↔ / ↔	↔ / ↔
MK 10 μM	↔ / ↔	↔ / ↔	↔ / ↔	↑ / ↔
MK+WY (10+10 μM)	↔ / ↔	↔ / ↔	↔ / ↔	↔ / ↔

↑ stimulatory effect; ↔ lack of effect.

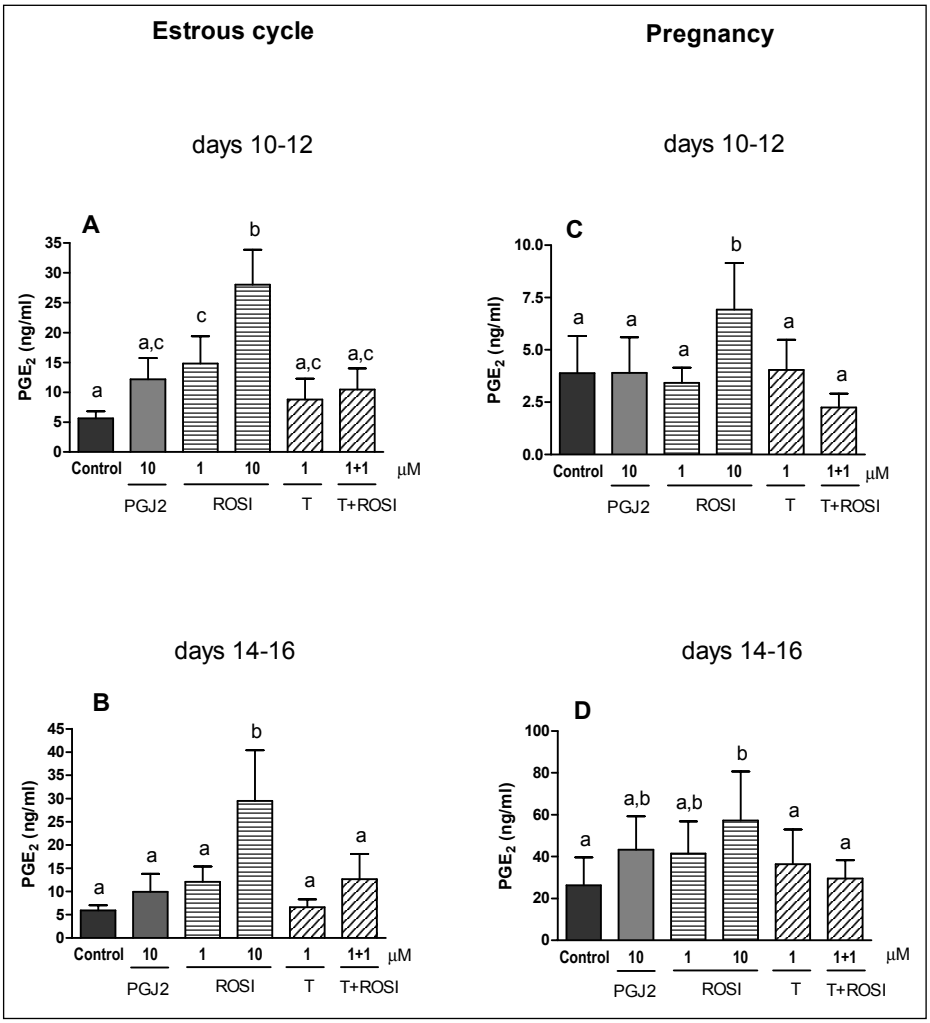

Fig. 1. The effect of PPARγ agonists: 15d-prostaglandin J_2 (PGJ_2, 10 μM), rosiglitazone (ROSI, 1 and 10 μM) and/or PPARγ antagonist T0070907 (T, 1 μM) on PGE_2 release by the endometrial tissue collected from gilts on days 10–12 and 14–16 of the estrous cycle (*A* and *B*) and days 10–12 and 14–16 of pregnancy (*C* and *D*). Tissue slices were incubated with the treatments or without (control) for 6 hours. Different letters designate significant statistical differences ($p<0.05$).

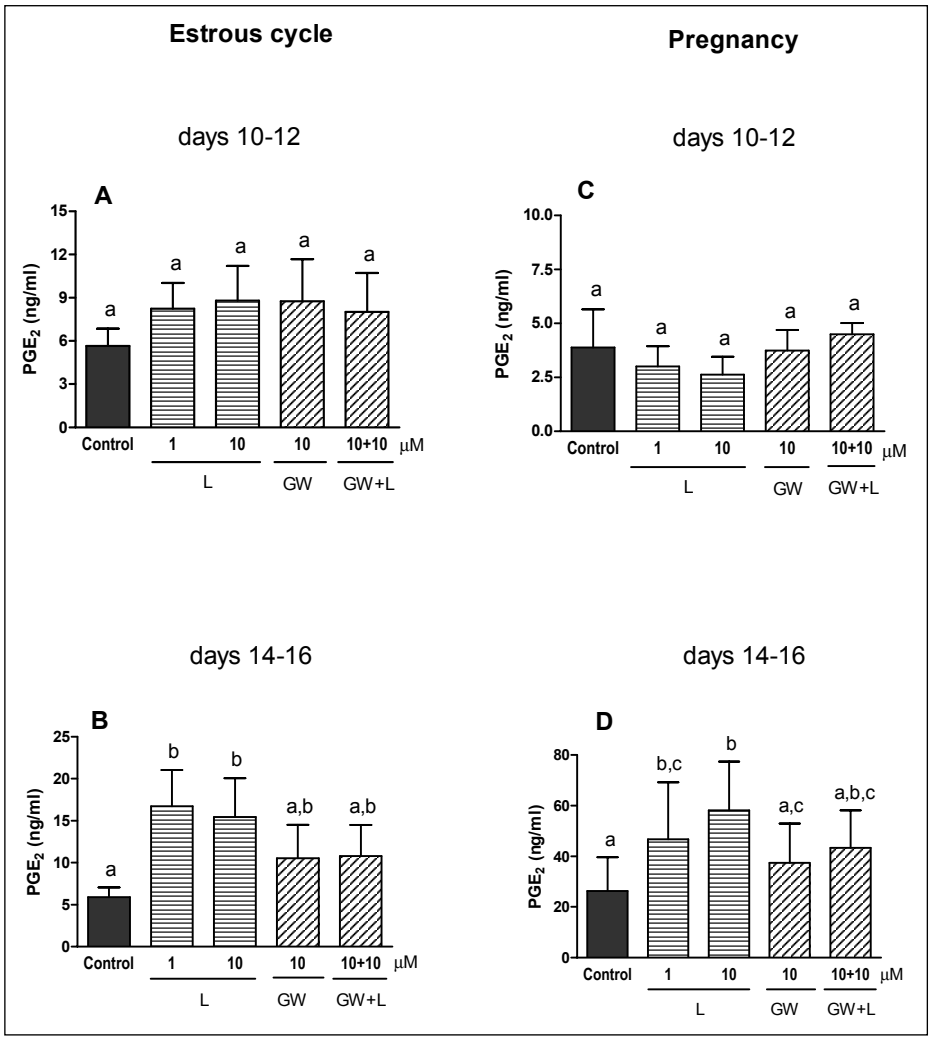

Fig. 2. The effect of PPARβ/δ agonist L-165,041 (L, 1 and 10 μM) and/or its antagonist GW 9662 (GW, 10 μM) on PGE_2 release by the endometrial tissue collected from gilts on days 10–12 and 14–16 of the estrous cycle (*A* and *B*) and days 10–12 and 14–16 of pregnancy (*C* and *D*). Tissue slices were incubated with the treatments or without (control) for 6 hours. Different letters designate significant statistical differences ($p<0.05$).

165,041 did not affect PGE_2 accumulation in culture media (*Fig. 2B* and *2D*). The tested ligands were ineffective on days 10–12 of the estrous cycle (*Fig. 2A*) and on days 10–12 of pregnancy (*Fig. 2C*).

A stimulatory effect ($p<0.05$) of a higher dose (10 μM) of WY-14643 (PPARα agonist) on PGE_2 release by the endometrium was noted on days 10–12 of the estrous cycle (*Fig. 3A*). However, using the lower dose (1 μM) of WY-14643 tended to increase ($p=0.07$, *Fig. 3A*) PGE_2 secretion by the endometrium on days 10–12 of the estrous cycle. The tested factor did not affect PGE_2 secretion by endometrial explants in the remaining groups of animals (*Fig. 3B-3D*). The presence of MK-886 (PPARα antagonist) increased ($p<0.05$) PGE_2 secretion by the tissue collected on days 14–16 of pregnancy (*Fig. 3D*) and it was ineffective in the remaining groups of pigs (*Fig. 3A*, *3B*, *3C*).

Prostaglandin E synthase (PGES) mRNA abundance

We observed a stimulatory ($p<0.05$) effect of both doses (1 and 10 μM) of rosiglitazone on PGES mRNA abundance in the endometrial tissue on days 10–12 of the estrous cycle (*Fig. 4A*, *Table 1*). The presence of PPARγ antagonist (T0070907) attenuated the stimulatory effect of rosiglitazone (*Fig. 4A*). Prostaglandin J_2 tended ($p=0.07$) to increase PGE_2 mRNA expression (*Fig. 4A*). Rosiglitazone at the higher dose (10 μM) enhanced ($p<0.05$) and at the lower dose (1 μM) tended to increase ($p=0.08$) PGES gene expression in the endometrium collected on days 14–16 of pregnancy (*Fig. 4D*). PPARγ ligands did not affect PGES mRNA abundance in porcine endometrium on days 10–12 of pregnancy (*Fig. 4C*) and on days 14–16 of the estrous cycle (*Fig. 4B*).

The activation of PPARβ by L-165,041 (1 and/or 10 μM) augmented ($p<0.05$) PGES mRNA level in the endometrium collected on days 10–12 of the estrous cycle (*Fig. 5A*) and during both stages of pregnancy (*Fig. 5C*, *5D*). The presence of GW9662 (PPARβ antagonist) abolished the stimulatory effect of L-165,041 (*Fig. 5A*). The tested ligands did not change PGES mRNA abundance in the tissue on days 14–16 of the estrous cycle (*Fig. 5B*). PPARα ligands did not affect PGES mRNA abundance in the endometrium in any of the experimental groups of animals (data not shown).

DISCUSSION

In the present study we investigated the *in vitro* effect of PPAR ligands on prostaglandin E_2 release and prostaglandin E synthase gene expression in the endometrium collected from cyclic (days 10–12 and 14–16) or pregnant (days 10–12 and 14–16) pigs. We found that all PPARs are involved in PGE_2 synthesis/release by the tissue. A stimulatory effect of rosiglitazone (PPARγ agonist) on PGE_2 accumulation was noted during both stages of the estrous cycle and both stages of

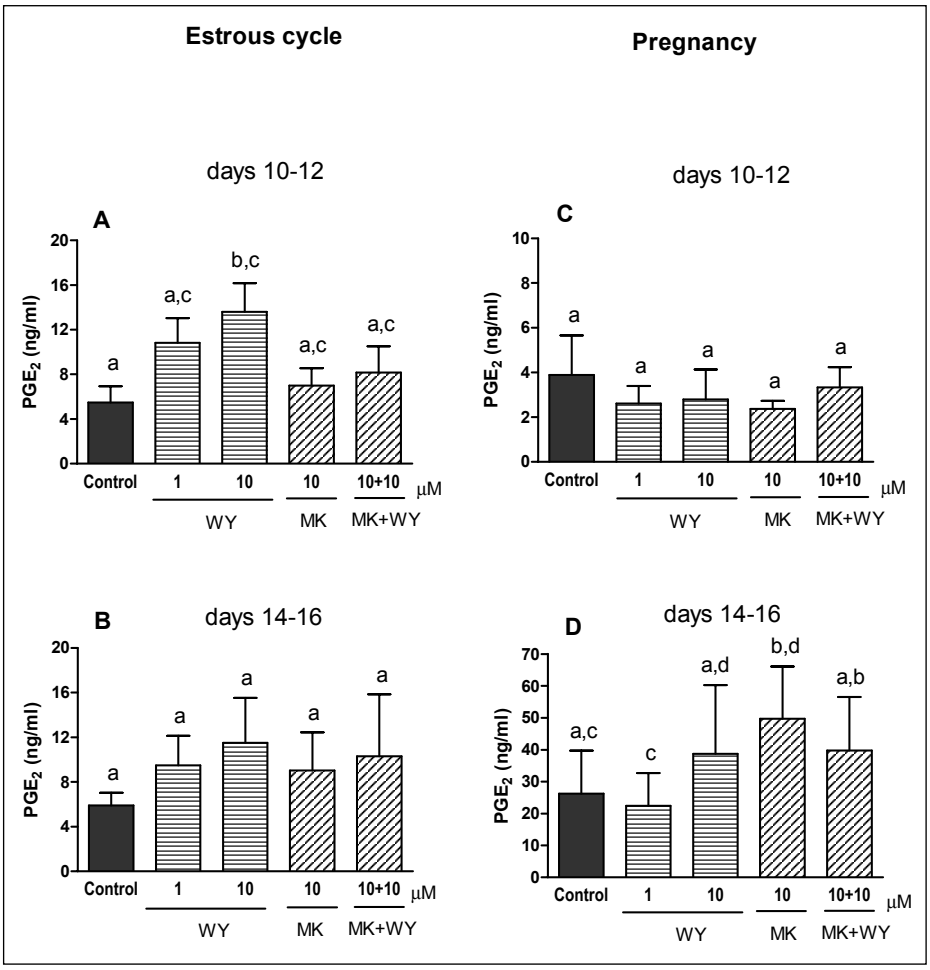

Fig. 3. The effect of PPARα agonist WY-14643 (WY, 1 and 10 μM) and/or its antagonist MK-886 (MK, 10 μM) on PGE_2 release by the endometrial tissue collected from gilts on days 10–12 and 14–16 of the estrous cycle (*A* and *B*) and days 10–12 and 14–16 of pregnancy (*C* and *D*). Tissue slices were incubated with the treatments or without (control) for 6 hours. Different letters designate significant statistical differences ($p<0.05$).

pregnancy whereas a higher PGES mRNA level was observed on days 10–12 of the estrous cycle and on days 14–16 of gestation when compared to the controls. This indicates that on days 10–12 of the estrous cycle and days 14–16 of pregnancy rosiglitazone acts in a more comprehensive way on both the release of PGE_2 and its own synthesis, supposedly to meet further requirements for PGE_2.

The activation of PPARβ by L-165,041 enhanced PGE_2 release by the endometrium on days 14–16 of the estrous cycle and on days 14–16 of pregnancy, but a significant increase in PGES mRNA abundance was noted on days 10–12 of the estrous cycle and during both stages of pregnancy. This shows that the PPARβ receptor mediates signal transduction as early as on days 10–12 of the estrous cycle by the stimulation of PGES gene expression for the increase in PGE_2 release on days 14–16 of the estrous cycle. Similarly, the same situation is observed in pregnant animals, where gene expression is stimulated earlier in pregnancy (days 10–12) and the final product - PGE_2 release is increased on days 14–16 of pregnancy. Stimulation of gene expression is sustained till days 14–16 of pregnancy, suggesting that the later phases of pregnancy require more PGE_2 for the implantation process. Thus, PPARβ participates in PGE_2 synthesis and release in relation to the implantation process. The importance of several other factors, including calcium, can not be ruled out (27).

In the present study, PPARα ligands were much less effective. A stimulatory effect of WY-14643 (agonist) and MK 886 (antagonist) on PGE_2 release was noted on days 10–12 of the estrous cycle and days 14–16 of pregnancy, respectively. We did not observe changes in PGES gene expressions in the endometrium exposed to PPARα ligands. This suggests that PPARα is only a mediator in the releasing process of PGE_2, supporting other PPAR receptors.

The endometrial synthesis of PGE_2 in pigs is regulated by cyclooxygenase-2 (COX-2) (28-30) and by specific microsomal PGE synthase (mPGES) (30, 31). COX-2, however, is a key enzyme catalyzing the synthesis of distinct prostaglandins comprising PGE_2 and $PGF_{2\alpha}$. No information concerning the existence of direct correlation between PGES/PGE_2 and PPARs in the female reproductive system is available. However, the role of PPARs in the modulation of COX-2 expression in different types of cells or tissues has been reported. For instance, rosiglitazone and 15d-PGJ_2 (PPARγ agonists) reduced gene and protein expression of COX-2 in human endometriotic/endometrial cells in the secretory phase of the menstrual cells (25), but the same agonists stimulated the expression in human WISH and primary amnion cells (23). Furthermore, WY-14643 (PPARα agonist) induced COX-2 in bovine endometrial stromal (32) and epithelial (24) cells but PPARβ agonists did not affect COX-2 expression in these cells (32). Stimulatory or inhibitory effects of PPAR ligands (linoleic acid, PGA_2, 15d-PGJ_2, non-steroidal anti-inflammatory drugs or WY-14643) on COX-2 gene expression or COX-2 activity have been demonstrated in mammary and colon epithelial cells (33). Although the results of many studies demonstrated the correlation between PPARs and COX-2 expression/activity, they do not provide a direct evidence on the PPARs involvement in PGE_2 production.

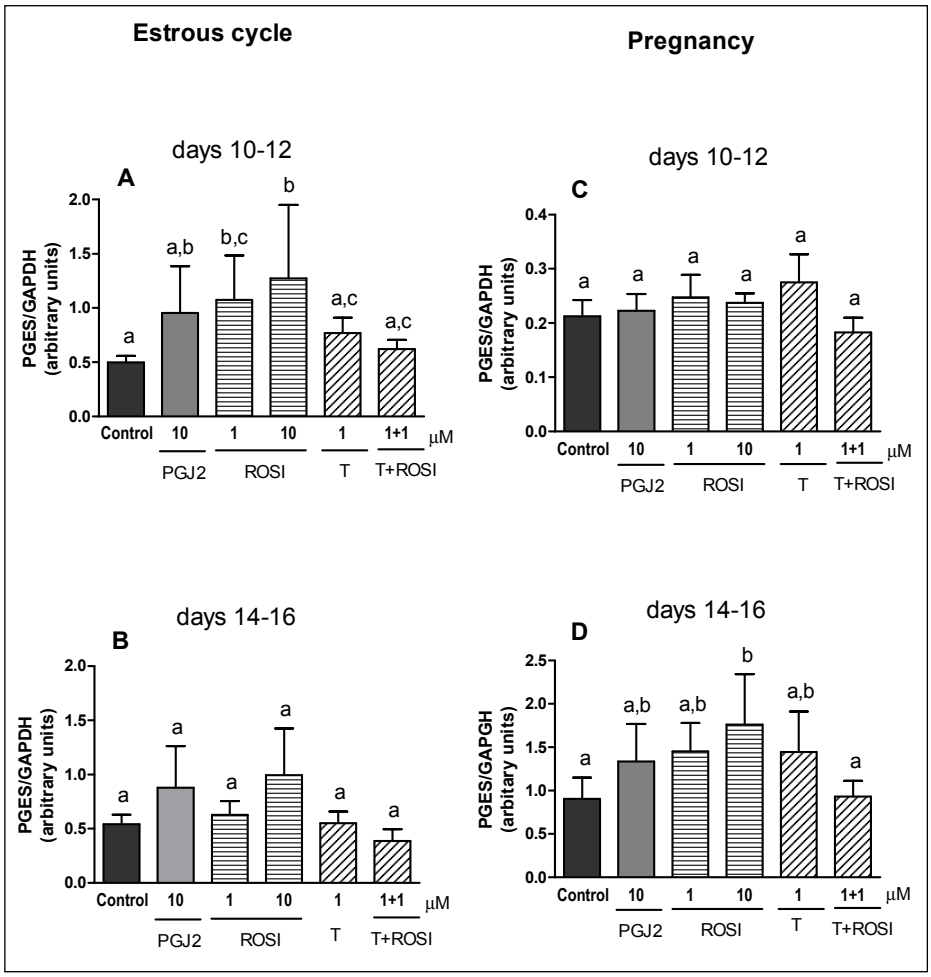

Fig. 4. Prostaglandin E synthase (PGES) mRNA expression in the endometrial tissue collected from gilts during the estrous cycle (10–12 and 14–16, *Fig. A* and *B*) or pregnancy (days 10–12 and 14–16, *Fig. C* and *D*). The tissue explants were incubated for 6 h without (control) or with PPARγ agonists: 15d-prostaglandin J_2 (PGJ_2; 10 µM) and rosiglitazone (ROSI; 1 and 10 µM) as well as PPARγ antagonist T0070907 (T, 1 µM). Different letters designate significant statistical differences ($p<0.05$).

There is only one study investigating prostaglandin synthesis/accumulation in cultured bovine endometrial (BEND) cells in response to PPAR ligands (24). MacLaren *et al*. (24) observed a stimulatory effect of ciglitazone (PPARγ agonist) and WY-14643 (PPARα agonist) on COX-2 expression and prostaglandins PGE_2 and $PGF_{2\alpha}$ accumulation in BEND cells treated for 3 hours with phorbol-ester (PdBu). In turn, carbaprostacyklin (PPARα/β agonist) did not change COX-2 mRNA abundance, but it increased both prostaglandins accumulation in these cells (24). Our recent results (Bogacka *et al*, submitted) indicate that ligands of all PPARs (α, β, γ) modulated prostaglandin $F_{2\alpha}$ release and mRNA expression of prostaglandin F synthase in porcine endometrium depending on the reproductive status of the animals. The activation of the PPARs by WY-14643 (PPARα agonist); L-165,041 (PPARβ/δ agonist); 15d-PGJ_2 or rosiglitazone (PPARγ agonists) increased $PGF_{2\alpha}$ secretion, but not PGFS gene expression, on days 10–12 and days 14–16 of the estrous cycle. During pregnancy (days 10–12 and 14–16), there was lack of change in $PGF_{2\alpha}$ release after treatment with PPARα and PPARγ ligands, but the increase in PGFS mRNA abundance was noted in the presence of WY-14643 (PPARα agonist), 15d-PGJ_2 (natural PPARγ agonist) and L-165,041 (PPARβ agonist). The above results suggest that a different pattern in $PGF_{2\alpha}$ synthesis/release during the early pregnancy and the luteal phase of the estrous cycle might protect the corpus luteum from degradation or might induce its regression, respectively.

The role of prostaglandin E_2 as a luteotropic/anti-luteolytic factor in pigs is well established (18) and PGE_2 protects the corpus luteum against luteolytic effect of prostaglandin $F_{2\alpha}$. Therefore, it is not surprising that in the present study the PPAR activators stimulated PGE_2 release by the endometrial explants collected from pregnant or cyclic pigs with fully active corpora lutea during the mid-luteal phase of the estrous cycle. However, it is unexpected that PPAR agonists induced PGE_2 release also during the late-luteal phase of the estrous cycle. It has been previously reported that during the estrous cycle, the endometrial secretion of $PGF_{2\alpha}$ markedly increased from day 13 to day 16 (34). Surprisingly, PGE_2 release was augmented on days 13–16 as well (34). In other experiment it has been also shown that the endometrial *in vitro* PGE_2 production increased on days 10–16 of the estrous cycle (35). On days 14–16 of the estrous cycle we observed a higher accumulation of PGE_2 in the culture media after treatment with PPARγ and PPARβ but PGES gene expression did not change. It is possible that the luteotropic PGE_2, released in large amounts during the late-luteal phase of the estrous cycle, might be converted to luteolytic $PGF_{2\alpha}$ by PGE2-9-oksoreductase, as reported previously in pigs (31, 36).

We conclude that PPARs are mediators of prostaglandin E_2 synthesis/release in porcine endometrium during the luteal phase of the estrous cycle and the time of periimplantation. Ligands of PPARγ or PPARβ modulated both the synthesis and accumulation of PGE_2 in porcine endometrium depending on the phase of the estrous cycle or pregnancy, whereas PPARα ligands were less effective in PGE_2 production.

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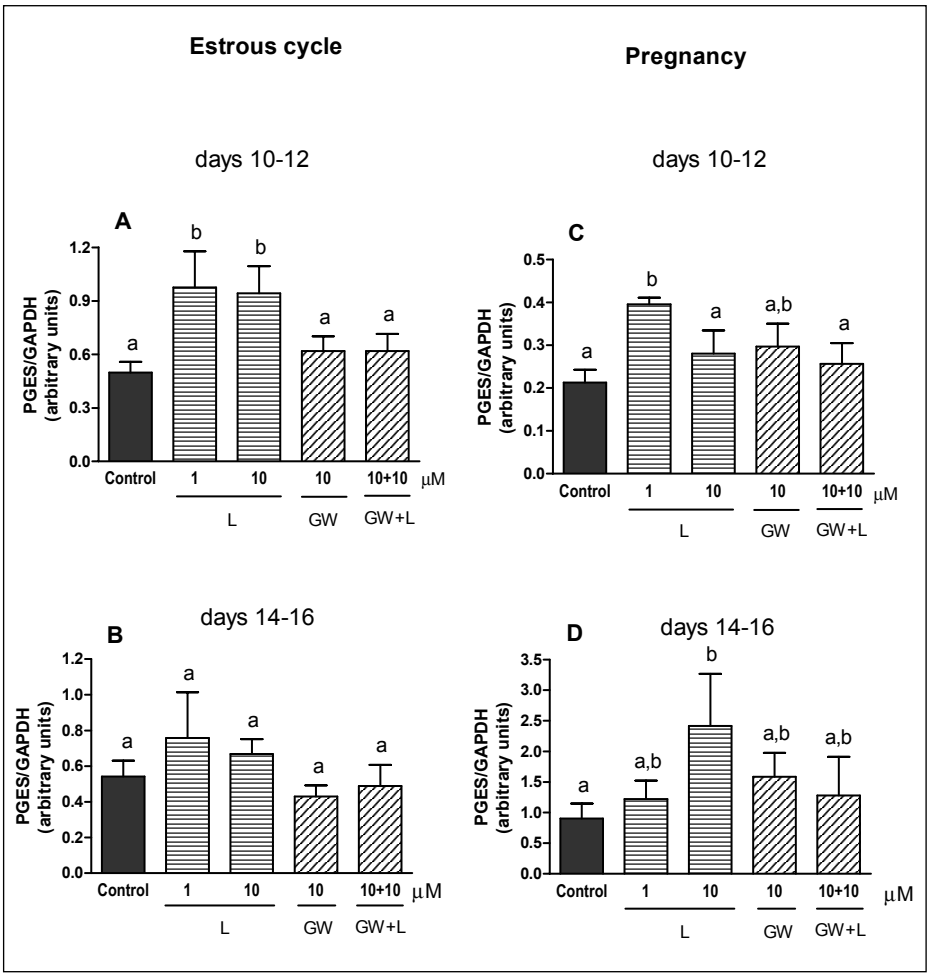

Fig. 5. Prostaglandin E synthase (PGES) mRNA expression in the endometrial tissue collected from gilts during the estrous cycle (10–12 and 14–16, *Fig. A* and *B*) or pregnancy (days 10–12 and 14–16, *Fig. C* and *D*). The tissue explants were incubated for 6 h without (control) or with PPARβ agonist L-165,041 (L, 1 and 10 μM) and/or its antagonist GW 9662 (GW, 10 μM). Different letters designate significant statistical differences ($p<0.05$).

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Conflict of interests: None declared.

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