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IMPAIRED LUTEAL PROGESTERONE SYNTHESIS OBSERVED IN GILTS WITH PMSG/hCG-INDUCED ESTRUS AFFECTS THE EXPRESSION OF STEROID, PROSTAGLANDIN, AND CYTOKINE RECEPTORS IN THE ENDOMETRIUM AND MYOMETRIUM DURING THE PERI-IMPLANTATION PERIOD*

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Abstract

The present study aimed to examine the effect of impaired progesterone (P_4) synthesis, observed in gilts with gonadotropin-induced estrus, on the uterine expression of receptors important for pregnancy establishment. Twenty prepubertal gilts received 750 IU PMSG and 500 IU hCG 72 h later, while 18 prepubertal gilts in the control group were observed daily for estrus behavior. Gilts were inseminated in their first estrus and slaughtered on days 10, 12, and 15 of pregnancy to collect endometrial and myometrial tissues for mRNA analysis using real-time PCR. As we previously described, gilts with PMSG/hCG-induced estrus showed decreased luteal P4 synthesis on days 10 and 12 of pregnancy. PMSG/hCG treatment did not affect P4 receptor mRNA expression in either uterine tissue. In the endometrium, a greater mRNA transcript abundance of estrogen receptors (*ESR1* and *ESR2*), androgen receptor (*AR*), prostaglandin (PG) E_2 receptors (*PTGER2* and *PTGER4*), $PGF_{2\alpha}$ receptor (*PTGFR*), interleukin 6 receptor (*IL6R*), and tumor necrosis factor α receptors (*TNFRSF1A* and *TNFRSF1B*) was detected in gilts with natural than with PMSG/hCG-induced estrus (P<0.05). In the myometrium, the mRNA expression of *AR*, *PTGER2*, and *PTGFR* was lower, while PGI_2 receptor (*PTGIR*) transcript abundance was elevated in the gilts treated with PMSG/hCG as compared with the control animals (P<0.05). In summary, a decreased luteal P_4 level during the peri-implantation period in gonadotropin-stimulated pigs affects endometrial and myometrial receptor expression, with the endometrium being more sensitive to impaired P_4 synthesis. Whether the observed changes alter uterine receptivity to local and systemic factors remains to be elucidated.

Key words: pig, progesterone, endometrium, myometrium, receptor expression

Progesterone (P_4) produced by the corpus luteum (CL) is the key regulator of reproductive events related to the establishment and maintenance of pregnancy in all mammalian species. Concentrations of circulating P_4 increase with the maturation of CLs, and sustained P_4 action on the uterus is crucial for the attainment of endometrial receptivity for conceptus implantation. Under the influence of P_4, several changes related to the secretory activity of the endometrium, as well as its susceptibility to local and systemic factors, occur during the luteal phase to facilitate embryo-maternal interactions (Johnson et al., 2021; Waclawik et al., 2017). In pigs, P_4-regulated genes in the endometrium include those related to vascular development and function, immune response, and prostaglandin (PG) synthesis (Bazer et al., 2012; Morawska et al., 2012; Szymanska and Blitek, 2016; Welter et al., 2004). Moreover, prolonged exposure of the uterus to P_4 causes down-regulation of progesterone receptor (PGR) expression in the luminal and glandular epithelium (Geisert et al., 1994; Steinhauser et al., 2017), which is a pre-requisite for conceptus implantation.

In domestic species, variability in P_4 synthesis and concentrations of this hormone in the blood affect both endometrial development (Ashworth and Bazer, 1989; Wiltbank et al., 2014) and conceptus survival (Inskeep, 2004; Jindal et al., 1997; Mao and Foxcroft, 1998). In cattle, low circulating P_4 alters the normal temporal changes in the endometrial transcriptome, which are associated with a reduced ability of the uterus to support conceptus development (Forde et al., 2011, 2012). In addition to endometrial receptivity, changes in both the contractile and secretory activities of the myometrium are important for pregnancy maintenance in the pig (Franczak, 2008; Markiewicz et al., 2016; Scheerboom et al., 1987). This tissue synthesizes PGs (Franczak et al., 2006 a) and steroids (Franczak, 2008; Franczak et al.,

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2014), which may act locally on both myometrial and endometrial tissues. Moreover, P_4 substantially modulates oxytocin-stimulated PGE_2 and $PGF_{2\alpha}$ release from the porcine myometrium during pregnancy (Franczak et al., 2006 b). Limited information, however, is available about the consequences of decreased luteal P_4 synthesis on the porcine uterus during the peri-implantation period.

Induced ovulation is of great interest in the commercial swine industry, as it is a useful tool for improving production efficiency and facilitating animal management (De Rensis and Kirkwood, 2016; Knox, 2015). Beside its undoubted practical significance, exogenous hormones applied to induce ovulation may also negatively affect some reproductive parameters. Specifically, estrus-induced prepubertal gilts do not express a normal estrous cycle, and if inseminated, they display a decreased conception rate or small litters, or even fail to maintain pregnancy (Knox et al., 2000; Webel and Day, 1982). The morphological and biochemical characteristics of ovarian follicle development differ between gonadotropin-treated and naturally ovulating gilts (Wiesak et al., 1990), and a greater proportion of gilts expressing follicular cysts was observed in response to exogenous gonadotropins (Breen and Knox, 2012). The hormonal induction of estrus may also affect CL development and function. A greater proportion of gilts with poorly formed CLs was observed after treatment with gonadotropins (Breen and Knox, 2012). Moreover, the CLs of gonadotropin-treated gilts showed an impaired luteal P_4 metabolic pathway and reduced sensitivity to luteotropic factors (Kineman et al., 1987 a, b).

We have previously demonstrated that injections of exogenous gonadotropins to stimulate ovulation in gilts resulted in the significant reduction of luteal P_4 production manifested by decreased expression of enzymes involved in P_4 synthesis in the CL and lower concentrations of P_4 in the luteal tissue on days 10 and 12 of pregnancy and in the blood serum on day 12 of pregnancy (Blitek et al., 2010 a, 2016). It is worthy to notice that impaired luteal P_4 synthesis led to the decreased endometrial expression of genes known as "receptivity markers", including homeobox A10 and prostaglandin endoperoxide synthase 2 (PTGS2), along with those encoding proteins directly involved in the process of implantation, i.e., transforming growth factor β1 and leukemia inhibitory factor (Blitek et al., 2010 a). Moreover, PG synthesis enzyme expression in the endometrium and/or conceptuses and concentrations of PGE_2 and $PGF_{2\alpha}$ in the uterine lumen or endometrial tissue differed between the control and PMSG/hCG-treated gilts (Blitek et al., 2010 b).

The key to successful pregnancy is highly coordinated reciprocal interactions among several molecules secreted by ovarian cells, conceptuses, and the uterus (Johnson et al., 2021; Ziecik et al., 2011). However, the ability of the tissue to respond to biological factors depends primarily on the availability of these factors' receptors. Here, we hypothesize that the reduced synthesis of luteal P_4 detected in PMSG/hCG-treated gilts on days 10 and 12 of gestation may affect the uterine expression of receptors important for pregnancy establishment. The current study aimed to examine endometrial and myometrial mRNA transcript abundance of steroid, PG, and cytokine receptors using early pregnant gilts with natural and gonadotropin-induced estrus (Blitek et al., 2010 a, 2016), which correspond to the control and decreased P_4 synthesis groups of gilts, respectively.

Material and methods

Animals and sample collection

All procedures involving the use of animals were conducted in accordance with the national guidelines for agricultural animal care and approved by the Local Ethics Committee for Experiments on Animals, University of Warmia and Mazury in Olsztyn, Poland (permission No. 23/2006).

Endometrial and myometrial tissue samples were collected from the same crossbred gilts (Polish Landrace × Duroc) that have been previously described (Blitek et al., 2010 a, 2016). Briefly, prepubertal gilts of similar age (165 to 175 days old) and weight (100 to 110 kg) were randomly assigned to two groups. Animals from the control group (gilts allowed to exhibit natural estrus; n = 18) were observed daily for estrus behavior and inseminated 24 and 48 h after the detection of their first estrus. Animals assigned to the PMSG/hCG group (n = 20; gilts with hormonally induced estrus) received i.m. injections of 750 IU PMSG (Folligon; Intervet, Boxmeer, The Netherlands) and 500 IU hCG (Chorulon; Intervet) 72 h later. These hormone-stimulated animals were inseminated 24 and 48 h after hCG injection. The day of the second insemination was recorded as the first day of pregnancy for all animals. Gilts from both groups were slaughtered on days 10 (the day before maternal recognition of pregnancy; n = 6 in the control group and n = 7 in the PMSG/hCG group), 12 (maternal recognition of pregnancy; n = 6/group), or 15 (the beginning of conceptus implantation; n = 6 in the control group and n = 4 in the PMSG/hCG group) of pregnancy. Days of pregnancy were confirmed based on the morphology of conceptuses flushed from both uterine horns of each gilt: day 10 (all conceptuses were spherical in shape with a diameter of 3–8 mm), day 12 (all conceptuses were filamentous), and day 15 (all conceptuses were elongated), according to Anderson (1978). Three gilts in the PMSG/hCG group were not pregnant and were not used for sample collection. As previously described in detail (Blitek et al., 2016), gilts from the gonadotropin-stimulated group showed a decreased expression of enzymes involved in P_4 synthesis in the CL and had lower concentrations of P_4 in the luteal tissue on days 10 and 12 of pregnancy compared with the control gilts. Moreover, concentrations of P_4 in the blood serum were lower in the PMSG/hCG-treated group than in the control gilts on day 12 of pregnancy (Blitek et al., 2010 a; 2016). The basal characteristics of the CLs and conceptuses as well as steroid and PG concentrations in these animals are provided in

Supplementary Table 1. The uterine horns were opened longitudinally on the antimesometrial site, and sections of the endometrium and myometrium were separately collected from the middle portion of the randomly selected uterine horn, snap frozen in liquid nitrogen, and stored at –80°C for further analyses.

Total RNA isolation and real-time PCR

Total RNA was extracted from endometrial and myometrial tissue samples using a Total RNA Prep Plus kit (A&A Biotechnology, Gdansk, Poland) and treated with DNase I (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. Samples were reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific), as described in detail previously (Blitek et al., 2010 a).

Diluted cDNA from RT-PCR was used to determine relative mRNA transcript abundance using an ABI Viia7 Sequence Detection System (Life Technologies Inc., Carlsbad, CA, USA). To evaluate the expression of *PGR*, *ESR1*, *ESR2*, *AR*, *PTGER2*, *PTGER4*, *PTGFR*, *PTGIR*, *IL6R, TNFRSF1A*, *TNFRSF1B*, *GAPDH*, and *HPRT1* mRNA, 15 ng of cDNA was amplified using TaqMan Gene Expression Assays (Applied Biosystems, Thermo Fisher Scientific). All abbreviations of the examined genes, their full names, and the ID numbers of the TaqMan probes are listed in Table 1. Each PCR reaction (10 µl) was performed in duplicate in 384-well plates using the following conditions: initial denaturation for 10 min at 95°C, followed by 40 cycles of denaturation for 15 s at 95°C and 60 s of annealing at 60°C. Data from real-time PCR were analyzed using the Miner method (Zhao and Fernald, 2005). The control reactions in the absence of reverse transcriptase were performed to test for genomic DNA contamination. For both RT-PCR and qPCR, no template controls with nuclease-free water were included to monitor possible reagent contamination. All data for each target gene were normalized against the geometric averaging of *GAPDH* and *HPRT1*.

Statistical analysis

All statistical analyses were conducted using GraphPad Prism v. 8.0 (GraphPad Software, Inc., San Diego, CA, USA). Prior to the analysis, all numerical data were tested for the normality and homogeneity of the variances. The data were analyzed using two-way ANOVA followed by a Bonferroni multiple comparison *post hoc* test. This analysis included the effect of day, PMSG/hCG treatment, and day by PMSG/hCG treatment interaction. All numerical data were expressed as mean ± SEM, and the means were considered to be statistically different at $P<0.05$.

Results

Effect of PMSG/hCG treatment on the mRNA transcript abundance of steroid receptors

Neither the day of pregnancy nor PMSG/hCG treatment affected the expression of *PGR* mRNA in the endometrium ($P=0.22$ and $P=0.89$, respectively) or in the myometrium ($P=0.82$ and $P=0.44$, respectively; Figure 1).

Estrogen receptor 1 mRNA expression in both the endometrium and the myometrium was affected by the day of gestation ($P<0.0001$) but not PMSG/hCG treatment ($P=0.15$ and $P=0.92$, respectively; Figure 1). Moreover, the day by PMSG/hCG treatment interaction ($P=0.02$) was detected in the endometrium. In this tissue, a lower expression of *ESR1* mRNA was detected on day 12 of gestation as compared with day 10 ($P<0.05$) in both groups of animals. A decrease in *ESR1* transcript abundance was also observed in the myometrium of control and PMSG/hCG-treated gilts between days 10 and 15 of gestation ($P<0.01$). Moreover, on day 10 of pregnancy, the endometrial expression of *ESR1* mRNA was greater in gilts with natural than with hormonally induced estrus ($P<0.05$).

Table 1. Full names of genes and the identity number of TaqMan probes used to examine relative mRNA transcript abundance

Abbreviation	Gene name	ID of TaqMan probe
PGR	Progesterone receptor	Ss03374440_m1
ESR1	Estrogen receptor 1	Ss03383398_u1
ESR2	Estrogen receptor 2	Ss03391479_m1
AR	Androgen receptor	Ss03822350_s1
PTGER2	Prostaglandin E receptor 2	Ss03374177_g1
PTGER4	Prostaglandin E receptor 4	Ss03377412_u1
PTGFR	Prostaglandin F receptor	Ss03393819_s1
PTGIR	Prostaglandin I2 receptor	–[1]
IL6R	Interleukin 6 receptor	Ss03394904_g1
TNFRSF1A	TNF receptor superfamily member 1A	Ss03391124_g1
TNFRSF1B	TNF receptor superfamily member 1B	Ss03385518_u1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Ss03375435_u1
HPRT1	Hypoxanthine phosphoribosyltransferase 1	Ss03388274_m1

[1]Designed by Applied Biosystems, Thermo Fisher Scientific; GenBank accession no. NC_010448.3.

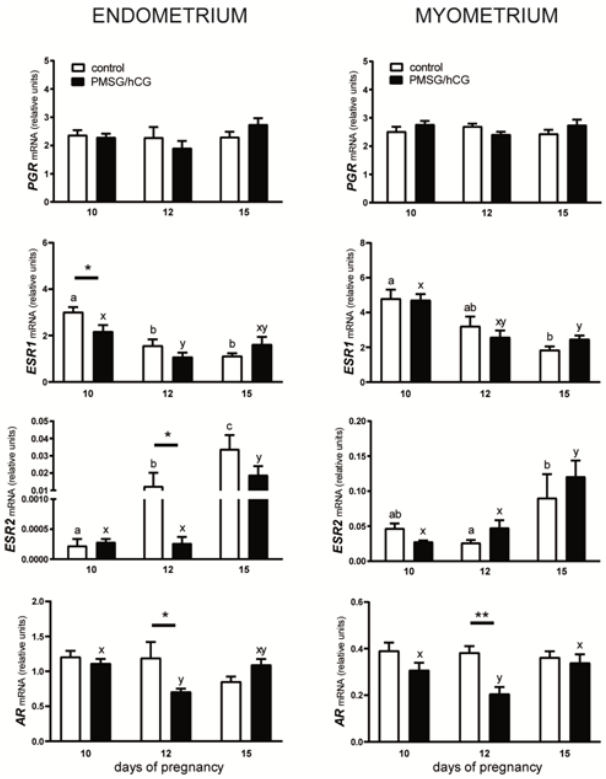


Figure 1. The mRNA transcript abundance of progesterone receptor (*PGR*), estrogen receptors (*ESR1* and *ESR2*), and androgen receptor (*AR*) in the endometrium and the myometrium of pregnant gilts with natural and PMSG/hCG-induced estrus. Values from real-time PCR for studied genes were normalized against geometric averaging of *GAPDH* and *HPRT1*. Values are expressed as the mean ± SEM (n = 4–7). Bars with various letters are different among groups (a, b and c for the control group, x and y for the PMSG/hCG group). Asterisks indicate differences between groups on particular day of pregnancy (*P<0.05; **P<0.01)

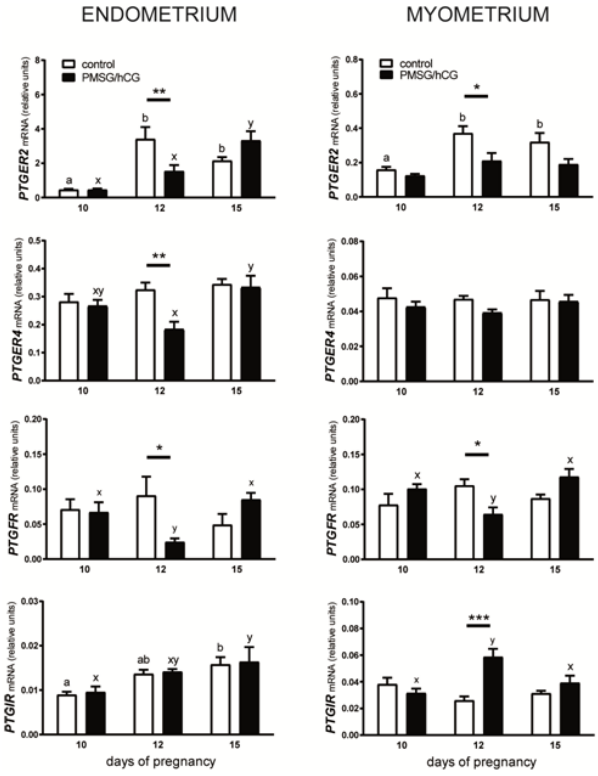


Figure 2. The mRNA transcript abundance of prostaglandin (PG) E_2 receptors (*PTGER2* and *PTGER4*), $PGF_{2\alpha}$ receptor (*PTGFR*), and PGI_2 receptor (*PTGIR*) in the endometrium and the myometrium of pregnant gilts with natural and PMSG/hCG-induced estrus. Values from real-time PCR for studied genes were normalized against geometric averaging of *GAPDH* and *HPRT1*. Values are expressed as the mean ± SEM (n = 4–7). Bars with various letters are different among groups (a and b for the control group, x and y for the PMSG/hCG group). Asterisks indicate differences between groups on particular day of pregnancy (*P<0.05; **P<0.01; ***P<0.001)

The day of pregnancy affected *ESR2* mRNA transcript abundance in both the endometrium and the myometrium (P<0.001; Figure 1). Moreover, the endometrial expression of *ESR2* mRNA was affected by gonadotropin treatment (P=0.048). A dramatic increase in *ESR2* transcript abundance in the endometrial tissue was observed in the control gilts between days 10 and 12 of pregnancy (11-fold change; P<0.01) and was still detected on day 15 (P<0.001 and P<0.05 compared with days 10 and 12, respectively). In the PMSG/hCG group, a greater expression of *ESR2* mRNA in the endometrium was observed on day 15 as compared with days 10 and 12 (P<0.01). The effect of PMSG/hCG treatment was apparent on day 12 of pregnancy in this tissue, when the concentration of *ESR2* transcripts was more than 40-fold lower in the gilts with PMSG/hCG-induced estrus than in the control gilts (P<0.05). In the myometrium, *ESR2* mRNA expression increased between days 12 and 15 in both the control and gonadotropin-treated gilts (P<0.05).

Neither the day of pregnancy nor PMSG/hCG treatment affected *AR* mRNA expression in the endometrium (P=0.13 and P=0.23, respectively; Figure 1). However, the day by PMSG/hCG treatment interaction (P=0.01) was detected in this tissue. In the myometrium, the induction of estrus with gonadotropins (P=0.001), but not the day of pregnancy (P=0.14), affected *AR* transcript abundance. In both uterine tissues, *AR* mRNA expression decreased between days 10 and 12 of pregnancy in the PMSG/hCG group of gilts (P<0.05) but not in the control animals. Moreover, the decreased expression of *AR* mRNA was observed on day 12 of gestation in the endometrium and myometrium of PMSG/hCG-treated gilts as compared with control gilts (P<0.05 and P<0.01, respectively).

Effect of PMSG/hCG treatment on the mRNA transcript abundance of prostaglandin receptors

Prostaglandin E receptor 2 mRNA transcript abundance in the endometrium and the myometrium was affected by the day of pregnancy (P<0.0001) and PMSG/hCG treatment (P=0.046 and P=0.001, respectively; Figure 2). In the control animals, the expression of *PTGER2* mRNA was greater on days 12 and 15 of gestation as compared with day 10 (P<0.05) in both tissues examined. In the PMSG/hCG-treated gilts, the increased *PTGER2* mRNA transcript abundance was detected on day 15 compared with days 10 and 12 in the endometrium (P<0.05). The effect of PMSG/hCG treatment was apparent on day 12 of pregnancy, when the decreased expression of *PTGER2* mRNA was observed in the gilts with gonadotropin-induced estrus as compared with the control animals in the endometrium (P<0.01) and the myometrium (P<0.05).

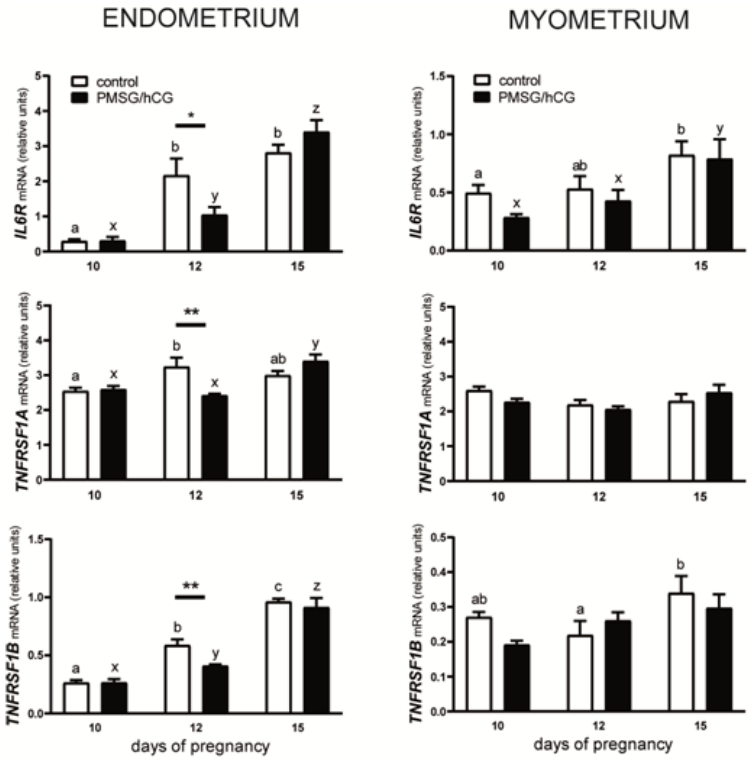


Figure 3. The mRNA transcript abundance of interleukin 6 receptor (*IL6R*) and tumor necrosis factor α receptors (*TNFRSF1A* and *TNFRSF1B*) in the endometrium and the myometrium of pregnant gilts with natural and PMSG/hCG-induced estrus. Values from real-time PCR for studied genes were normalized against geometric averaging of *GAPDH* and *HPRT1*. Values are expressed as the mean ± SEM (n = 4–7). Bars with various letters are different among groups (a, b and c for the control group, x, y and z for the PMSG/hCG group). Asterisks indicate differences between groups on particular day of pregnancy (*P<0.05; **P<0.01)

The day of pregnancy (P=0.012), the PMSG/hCG treatment (P=0.016), and the day by PMSG/hCG treatment interaction (P=0.03) affected *PTGER4* transcript abundance in the endometrium but not in the myometrium (Figure 2). Similar to *PTGER2* mRNA, a lower expression of *PTGER4* mRNA was detected in the endometrium of PMSG/hCG-treated gilts on day 12 compared with day 15 (P<0.01) and also compared with day 12 control gilts (P<0.01).

Prostaglandin F receptor mRNA showed similar profiles of expression in the endometrium and the myometrium, with decreased transcript abundance found in the gonadotropin-induced gilts on day 12 as compared with days 10 and 15 (P<0.05) and as compared with the control gilts (P<0.05; Figure 2).

The expression of *PTGIR* mRNA in the endometrium was affected by the day of pregnancy (P<0.001; Figure 2). In both groups of animals, the endometrial expression of *PTGIR* mRNA increased between days 10 and 15 of pregnancy (P<0.05). In contrast to the endometrium, *PTGIR* mRNA expression in the myometrium was affected by gonadotropin treatment (P=0.004), and this effect was clearly visible on day 12 of gestation, when *PTGIR* transcript abundance was more than two-fold greater in the PMSG/hCG-treated gilts than in the control gilts (P<0.001).

Effect of PMSG/hCG treatment on the mRNA transcript abundance of cytokine receptors

The day of pregnancy significantly affected *IL6R* mRNA expression in both the endometrium (P<0.0001) and myometrium (P=0.0003; Figure 3). Moreover, the effect of PMSG/hCG treatment was detected in the endometrium (P=0.04). Endometrial abundance of *IL6R* transcripts increased between days 10 and 12 (P<0.001) in the gilts with natural estrus and between days 10 and 12 (P<0.01), followed by a further increase on day 15 (P<0.001), in the gilts with gonadotropin-induced estrus. Moreover, on day 12 of pregnancy, *IL6R* mRNA expression in the endometrium was two-fold lower in the PMSG/hCG group of gilts as compared with the control animals (P<0.05). In the myometrium, a greater abundance of *IL6R* transcripts was detected on day 15 of gestation as compared with day 10 in both groups of gilts (P<0.05).

The expression of *TNFRSF1A* mRNA in the endometrium was affected by the day of pregnancy (P=0.001) and by the day by PMSG/hCG treatment interaction (P=0.001; Figure 3). In this tissue, an increase in *TNFRSF1A* transcript abundance was observed between days 10 and 12 of pregnancy in the control gilts (P<0.01), while the gonadotropin-induced animals showed the highest expression of this mRNA on day 15 (P<0.001). Moreover, a lower abundance of *TNFRSF1A* mRNA transcripts in gilts with PMSG/hCG-induced estrus as compared with control gilts was detected in the endometrium on day 12 of pregnancy (P<0.01). In contrast, neither the day (P=0.07) nor PMSG/hCG treatment (P=0.55) affected the mRNA expression of *TNFRSF1A* in the myometrium.

The day of pregnancy and the PMSG/hCG treatment affected *TNFRSF1B* mRNA expression in the endometrium (P<0.0001 and P=0.003, respectively) and myometrium (P=0.004 and P=0.048, respectively; Figure 3). A gradual increase in *TNFRSF1B* transcript abundance in the endometrial tissue of both the control and PMSG/hCG-treated animals was observed between days 10 and 15 of pregnancy (P<0.001). The effect of PMSG/hCG treatment was detected on day 12 of gestation, when *TNFRSF1B* mRNA expression in the endometrium was greater in the control gilts as compared with the gonadotropin-induced animals (P<0.01). In the myometrium, a greater abundance of *TNFRSF1B* transcripts was found in the control group on day 15 of pregnancy as compared with day 12 (P<0.05).

Discussion

Successful pregnancy establishment in all mammalian species is considerably influenced by P_4. In cattle, decreased concentrations of circulating P_4 have been implicated in low pregnancy rates associated with an impaired ability of the uterus to support embryo development due to altered endometrial gene expression (Forde et al., 2011, 2012; Rizos et al., 2010). In the current study, we used early pregnant gilts with PMSG/hCG-induced estrus accompanied by impaired luteal P_4 synthesis to test the hypothesis that insufficient P_4 concentrations may affect endometrial and myometrial receptivity; in particular, the mRNA transcript abundance of steroid, PG, and cytokine receptors was examined. As we previously described (Blitek et al., 2010 a, 2016), animals with gonadotropin-induced estrus demonstrated decreased luteal P_4 synthesis compared with the control group, which was apparent on days 10 and 12 of pregnancy. Moreover, significant differences between the gonadotropin-treated and control animals were observed on days 12 and 15 of pregnancy with regard to PG synthesis and concentrations in the uterus (Blitek et al., 2010 b). Our present results clearly show that impaired synthesis of luteal P_4 observed in PMSG/hCG-treated gilts had no effect on *PGR* mRNA expression in the uterine tissues but negatively affected the endometrial mRNA expression of estrogen, androgen, PGE_2, $PGF_{2\alpha}$, IL6, and TNFα receptors, which was primarily visible on day 12 of gestation. The response of the myometrial tissue to decreased P_4 synthesis was more differentiated because both the inhibitory and stimulatory effects of PMSG/hCG treatment were detected. These results broaden the knowledge about P_4-modulated mRNA transcripts during endometrial preparation for implantation in pigs and are the first demonstrating that low P_4 may contribute to changes in the transcriptome in the myometrium of farm animals.

In pigs, the loss of PGR protein from the endometrial epithelium is observed on days 10 to 12 after ovulation (Geisert et al., 1994; Steinhauser et al., 2017). As we reported here, the expression of *PGR* mRNA in the endometrial tissue did not change in either group of animals.

Such an observation is in agreement with previous data showing relatively stable *PGR* transcript abundance in the porcine endometrium on days 9 to 25 of pregnancy (Steinhauser et al., 2017). However, *PGR* mRNA expression did not differ between gilts with control and impaired P_4 synthesis (current results), which seems to contradict the theory regarding the negative correlation between concentrations of circulating P_4 and the abundance of PGR in the endometrium of pigs (Bazer et al., 2012; Geisert et al., 1994). Similarly, no differences in *PGR* mRNA expression in the endometrium were detected on day 13 in cyclic beef heifers with normal and low concentrations of circulating P_4 (Forde et al., 2011). However, the mechanisms controlling the decline in PGR expression in pigs are activated before day 6 of pregnancy (Mathew et al., 2011), while CLs and blood samples for P_4 analyses in the present study were collected after day 9 of pregnancy (Blitek et al., 2016). Therefore, we can only speculate that the amount of P4 secreted before day 6 by CLs of gonadotropin-treated and control gilts was similar and sufficient to induce PGR down-regulation in both groups of animals. Additionally, it should be noted that the maintenance of PGR expression in the endometrial stroma (Spencer and Bazer, 1995; Steinhauser et al., 2017) may hinder changes in PGR expression in the endometrial epithelium.

As we showed here, the abundant *PGR* transcripts in the myometrial tissue were similar to those detected in the endometrium and were not affected by either the day of pregnancy or the reduced P_4 synthesis observed in the PMSG/hCG-treated gilts. This indicates that *PGR* mRNA expression in the myometrium may not be directly controlled by luteal P_4. To support this statement, the PGR protein was localized in the myometrial cells of ovariectomized gilts injected with both P_4 or corn oil vehicle (Steinhauser et al., 2017). Moreover, steady-state levels of *PGR* mRNA transcripts in the myometrium were detected in cyclic and pregnant ewes on days 11 to 15 after estrus (Spencer and Bazer, 1995).

In addition to P_4, ovarian estrogens influence morphological and physiological changes in the uterus, acting via its nuclear receptors, ESR1 and ESR2. In pigs, conceptus-derived estradiol-17β (E_2) is the main pregnancy recognition signal, and it significantly affects uterine functions during early pregnancy (Johnson et al., 2021; Waclawik et al., 2017; Ziecik et al., 2011). As we demonstrated previously, concentrations of E_2 in the uterine lumen did not differ between gilts with natural and PMSG/hCG-induced estrus (Blitek et al., 2010 a; Supplementary Table 1), indicating no effects of impaired P_4 synthesis on conceptus development and their ability to synthesize E_2. The present analyses revealed that the abundance of *ESR2* transcripts increased substantially from day 12 in the endometrium of the control gilts, while in the PMSG/hCG-treated gilts, the up-regulation of *ESR2* mRNA expression was delayed and observed on day 15. Such delay in *ESR2* mRNA expression and lower abundance of *ESR1* transcripts in the endometrium of gilts with lower P_4 concentrations in the CL on days 10 and 12 of pregnancy indicate that sustained P_4 synthesis may be required for the development of proper endometrial receptivity to E_2 action. In contrast to the endometrium, *ESR2* mRNA expression in the myometrium was not affected by gonadotropin treatment.

Compared with E_2 and P_4 receptor expression, there is little data concerning the regulation of AR expression in the porcine uterus. The marked up-regulation of *AR* mRNA expression in the porcine endometrium on day 12 of pregnancy and the potent stimulatory effect of E_2 on *AR* transcript abundance *in vitro* have been previously described (Kowalski et al., 2004). In the present study, *AR* mRNA expression in the endometrium and myometrium did not change during the studied period of pregnancy in control gilts, but a lower mRNA expression of these receptors was found on day 12 in PMSG/hCG-treated animals expressing low P_4 concentrations. Thus, AR expression in the porcine uterus seems to be regulated not only by E_2 from conceptuses (Kowalski et al., 2004) but also by P_4 from the CL (present data).

The PGs are important regulators of the CL lifespan, and $PGF_{2\alpha}$ is responsible for luteal regression in non-pregnant animals (McCracken et al., 1999), while PGE_2 functions as a luteotropic and antiluteolytic factor promoting CL maintenance during pregnancy (Ziecik et al., 2018). Additionally, PGs act locally to modulate uterine functions (Kennedy et al., 2007; Waclawik et al., 2009). As we previously demonstrated, gilts with PMSG/hCG-induced estrus that was accompanied by reduced luteal P_4 synthesis on days 10 and 12 of pregnancy had decreased endometrial expression of the key enzymes involved in PGE_2 synthesis, i.e. PTGS2 and PGE synthase, on day 12 of pregnancy and lower levels of PGE_2 in the uterine lumen observed on day 15 (Blitek et al., 2010 a, b). Moreover, much lower concentrations of $PGF_{2\alpha}$ in endometrial tissue and blood serum were detected in the pregnant gilts with low P_4 compared with the control animals on days 12 and/or 15 of pregnancy (Blitek et al., 2010 b). In the present study, insufficient P_4 synthesis in the gilts with gonadotropin-induced estrus negatively affected the expression of *PTGER2*, *PTGER4*, and *PTGFR* mRNA in the endometrium and/or myometrium. This indicates that sustained P_4 production during the peri-implantation period in pigs is required not only for the proper synthesis of PGE_2 and $PGF_{2\alpha}$ but also for the expression of the PG receptor system.

To our knowledge, this is the first report demonstrating the profiles of the mRNA transcript abundance of PGE_2 and $PGF_{2\alpha}$ receptors in the myometrium of early pregnant pigs. In the control animals, *PTGFR* mRNA expression did not vary during the examined period of gestation, while *PTGER2* mRNA expression increased markedly after day 10. Moreover, levels of both types of receptors were decreased during the maternal recognition of pregnancy in the PMSG/hCG-treated gilts. In the porcine myometrium, PTGER2 has been described as a relaxant, and PTGFR has been reported as a contrac-

tile receptor (Cao et al., 2005). However, both PGE_2 and $PGF_{2\alpha}$ increase the tension of the contractions of the porcine myometrium during early pregnancy (Markiewicz et al., 2016). Therefore, the decreased mRNA expression of PG receptors in gilts with impaired luteal P_4 synthesis may result in abnormal myometrial contractility during conceptus elongation and initial implantation.

PGI_2, commonly known as prostacyclin, has been primarily described as a potent vasodilator and inhibitor of platelet aggregation in the vascular system but is also involved in pregnancy establishment and implantation in various species (Cammas et al., 2006; Lim et al., 1999; Morawska et al., 2012). Specifically, PGI_2, acting via its membrane receptor, PTGIR, stimulates the expression of pro-angiogenic factors in the porcine endometrium (Blitek et al., 2015). In the present study, endometrial *PTGIR* expression was not affected by PMSG/hCG treatment. However, concentrations of *PTGIR* transcripts in the myometrium were greater in the gilts with gonadotropin-induced estrus than in the control group. PGI_2 is a smooth muscle relaxant in the uteri of non-pregnant women and pigs (Cao et al., 2002; Lumsden and Baird, 1986). P_4, in turn, promotes uterine quiescence during pregnancy (Wray et al., 2015). Moreover, the supplementation of early pregnant gilts with P_4 stimulates *PTGIR* mRNA expression in the CL (Szymanska and Blitek, 2020). Therefore, the up-regulation of *PTGIR* mRNA in the myometrium of PMSG/hCG gilts is surprising, but it may point to the activation of some local mechanisms in the uterus to compensate for reduced P_4 synthesis and prevent contractions. This suggestion, however, requires more detailed research.

The period of the maternal recognition of pregnancy and early implantation is accompanied by the presence of cytokines in the porcine uterus (Edwards et al., 2012; Geisert et al., 2014). Among these, IL6 and TNFα contribute to local inflammatory reactions related to conceptus implantation (Hunt et al., 1996; Prins et al., 2012). In the present study, the abundance of *IL6R*, *TNFRSF1A*, and *TNFRSF1B* transcripts in the endometrium increased after day 10 of gestation. Furthermore, we observed a negative effect of PMSG/hCG treatment on the expression of all three types of receptors in the endometrium that was apparent on day 12 of gestation. IL6 and TNFα are important regulators of cell proliferation, differentiation, and apoptosis (Hunt et al., 1996; Lee, 1992; Yoo et al., 2017) and substantially modulate PGE_2 and/or PGF2α synthesis in the porcine endometrium (Blitek and Ziecik, 2006; Franczak et al., 2012; Waclawik et al., 2010). Therefore, the reduced mRNA expression of *IL6R*, *TNFRSF1A*, and *TNFRSF1B* during early pregnancy may negatively affect endometrial preparation for implantation. In contrast to the endometrium, estrus induction with PMSG/hCG did not alter the mRNA expression of IL6 and TNFα receptors in the myometrium, which points to other than P_4-dependent mechanisms controlling cytokine receptor transcript abundance in this tissue.

In conclusion, the use of early pregnant gilts with natural and PMSG/hCG-induced estrus which exhibited normal and impaired luteal P_4 synthesis, respectively, allowed us to demonstrate that uterine mRNA expression of estrogen, androgen, PGE_2, $PGF_{2\alpha}$, IL6, and TNFα receptors may be regulated by P_4 of CL origin. Endometrial tissue seems to be more sensitive to decreased P_4 synthesis than the myometrium. Moreover, the differences in the mRNA expression of examined receptors were apparent primarily on day 12 of pregnancy (present data), when both luteal and circulating concentrations of P_4 were lower in PMSG/hCG-induced than control animals (Blitek et al., 2016). Whether the observed changes may alter embryo-maternal interactions and conceptus implantation remains to be elucidated. As we showed previously (Blitek et al., 2010 a; Supplementary Table 1), neither the number of conceptuses nor their developmental stage, at least until day 15 of pregnancy, were affected by impaired P_4 synthesis in the CL. The major limitation of this study is that the experimental design does not allow for the determination of the exact mechanisms explaining how decreased P_4 synthesis affects endometrial and myometrial receptivity. Therefore, further studies should be undertaken to identify these regulations. Moreover, the examination of the long-term consequences of impaired but still occurring P_4 synthesis on conceptus implantation and placenta efficiency would be of great benefit for understanding pig reproduction.

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