

The Alterations of Endometrial and Myometrial Receptivity in Early Pregnant Gilts in Response to Estrus Induction With PMSG/hCG

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Research

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Abstract

Background: The hormonal control of ovulation has become a standard procedure in the swine industry. However, exogenous gonadotropins can be detrimental to reproductive function, affecting follicle development, corpus luteum formation, and embryo development and survival. Much less is known about uterine receptivity in gilts with induced estrus. Therefore, our objective was to determine the effect of estrus induction with pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) on the expression of steroid, prostaglandin, cytokine, and oxytocin receptors, as well as nuclear factor kappa B subunit 1 (*NFKB1*), peroxisome proliferator activated receptor gamma (*PPARG*), and gap junction protein alpha 1 (*GJA1*), in the endometrium and myometrium of early pregnant gilts. Twenty prepubertal gilts received 750 IU PMSG and 500 IU hCG 72 h later, while eighteen prepubertal gilts in the control group were observed daily for estrus behavior. All gilts were inseminated in their first estrus and slaughtered on days 10, 12, and 15 of pregnancy to collect uterine tissues for mRNA expression analyses using real-time PCR.

Results: Estrus induction did not affect progesterone receptor expression in either uterine tissue. In the endometrium, greater mRNA expression of estrogen receptors (*ESR1* and *ESR2*), androgen receptor (*AR*), prostaglandin (PG) E2 receptors (*PTGER2* and *PTGER4*), PGF2 α receptor (*PTGFR*), interleukin 6 receptor (*IL6R*), tumor necrosis factor α receptors (*TNFRSF1A* and *TNFRSF1B*), and oxytocin receptor (*OXTR*) was detected in the control than in the PMSG/hCG-treated gilts ($P < 0.05$). In the myometrium, concentrations of *AR*, *PTGER2*, *PTGFR*, and *NFKB1* transcripts were lower, while PGI2 receptor and *PPARG* transcripts were elevated in gilts with gonadotropin-induced estrus as compared with naturally ovulated gilts ($P < 0.05$). Furthermore, the administration of PMSG/hCG resulted in the greater expression of *GJA1* mRNA in both the endometrium and myometrium of day 15 pregnant gilts ($P < 0.05$).

Conclusions: Estrus induction with PMSG/hCG in prepubertal gilts may affect steroid, prostaglandin, cytokine, and oxytocin receptor expression in the endometrium and myometrium, thereby altering uterine receptivity to local or systemic factors. This may, in turn, contribute to disorders in embryo-maternal interactions and the process of implantation.

Background

Induced ovulation is of great interest in the commercial swine industry being a useful tool for improving production efficiency and facilitating animal management. It ensures fixed-time artificial insemination and may serve as a gateway technology for the use of cryopreserved boar semen and sex-sorted semen [1, 2]. Additionally, control of estrus is a fundamental technique in the preparation of embryo donors and recipients in the application of transgenesis, nuclear transfer, and other reproductive biotechniques. Several protocols for the hormonal control of ovulation are available for gilts and sows, and the particular approach depends primarily upon the maturity of the female. In prepubertal gilts, a treatment protocol based on a combination of pregnant mare serum gonadotropin (PMSG), used to stimulate follicular

growth, followed by human chorionic gonadotropin (hCG), used to trigger ovulation [3, 4], is commonly applied.

Until now, the majority of studies dedicated to analyzing the effect of the hormonal control of ovulation in swine have focused on parameters describing follicle development, time to and duration of ovulation, variability in oocyte maturation and early embryo cell number, as well as gonadotropin and steroid hormone profiles [3, 4]. Specifically, estrus-induced prepubertal gilts do not express a normal estrous cycle, and if inseminated, they display a decreased conception rate, small litters, or even fail to maintain pregnancy [3, 5]. Relationships among the concentrations of estrogens, prolactin, and luteinizing hormone in the blood serum vary in prepubertal gilts with induced ovulation as compared with mature females [6]. Both the morphological and biochemical characteristics of ovarian follicle development differ between gonadotropin-treated and naturally ovulating gilts [7], and a greater proportion of gilts expressing follicular cysts were observed in response to exogenous gonadotropins [8]. Moreover, a higher number of degenerated embryos and a lower number of blastocysts hatched *in vitro* were found in pregnant gilts that were hormonally induced to ovulate [9].

The hormonal induction of estrus may also affect corpus luteum (CL) development and function. A greater proportion of gilts with poorly formed CLs was observed after treatment with gonadotropins [8]. Moreover, the CLs of gonadotropin-treated gilts showed impaired luteal progesterone (P4) metabolic pathway and reduced sensitivity to luteotropic factors [10, 11]. Lower concentrations of circulating P4 in gilts inseminated during gonadotropin-induced first estrus as compared with gilts inseminated during natural first estrus were accompanied by the reduced expression of enzymes involved in P4 synthesis and decreased levels of this steroid in the luteal tissue [12]. Because luteal P4 is absolutely essential for the establishment of pregnancy [13], impaired CL development and/or its secretory activity may influence uterine preparation for implantation.

Under the influence of P4, 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 [14, 15]. P4-regulated genes in the porcine endometrium include those related to vascular development and function, immune response, and prostaglandin (PG) synthesis [13, 16–18]. Thus, disturbances in endometrial preparation for implantation may result in pregnancy failure [19, 20]. However, limited information is available about the consequences of estrus induction with exogenous hormones on the uterus. As we previously reported, the reduced luteal P4 synthesis detected in prepubertal gilts stimulated to ovulate with PMSG/hCG resulted in the decreased endometrial expression of genes known as “receptivity markers”, including homeobox A10 and prostaglandin endoperoxide synthase 2, and also those encoding proteins directly involved in the process of implantation, i.e., transforming growth factor β 1 and leukemia inhibitory factor [21]. Moreover, PG synthesis enzyme expression in the endometrium and/or conceptuses and concentrations of PGE2 and PGF2 α in the uterine lumen or endometrial tissue differed between gonadotropin-treated and naturally-ovulated pregnant gilts [22].

In addition to endometrial receptivity, changes in both the contractile and secretory activities of the myometrium are important for pregnancy establishment and maintenance in the pig [23–25]. This tissue synthesizes PGs [26] and steroids [25, 27], which may act locally on both myometrial and endometrial tissues. Moreover, P4 substantially modulates oxytocin-stimulated PGE2 and PGF2 α release from the porcine myometrium during pregnancy [28] and inhibits oxytocin-induced myometrial contractility [29]. However, the consequences of decreased luteal P4 synthesis on the myometrium have not yet been examined in domestic species, including the pig.

Here, we hypothesize that the reduced synthesis of luteal P4 in pregnant gilts with PMSG/hCG-induced estrus may affect endometrial and/or myometrial preparation for pregnancy establishment. The key to successful pregnancy is highly coordinated reciprocal interactions among several molecules secreted by ovarian cells, conceptuses, and the uterus [14, 30]; however, the ability of the tissue to respond to biological factors depends primarily on the availability of these factors' receptors. Therefore, in the current study, we used endometrial and myometrial tissue samples of early pregnant gilts with natural and gonadotropin-induced first estrus [12] to examine the expression of steroid, prostaglandin, cytokine, and oxytocin receptors. Additionally, we analyzed the expression of nuclear factor kappa B subunit 1 (*NFKB1*), peroxisome proliferator activated receptor gamma (*PPARG*), and gap junction protein alpha 1 (*GJA1*, known as connexin 43), which have also been shown to be important for uterine function [31–33].

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.

Endometrial and myometrial tissue samples were collected from the same crossbred gilts (Polish Landrace x Duroc) that have been previously described [12, 21]. Briefly, prepubertal gilts of similar age (165–175 days old) and weight (100–110 kg) were randomly assigned into two groups. Animals from Group I (control gilts allowed to exhibit natural estrus) were observed daily for estrus behavior. Gilts were considered to be in estrus when they showed a standing response to the back pressure test during boar exposure. Gilts that expressed estrus within 3 to 4 weeks after assignment into Group I (n = 18) were inseminated 24 and 48 h after the detection of their first estrus. Animals from Group II (n = 20; gilts with hormonally-induced estrus) received i.m. injections of 750 IU PMSG (Folligon; Intervet, Boxmeer, The Netherlands) on the third day after their assignment into this group 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 in all animals. Gilts from both groups were slaughtered on days 10 (the day before maternal recognition of pregnancy; n = 6–7/group), 12 (maternal recognition of pregnancy; n = 6/group), or 15 (the beginning of conceptus implantation; n = 4–6/group) of pregnancy. Due to the experimental procedure, gilts from Group I were 2 to 3 weeks older

at slaughter than gilts from Group II. Concentrations of P4 in the luteal tissue and blood serum were lower in PMSG/hCG-treated than control gilts, as we reported earlier [12, 21]. After slaughter, both uterine horns of each gilt were flushed with phosphate-buffered saline to obtain conceptuses. Days of pregnancy were confirmed based on the morphology of conceptuses: 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). Then, 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 details previously [21].

Diluted cDNA from RT-PCR was used to determine relative mRNA expression using an ABI Viiia7 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*, *NFKB1*, *OXTR*, *PPARG*, *GJA1*, *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 number of 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 obtained by real-time PCR were analyzed using the Miner method [34]. The control reactions in the absence of reverse transcriptase were performed to test for genomic DNA contamination. All data for each target gene were normalized against geometric averaging of *GAPDH* and *HPRT1*.

Statistical analyses

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 normality and homogeneity of the variances. The data were analyzed using two-way ANOVA followed by 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 estrus induction on the mRNA expression of steroid receptors

Neither the day of pregnancy nor the treatment with gonadotropins 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; Fig. 1).

ESR1 mRNA expression in both the endometrium and the myometrium was affected by the day of gestation ($P < 0.0001$) but not estrus induction with gonadotropins ($P= 0.15$ and $P= 0.92$, respectively; Fig. 1). In the endometrium, a lower concentration 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 animals 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 ($P < 0.05$).

The day of pregnancy affected *ESR2* mRNA expression in both the endometrium and the myometrium ($P < 0.001$; Fig. 1). Moreover, the endometrial expression of *ESR2* mRNA was affected by the treatment of gilts with gonadotropins ($P= 0.048$). A dramatic increase in *ESR2* transcript abundance in the endometrial tissue was observed in gilts with natural estrus 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 gonadotropin-treated animals, 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 estrus induction was apparent on day 12 of pregnancy in this tissue, when the concentration of *ESR2* transcripts was more than 40-fold lower in PMSG/hCG-treated gilts than in control gilts ($P < 0.05$). In the myometrium, *ESR2* mRNA expression increased between days 12 and 15 in both control and gonadotropin-induced gilts ($P < 0.05$).

Neither the day of pregnancy nor treatment with PMSG/hCG affected *AR* mRNA expression in the endometrium ($P= 0.13$ and $P= 0.23$, respectively; Fig. 1). However, the day by treatment interaction ($P= 0.01$) was detected in this tissue. In the myometrium, the induction of estrus ($P= 0.001$) but not the day of pregnancy ($P= 0.14$) or the day by treatment interaction ($P= 0.08$) affected *AR* transcript abundance. In both uterine tissues, *AR* mRNA expression decreased between days 10 and 12 of pregnancy in PMSG/hCG-treated ($P < 0.05$) but not in control gilts. Moreover, the decreased expression of *AR* mRNA was observed on day 12 of gestation in the endometrium and myometrium of gilts treated with gonadotropins as compared with those expressing natural estrus ($P < 0.05$ and $P < 0.01$, respectively).

Effect of estrus induction on the mRNA expression of prostaglandin receptors

PTGER2 mRNA expression in the endometrium and the myometrium was affected by the day of pregnancy ($P < 0.0001$) and estrus induction with PMSG/hCG ($P= 0.046$ and $P= 0.001$, respectively; Fig. 2). Moreover, the day by treatment interaction ($P= 0.002$) was detected in the endometrium. In naturally ovulated animals, concentrations of *PTGER2* transcripts were greater on days 12 and 15 of gestation as compared with day 10 ($P < 0.05$) in both tissues examined. In gonadotropin-treated gilts, the increased expression of *PTGER2* mRNA was detected on day 15 compared with days 10 and 12 in the

endometrium ($P < 0.05$), but not in the myometrium. The effect of gonadotropin treatment was apparent on day 12 of pregnancy, when the decreased expression of *PTGER2* mRNA was observed in gilts with induced estrus as compared with control animals in the endometrium ($P < 0.01$) and myometrium ($P < 0.05$).

The day of pregnancy ($P = 0.012$), the treatment of gilts with gonadotropins ($P = 0.016$), and the day by treatment interaction ($P = 0.03$) affected *PTGER4* mRNA expression in the endometrium, but not in the myometrium (Fig. 2). Similar to *PTGER2* mRNA, lower concentrations of *PTGER4* transcripts were detected in the endometrium of gonadotropin-induced pregnant gilts on day 12 compared with day 15 ($P < 0.01$) and also compared with day 12 pregnant gilts with natural estrus ($P < 0.01$).

Neither the day of pregnancy nor estrus induction with PMSG/hCG affected *PTGFR* mRNA expression in either examined uterine tissue (Fig. 2). However, the day by treatment interaction was detected in the endometrium ($P = 0.006$) and myometrium ($P = 0.003$). *PTGFR* mRNA showed similar profiles of expression in both tissues, with decreased levels found in gonadotropin-induced gilts on day 12 as compared with days 10 and 15 ($P < 0.05$) and also as compared with gilts expressing natural estrus ($P < 0.05$).

The expression of *PTGIR* mRNA in the endometrium was affected by the day of pregnancy ($P < 0.001$) but not by the treatment of gilts with PMSG/hCG ($P = 0.65$; Fig. 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 the treatment of gilts with gonadotropins ($P = 0.004$) and the day by treatment interaction ($P = 0.003$), but not by the day of pregnancy ($P = 0.16$). The effect of estrus induction in this tissue was clearly visible on day 12 of gestation, when *PTGIR* transcript abundance was more than two-fold greater in PMSG/hCG-treated than in control gilts ($P < 0.001$).

Effect of estrus induction on the mRNA expression of cytokine receptors and NF κ B1

The day of pregnancy significantly affected *IL6R* mRNA expression in both the endometrium ($P < 0.0001$) and myometrium ($P = 0.0003$; Fig. 3). Moreover, the effect of estrus induction with gonadotropins and the day by gonadotropin treatment interaction were detected in the endometrium ($P = 0.04$ and $P = 0.03$, respectively). Endometrial concentrations of *IL6R* transcripts increased between days 10 and 12 ($P < 0.001$) in 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 gilts with induced estrus. Moreover, on day 12 of pregnancy, *IL6R* mRNA expression in the endometrium was two-fold lower in gilts that were hormonally induced to ovulate as compared with control animals ($P < 0.05$). In the myometrium, greater concentrations of *IL6R* transcripts were 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 ($P = 0.001$), but not estrus induction ($P = 0.32$;

Fig. 3). In this tissue, an increase in *TNFRSF1A* transcript concentration was observed between days 10 and 12 of pregnancy in gilts with natural estrus ($P < 0.01$), while gonadotropin-stimulated gilts expressed the highest level of this mRNA on day 15 ($P < 0.001$). Moreover, lower levels of *TNFRSF1A* mRNA in PMSG/hCG-treated as compared with control gilts were detected in the endometrium on day 12 of pregnancy ($P < 0.01$). By contrast, neither the day ($P = 0.07$) nor treatment with hormones ($P = 0.55$) affected the expression of *TNFRSF1A* mRNA in the myometrium.

The day of pregnancy and treatment with gonadotropins 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; Fig. 3). A gradual increase in *TNFRSF1B* transcript abundance in the endometrial tissue of both control and gonadotropin-treated animals was observed between days 10 and 15 of pregnancy ($P < 0.001$). The effect of estrus induction was detected on day 12 of gestation, when *TNFRSF1B* mRNA expression in the endometrium was lower in gonadotropin-treated than in control gilts ($P < 0.01$). In the myometrium, a greater abundance of *TNFRSF1B* transcripts was found on day 15 of pregnancy as compared with day 12 ($P < 0.05$) in the control group.

The day of pregnancy ($P = 0.003$) but not treatment with PMSG/hCG ($P = 0.07$) affected the expression of *NFKB1* mRNA in the endometrium (Fig. 3). In both groups of animals, concentrations of *NFKB1* transcripts increased between days 10 and 12 of pregnancy ($P < 0.05$). In the myometrium, both the day of pregnancy ($P = 0.004$) and induction of estrus with hormones ($P = 0.048$) affected the expression of *NFKB1* mRNA. Lower concentrations of *NFKB1* transcripts in the myometrium were detected in gonadotropin-treated gilts on day 12 of pregnancy as compared with day 15 ($P < 0.05$) and also as compared with day 12 pregnant gilts with natural estrus ($P < 0.05$).

Effect of estrus induction on the mRNA expression of OXTR, PPARG, and GJA1

The expression of *OXTR* mRNA in the endometrium was affected by the day of pregnancy ($P = 0.03$) and treatment with gonadotropins ($P = 0.04$; Fig. 4). Moreover, a day by treatment interaction was detected ($P = 0.04$). Greater concentration of *OXTR* transcripts in this tissue was found on day 15 of pregnancy as compared with day 10 in the control group and as compared with day 12 in gonadotropin-treated gilts ($P < 0.05$). Moreover, the two-fold lower endometrial expression of *OXTR* mRNA was observed on day 12 in gilts with induced estrus as compared with naturally ovulated animals ($P < 0.05$). In the myometrium, *OXTR* mRNA expression was affected by the day of pregnancy ($P = 0.002$), but not treatment with PMSG/hCG ($P = 0.78$). The level of *OXTR* transcripts increased between days 10 and 15 of gestation in gilts subjected to the PMSG/hCG treatment protocol ($P < 0.01$).

The day of pregnancy but not treatment with PMSG/hCG affected *PPARG* mRNA expression in the endometrium ($P < 0.0001$ and $P = 0.79$, respectively) and myometrium ($P = 0.0004$ and $P = 0.35$, respectively; Fig. 4). Moreover, the day by treatment interaction was detected in the myometrium ($P = 0.04$). In the endometrium, concentrations of *PPARG* transcripts increased between days 10 and 15 in both groups of animals ($P < 0.001$). In the myometrium, the increased expression of *PPARG* mRNA was

detected on day 15 in gonadotropin-treated gilts as compared with days 10 and 12 ($P < 0.01$) and also as compared with day 15 pregnant gilts with natural estrus ($P < 0.05$).

The expression of *GJA1* mRNA in the endometrium was affected by the day of pregnancy ($P < 0.0001$) and treatment with gonadotropins ($P = 0.007$; Fig. 4). Greater concentrations of *GJA1* transcripts in endometrial tissue were found on day 12 of gestation as compared with day 10 in the control group ($P < 0.01$) and on days 12 and 15 as compared with day 10 in gonadotropin-treated gilts ($P < 0.01$). In the myometrium, *GJA1* mRNA expression was affected by the day of pregnancy ($P = 0.001$) but not treatment with PMSG/hCG ($P = 0.37$). Moreover, the day by treatment interaction was detected in the myometrium ($P = 0.008$). In gilts with natural estrus, the myometrial expression of *GJA1* mRNA decreased on day 12 of pregnancy as compared with day 10 ($P < 0.05$). In gilts with PMSG/hCG-induced estrus, concentrations of *GJA1* transcripts in this tissue were greater on day 15 as compared with days 10 and 12 of gestation ($P < 0.05$). The effect of gonadotropin treatment was evident in both tissues on day 15 of pregnancy, when the expression of *GJA1* mRNA was significantly elevated by PMSG/hCG treatment ($P < 0.05$).

Discussion

Successful pregnancy establishment in all mammalian species is considerably influenced by P4. In cows, low concentrations of circulating P4 are associated with altered endometrial gene expression and a reduced ability of the uterus to support embryo development [35, 36]. In the current study, we used early pregnant gilts with PMSG/hCG-induced estrus accompanied by impaired luteal P4 synthesis, which were previously described [12, 21], to test the hypothesis that insufficient P4 concentrations alter both endometrial and myometrial receptivity; in particular, the expression levels of steroid, PG, cytokine, and oxytocin receptors were examined. Our results clearly showed that the low concentrations of P4 observed in the luteal tissue and blood serum of gilts with gonadotropin-induced estrus [12] had no effect on *PGR* expression in the uterus but negatively affected the endometrial expression of genes encoding estrogen, androgen, PGE2, PGF2 α , IL6, TNF α , and oxytocin receptors, which was primarily visible on day 12 of gestation. The response of the myometrial tissue was more differentiated because both inhibitory and stimulatory effects were detected. These results are the first demonstrating that estrus induction with PMSG/hCG, accompanied by reduced P4 synthesis during the peri-implantation period, contributes to changes in the transcriptome in the myometrium of farm animals.

The uterus is the main target of luteal P4, and P4 receptors are expressed in the porcine uterus during both the estrous cycle and pregnancy [37]. Interestingly, the loss of PGR protein from the luminal epithelium of the endometrium is a prerequisite for pregnancy establishment in various species [13, 16], and this phenomenon is observed on days 10 to 12 after ovulation in pigs [38]. As we reported here, the concentrations of *PGR* mRNA in the endometrial tissue did not change between days 10 and 15 of pregnancy 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 [37]. However, *PGR* mRNA expression did not differ between gilts with natural and gonadotropin-induced estrus (current results), which seems to contradict the theory regarding the negative correlation between

concentrations of circulating P4 and the abundance of PGR in the endometrium of the pig [16, 38]. 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 P4 [36]. The lack of differences in endometrial *PGR* expression in gilts with normal and reduced luteal P4 synthesis observed in the present study suggests that concentrations of circulating P4 in gonadotropin-treated gilts were sufficient to induce PGR down-regulation. Moreover, the mechanisms controlling the decline in PGR expression in pigs are activated before day 6 of pregnancy because the blocking of P4 action with P4 antagonist on days 3 to 5 but not on days 6 and 7 of pregnancy elevates *PGR* mRNA levels in the endometrium and prevent PGR protein down-regulation [39]. In the current study, CLs and blood samples for P4 analyses were collected after day 9 of pregnancy [12]; therefore, we can only speculate that the amount of P4 secreted before day 6 by CLs of PMSG/hCG-treated and control gilts were similar.

As previously demonstrated, the highest *PGR* mRNA expression in the myometrium was detected at estrus, followed by much lower levels on days 11 and 19 of pregnancy in mature sows [40]. In fact, PGR protein is localized within in the myometrium on days 9 to 85 in pregnant gilts [37]. In the present study, the concentrations of *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 PMSG/hCG treatment. This indicates that *PGR* mRNA expression in the myometrium is not directly controlled by luteal P4. To support this statement, PGR protein was localized in the myometrial cells of ovariectomized gilts injected with both P4 and corn oil vehicle [37].

In addition to P4, ovarian estrogens influence morphological and physiological changes in the uterus [20, 41, 42]. In pigs, conceptus-derived estradiol-17 β (E2) is the main pregnancy recognition signal, and it significantly affects uterine functions during early pregnancy [14, 16]. Both endometrial and myometrial cells of the porcine uterus express the estrogen receptor proteins ER α and ER β , with ER α being the dominant form [43]. Our present results also showed that *ESR1* mRNA is much more abundantly expressed than *ESR2* in both tissues. Furthermore, *ESR1* showed similar expression profiles in the endometrium and myometrium, which decreased gradually during the examined pregnancy period. Such results are consistent with previously published profiles of *ESR1* mRNA and ER α protein expression in pregnant sows [40, 43]. In contrast to *ESR1*, *ESR2* mRNA expression in the endometrium and myometrium increased during the studied days of early pregnancy. In the endometrium of naturally ovulating gilts, concentrations of *ESR2* transcripts increased substantially from day 12, while in 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 concentrations of *ESR1* transcripts in the endometrium of gilts with gonadotropin-induced estrus indicate that sustained P4 synthesis is required for the development of the proper endometrial receptivity to E2 action.

In contrast to the endometrium, *ESR2* mRNA expression in the myometrium was not affected by estrus induction with gonadotropins. Although a similar expression of ER β on days 10–11 and 18–19 in the endometrium and myometrium of pregnant sows was reported [40, 43], the transient up-regulation of *ESR2* mRNA may occur on days 12 and 15 (current data) in response to conceptus-derived E2, followed

by a decrease on day 19 to a level comparable to that observed on day 11 [40]. The potential regulation of ER β expression by E2 was suggested for porcine peri-implantation conceptuses [44].

Compared with E2 and P4, there is little data concerning regulation of AR expression in the porcine uterus. Both endometrial and myometrial cells of the uteri of cyclic and pregnant gilts express AR [45]. Further studies have demonstrated the marked up-regulation of *AR* mRNA expression in the porcine endometrium on day 12 of pregnancy and a potent stimulatory effect of E2 on *AR* transcript abundance *in vitro* [46]. In the present study, *AR* mRNA expression in the endometrium and myometrium did not change during the studied period of pregnancy in gilts with natural estrus, but lower levels of these receptors were found on day 12 in PMSG/hCG-induced animals. Because gonadotropin-treated gilts showed decreased luteal P4 synthesis [12], AR expression in the porcine uterus seems to be regulated not only by E2 from conceptuses [46] but also by P4 from the CL (present data). It has been demonstrated that the androgens present in the pig uterus may modulate the biological effect of E2 in the endometrium [46]. Therefore, lower levels of *AR* mRNA in PMSG/hCG-treated gilts may influence E2-dependent gene expression and uterine growth, which are required for successful implantation.

The PGs are important regulators of the CL lifespan, and PGF2 α is responsible for luteal regression in non-pregnant animals [47], while PGE2 functions as a luteotropic and antiluteolytic factor promoting CL maintenance during pregnancy [48]. Additionally, PGs act locally to modulate uterine functions [49, 50]. In pregnant gilts, PGE2 and PGF2 α activate their membrane receptors expressed in the endometrium to regulate vascular permeability, angiogenesis, immune response, and implantation [15, 51]. As we previously demonstrated, the treatment of gilts with PMSG/hCG to induce estrus resulted in the decreased expression of enzymes involved in PGE2 synthesis in the endometrium on day 12 of pregnancy and lower levels of PGE2 in the uterine lumen observed on day 15 [12, 22]. Moreover, much lower concentrations of PGF2 α in endometrial tissue and blood plasma were detected in pregnant gilts with gonadotropin-induced estrus [22]. In the present study, insufficient P4 synthesis in gilts with precocious estrus induction negatively affected the expression of *PTGER2*, *PTGER4*, and *PTGFR* mRNA in the endometrium and/or myometrium. It indicates that sustained P4 synthesis during the peri-implantation period in pigs is required not only for the proper synthesis of PGE2 and PGF2 α but also for the expression of PG receptor system. Considering the important role of PGs during early pregnancy, the decreased expression of PG receptors in the endometrium may influence embryo-maternal interactions and conceptus implantation in the pig.

To our knowledge, this is the first report demonstrating the profiles of PGE2 and PGF2 α receptor expression in the myometrium of early pregnant pigs. In gilts with natural estrus, *PTGFR* mRNA levels 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 lower at the maternal recognition of pregnancy in gonadotropin-treated than in naturally ovulating gilts. In the porcine myometrium, *PTGER2* has been described as a relaxant, and *PTGFR* has been reported as a contractile receptor [52]. However, both PGE2 and PGF2 α increase the tension of the contractions of the porcine myometrium during early

pregnancy [24]. Therefore, the decreased expression of PG receptors in gilts with impaired luteal P4 may result in abnormal myometrial contractility during conceptus elongation and initial implantation.

PGI₂ has been primarily described as a potent vasodilator and inhibitor of platelet aggregation in the vascular system [53]. PGI₂ is also involved in pregnancy establishment and implantation in various species [17, 54–56]. Specifically, PGI₂, acting via its membrane receptor, PTGIR, stimulates the expression of pro-angiogenic factors in the porcine endometrium [57]. 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 gilts with induced estrus, characterized by lower P4 synthesis, than in the control group. PGI₂ is a smooth muscle relaxant in the uteri of non-pregnant women and pigs [58, 59]. P4, in turn, promotes uterine quiescence during pregnancy [60]. Moreover, the supplementation of early pregnant gilts with P4 stimulates *PTGIR* mRNA expression in the CL [61]. Therefore, the up-regulation of *PTGIR* mRNA in the myometrium of PMSG/hCG-induced gilts is surprising, but it may point to the activation of some local mechanisms in the uterus to compensate for reduced P4 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 [62, 63]. Among these, IL6 and TNF α contribute to local inflammatory reactions related to conceptus implantation [64, 65]. Both the endometrium and myometrium of the pig uterus express *IL6R* and *TNFR* mRNA during pregnancy [27, 66, 67]. In the present study, concentrations of *IL6R*, *TNFRSF1A*, and *TNFRSF1B* transcripts in the endometrium increased after day 10 of gestation, which is partly consistent with previously published data showing a stimulatory effect of conceptus estrogens on the expression of TNF α receptors [66] but not on IL6R [67]. Furthermore, we observed a negative effect of estrus induction on the expression of all three types of receptors. IL6 and TNF α are important regulators of cell proliferation, differentiation, and apoptosis [64, 68, 69] and also substantially modulate PGE₂ and/or PGF α synthesis in the porcine endometrium [70–72]. Therefore, the reduced expression of *IL6R*, *TNFRSF1A*, and *TNFRSF1B* during early pregnancy may be detrimental to endometrial preparation for implantation.

In the myometrium, greater *IL6R* and *TNFRSF1B* mRNA expression was observed on day 15 of pregnancy. This implies a possible role of IL6 and TNF α in this tissue during the period of early implantation. Notably, the incubation of myometrial explants collected on day 15–16 of pregnancy, but not the estrous cycle, with IL6 and TNF α resulted in the elevated secretion of E₂, supplementing, in this way, the amount of estrogens synthesized by the endometrium and conceptuses [27]. Estrus induction with PMSG/hCG did not affect IL6 and TNF α receptor expression in the myometrium, which points to other than P4-dependent mechanisms controlling *IL6R* and *TNFRSF1B* abundance in this tissue.

A number of cytokines released by developing porcine conceptuses activate inducible transcription factors, including NF κ B, which are thought to be involved in pro-inflammatory and immune responses in the maternal uterus [73]. The results of our current research showed an increase in *NFKB1* mRNA expression in the endometrium between days 10 and 12 of pregnancy in both groups of animals. Such

up-regulation of *NFKB1* is in line with the role of this factor as a receptivity marker in the porcine endometrium [74] and coincides with the elevated secretion of IL1 β by elongating pig conceptuses, which has been shown to activate NF κ B in the endometrium [63]. Estrus induction with gonadotropins had no effect on *NFKB1* mRNA expression in the endometrium, which is consistent with the previously described lack of correlation between P4 action and NF κ B activation in this tissue [39]. By contrast, the myometrial expression of *NFKB1* mRNA was lower on day 12 in PMSG/hCG-treated gilts as compared with the control animals, indicating a transient inhibition of inflammatory response in the myometrium of gonadotropin-stimulated gilts.

Oxytocin is involved in luteal regression in both ruminants and pigs [47], and its stimulatory effect on luteolytic PGF2 α secretion from the porcine endometrium has been documented [75]. Interestingly, *OXTR* was found in the endometrium and myometrium of pigs not only during the estrous cycle but also during early pregnancy [76]. In the current study, greater *OXTR* mRNA expression in the endometrium was detected around the time of conceptus implantation (day 15). This was consistent with elevated levels of oxytocin detected on days 10 and 14 in the uterine lumen of pregnant gilts [77]. Moreover, oxytocin stimulates the synthesis of PGE2 in porcine endometrial epithelial cells collected on days 11–12 of pregnancy but not on the corresponding days of the estrous cycle [70]. Therefore, the lower concentrations of *OXTR* transcripts detected in the endometrium of gilts with PMSG/hCG-induced estrus (present data) may participate in the disturbed PGE2 synthesis reported previously for these animals [22].

Although oxytocin plays an important role during parturition, much less is known about the role of this hormone in the myometrium of early pregnant pigs. Its involvement, however, in the regulation of PGF2 α secretion from the myometrial slices collected on days 15–16 of pregnancy has been demonstrated previously [78]. Nevertheless, the results of our present study showed that concentrations of *OXTR* transcripts in the porcine myometrium were not affected by gonadotropin treatment to induce estrus.

The PPAR γ isoform belongs to the nuclear receptor family of transcription regulators and its activation in the reproductive tract has been observed during steroidogenesis, angiogenesis, apoptosis, and tissue remodeling [79, 80]. The presence and activation of PPAR γ in the porcine endometrium has been previously documented [33, 81]. No effect of estrus induction on *PPARG* mRNA was observed in the endometrium indicating no correlation between luteal P4 and *PPARG* transcript abundance in this tissue. Similarly, no difference in endometrial *PPARG* expression was found in beef heifers with normal and low concentrations of circulating P4 [36]. To our knowledge, this is the first study demonstrating *PPARG* mRNA expression in the myometrium of early pregnant gilts. Relatively stable *PPARG* mRNA expression was detected during the peri-implantation period in gilts with natural estrus. Interestingly, greater *PPARG* mRNA expression in gonadotropin-treated as compared to control gilts was found in this tissue on day 15. Because PPAR γ is primarily involved in glucose homeostasis, such up-regulation may be attributed to the higher energy demands of the myometrium in gonadotropin-treated gilts.

The key element involved in the regulation of embryo-maternal interactions and myometrial activity is cell-to-cell communication mediated via gap junctions. Among the gap junction proteins, connexin 43

was localized in both the endometrium and myometrium of cyclic and pregnant gilts [82]. Greater expression of *GJA1* mRNA was found in the porcine endometrium on day 12 of pregnancy as compared with day 12 of the estrous cycle [83]. The results of our present study showed that *GJA1* mRNA expression increased in the endometrium and decreased in the myometrium between days 10 and 12 of pregnancy in gilts with natural estrus. This indicates a distinct response of both tissues to conceptus signals. Although *GJA1* is more expressed in the myometrium than in the endometrium, both tissues responded with greater concentrations of *GJA1* transcripts in PMSG/hCG-induced gilts on day 15. Such results are consistent with a negative correlation between the number of connexin 43 gap junctions in the myometrium and P4 concentrations in this tissue, as demonstrated in non-pregnant gilts [84]. However, the consequences of the elevated expression of *GJA1* during early pregnancy remain to be elucidated.

Conclusions

Estrus induction with PMSG/hCG in premature gilts resulted in the decreased expression of estrogen, androgen, PGE₂, PGF₂ α , IL6, TNF α , and oxytocin receptors on day 12 of pregnancy as compared with naturally ovulating gilts. The response of the myometrium to exogenous gonadotropins was more differentiated because both down- and up-regulated levels of examined transcripts were detected. The major limitation of this study is that the experimental design does not allow for the determination of the exact mechanisms explaining how PMSG/hCG affects endometrial and myometrial receptivity. However, it is assumed that, in addition to the direct effect of insufficient P4 synthesis observed in gonadotropin-treated gilts [12] on the expression of examined receptors, some locally produced uterine factors, the activation of which could be altered due to precocious estrus induction, may participate in the abnormal development of endometrial and/or myometrial receptivity. Therefore, further studies should be undertaken to identify the uterine factors that may be involved in the modulation of endometrial and myometrial function in PMSG/hCG-treated pigs.

Abbreviations

AR: androgen receptor; cDNA: complementary DNA; CL: corpus luteum; E2: estradiol-17 β ; ESR1; estrogen receptor 1; ESR2: estrogen receptor 2; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GJA1: gap junction protein alpha 1; hCG; human chorionic gonadotropin; HPRT1: hypoxanthine phosphoribosyltransferase 1; IL1 β : interleukin 1 β ; IL6R: interleukin 6 receptor; mRNA: messenger RNA; i.m.: intramuscular; NFkB1: nuclear factor kappa B subunit 1; OXTR: oxytocin receptor; P4: progesterone; PGE₂: prostaglandin E₂; PGF₂ α : prostaglandin F₂ α ; PGI₂: prostaglandin I₂; PGR: P4 receptor; PMSG: pregnant mare serum gonadotropin; PPAR γ : peroxisome proliferator activated receptor, isoform γ ; PTGER2: PGE₂ receptor 2; PTGER4: PGE₂ receptor 4; PTGFR: PGF receptor; PTGIR: PGI₂ receptor; RT-PCR: reverse-transcriptase polymerase chain reaction; SEM: standard error of mean; TNF: tumor necrosis factor; TNFRSF1A: TNF receptor superfamily member 1A; TNFRSF1B: TNF receptor superfamily member 1B.

Declarations

Ethics approval and consent to participate

All procedures involving animals such as welfare and ethical issues were approved by the Local Ethics Committee for Experiments on Animals, University of Warmia and Mazury in Olsztyn, Poland.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AB and MS formulated the hypothesis and designed the study; MS conducted laboratory analysis; AB performed the statistical analysis of the obtained data; AB and MS discussed the results and formulated conclusions; AB prepared the original draft of the manuscript; MS reviewed and edited the manuscript. Both authors read and approved the final version of the manuscript.

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References

1. Knox RV. Recent advancements in the hormonal stimulation of ovulation in swine. *Vet Med (Auckl)*. 2015;6:309–20.

2. De Rensis F, Kirkwood RN. Control of estrus and ovulation: Fertility to timed insemination of gilts and sows. *Theriogenology*. 2016;86:1460–6.
3. Weibel S, Day B. The control of ovulation. In: Cole D, Foxcroft G, editors. *Control of Pig Reproduction*. London: Butterworth Scientific; 1982. pp. 197–210.
4. Knox RV. PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Factors influencing follicle development in gilts and sows and management strategies used to regulate growth for control of estrus and ovulation¹. *J Anim Sci*. 2019;97:1433-45.
5. Knox RV, Tudor KW, Rodriguez-Zas SL, Robb JA. Effect of subcutaneous vs intramuscular administration of P.G. 600 on estrual and ovulatory responses of prepubertal gilts. *J Anim Sci*. 2000;78:1732–7.
6. Pinkert CA, Rampacek GB, Kraeling RR. Serum concentrations of prolactin, oestrogen and LH during the perioestrous period in prepubertal gilts induced to ovulate and mature gilts. *J Reprod Fertil*. 1988;83:471–8.
7. Wiesak T, Hunter MG, Foxcroft GR. Differences in follicular morphology, steroidogenesis and oocyte maturation in naturally cyclic and PMSG/hCG-treated prepubertal gilts. *J Reprod Fertil*. 1990;89:633–41.
8. Breen SM, Knox RV. The impact of dose of FSH (Folltropin) containing LH (Lutropin) on follicular development, estrus and ovulation responses in prepubertal gilts. *Anim Reprod Sci*. 2012;132:193–200.
9. Ziecik AJ, Biallowicz M, Kaczmarek M, Demianowicz W, Rioperez J, Wasielek M, et al. Influence of estrus synchronization of prepubertal gilts on embryo quality. *J Reprod Dev*. 2005;51:379–84.
10. Kineman RD, Kraeling RR, Rampacek GB, Hausman GJ. Comparison of induced corpora lutea from prepubertal gilts and spontaneous corpora lutea from mature gilts: hydroxysteroid dehydrogenase activity. *J Anim Sci*. 1987;64:237–40.
11. Kineman RD, Rampacek GB, Kraeling RR, Fiorello-Stocks NA, Wilson RL. Comparison of induced corpora lutea from prepubertal gilts and spontaneous corpora lutea from mature gilts: in vitro progesterone production. *J Anim Sci*. 1987;64:526–32.
12. Blitek A, Szymanska M, Pieczywek M, Morawska-Pucinska E. Luteal P4 synthesis in early pregnant gilts after induction of estrus with PMSG/hCG. *Anim Reprod Sci*. 2016;166:28–35.
13. Spencer TE, Johnson GA, Burghardt RC, Bazer FW. Progesterone and placental hormone actions on the uterus: insights from domestic animals. *Biol Reprod*. 2004;71:2–10.
14. Bazer FW, Johnson GA. Pig blastocyst-uterine interactions. *Differentiation*. 2014;87:52–65.
15. Waclawik A, Kaczmarek MM, Blitek A, Kaczynski P, Ziecik AJ. Embryo-maternal dialogue during pregnancy establishment and implantation in the pig. *Mol Reprod Dev*. 2017;84:842–55.
16. Bazer FW, Song G, Kim J, Dunlap KA, Satterfield MC, Johnson GA, et al. Uterine biology in pigs and sheep. *J Anim Sci Biotechnol*. 2012;3:23.

17. Morawska E, Kaczmarek MM, Blitek A. Regulation of prostacyclin synthase expression and prostacyclin content in the pig endometrium. *Theriogenology*. 2012;78:2071–86.
18. 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.
19. Cavagna M, Mantese JC. Biomarkers of endometrial receptivity—a review. *Placenta*. 2003;24(Suppl B):39–47.
20. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. *Hum Reprod Update*. 2006;12:731–46.
21. Blitek A, Kaczmarek MM, Kiewisz J, Ziecik AJ. Endometrial and conceptus expression of HoxA10, transforming growth factor beta1, leukemia inhibitory factor, and prostaglandin H synthase-2 in early pregnant pigs with gonadotropin-induced estrus. *Domest Anim Endocrinol*. 2010;38:222–34.
22. Blitek A, Waclawik A, Kaczmarek MM, Kiewisz J, Ziecik AJ. Effect of estrus induction on prostaglandin content and prostaglandin synthesis enzyme expression in the uterus of early pregnant pigs. *Theriogenology*. 2010;73:1244–56.
23. Scheerboom JE, Van Adrichem PW, Taverne MA. Uterine motility of the sow during the oestrous cycle and early pregnancy. *Vet Res Commun*. 1987;11:253–69.
24. Markiewicz W, Bogacki M, Blitek M, Jaroszewski JJ. Comparison of the porcine uterine smooth muscle contractility on days 12–14 of the estrous cycle and pregnancy. *Acta Vet Scand*. 2016;58:20.
25. Franczak A. Endometrial and myometrial secretion of androgens and estrone during early pregnancy and luteolysis in pigs. *Reprod Biol*. 2008;8:213–28.
26. Franczak A, Kotwica G, Kurowicka B, Oponowicz A, Wocławek-Potocka I, Petroff BK. Expression of enzymes of cyclooxygenase pathway and secretion of prostaglandin E2 and F2alpha by porcine myometrium during luteolysis and early pregnancy. *Theriogenology*. 2006;66:1049–56.
27. Franczak A, Wojciechowicz B, Kolakowska J, Kotwica G. The effect of interleukin-1 β , interleukin-6, and tumor necrosis factor- α on estradiol-17 β release in the myometrium: the in vitro study on the pig model. *Theriogenology*. 2014;81:266–74.
28. Franczak A, Kurowicka B, Oponowicz A, Petroff BK, Kotwica G. The effect of progesterone on oxytocin-stimulated intracellular mobilization of Ca²⁺ and prostaglandin E2 and F2alpha secretion from porcine myometrial cells. *Prostaglandins Other Lipid Mediat*. 2006;81:37–44.
29. 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;5:151–69.
30. Ziecik AJ, Waclawik A, Kaczmarek MM, Blitek A, Jalali BM, Andronowska A. Mechanisms for the establishment of pregnancy in the pig. *Reprod Domest Anim*. 2011;46(Suppl 3):31–41.
31. Grümmer R, Reuss B, Winterhager E. Expression pattern of different gap junction connexins is related to embryo implantation. *Int J Dev Biol*. 1996;40:361–7.

32. Geisert R, Fazleabas A, Lucy M, Mathew D. Interaction of the conceptus and endometrium to establish pregnancy in mammals: role of interleukin 1 β . *Cell Tissue Res.* 2012;349:825–38.
33. 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.
34. Zhao S, Fernald RD. Comprehensive algorithm for quantitative real-time polymerase chain reaction. *J Comput Biol.* 2005;12:1047–64.
35. Rizos D, Carter F, Besenfelder U, Havlicek V, Lonergan P. Contribution of the female reproductive tract to low fertility in postpartum lactating dairy cows. *J Dairy Sci.* 2010;93:1022–9.
36. Forde N, Beltman ME, Duffy GB, Duffy P, Mehta JP, O'Gaora P, et al. Changes in the endometrial transcriptome during the bovine estrous cycle: effect of low circulating progesterone and consequences for conceptus elongation. *Biol Reprod.* 2011;84:266–78.
37. Steinhauser CB, Bazer FW, Burghardt RC, Johnson GA. Expression of progesterone receptor in the porcine uterus and placenta throughout gestation: correlation with expression of uteroferrin and osteopontin. *Domest Anim Endocrinol.* 2017;58:19–29.
38. Geisert RD, Pratt TN, Bazer FW, Mayes JS, Watson GH. Immunocytochemical localization and changes in endometrial progesterone receptor protein during the porcine oestrous cycle and early pregnancy. *Reprod Fertil Dev.* 1994;6:749–60.
39. Mathew DJ, Sellner EM, Green JC, Okamura CS, Anderson LL, Lucy MC, et al. Uterine progesterone receptor expression, conceptus development, and ovarian function in pigs treated with RU 486 during early pregnancy. *Biol Reprod.* 2011;84:130–9.
40. Sukjumlom S, Persson E, Dalin AM, Janson V, Sahlin L. Messenger RNA levels of estrogen receptors alpha and beta and progesterone receptors in the cyclic and inseminated/early pregnant sow uterus. *Anim Reprod Sci.* 2009;112:215–28.
41. Paria BC, Song H, Dey SK. Implantation: molecular basis of embryo-uterine dialogue. *Int J Dev Biol.* 2001;45:597–605.
42. Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW, et al. Developmental biology of uterine glands. *Biol Reprod.* 2001;65:1311–23.
43. Knapczyk-Stwora K, Durlej M, Duda M, Czernichowska-Ferreira K, Tabecka-Lonczynska A, Slomczynska M. Expression of oestrogen receptor α and oestrogen receptor β in the uterus of the pregnant swine. *Reprod Domest Anim.* 2011;46:1–7.
44. Kowalski AA, Graddy LG, Vale-Cruz DS, Choi I, Katzenellenbogen BS, Simmen FA, et al. Molecular cloning of porcine estrogen receptor-beta complementary DNAs and developmental expression in periimplantation embryos. *Biol Reprod.* 2002;66:760–9.
45. Cárdenas H, Pope WF. Distribution and changes in amounts of the androgen receptor in the pig uterus during the estrous cycle, early pregnancy and after treatment with sex steroids. *J Endocrinol.* 2003;177:461–9.

46. Kowalski AA, Vale-Cruz DS, Simmen FA, Simmen RC. Uterine androgen receptors: roles in estrogen-mediated gene expression and DNA synthesis. *Biol Reprod.* 2004;70:1349–57.
47. McCracken JA, Custer EE, Lamsa JC. Luteolysis: a neuroendocrine-mediated event. *Physiol Rev.* 1999;79:263–323.
48. Ziecik AJ, Przygodzka E, Jalali BM, Kaczmarek MM. Regulation of the porcine corpus luteum during pregnancy. *Reproduction.* 2018;156:R57–67.
49. Kennedy TG, Gillio-Meina C, Phang SH. Prostaglandins and the initiation of blastocyst implantation and decidualization. *Reproduction.* 2007;134:635–43.
50. Waclawik A, Jabbour HN, Blitek A, Ziecik AJ. Estradiol-17beta, prostaglandin E2 (PGE2), and the PGE2 receptor are involved in PGE2 positive feedback loop in the porcine endometrium. *Endocrinology.* 2009;150:3823–32.
51. Kaczynski P, Kowalewski MP, Waclawik A. Prostaglandin F2 α promotes angiogenesis and embryo-maternal interactions during implantation. *Reproduction.* 2016;151:539–52.
52. 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;75:105–22.
53. Gryglewski RJ. Prostacyclin among prostanoids. *Pharmacol Rep.* 2008;60:3–11.
54. Lim H, Gupta RA, Ma WG, Paria BC, Moller DE, Morrow JD, et al. Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARdelta. *Genes Dev.* 1999;13:1561–74.
55. 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;131:917–27.
56. Morawska-Pucinska E, Szymanska M, Blitek A. Expression profile and role of prostacyclin receptor (PTGIR) in peri-implantation porcine conceptuses. *Theriogenology.* 2014;82:546–56.
57. 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;84:969–82.
58. Lumsden MA, Baird DT. The effect of intrauterine administration of prostacyclin on the contractility of the non-pregnant uterus in vivo. *Prostaglandins.* 1986;31:1011–22.
59. 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;442:115–23.
60. Wray S, Burdyga T, Noble D, Noble K, Borysova L, Arrowsmith S. Progress in understanding electro-mechanical signalling in the myometrium. *Acta Physiol (Oxf).* 2015;213:417–31.
61. Szymanska M, Blitek A. In vivo response of the corpus luteum to progesterone treatment of gilts during early gestation. *Anim Reprod Sci.* 2020;221:106583.

62. Edwards AK, Wessels JM, Kerr A, Tayade C. An overview of molecular and cellular mechanisms associated with porcine pregnancy success or failure. *Reprod Domest Anim.* 2012;47(Suppl 4):394–401.
63. Geisert RD, Lucy MC, Whyte JJ, Ross JW, Mathew DJ. Cytokines from the pig conceptus: roles in conceptus development in pigs. *J Anim Sci Biotechnol.* 2014;5:51.
64. Hunt JS, Chen HL, Miller L. Tumor necrosis factors: pivotal components of pregnancy? *Biol Reprod.* 1996;54:554–62.
65. Prins JR, Gomez-Lopez N, Robertson SA. Interleukin-6 in pregnancy and gestational disorders. *J Reprod Immunol.* 2012;95:1–14.
66. Yu Z, Gordon JR, Kendall J, Thacker PA. Elevation in tumour necrosis factor-alpha (TNF-alpha) messenger RNA levels in the uterus of pregnant gilts after oestrogen treatment. *Anim Reprod Sci.* 1998;50:57–67.
67. Yoo I, Han J, Kim M, Jang H, Sa S, Choi SH, et al. Expression and regulation of interleukin 6 and its receptor at the maternal-conceptus interface during pregnancy in pigs. *Theriogenology.* 2017;96:85–91.
68. Lee FD. The role of interleukin-6 in development. *Dev Biol.* 1992;151:331–8.
69. Blitek A, Morawska E, Ziecik AJ. Regulation of expression and role of leukemia inhibitory factor and interleukin-6 in the uterus of early pregnant pigs. *Theriogenology.* 2012;78:951–64.
70. Waclawik A, Blitek A, Ziecik AJ. Oxytocin and tumor necrosis factor alpha stimulate expression of prostaglandin E2 synthase and secretion of prostaglandin E2 by luminal epithelial cells of the porcine endometrium during early pregnancy. *Reproduction.* 2010;140:613–22.
71. Blitek A, Ziecik AJ. Role of tumour necrosis factor alpha in stimulation of prostaglandins F(2alpha) and E(2) release by cultured porcine endometrial cells. *Reprod Domest Anim.* 2006;41:562–7.
72. Franczak A, Zmijewska A, Kurowicka B, Wojciechowicz B, Petroff BK, Kotwica G. The effect of tumor necrosis factor α (TNF α), interleukin 1 β (IL1 β) and interleukin 6 (IL6) on endometrial PGF2 α synthesis, metabolism and release in early-pregnant pigs. *Theriogenology.* 2012;77:155–65.
73. Hayden MS, Ghosh S. NF- κ B, the first quarter-century: remarkable progress and outstanding questions. *Genes Dev.* 2012;26:203–34.
74. Ross JW, Ashworth MD, Mathew D, Reagan P, Ritchey JW, Hayashi K, et al. Activation of the transcription factor, nuclear factor kappa-B, during the estrous cycle and early pregnancy in the pig. *Reprod Biol Endocrinol.* 2010;8:39.
75. Kieborz KR, Silvia WJ, Edgerton LA. Changes in uterine secretion of prostaglandin F2 alpha and luteal secretion of progesterone in response to oxytocin during the porcine estrous cycle. *Biol Reprod.* 1991;45:950–4.
76. Franczak A, Ciereszko R, Kotwica G. Oxytocin (OT) action in uterine tissues of cyclic and early pregnant gilts: OT receptors concentration, prostaglandin F(2)alpha secretion, and phosphoinositide hydrolysis. *Anim Reprod Sci.* 2005;88:325–39.

77. Vallet JL, Christenson RK, Trout WE, Klemcke HG. Conceptus, progesterone, and breed effects on uterine protein secretion in swine. *J Anim Sci.* 1998;76:2657–70.
78. Franczak A, Woławek-Potocka I, Oponowicz A, Kurowicka B, Kotwica G. Oxytocin stimulates prostaglandin F₂α secretion and prostaglandin F synthase protein expression in porcine myometrial tissue. *Reprod Biol.* 2004;4:177–84.
79. Froment P, Gizard F, Defever D, Staels B, Dupont J, Monget P. Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition. *J Endocrinol.* 2006;189:199–209.
80. Toth B, Hornung D, Scholz C, Djalali S, Friese K, Jeschke U. Peroxisome proliferator-activated receptors: new players in the field of reproduction. *Am J Reprod Immunol.* 2007;58:289–310.
81. Bogacka I, Bogacki M. The quantitative expression of peroxisome proliferator activated receptor (PPAR) genes in porcine endometrium through the estrous cycle and early pregnancy. *J Physiol Pharmacol.* 2011;62:559–65.
82. Lenhart JA, Ryan PL, Ohleth KM, Bagnell CA. Expression of connexin-26, -32, and -43 gap junction proteins in the porcine cervix and uterus during pregnancy and relaxin-induced growth. *Biol Reprod.* 1999;61:1452–9.
83. Zeng S, Bick J, Ulbrich SE, Bauersachs S. Cell type-specific analysis of transcriptome changes in the porcine endometrium on Day 12 of pregnancy. *BMC Genom.* 2018;19:459.
84. Karasinski J, Galas J, Semik D, Fiertak A, Bilinska B, Kilarski WM. Changes of connexin43 expression in non-pregnant porcine myometrium correlate with progesterone concentration during oestrous cycle. *Reprod Domest Anim.* 2010;45:959–66.

Table

Table 1 Full names of genes and the number of TaqMan probes used to examine relative mRNA expression

Abbreviation	Gene name	No. of TaqMan probe
<i>PGR</i>	Progesterone receptor	Ss03374440_m1
<i>ESR1</i>	Estrogen receptor 1	Ss03383398_u1
<i>ESR2</i>	Estrogen receptor 2	Ss03391479_m1
<i>AR</i>	Androgen receptor	Ss03822350_s1
<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>PTGIR</i>	Prostaglandin I2 receptor	_a
<i>IL6R</i>	Interleukin 6 receptor	Ss03394904_g1
<i>TNFRSF1A</i>	TNF receptor superfamily member 1A	Ss03391124_g1
<i>TNFRSF1B</i>	TNF receptor superfamily member 1B	Ss03385518_g1
<i>NFKB1</i>	Nuclear factor kappa B subunit 1	Ss03388579_m1
<i>OXTR</i>	Oxytocin receptor	Ss03382363_ul
<i>PPARG</i>	Peroxisome proliferator activated receptor gamma	Ss03394829_m1
<i>GJA1</i>	Gap junction protein alpha 1 (connexin 43)	Ss03375693_ul
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	Ss03375435_u1
<i>HPRT1</i>	Hypoxanthine phosphoribosyltransferase 1	Ss03388274_m1

^aDesigned by Applied Biosystems, Thermo Fisher Scientific; GeneBank accession no. NC_010448.3

Figures

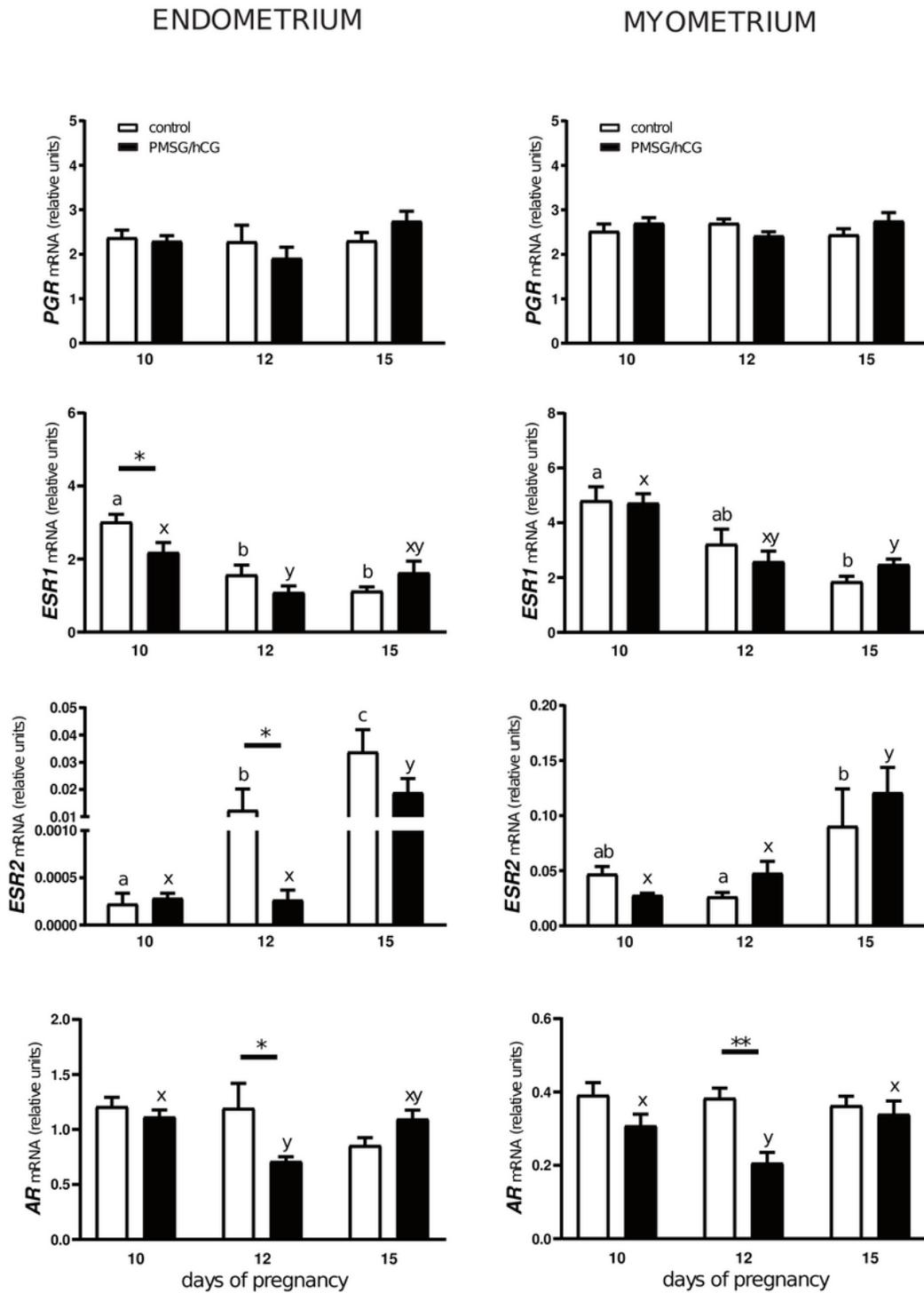


Figure 1

The mRNA expression of progesterone receptor (PGR), estrogen receptors (ESR1 and ESR2), and androgen receptor (AR) in the endometrium (left panel) and the myometrium (right panel) of pregnant gilts with natural (control) and PMSG/hCG-induced estrus. Values from real-time PCR for studied genes were normalized against geometric averaging of GAPDH and HPRT1 genes. 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 PMSG/hCG-treated gilts). Asterisks indicate differences between groups on particular day of pregnancy (* $P < 0.05$, ** $P < 0.01$). Note: PMSG: pregnant mare serum gonadotropin, hCG: human chorionic gonadotropin, SEM: standard error of the mean.

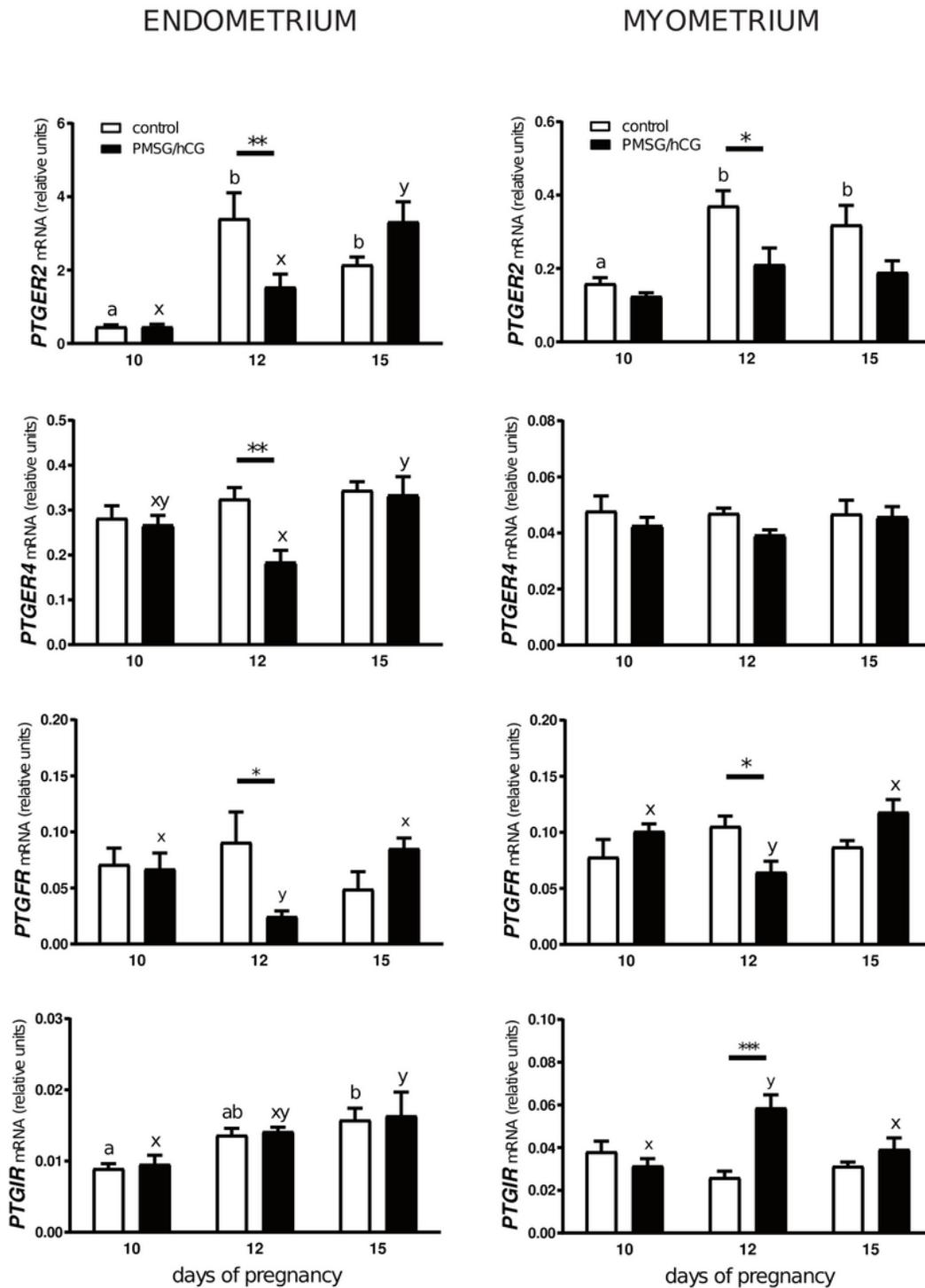


Figure 2

The mRNA expression of prostaglandin (PG) E2 receptors (PTGER2 and PTGER4), PGF2 α receptor (PTGFR), and PGI2 receptor (PTGIR) in the endometrium (left panel) and the myometrium (right panel) of

pregnant gilts with natural (control) and PMSG/hCG-induced estrus. Values from real-time PCR for studied genes were normalized against geometric averaging of GAPDH and HPRT1 genes. 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 PMSG/hCG-treated gilts). Asterisks indicate differences between groups on particular day of pregnancy (* P < 0.05, ** P < 0.01, *** P < 0.001). Note: PMSG: pregnant mare serum gonadotropin, hCG: human chorionic gonadotropin, SEM: standard error of the mean.

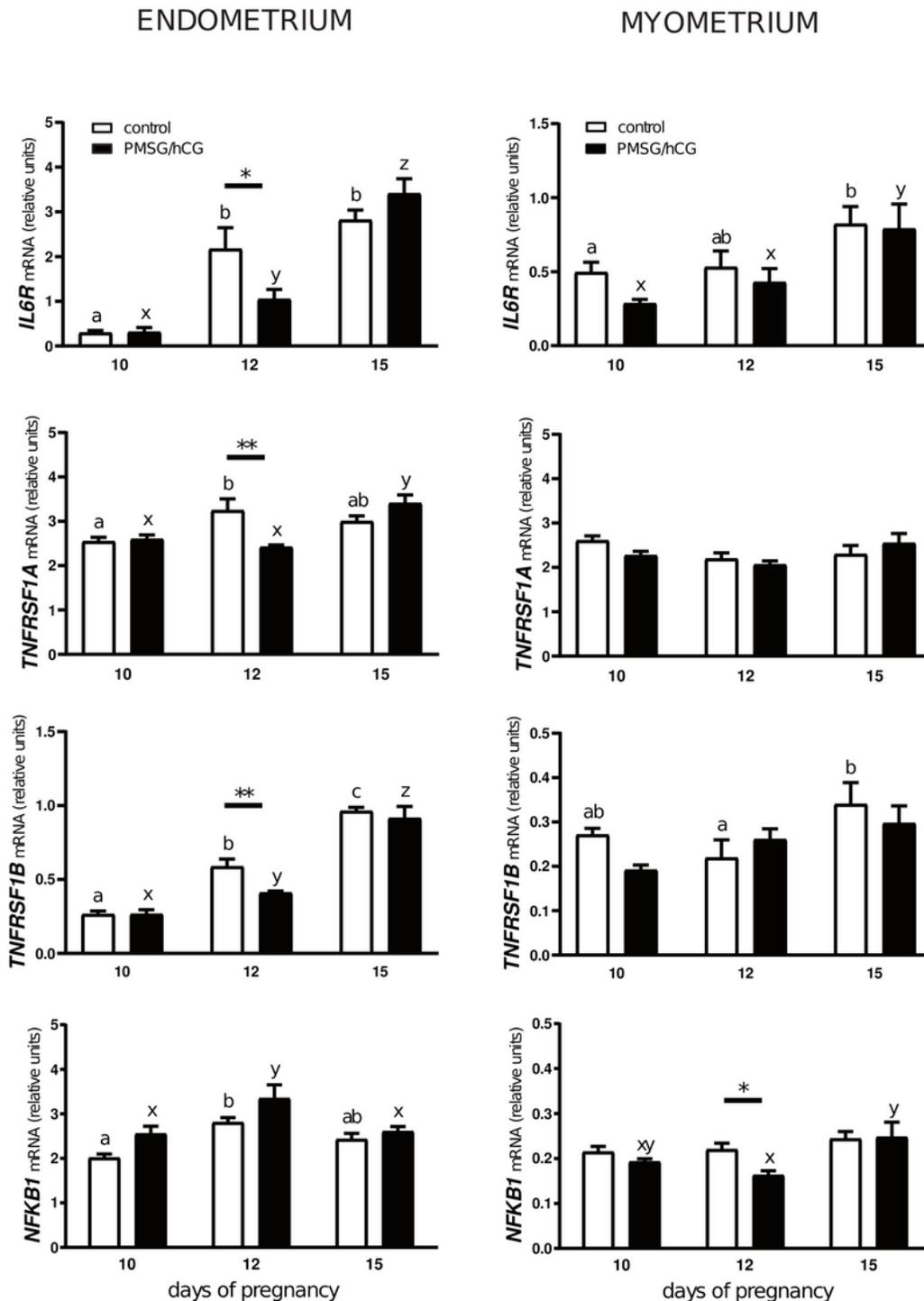


Figure 3

The mRNA expression of interleukin 6 receptor (IL6R), tumor necrosis factor α receptors (TNFRSF1A and TNFRSF1B), and nuclear factor kappa B subunit 1 (NFKB1) in the endometrium (left panel) and the myometrium (right panel) of pregnant gilts with natural (control) and PMSG/hCG-induced estrus. Values from real-time PCR for studied genes were normalized against geometric averaging of GAPDH and HPRT1 genes. 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 PMSG/hCG-treated gilts). Asterisks indicate differences between groups on particular day of pregnancy (* P < 0.05, ** P < 0.01). Note: PMSG: pregnant mare serum gonadotropin, hCG: human chorionic gonadotropin, SEM: standard error of the mean.

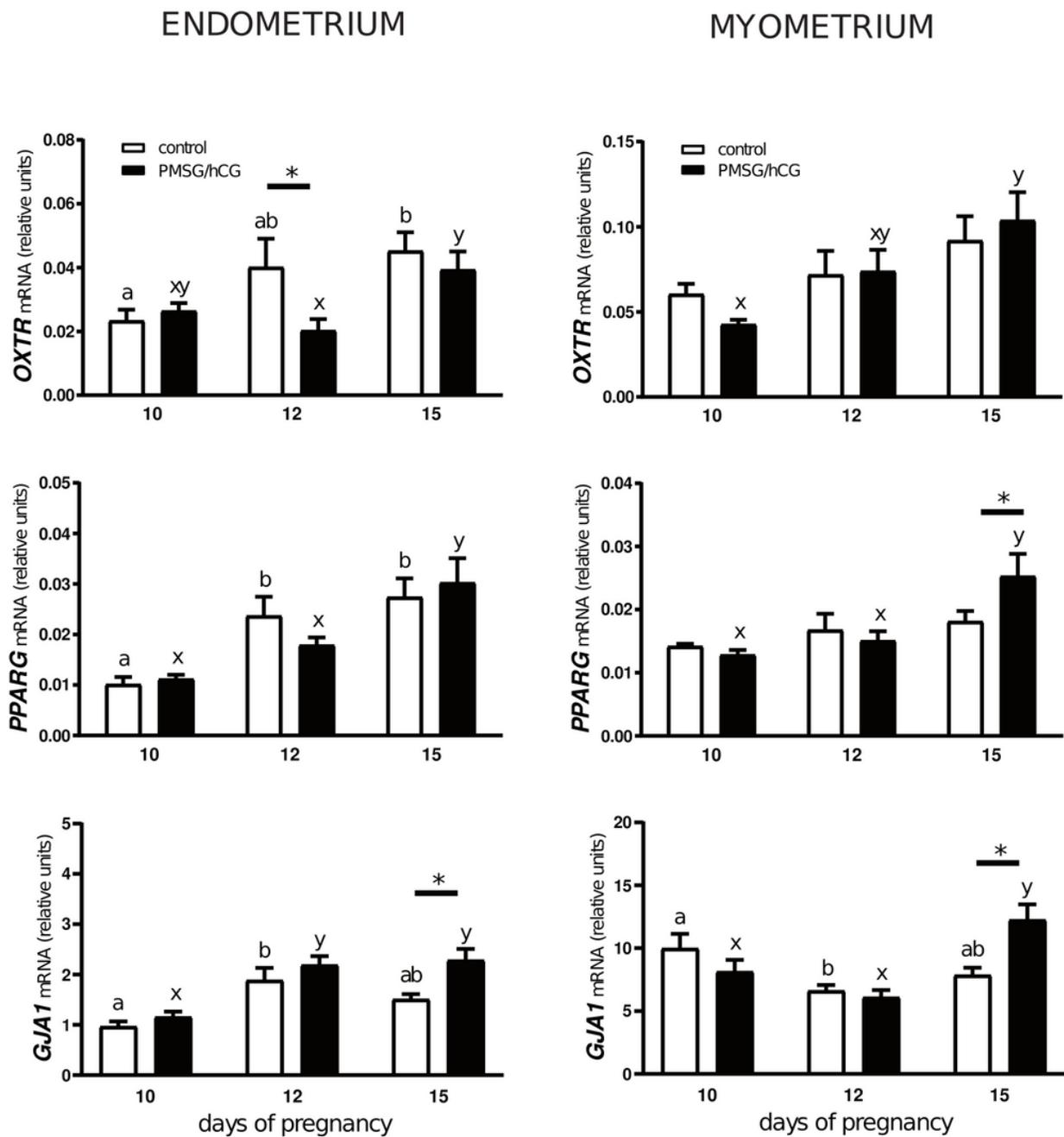


Figure 4

The mRNA expression of oxytocin receptor (OXTR), peroxisome proliferator activated receptor gamma (PPARG), and gap junction protein alpha 1 (GJA1) in the endometrium (left panel) and the myometrium (right panel) of pregnant gilts with natural (control) and PMSG/hCG-induced estrus. Values from real-time PCR for studied genes were normalized against geometric averaging of GAPDH and HPRT1 genes. 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 PMSG/hCG-treated gilts). Asterisk indicates differences between groups on particular day of pregnancy (* P < 0.05). Note: PMSG: pregnant mare serum gonadotropin, hCG: human chorionic gonadotropin, SEM: standard error of the mean.