




## Article

# Prostacyclin Synthesis and Prostacyclin Receptor Expression in the Porcine Myometrium: Prostacyclin Potential to Regulate Fatty Acid Transporters, Cytokines and Contractility-Related Factors

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**Simple Summary:** Prostacyclin (prostaglandin I<sub>2</sub>; PGI<sub>2</sub>) is an important modulator of vascular functions and is involved in various reproductive processes. PGI<sub>2</sub> was also described as a modulator of uterine contractility in several species, including the pig. However, its synthesis and role in the myometrium of the porcine uterus are still not fully described. The objective of this study was to evaluate profiles of PGI<sub>2</sub> synthesis and PGI<sub>2</sub> receptor expression in the myometrium of gilts throughout the estrous cycle and during early pregnancy and to investigate the *in vitro* effect of PGI<sub>2</sub> on the mRNA expression of factors engaged in smooth muscle contraction, nutrient transport, prostaglandin synthesis and action, and inflammatory response. The obtained results showed that the synthesis of PGI<sub>2</sub> changes in the myometrium of pigs during both the estrous cycle and early pregnancy, resulting in much greater concentrations of PGI<sub>2</sub> in cyclic than in pregnant gilts. Moreover, PGI<sub>2</sub> stimulated the expression of fatty acid transporters and contractility-related calponin 1 and caldesmon 1, whereas it decreased cytokine expression. This study indicates that PGI<sub>2</sub> may participate in the regulation of myometrial functions modulating the availability of factors involved in smooth muscle activity and inflammatory reaction in the uterus of pigs.



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**Abstract:** Although prostacyclin (PGI<sub>2</sub>) has been well described as a regulator of smooth muscle activity, limited data are available concerning its role in the myometrium of pigs. The present research aimed to examine profiles of PGI<sub>2</sub> synthase (PTGIS) and PGI<sub>2</sub> receptor (PTGIR) expression and 6-keto PGF<sub>1</sub>α (a PGI<sub>2</sub> metabolite) concentrations in the myometrium of gilts throughout the estrous cycle and during early pregnancy using qPCR, Western blot, and/or ELISA methods. Furthermore, myometrial explants were exposed to iloprost (a stable PGI<sub>2</sub> analog) to investigate the effect of PGI<sub>2</sub> on the mRNA expression of factors engaged in smooth muscle contraction, nutrient transport, prostaglandin synthesis and action, and inflammatory response. *PTGIS* mRNA expression was greater in cyclic than in pregnant gilts on days 11–12 after estrus and was accompanied by greater concentrations of 6-keto PGF<sub>1</sub>α detected in cyclic than in pregnant animals on days 11–20. Iloprost stimulated fatty acid transporters and contractility-related calponin 1 and caldesmon 1 mRNA expression and decreased interleukin 1β and tumor necrosis factor transcript abundance. The obtained results indicate a physiologically relevant role of PGI<sub>2</sub> during the estrous cycle in the porcine myometrium with its importance for regulating the expression of contractility-, nutrient transport- and inflammatory response-related factors.

**Keywords:** pig; myometrium; the estrous cycle; pregnancy; prostacyclin; prostacyclin receptor; contractility-related factors; nutrient transporters; prostaglandin receptors; cytokines

## 1. Introduction

The uterus is a complex organ composed of various cell types that form the endometrium and the myometrium. It has been well described that the successful outcome

of the reproductive processes requires proper remodeling and secretory activity of the uterine endometrium [1–3]. However, the role of the myometrium as a local regulator of uterine functions should not be neglected. Both the contractile and secretory activities of the myometrium are important for the estrous cycle regulation and pregnancy maintenance in pigs [4–6]. Ovarian steroids appear as the primary modulators of myometrial function [7,8]. In addition, several endocrine and locally produced factors, including prostanoids, may act on the myometrial tissue [7–11].

Prostaglandin I<sub>2</sub> (PGI<sub>2</sub>), commonly known as prostacyclin, belongs to the prostanoid family of lipid mediators. Prostanoids are derivatives of the 20-carbon omega-6 fatty acid, arachidonic acid (AA). Prostaglandin (PG) endoperoxide synthase (PGHS; generally known as cyclooxygenase) catalyzes the conversion of AA to unstable PGH<sub>2</sub> that is subsequently transformed into PGE<sub>2</sub>, PGF<sub>2</sub>α, PGD<sub>2</sub>, PGI<sub>2</sub>, or thromboxane A<sub>2</sub> by specific synthases [12,13]. The formation of PGI<sub>2</sub> is catalyzed by prostaglandin I<sub>2</sub> synthase (PTGIS), which is an enzyme located at the membrane of the endoplasmic reticulum [14]. This enzyme was primarily localized in endothelial cells, but its expression was also demonstrated in other cell types in the body [13,15,16]. PGI<sub>2</sub> has a very short half-life in biological fluids and rapidly undergoes degradation to form an inactive 6-keto PGF<sub>1</sub>α. As a result of this, PGI<sub>2</sub> exerts mainly auto- and paracrine actions on target cells [17,18].

The effects of PGI<sub>2</sub> are mediated by a G protein-coupled membrane receptor, PTGIR, which possesses a typical seven transmembrane structure [17]. The linking of PGI<sub>2</sub> to the G<sub>s</sub> subunit of PTGIR results in the activation of adenylate cyclase and the formation of cAMP. This classical PGI<sub>2</sub> signaling is engaged in the regulation of vascular functions and smooth muscle relaxation [13,17,19]. Mice lacking *Ptgir* expression exhibited an increased predisposition to thrombosis and impaired inflammatory response [20].

Among livestock species, profiles of PTGIS and PTGIR expression were documented in the endometrium of cyclic and/or pregnant cows [21], ewes [22], and pigs [23,24]. In the porcine endometrium, activation of PTGIR with iloprost, a stable analog of PGI<sub>2</sub>, results in an increased expression of pro-angiogenic genes [24]. PTGIR is also differentially expressed during the estrous cycle and early pregnancy in the corpus luteum of the pig and PGI<sub>2</sub> analogs, iloprost and carbaprostacyclin, affect progesterone production in luteal cells acting through PTGIR [25]. Moreover, PGI<sub>2</sub> may support conceptus implantation in this species because it promotes the attachment and proliferation of porcine trophoblast cells [26].

Although PGI<sub>2</sub> has been well described as a modulator of muscle contractility [17] and the porcine myometrium is a target for PGI<sub>2</sub> [27,28], only limited data are available regarding PGI<sub>2</sub> synthesis and its receptor system expression in the myometrium of pigs. Moreover, the possible impact of PGI<sub>2</sub> on the mRNA transcript abundance in the myometrial tissue has not been described for this species. Thus, the present research was undertaken to examine profiles of PTGIS and PTGIR expression and PGI<sub>2</sub> concentration in the myometrium of cyclic and early pregnant gilts. Moreover, the effect of PGI<sub>2</sub> on the mRNA expression of selected genes encoding proteins implicated in contractility, nutrient transport, PG synthesis and action, and/or inflammatory response was determined *in vitro*. In particular, gap junction protein alpha 1, calponin 1, caldesmon 1, and oxytocin receptor were chosen as contractility-related genes, while tumor necrosis factor, interleukin (IL) 1 beta, C-X-C motif chemokine ligand 8 (encodes IL8) and nuclear factor kappa B subunit 1 were selected as inflammatory response-related genes. Moreover, solute carrier family 5 member 1 and solute carrier family 38 member 1 were chosen as glucose and amino acid transporters, respectively, while CD36 and solute carrier family 27 member 4 were examined as genes encoding fatty acid transporters. Genes encoding proteins crucial for PG synthesis, namely prostaglandin-endoperoxide synthase 2, and action, *i.e.*, PGE<sub>2</sub> receptors type 2 and 4 and PGF receptor, were also selected for mRNA examination.

## 2. Materials and Methods

### 2.1. Animals and Sample Collection

For ex vivo analyses of myometrial PGI<sub>2</sub> synthesis and PTGIR expression, uteri were obtained from 58 crossbred gilts (Polish Landrace × Duroc) of similar age, weight, and genetic background originating from one commercial herd. After exhibiting two estrous cycles of normal length, females were divided into two groups, cyclic and pregnant, and further observed for the next estrus behavior. Animals assigned to the cyclic group were slaughtered on days 3–4 ( $n = 6$ ), 6–7 ( $n = 6$ ), 11–12 ( $n = 6$ ), 15–16 ( $n = 5$ ) and 19–20 ( $n = 5$ ) of their third estrous cycle. The day of the estrous cycle was confirmed by macroscopic examination of the ovaries, as outlined earlier [29]. Gilts assigned to be pregnant were bred 12 and 24 h after recognition of the estrus. The day of the second breeding was deemed the first day of pregnancy. Gilts were slaughtered on days 11–12 ( $n = 7$ ), 15–16 ( $n = 6$ ), 19–20 ( $n = 6$ ), 25 ( $n = 5$ ) and 30 ( $n = 6$ ) of pregnancy. The day of pregnancy was verified by the morphology of conceptuses flushed from uterine horns (days 11 to 16) or by the morphology and size of embryos/fetuses attached to the uterus (days 19 to 30) [30,31]. Myometrial tissue was separated from the endometrial tissue, snap-frozen in liquid nitrogen, and maintained at  $-80\text{ }^{\circ}\text{C}$  for further analyses.

For in vitro experiments, randomly selected uterine horns were collected from additional six cyclic gilts slaughtered on days 12–14 after estrus and transported to the laboratory in sterile, ice-cold PBS supplemented with antibiotics (100 IU/mL penicillin and 100  $\mu\text{g}/\text{mL}$  streptomycin; P4333, Sigma-Aldrich, St. Louis, MO, USA).

### 2.2. Incubation of Myometrial Explants

In order to study the effect of PGI<sub>2</sub> on the abundance of mRNA transcripts of selected genes, myometrial explants were used. Briefly, a 10 to 20 cm long fragment was cut off from each of six uterine horns, which were transported to the laboratory, and opened longitudinally. Then, the myometrial tissue was separated from the endometrium using scissors, washed with sterile PBS, and cut with a scalpel blade into small pieces (20 to 25 mg). A total of 110–120 mg of the myometrial tissue was placed into glass vials (5 explants per vial) containing 2 mL of incubation medium (Dulbecco's Modified Eagle's Medium [D2902, Sigma-Aldrich] supplemented with antibiotics and 25 mM HEPES [H4034, Sigma-Aldrich]) and pre-incubated for 2 h. Then, the pre-incubation media were removed, and myometrial explants were incubated for 8 h in an incubation medium enriched with 0.1% BSA (81-003-3, Millipore, Kankakee, IL, USA) with vehicle (0.01% ethanol; control) or with 1  $\mu\text{M}$  of iloprost (a stable analog of PGI<sub>2</sub>; 18215, Cayman Chemicals, Ann Arbor, MI, USA). After incubation, myometrial explants were washed with ice-cold PBS, snap-frozen in liquid nitrogen, and maintained at  $-80\text{ }^{\circ}\text{C}$  until RNA isolation. The treatment was carried out in duplicate using myometrial tissue from six gilts.

### 2.3. Total RNA Isolation and Real-Time PCR

Total RNA was extracted from the myometrial tissue collected at slaughter and from myometrial explants after incubation using a Total RNA Mini kit (A&A Biotechnology, Gdansk, Poland) and treated with DNase I (Sigma-Aldrich) in accordance with the manufacturer's instructions. RNA samples (1  $\mu\text{g}$ ) were reverse transcribed using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Waltham, MA, USA) according to the manufacturer's protocol.

Diluted cDNA from RT-PCR was used to analyze the mRNA transcript abundance of selected genes with an ABI Vii7 Sequence Detection System (Life Technologies Inc., Carlsbad, CA, USA). In order to determine the mRNA expression of genes: *PTGIS*, *PTGIR*, *GJA1*, *OXTR*, *CNN1*, *CALD1*, *SLC5A1*, *SLC38A1*, *CD36*, *SLC27A4*, *PTGS2*, *PTGER2*, *PTGER4*, *PTGFR*, *TNF*, *IL1B*, *CXCL8*, *NFKB1*, *GAPDH*, *HPRT1*, and *ACTB*, 15 ng of cDNA was amplified using TaqMan Gene Expression Assays (Applied Biosystems). All abbreviations of the studied genes, their full names, and the ID numbers of the TaqMan probes are outlined in Table 1. Each PCR reaction (10  $\mu\text{L}$ ) was carried out in duplicates in a 384-well

plate using the following conditions: initial denaturation for 10 min at 95 °C, followed by 45 cycles of 15 s of denaturation at 95 °C and then 60 s of annealing at 60 °C. The control reactions in the absence of reverse transcriptase were conducted to test for genomic DNA contamination. For both RT-PCR and qPCR, no template controls with nuclease-free water were performed to check for possible reagent contamination. Data from real-time PCR were analyzed using the PCR Miner algorithm [32], as previously described [33]. In order to select the most stable reference genes among *GAPDH*, *HPRT1*, and *ACTB*, NormFinder software was applied [34]. All expression data for each target gene were normalized against geometric averaging of *GAPDH* and *HPRT1*.

**Table 1.** Full names of genes exploited to examine the mRNA transcript abundance with real-time PCR.

Abbreviation	Gene Name	ID of TaqMan Probe
<i>PTGIS</i>	Prostaglandin I synthase	Ss03374149_m1
<i>PTGIR</i>	Prostaglandin I receptor	- <sup>1</sup>
<i>GJA1</i>	Gap junction protein alpha 1	Ss03375693_u1
<i>OXTR</i>	Oxytocin receptor	Ss03382363_u1
<i>CNN1</i>	Calponin 1	Ss03392449_g1
<i>CALD1</i>	Caldesmon 1	Ss06890042_m1
<i>SLC5A1</i>	Solute carrier family 5 member 1	Ss03374375_ml
<i>SLC38A1</i>	Solute carrier family 38 member 1	- <sup>1</sup>
<i>CD36</i>	CD 36 molecule	Ss03388549_m1
<i>SLC27A4</i>	Solute carrier family 27 member 4	Ss04329560_m1
<i>PTGS2</i>	Prostaglandin endoperoxide synthase 2	Ss03394692_m1
<i>PTGER2</i>	Prostaglandin E receptor 2	Ss03374177_g1
<i>PTGER4</i>	Prostaglandin E receptor 4	Ss03377412_u1
<i>PTGFR</i>	Prostaglandin F receptor	Ss03393819_s1
<i>TNF</i>	Tumor necrosis factor	Ss03391318_g1
<i>IL1B</i>	Interleukin 1 beta	Ss04321151_m1
<i>CXCL8</i>	C-X-C motif chemokine ligand 8	Ss03392437_m1
<i>NFKB1</i>	Nuclear factor kappa B subunit 1	Ss03388579_m1
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	Ss03375435_u1
<i>HPRT1</i>	Hypoxanthine phosphoribosyltransferase 1	Ss03388274_m1
<i>ACTB</i>	Actin beta	Ss03376081_u1

<sup>1</sup> Designed by Applied Biosystems, Thermo Fisher Scientific; for *PTGIR*: GeneBank accession no. NC\_010448.3; for *SLC38A1*: GeneBank accession no. NC\_010447.5.

#### 2.4. Preparation of Myometrial Tissue Homogenates

Myometrial tissue of cyclic and pregnant gilts was homogenized utilizing an ice-cold homogenization buffer (50 mM Tris-HCl, pH 8.0; 150 mM NaCl, 1% Triton X-100, 1 mM EDTA) supplemented with 10 µL/mL Protease Inhibitor Cocktail (P8340; Sigma-Aldrich) and centrifuged for 10 min at 800× *g* at 4 °C. Subsequently, a portion (0.2 µL) of each supernatant was collected, stored at −80 °C, and used for Western blot analysis of *PTGIS* and *PTGIR* proteins. The rest of the supernatants were centrifuged for an additional 1 h at 100,000× *g* at 4 °C to obtain cytosolic fraction, which was used for enzyme-linked immunosorbent assay (ELISA) of 6-keto PGF1α. Total protein concentrations in tissue homogenates were further determined [35].

#### 2.5. Western Blot Analysis

Myometrial tissue samples (10 µg for both *PTGIS* and *PTGIR*) were dissolved in SDS gel-loading buffer (50 mM Tris-HCl, pH 6.8; 4% SDS, 20% glycerol, and 2% β-mercaptoethanol), heated to 95 °C for 5 min, and separated on 8% (for *PTGIS*) or 10% (for *PTGIR*) SDS-PAGE. Separated proteins were electroblotted onto 0.45 µm pore size polyvinylidene difluoride membrane in transfer buffer (20 mM Tris-HCl, pH 8.2; 150 mM glycine, 20% methanol). Then, nonspecific binding sites were blocked using 5% nonfat

dry milk in TBS-T (Tris-buffered saline, containing 0.1% Tween-20) for 1.5 h at room temperature. After blocking, membranes were incubated overnight with primary antibodies: rabbit polyclonal to PTGIS (dilution 1:100; 160640, Cayman Chemicals) or rabbit polyclonal to PTGIR (1:200 dilution; 10005518, Cayman Chemicals) at 4 °C. After that, membranes were washed with TBS-T buffer and incubated with anti-rabbit IgG-alkaline phosphatase antibody (1:20,000 dilution; A8025; Sigma-Aldrich) at room temperature for 1 h. Immune complexes were visualized using a standard alkaline phosphatase visualization procedure. All images were captured with ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA), and protein bands were quantitated using Image Lab 6.0 Software (Bio-Rad). As internal controls for protein loading, rabbit polyclonal to ACTB antibody (1:3000 dilution; ab8227, abcam, Cambridge, UK; for PTGIS) or rabbit polyclonal to GAPDH antibody (1:2000 dilution; ab9485, abcam; for PTGIR) were used.

### 2.6. ELISA of 6-Keto PGF1 $\alpha$

In order to analyze concentrations of PGI<sub>2</sub> metabolite in myometrial tissue homogenates, a 6-keto PGF1 $\alpha$  ELISA kit (515211; Cayman Chemicals) was used according to the manufacturer's instructions. Myometrial tissue samples were diluted 1:400 before the assay. The sensitivity of this assay was 1.6 pg/mL, and intra- and inter-assay coefficients of variation were 7.8% and 9.1%, respectively. Levels of 6-keto PGF1 $\alpha$  in the myometrium were standardized per total protein content.

### 2.7. Statistical Analysis

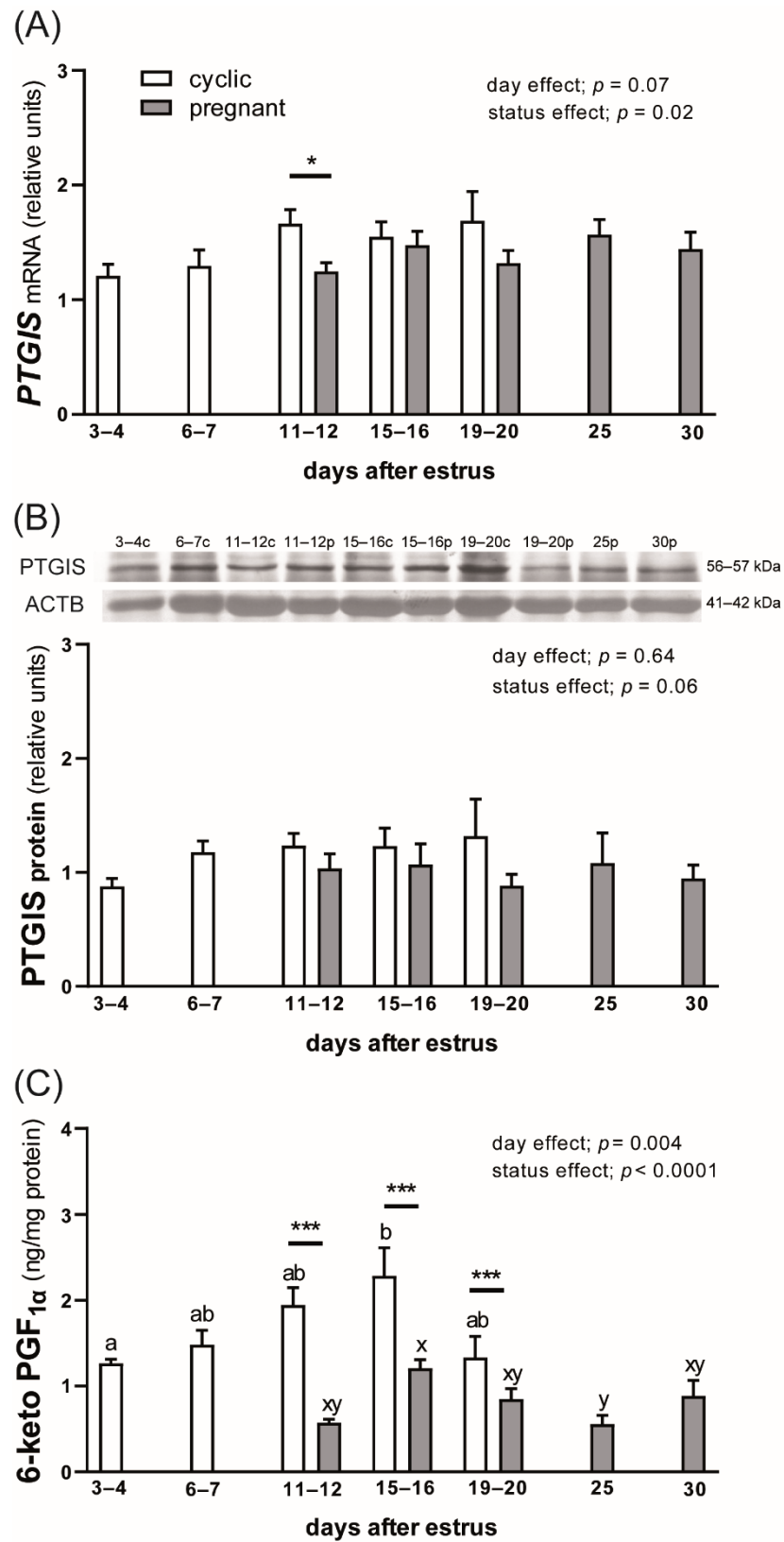
All statistical analyses were conducted using GraphPad PRISM v. 9.3.1 (GraphPad Software, Inc., San Diego, CA, USA). In order to test the profiles of PTGIS and PTGIR mRNA and protein expression and the concentrations of 6-keto PGF1 $\alpha$  in the myometrium of cyclic and pregnant gilts, two-way ANOVA followed by Tukey's post hoc test was used. This analysis included the effect of the day after estrus and the reproductive status of animals (cyclic vs. pregnant). In order to evaluate the effect of iloprost on the mRNA transcript abundance of selected genes, a paired *t*-test was used. All numerical data are presented as the mean  $\pm$  SEM. Means were considered to be statistically different at  $p \leq 0.05$ , with a tendency for significance at  $p$  ranging from 0.06 to 0.07.

## 3. Results

### 3.1. PTGIS mRNA and Protein Expression and 6-Keto PGF1 $\alpha$ Concentrations in the Myometrial Tissue of Cyclic and Early Pregnant Gilts

PTGIS protein showed stable profiles of expression in the myometrial tissue during examined periods of both the estrous cycle and early pregnancy ( $p = 0.64$ ; Figure 1B), while the day after estrus tended to affect PTGIS mRNA expression ( $p = 0.07$ ; Figure 1A). Moreover, the reproductive status of animals affected the abundance of PTGIS transcripts ( $p = 0.02$ ; Figure 1A). On days 11–12 after estrus, gilts from the pregnant group showed lower expression of PTGIS mRNA compared with cyclic animals ( $p < 0.05$ ). Moreover, a tendency to decrease PTGIS protein levels in pregnant gilts compared with cyclic animals was detected ( $p = 0.06$ ; Figure 1B).

Concentrations of 6-keto PGF1 $\alpha$  in the cytosolic fraction of the myometrial tissue were affected by both the day after estrus ( $p = 0.004$ ) and by the reproductive status of animals ( $p < 0.0001$ ; Figure 1C). During the estrous cycle, the content of 6-keto PGF1 $\alpha$  in the myometrium increased between days 3–4 and 15–16 ( $1.26 \pm 0.05$  vs.  $2.14 \pm 0.36$  ng/mg protein;  $p < 0.05$ ). During pregnancy, a decrease in the concentration of 6-keto PGF1 $\alpha$  was detected on day 25 compared with days 15–16 ( $0.56 \pm 0.1$  vs.  $1.21 \pm 0.36$  ng/mg protein;  $p < 0.01$ ). The effect of the reproductive status on the content of PGI<sub>2</sub> metabolite in the myometrial tissue was observed on days 11 to 20 after estrus when concentrations of 6-keto PGF1 $\alpha$  were lower in pregnant than in cyclic gilts ( $p < 0.001$ ).

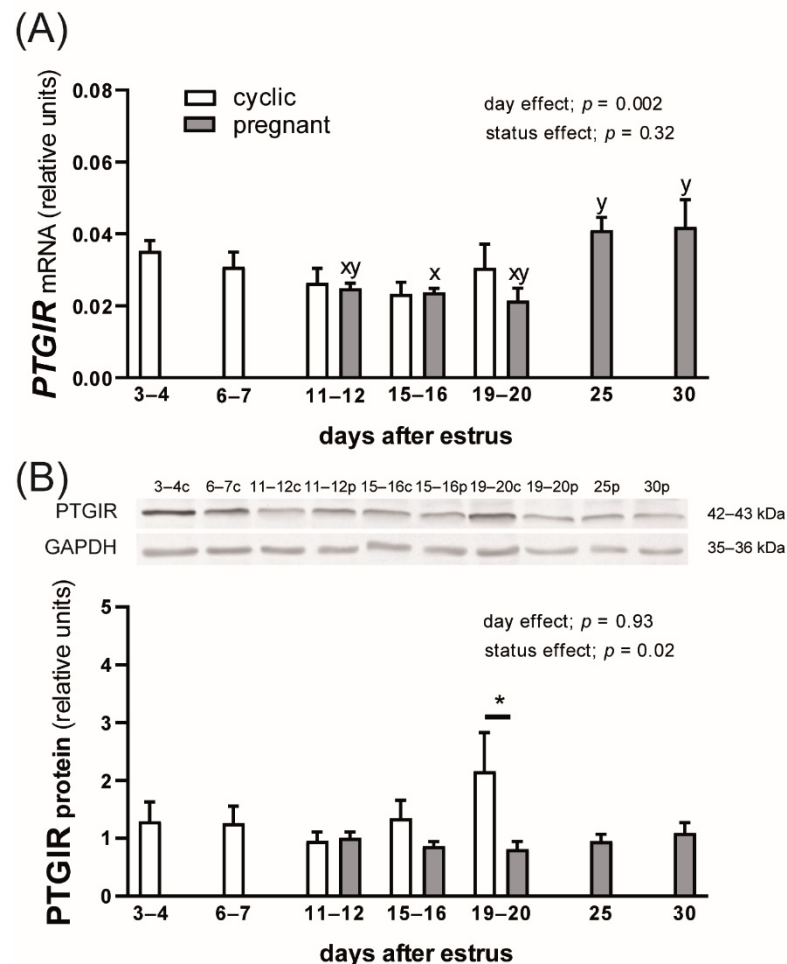


**Figure 1.** Expression of prostaglandin I<sub>2</sub> (PGI<sub>2</sub>; prostacyclin) synthase (PTGIS) mRNA (A) and protein (B) and concentrations of 6-keto PGF<sub>1α</sub> (a PGI<sub>2</sub> metabolite; C) in the myometrium of cyclic and early pregnant gilts. Values from real-time PCR for *PTGIS* were normalized to geometric averaging of *GAPDH* and *HPRT1* gene expression. Values from densitometric analyses of PTGIS protein were normalized to ACTB. Representative blots are presented (c—cyclic, p—pregnant; original western

blots are included in Figure S1). Data are expressed as the mean  $\pm$  SEM ( $n = 5-7$ ). Bars marked with various letters (ab—for cyclic gilts; xy—for pregnant gilts) are different among groups. Asterisks specify differences between cyclic and pregnant animals on particular days after estrus (\*  $p < 0.05$ ; \*\*\*  $p < 0.001$ ).

### 3.2. PTGIR mRNA and Protein Expression in the Myometrial Tissue of Cyclic and Early Pregnant Gilts

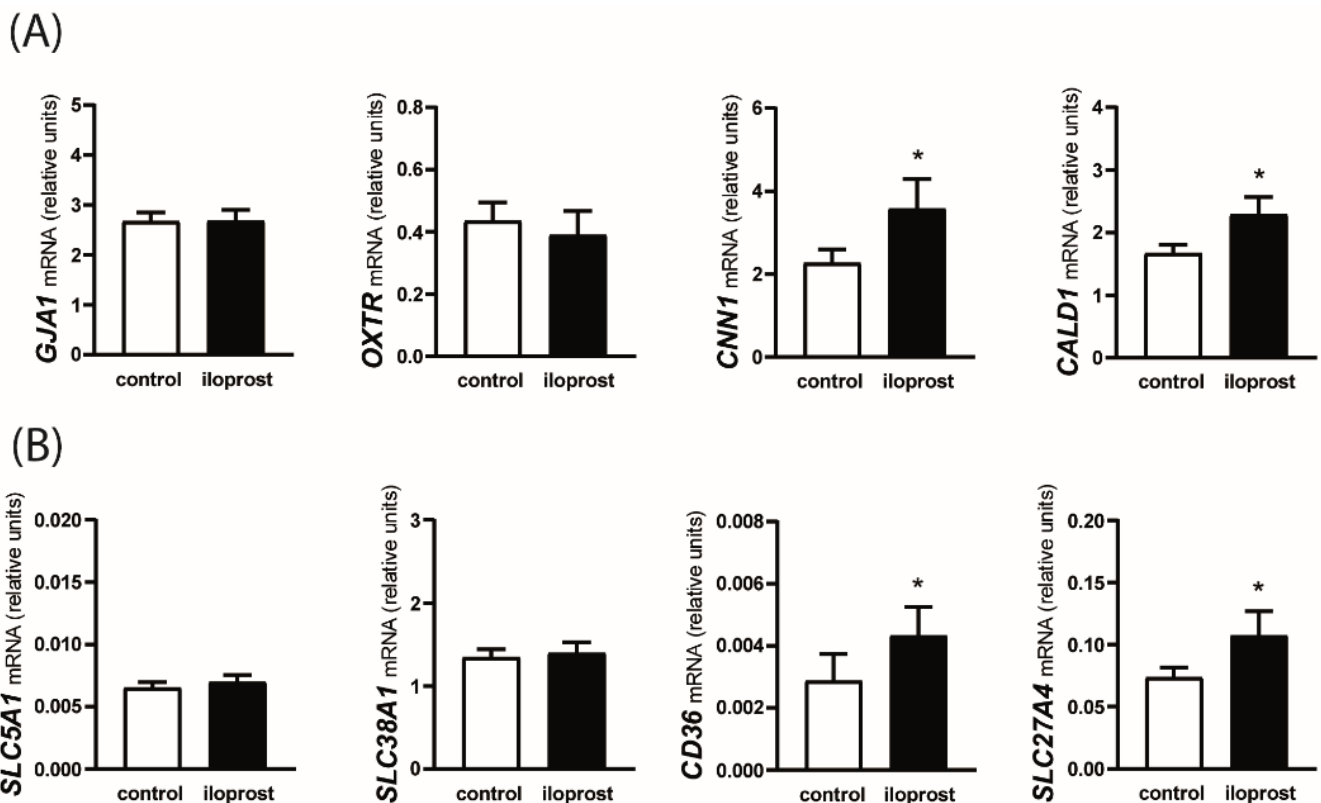
The day after estrus but not the reproductive status of animals affected *PTGIR* mRNA expression in the porcine myometrium ( $p = 0.002$  and  $p = 0.32$ , respectively; Figure 2A). A greater abundance of *PTGIR* transcripts was detected in the myometrial tissue on days 25 and 30 compared with days 15–16 of gestation ( $p < 0.05$ ). *PTGIR* protein level was affected by the reproductive status of animals ( $p = 0.02$ ) but not by the day after estrus ( $p = 0.93$ ; Figure 2B). On days 19–20 after estrus, pregnant gilts showed lower concentrations of *PTGIR* protein compared with cyclic animals ( $p < 0.05$ ).



**Figure 2.** Prostaglandin I2 receptor (*PTGIR*) mRNA (A) and protein (B) expression in the myometrium of cyclic and early pregnant gilts. Values from real-time PCR for *PTGIR* were normalized to geometric averaging of *GAPDH* and *HPRT1* gene expression. Values from densitometric analyses of *PTGIR* protein were normalized to *GAPDH*. Representative blots are presented (c—cyclic, p—pregnant; original western blots are included in Figure S1). Data are expressed as the mean  $\pm$  SEM ( $n = 5-7$ ). Bars marked with various letters are different among the group of pregnant females. Asterisk specifies the difference between cyclic and pregnant animals on days 19–20 after estrus (\*  $p < 0.05$ ).

### 3.3. Effect of Iloprost on the mRNA Transcript Abundance of Selected Genes in Myometrial Explants

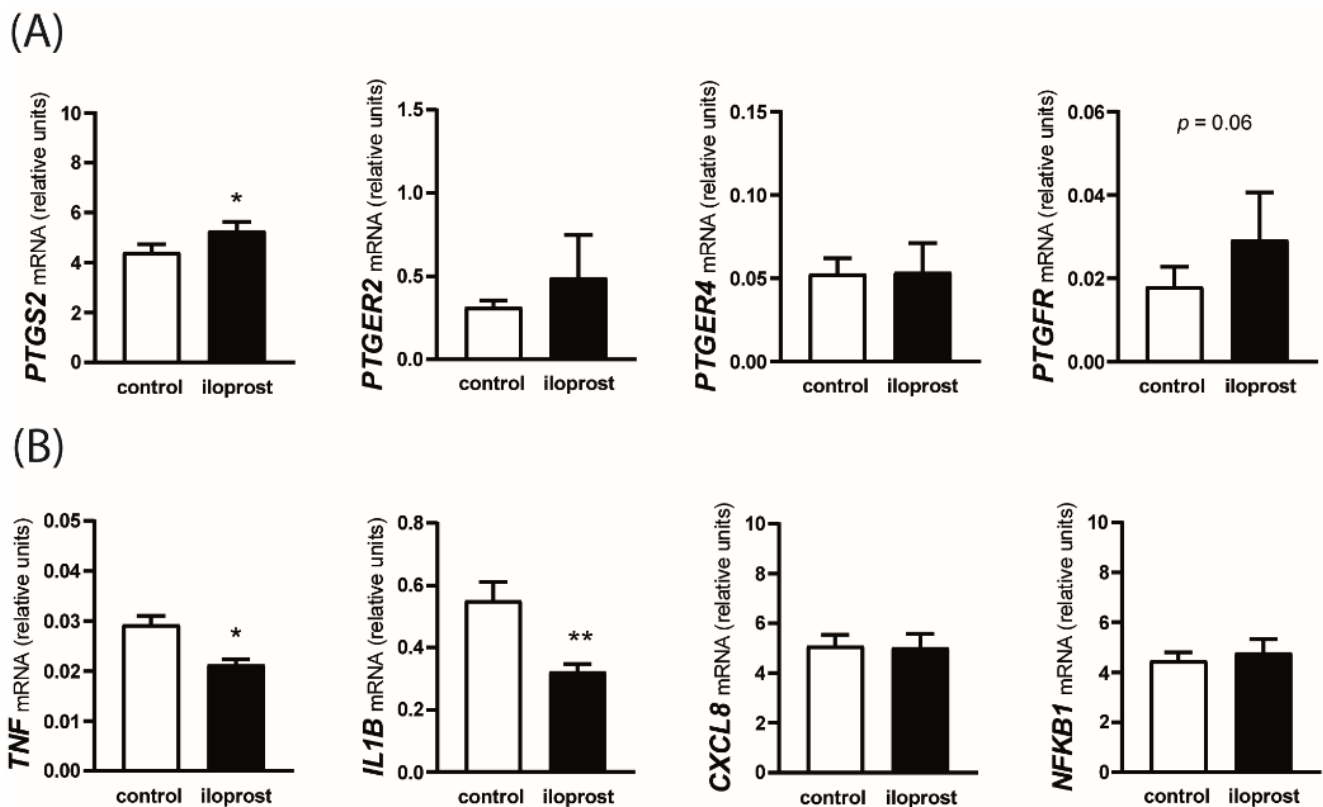
The treatment of myometrial explants with iloprost increased the mRNA expression of *CNN1* and *CALD1* ( $p < 0.05$ ) but did not affect the relative abundance of *GJA1* and *OXTR* transcripts (Figure 3A). Among nutrient transporter encoding genes, iloprost stimulated the mRNA expression of *CD36* and *SLC27A4* ( $p < 0.05$ ), while the expression of *SLC5A1* and *SLC38A1* mRNA did not differ between control and iloprost-treated myometrial explants (Figure 3B).



**Figure 3.** Effect of prostaglandin I<sub>2</sub> (PGI<sub>2</sub>; prostacyclin) analog, iloprost, on the mRNA expression of contractility-related genes (panel A; *GJA1*, *OXTR*, *CNN1*, and *CALD1*) and genes encoding transporters (panel B) of glucose (*SLC5A1*), amino acids (*SLC38A1*), and fatty acids (*CD36* and *SLC27A4*) in the myometrium. Myometrial explants were incubated for 8 h without (control) or with 1  $\mu$ M of iloprost. Values from real-time PCR for each studied mRNA transcript were normalized to geometric averaging of *GAPDH* and *HPRT1* mRNA expression. Data are expressed as the mean  $\pm$  SEM ( $n = 6$ ). Asterisk specifies the difference compared with the control value ( $* p < 0.05$ ).

As shown in Figure 4A, the addition of iloprost to the incubation medium resulted in a greater expression of *PTGS2* mRNA ( $p < 0.05$ ) and tended to increase *PTGFR* mRNA expression ( $p = 0.06$ ). The relative abundance of *PTGER2* and *PTGER4* transcripts were not affected by the presence of iloprost. Among inflammatory response-related genes, iloprost decreased *TNF* and *IL1B* mRNA expression ( $p < 0.05$  and  $p < 0.01$ , respectively) and had no effect on *CXCL8* and *NFKB1* mRNA expression (Figure 4B).





**Figure 4.** Effect of prostaglandin I<sub>2</sub> (PGI<sub>2</sub>; prostacyclin) analog, iloprost, on the mRNA expression of genes involved in PG synthesis (panel A; *PTGS2*) and action (panel A; *PTGER2*, *PTGER4*, and *PTGFR*) and genes involved in the inflammatory response (panel B; *TNF*, *IL1B*, *CXCL8*, and *NFKB1*) in the myometrium. Myometrial explants were incubated for 8 h without (control) or with 1  $\mu$ M of iloprost. Values from real-time PCR for each studied mRNA transcript were normalized to geometric averaging of *GAPDH* and *HPRT1* mRNA expression. Data are expressed as the mean  $\pm$  SEM ( $n = 6$ ). Asterisks specify the difference compared with the control value (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).

#### 4. Discussion

To the best of our knowledge, the present study is the first to demonstrate profiles of PGI<sub>2</sub> concentrations and PGI<sub>2</sub> receptor expression throughout the estrous cycle and during the peri-implantation period in the myometrium of livestock species. The results of the present study revealed increased synthesis of PGI<sub>2</sub> and the expression of its membrane receptor in the myometrium of cyclic compared with pregnant gilts. Moreover, PGI<sub>2</sub> was shown as a modulator of the myometrial expression of contractility-related genes, fatty acid transporters, PG synthesis enzymes, and cytokines.

The expression of the terminal enzyme of the PGI<sub>2</sub> synthesis pathway, PTGIS, was shown in the myometrium of pregnant and/or non-pregnant sheep [22,36] and humans [37,38]. Moreover, PTGIS protein was localized in the smooth muscle cells of the porcine myometrium [28]. The results of the present study revealed that PTGIS was abundantly expressed throughout the estrous cycle and during early pregnancy in the myometrial tissue. However, PTGIS mRNA and protein expression did not vary significantly on particular days of the estrous cycle or gestation. Interestingly, *PTGIS* mRNA transcript abundance was greater, and PTGIS protein content tended to be greater in cyclic than in pregnant gilts. Steady-state levels of PTGIS expression during examined periods were accompanied by dynamic changes in 6-keto PGF<sub>1</sub> $\alpha$  concentration detected in the myometrial tissue throughout the estrous cycle and during early pregnancy. Thus, elevated content of PGI<sub>2</sub> in the myometrial tissue of cyclic gilts results from a greater PTGIS activity rather than a greater abundance of this enzyme.

Increased myometrial synthesis of PGI<sub>2</sub> during the estrous cycle as compared with respective days of pregnancy observed in the present study seems somewhat surprising because PGI<sub>2</sub> is a well-known relaxant of smooth muscles [17,39], and the quiescent uterus is important for developing embryos/fetuses. In the myometrium of pregnant rats, the most intense synthesis of PGI<sub>2</sub> was detected in the area remarkably stretched by the growing embryos, and spontaneous contractility was decreased in regions with a lower myometrial synthesis of PGI<sub>2</sub> [40]. Thus, we expected that in pigs, elevated synthesis of PGI<sub>2</sub> during the peri-implantation period would occur as part of some local changes ensuring the most favorable conditions for implanting conceptuses. The present results, however, showed a substantial increase in 6-keto PGF<sub>1</sub>α concentrations on days 15–16 of the estrous cycle compared with days 3–4 after estrus. Moreover, much greater concentrations of PGI<sub>2</sub> metabolite were detected on days 11 to 20 of the estrous cycle compared with respective days of pregnancy. These results indicate a role of PGI<sub>2</sub> in the porcine myometrium during luteolysis and pre-ovulatory period rather than during early pregnancy. Because much more attention has been attributed to the role of PGI<sub>2</sub> in the myometrium during late pregnancy and labor [38,39,41] than during the estrous or menstrual cycle, it is difficult to assess whether increased synthesis and/or accumulation of PGI<sub>2</sub> in this tissue during the late luteal phase is peculiar only to pigs. On the other hand, a similar phenomenon was described for women because PGI<sub>2</sub> concentrations increase during late pregnancy and PGI<sub>2</sub> is the most prominent PG at the time of parturition, while PGI<sub>2</sub> shows relaxant effects on the myometrium during labor (for review: [7]).

PGI<sub>2</sub> acts on target cells primarily via activation of its specific membrane receptor, PTGIR [17]. As we demonstrated in the present study, *PTGIR* mRNA level did not change during the estrous cycle but increased on days 25 and 30 of gestation compared with days 15–16. PTGIR protein level did not vary during both studied periods after estrus, but similar to PGI<sub>2</sub> metabolite, it was greater in cyclic than in pregnant gilts on days 19–20 after estrus. Our present results confirm previous data of Jana and co-workers [28], who localized PTGIR protein in the muscular and vascular cells of the porcine myometrium but are the first to show expression patterns of myometrial PTGIR during the estrous cycle and early pregnancy in pigs. Similar to data on PTGIS expression, only limited papers are available regarding PGI<sub>2</sub> receptor expression in the uterine myometrium. PTGIR protein was localized in myometrial smooth muscle cells in non-pregnant [42] and pregnant [43] women. Moreover, PGI<sub>2</sub> binding sites, along with a possible relaxing effect of PGI<sub>2</sub> on the uterine vasculature, were reported for non-pregnant bovine myometrial tissue [44]. Greater content of PTGIR protein in the myometrium of cyclic than pregnant gilts observed in the current study on days 19–20 points to a possible role of PGI<sub>2</sub> as a modulator of uterine motility during the peri-ovulatory period.

In order to determine the possible role of PGI<sub>2</sub> in the porcine myometrium, tissue explants were exposed to iloprost, a stable analog of PGI<sub>2</sub>, and examined for mRNA transcript abundance of selected genes; in particular, genes involved in contractility, nutrient transport, inflammatory response, and PG synthesis and action were studied. These processes were previously reported as important for the proper function of the myometrium [7,8,10,11,45].

The activation of the myometrial tissue depends on the coordinated expression of several contraction-related proteins, including connexin 43 (encoded by *GJA1*) and oxytocin [7]. Both proteins are important for myometrial contractility during labor and delivery in women [8]. Moreover, connexin 43 and oxytocin receptors were detected in the myometrium of cyclic and/or pregnant gilts [46,47], and oxytocin was shown as an effective stimulator of the myometrial contractility during luteolysis [48]. Of interest, PGI<sub>2</sub> stimulated connexin 43 expression and enhanced oxytocin-induced contractility in human myometrial tissue [41]. Our present results, however, showed that the PGI<sub>2</sub> analog did not affect *GJA1* and *OXTR* mRNA expression in the myometrium of pigs. It indicates that PGI<sub>2</sub> does not participate in the preparation of myometrial tissue for oxytocin-inducing contractions before luteolysis in this species.

Smooth muscle contractions result from actin and myosin interactions. Moreover, thin filament-related proteins, calponin and caldesmon, modulate the actomyosin complex [49]. In the current experiment, the mRNA expression of both *CNN1* and *CALD1* was stimulated by iloprost. These results are consistent with previously demonstrated greater expression of calponin and caldesmon mRNA and protein in human myometrial tissue exposed to iloprost [41]. Although Fetalvero and co-workers [41] suggested a role for both proteins as important for the contractile response of the myometrium during initiation and progression of the parturition, calponin and caldesmon inhibit the activity of actin-induced myosin adenosine triphosphatase and were proposed as inhibitory regulators of smooth muscle contractions [50,51]. Thus, iloprost-induced myometrial *CNN1* and *CALD1* mRNA expression observed in the present study point to relaxant rather than contractile action of PGI<sub>2</sub> in this tissue. A possible dual role for PGI<sub>2</sub> was suggested in the myometrium of women, including myometrial quiescence and activation [52]. Accordingly, both inhibitory and stimulatory effects of PGI<sub>2</sub> on the myometrial contractility in cyclic gilts were reported [27,28].

Cellular growth and activity require the supply of various nutrients. Myometrial smooth muscle cells use both glucose and fatty acids as major energy substrates [45]. Moreover, amino acids are important for the proper functioning of the myometrium [53]. Increased amino acid catabolism and fatty acid oxidation were observed in the myometrium during labor in women [54]. A crucial rate-limiting process in glucose, fatty acids, and amino acid utilization involves their transport across the cell membrane. A number of glucose and amino acid transporters, including *SLC5A1* and *SLC38A1*, respectively, were reported in the endometrium of the porcine uterus [55]. The results of the present research showed, for the first time, the mRNA expression of both *SLC5A1* and *SLC38A1* in the porcine myometrium and demonstrated that their abundance in this tissue did not change in response to iloprost. Similarly, another analog of PGI<sub>2</sub>, carbaprostacyclin, did not affect the expression of glucose and amino acid transporters in porcine endometrial cells [56]. Of interest is the addition of iloprost stimulated *CD36* (also known as fatty acid translocase; FAT/CD36) and *SLC27A4* mRNA expression in the myometrial explants (present data). These results indicate that PGI<sub>2</sub> may support fatty acid delivery into the myometrial cells to enhance the energy status of this tissue.

Endometrium-derived PGE<sub>2</sub> and PGF<sub>2</sub>α acting via their membrane receptors, PTGER and PTGFR, respectively, regulate various reproductive events during the estrous cycle and early gestation [3,57]. The uterine myometrium also synthesizes both PGs and expresses their receptors [7,9,10]. In the myometrium of pigs, PTGER<sub>2</sub> was shown as a relaxant, while PTGFR was described as a contractile receptor [58]. In the current study, iloprost did not affect *PTGER2* and *PTGER4* mRNA expression but tended to increase *PTGFR* transcript abundance. It indicates that PGI<sub>2</sub> may affect the sensitivity of the myometrium to contractile PGF<sub>2</sub>α; however, this suggestion requires further research. PGHS<sub>2</sub> protein (encoded by *PTGS2* gene), the rate-limiting enzyme in PG synthesis, was detected in the myometrium of gilts [58], and its expression increases in response to oxytocin [59]. As we showed in the present study, *PTGS2* mRNA transcript abundance in myometrial explants was stimulated by iloprost treatment. It points to the possible function of PGI<sub>2</sub> in the sustained generation of PGs in this tissue.

The intense remodeling of the endometrium occurring during each estrous cycle involves the modulation of synthesis and/or secretion of various cytokines and inflammatory mediators [11]. As we demonstrated here, iloprost inhibited *IL1B1* and *TNF* mRNA expression in the porcine myometrium. Such a result is consistent with the previously suggested anti-inflammatory action of PGI<sub>2</sub> since iloprost suppressed pro-inflammatory cytokine expression in monocytes and dendritic cells [60–62]. Enhanced inflammatory response and increased pro-inflammatory cytokine content play a role in the transformation of the myometrial tissue from a quiescent to a contractile state before parturition [8,63]. Therefore, the inhibitory effect of iloprost on *IL1B1* and *TNF* transcript abundance in the porcine myometrium may indicate that PGI<sub>2</sub> alleviates pro-inflammatory reactions in the

myometrium and protects the uterus from excessive contractility. However, this hypothesis requires further verification.

## 5. Conclusions

In summary, this is the first demonstration of PTGIS expression and PGI<sub>2</sub> concentration profiles in the porcine myometrium during the estrous cycle and early pregnancy. Increased synthesis of PGI<sub>2</sub> on days 11 to 20 of the estrous cycle compared with respective days of pregnancy indicates a physiologically relevant role of PGI<sub>2</sub> in the porcine myometrium during the luteolysis and pre-ovulatory period. Moreover, our data showed day- and reproductive status-dependent changes in the abundance of PGI<sub>2</sub> receptor transcripts in the myometrium of pigs. Furthermore, a stable agonist of PGI<sub>2</sub> stimulated mRNA expression of thin-filament-related proteins, fatty acid transporters, and PG synthesis enzyme and decreased the mRNA expression of cytokines in the myometrial explants in vitro. Therefore, in addition to the direct regulatory action of PGI<sub>2</sub> on myometrial contractility [27,28], this prostanoid may modulate the availability of factors involved in smooth muscle activity and inflammatory reaction (present data) in the uterus of pigs.

Our findings broaden the knowledge about the significance of PGI<sub>2</sub> in the reproductive tract of pigs and provide valuable insights into the mechanisms regulating myometrial function. Uterine motility is important for the secretory activity of the endometrium during luteolysis, as well as for gamete and embryo transfer in the uterine lumen. Identification of factors affecting the secretory and contractile functions of the myometrium would be of great benefit for the control of pig reproduction. Therefore, further, more extensive studies are required to evaluate the importance of intense PGI<sub>2</sub> synthesis during the estrous cycle in pigs.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/ani12172237/s1>, Figure S1: Blots used for quantitative analyses of respective bands.

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## References

1. Paria, B.C.; Song, H.; Dey, S.K. Implantation: Molecular basis of uterine dialogue. *Int. J. Dev. Biol.* **2001**, *45*, 597–605. [PubMed]
2. Bazer, F.W.; Song, G.; Kim, J.; Dunlap, K.A.; Satterfield, M.C.; Johnson, G.A.; Burghardt, R.C.; Wu, G. Uterine biology of pigs and sheep. *J. Anim. Sci. Biotechnol.* **2012**, *3*, 23. [CrossRef] [PubMed]
3. Waclawik, A.; Kaczmarek, M.M.; Blitek, A.; Kaczynski, P.; Ziecik, A.J. Embryo-maternal dialogue during pregnancy establishment and implantation in the pig. *Mol. Reprod. Dev.* **2017**, *84*, 842–855. [CrossRef] [PubMed]
4. Scheerboom, J.E.; van Adrichem, P.W.; Taverne, M.A. Uterine motility of the sow during the oestrous cycle and early pregnancy. *Vet. Res. Commun.* **1987**, *11*, 253–269. [CrossRef]

5. Franczak, A. Endometrial and myometrial secretion of androgens and estrone during early pregnancy and luteolysis in pigs. *Reprod. Biol.* **2008**, *8*, 213–228. [[CrossRef](#)]
6. Markiewicz, W.; Bogacki, M.; Blitek, M.; Jaroszewski, J.J. Comparison of the porcine uterine smooth muscle contractility on days 12–14 of the estrous cycle and pregnancy. *Acta Vet. Scand.* **2016**, *58*, 20. [[CrossRef](#)]
7. Hertelendy, F.; Zakar, T. Regulation of myometrial smooth muscle functions. *Curr. Pharm. Des.* **2004**, *10*, 2499–2517. [[CrossRef](#)]
8. Mendelson, C.R.; Gao, L.; Montalbano, A.P. Multifactorial regulation of myometrial contractility during pregnancy and parturition. *Front. Endocrinol.* **2019**, *10*, 714. [[CrossRef](#)]
9. Franczak, A.; Bogacki, M. Local and systemic effects of the embryos on the uterine tissues during early pregnancy in pigs. *J. Reprod. Dev.* **2009**, *55*, 262–272. [[CrossRef](#)]
10. Mitchell, M.D.; Adamson, S.; Coulam, C.; Romero, R.J.; Lundin-Schiller, S.; Trautman, M.S. The roles and regulation of prostaglandins within the uterus. In *Control of Uterine Contractility*, 1st ed.; Garfield, R.E., Tabb, T.N., Eds.; CRC Press: Boca Raton, FL, USA, 1994; pp. 255–283.
11. Krzymowski, T.; Stefanczyk-Krzymowska, S. The role of the endometrium in endocrine regulation of the animal oestrous cycle. *Reprod. Domest. Anim.* **2008**, *43*, 80–91. [[CrossRef](#)]
12. Smith, W.L.; Garavito, R.M.; DeWitt, D.L. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J. Biol. Chem.* **1996**, *271*, 33157–33160. [[CrossRef](#)]
13. Helliwell, R.J.A.; Adams, L.F.; Mitchell, M.D. Prostaglandin synthases: Recent developments and a novel hypothesis. *Prostaglandins Leukot. Essent. Fatty Acids* **2004**, *70*, 101–113. [[CrossRef](#)] [[PubMed](#)]
14. Wu, K.K.; Liou, J.Y. Cellular and molecular biology of prostacyclin synthase. *Biochem. Biophys. Res. Commun.* **2005**, *338*, 45–52. [[CrossRef](#)]
15. Tanabe, T.; Hara, S.; Miyata, A.; Brugger, R.; Ullrich, Y. Molecular cloning of prostacyclin synthase from bovine endothelial cells. *Adv. Exp. Med. Biol.* **1997**, *400A*, 183–187. [[CrossRef](#)]
16. Spisni, E.; Griffoni, C.; Santi, S.; Riccio, M.; Marulli, R.; Bartolini, G.; Toni, M.; Ullrich, V.; Tomasi, V. Colocalization prostacyclin (PGI<sub>2</sub>) synthase—caveolin-1 in endothelial cells and new roles for PGI<sub>2</sub> in angiogenesis. *Exp. Cell Res.* **2001**, *266*, 31–43. [[CrossRef](#)]
17. Narumiya, S.; Sugimoto, Y.; Ushikubi, F. Prostanoid receptors: Structures, properties, and functions. *Physiol. Rev.* **1999**, *79*, 1193–1226. [[CrossRef](#)]
18. Wise, H. Multiple signaling options for prostacyclin. *Acta Pharmacol. Sin.* **2003**, *24*, 625–630.
19. Turcato, S.; Clapp, L.H. Effects of the adenylyl cyclase inhibitor SQ22536 on iloprost-induced vasorelaxation and cyclic AMP elevation in isolated guinea-pig aorta. *Br. J. Pharmacol.* **1999**, *126*, 845–847. [[CrossRef](#)]
20. Murata, T.; Ushikubi, F.; Matsuoka, T.; Hirata, M.; Yamasaki, A.; Sugimoto, Y.; Ichikawa, A.; Aze, Y.; Tanaka, T.; Yoshida, N.; et al. Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature* **1997**, *388*, 678–682. [[CrossRef](#)]
21. Ulbrich, S.E.; Schulke, K.; Groebner, A.E.; Reichenbach, H.D.; Angioni, C.; Geisslinger, G.; Meyer, H.H.D. Quantitative characterization of prostaglandins in the uterus of early pregnant cattle. *Reproduction* **2009**, *138*, 371–382. [[CrossRef](#)]
22. Cammas, L.; Reinaud, P.; Bordas, N.; Dubois, O.; Germain, G.; Charpigny, G. Developmental regulation of prostacyclin synthase and prostacyclin receptors in the ovine uterus and conceptus during the peri-implantation period. *Reproduction* **2006**, *131*, 917–927. [[CrossRef](#)] [[PubMed](#)]
23. Morawska, E.; Kaczmarek, M.M.; Blitek, A. Regulation of prostacyclin synthase expression and prostacyclin content in the pig endometrium. *Theriogenology* **2012**, *78*, 2071–2086. [[CrossRef](#)] [[PubMed](#)]
24. Blitek, A.; Szymanska, M.; Morawska-Pucinska, E.; Malysz-Cymborska, I.; Andronowska, A. Prostacyclin receptor (PTGIR) in the porcine endometrium: Regulation of expression and role in luminal epithelial and stromal cells. *Theriogenology* **2015**, *84*, 969–982. [[CrossRef](#)]
25. Szymanska, M.; Blitek, A. Prostacyclin synthesis and prostacyclin receptor expression in the porcine corpus luteum: Evidence for a luteotropic role in vitro. *Biol. Reprod.* **2019**, *100*, 162–174. [[CrossRef](#)]
26. Morawska-Pucinska, E.; Szymanska, M.; Blitek, A. Expression profile and role of prostacyclin receptor (PTGIR) in peri-implantation porcine conceptuses. *Theriogenology* **2014**, *82*, 546–556. [[CrossRef](#)]
27. Cao, J.; Shayibuzhati, M.; Tajima, T.; Kitazawa, T.; Taneike, T. In vitro pharmacological characterization of the prostanoid receptor population in the non-pregnant porcine myometrium. *Eur. J. Pharmacol.* **2002**, *442*, 115–123. [[CrossRef](#)]
28. Jana, B.; Jaroszewski, J.J.; Czarzasta, J.; Wlodarczyk, M.; Markiewicz, W. Synthesis of prostacyclin and its effect on the contractile activity of the inflamed porcine uterus. *Theriogenology* **2013**, *79*, 470–485. [[CrossRef](#)]
29. Akins, E.L.; Morrisette, M.C. Gross ovarian changes during estrous cycle of swine. *Am. J. Vet. Res.* **1968**, *29*, 1953–1957. [[PubMed](#)]
30. Anderson, L.L. Growth, protein content and distribution of early pig embryos. *Anat. Rec.* **1978**, *190*, 143–153. [[CrossRef](#)]
31. Vallet, J.L.; Miles, J.R.; Freking, B.A. Development of the pig placenta. In *Control of Pig Reproduction VIII*; Rodriguez-Martinez, H., Vallet, J.L., Ziecik, A.J., Eds.; Nottingham University Press: Nottingham, UK, 2009; pp. 265–279.
32. Zhao, S.; Fernald, R.D. Comprehensive algorithm for quantitative real-time polymerase chain reaction. *J. Comput. Biol.* **2005**, *12*, 1047–1064. [[CrossRef](#)]

33. Szymanska, M.; Blitek, A. Endometrial and conceptus response to exogenous progesterone treatment in early pregnant gilts following hormonally-induced estrus. *Anim. Reprod. Sci.* **2016**, *174*, 56–64. [[CrossRef](#)] [[PubMed](#)]
34. Andersen, C.L.; Jensen, J.L.; Ørntoft, T.F. Normalization of real-time quantitative reverse transcription-PCR data: A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res.* **2004**, *64*, 5245–5250. [[CrossRef](#)] [[PubMed](#)]
35. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [[CrossRef](#)]
36. Wu, W.X.; Ma, X.H.; Nathanielsz, P.W. Changes in prostacyclin synthase in pregnant sheep myometrium, endometrium, and placenta at spontaneous term. labor and regulation by estradiol and progesterone. *Am. J. Obstet. Gynecol.* **1999**, *180*, 744–749. [[CrossRef](#)]
37. Moonen, P.; Klok, G.; Keirse, M.J. Increase in concentrations of prostaglandin endoperoxide synthase and prostacyclin synthase in human myometrium in late pregnancy. *Prostaglandins* **1984**, *28*, 309–321. [[CrossRef](#)]
38. Giannoulis, D.; Alfaidy, N.; Holloway, A.C.; Gibb, W.; Sun, M.; Lye, S.J.; Challis, J.R. Expression of prostaglandin I(2) synthase, but not prostaglandin E synthase, changes in myometrium of women at term pregnancy. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 5274–5282. [[CrossRef](#)]
39. Omini, C.; Folco, G.C.; Pasargiklian, R.; Fano, M.; Berti, F. Prostacyclin (PGI<sub>2</sub>) in pregnant human uterus. *Prostaglandins* **1979**, *17*, 113–120. [[CrossRef](#)]
40. Kawano, M.; Mori, N. The significance of prostacyclin produced by pregnant rat myometrium: The relationship between myometrial prostacyclin producing activity and passive stretch of myometrium by growing conceptus. *Prostaglandins* **1988**, *35*, 305–325. [[CrossRef](#)]
41. Fetalvero, K.M.; Zhang, P.; Shyu, M.; Young, B.; Hwa, J.; Young, R.C.; Martin, K.A. Prostacyclin primes pregnant human myometrium for an enhanced contractile response in parturition. *J. Clin. Investig.* **2008**, *118*, 3966–3979. [[CrossRef](#)]
42. Battersby, S.; Critchley, H.O.D.; de Brum-Fernandes, A.J.; Jabbour, H.N. Temporal expression and signalling of prostacyclin receptor in the human endometrium across the menstrual cycle. *Reproduction* **2004**, *127*, 79–86. [[CrossRef](#)]
43. Leonhardt, A.; Glaser, A.; Wegmann, M.; Hackenberg, R.; Nüsing, R.M. Expression of prostanoid receptors in human lower segment pregnant myometrium. *Prostaglandins Leukot. Essent. Fatty Acids* **2003**, *69*, 307–313. [[CrossRef](#)]
44. Chegini, N.; Rao, C.V. The presence of prostacyclin binding sites in nonpregnant bovine uterine tissue. *Prostaglandins Leukot. Essent. Fatty Acids* **1989**, *38*, 75–79. [[CrossRef](#)]
45. Knapp, P.; Chabowski, A.; Posmyk, R.; Gorski, J. Expression of the energy substrate transporters in the uterine fibroids. *Prostaglandins Other Lipid Mediat.* **2016**, *123*, 9–15. [[CrossRef](#)]
46. Karasinski, J.; Galas, J.; Semik, D.; Fiertak, A.; Bilinska, B.; Kilarski, W.M. Changes of connexin43 expression in non-pregnant porcine myometrium correlate with progesterone concentration during oestrous cycle. *Reprod. Domest. Anim.* **2010**, *45*, 959–966. [[CrossRef](#)]
47. Oponowicz, A.; Franczak, A.; Kurowicka, B.; Kotwica, G. Relative transcript abundance of oxytocin receptor gene in porcine uterus during luteolysis and early pregnancy. *J. Appl. Genet.* **2006**, *47*, 345–351. [[CrossRef](#)]
48. Kurowicka, B.; Franczak, A.; Oponowicz, A.; Kotwica, G. In vitro contractile activity of porcine myometrium during luteolysis and early pregnancy: Effect of oxytocin and progesterone. *Reprod. Biol.* **2005**, *5*, 151–169.
49. Cornwell, T.L.; Li, J.; Sellak, H.; Miller, R.T.; Word, R.A. Reorganization of myofibrillar proteins and decreased cGMP-dependent protein kinase in the human uterus during pregnancy. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 3981–3988. [[CrossRef](#)]
50. Sobue, K.; Sellers, J.R. Caldesmon, a novel regulatory protein in smooth muscle and nonmuscle actomyosin systems. *J. Biol. Chem.* **1991**, *266*, 12115–12118. [[CrossRef](#)]
51. Winder, S.J.; Allen, B.G.; Clement-Chomienne, O.; Walsh, M.P. Regulation of smooth muscle actin-myosin interaction and force by calponin. *Acta Physiol. Scand.* **1998**, *164*, 415–426. [[CrossRef](#)]
52. Taggart, M.J.; Europe-Finner, G.N.; Mitchell, B.F. Possible dual roles for prostacyclin in human pregnancy and labor. *J. Clin. Investig.* **2008**, *118*, 3829–3832. [[CrossRef](#)]
53. Song, W.; Zhang, Z.; Jiang, Y.; Cao, Y.; Zhang, B.; Wang, Y.; Shi, H.; Zhu, L. Integrative metabolomics profiling reveals aberrations in myometrium associated with adenomyosis: A pilot study. *Reprod. Biol. Endocrinol.* **2022**, *20*, 49. [[CrossRef](#)] [[PubMed](#)]
54. Qian, X.; Wang, L.; Lin, B.; Luo, Y.; Chen, Y.; Liu, H. Maternal myometrium metabolomics profiles in labor: Preliminary results. *Gynecol. Obstet. Investig.* **2021**, *86*, 88–93. [[CrossRef](#)] [[PubMed](#)]
55. Bazer, F.W.; Kim, J.; Ka, H.; Johnson, G.A.; Wu, G.; Song, G. Select nutrients in the uterine lumen of sheep and pigs affect conceptus development. *J. Reprod. Dev.* **2012**, *58*, 180–188. [[CrossRef](#)] [[PubMed](#)]
56. Blitek, A.; Szymanska, M. Regulation of expression and role of peroxisome proliferator-activated receptors (PPARs) in luminal epithelial and stromal cells of the porcine endometrium. *Theriogenology* **2019**, *127*, 88–101. [[CrossRef](#)]
57. Sales, K.J.; Jabbour, H.N. Cyclooxygenase enzymes and prostaglandins in reproductive tract physiology and pathology. *Prostaglandins Other Lipid Mediat.* **2003**, *71*, 97–117. [[CrossRef](#)]
58. Cao, J.; Yosida, M.; Kitazawa, T.; Taneike, T. Uterine region-dependent differences in responsiveness to prostaglandins in the non-pregnant porcine myometrium. *Prostaglandins Other Lipid Mediat.* **2005**, *75*, 105–122. [[CrossRef](#)]

59. Franczak, A.; Kotwica, G.; Kurowicka, B.; Oponowicz, A.; Woclawek-Potocka, I.; Petroff, B.K. Expression of enzymes of cyclooxygenase pathway and secretion of prostaglandin E2 and F2alpha by porcine myometrium during luteolysis and early pregnancy. *Theriogenology* **2006**, *66*, 1049–1056. [[CrossRef](#)]
60. Zhou, W.; Hashimoto, K.; Goleniewska, K.; O'Neal, J.F.; Ji, S.; Blackwell, T.S.; Fitzgerald, G.A.; Egan, K.M.; Geraci, M.W.; Peebles, R.S., Jr. Prostaglandin I2 analogs inhibit proinflammatory cytokine production and T cell stimulatory function of dendritic cells. *J. Immunol.* **2007**, *178*, 702–707.710. [[CrossRef](#)]
61. Strassheim, D.; Riddle, S.R.; Burke, D.L.; Geraci, M.W.; Stenmark, K.R. Prostacyclin inhibits IFN-gamma-stimulated cytokine expression by reduced recruitment of CBP/p300 to STAT1 in a SOCS-1-independent manner. *J. Immunol.* **2009**, *183*, 6981–6988. [[CrossRef](#)]
62. Müller, T.; Dürk, T.; Blumnethal, B.; Herouy, Y.; Soricther, S.; Grimm, M.; Panther, E.; 000. Iloprost has potent anti-inflammatory properties on human monocyte-derived dendritic cells. *Clin. Exp. Allergy* **2010**, *40*, 1214–1221. [[CrossRef](#)]
63. Sivarajasingam, S.P.; Imami, N.; Johnson, M.R. Myometrial cytokines and their role in the onset of labour. *J. Endocrinol.* **2016**, *231*, R101–R119. [[CrossRef](#)]