Animal Reproduction Science 174 (2016) 56–64

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Endometrial and conceptus response to exogenous progesterone treatment in early pregnant gilts following hormonally-induced estrus

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ARTICLE INFO

Article history:
Received 29 April 2016
Received in revised form 26 August 2016
Accepted 6 September 2016
Available online 9 September 2016

Keywords:
Estrus induction
Pregnancy
Conceptus
Endometrial receptivity
Progesterone injections
Pig

ABSTRACT

The aim of the study was to determine how supplementation with exogenous progesterone (P_4) affects the expression of genes important for endometrial receptivity and conceptus development during the *peri*-implantation period in pigs. Gilts with PMSG/hCG-induced first estrus received daily injections of corn oil (CO; $n = 7$) or P_4 ($n = 7$) on days 3 to 10 after insemination. The dose of P_4 was 25 mg/100 kg BW on days 3 and 4 of gestation, then increased to 50 mg/100 kg BW on days 5 to 10. Blood samples were collected each day to monitor concentrations of circulating P_4. Animals were slaughtered on day 12 of pregnancy to obtain endometrial tissue, conceptuses, and uterine luminal flushing (ULF). Gilts in the P_4 group had consistently greater P_4 concentrations in the blood compared with controls. Treatment of gilts with P_4 increased uterine weight and enhanced total protein content and 6-keto $PGF_{1\alpha}$ (a PGI_2 metabolite) content in the uterine lumen. Moreover, injections of gilts with P_4 increased the expression of prostaglandin endoperoxide synthase 2, microsomal PGE_2 synthase and vascular endothelial growth factor A mRNA in the endometrium, but had no effect on gene expression in conceptuses. These results indicate that P_4 supplementation stimulated the secretory activity of the endometrium and increased the expression of genes responsible for vascular function and luteotropic PGE_2 synthesis, which are important components of successful pregnancy establishment in pigs. However, no effect of P_4 treatment was detected in conceptuses.

1. Introduction

Progesterone (P_4) is an essential hormone of pregnancy in all mammals and plays a pivotal role in the establishment and maintenance of pregnancy. P_4 acts on the endometrium to affect the expression of several genes encoding proteins that regulate conceptus growth and attachment during implantation. Developing conceptuses, in turn, secrete molecules that act in an autocrine or paracrine manner to improve their own survival in the uterus. Among the factors involved in embryo-maternal communication are prostaglandins (PG), growth factors, cytokines and their receptors (Bazer and Johnson, 2014; Bazer et al., 2012; Spencer et al., 2004; Ziecik et al., 2011).

Variability in P_4 synthesis and concentrations in the blood during early pregnancy can affect endometrial development (Ashworth and Bazer, 1989), as well as embryonic survival (Jindal et al., 1997; Mao and Foxcroft, 1998). Low concentrations of circulating P_4 alter the expression of endometrial genes that potentially contribute to histotroph composition and conceptus elongation in cattle (Forde et al., 2011, 2012). In the pig, lower concentrations of P_4 in

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http://dx.doi.org/10.1016/j.anireprosci.2016.09.005
0378-4320/

luteal tissue and blood serum were observed in early pregnant gilts with PMSG/hCG-induced first estrus (Blitek et al., 2010a, 2016). This was accompanied by decreased expression of homeobox A10 (HOXA10), leukemia inhibitory factor (LIF), transforming growth factor (TGF) β1 and PG synthesis enzyme genes in the endometrium (Blitek et al., 2010a, 2010c). Therefore, in the current study we tested the hypothesis that supplementing P_4 during early pregnancy in gilts will improve endometrial receptivity for implantation and conceptus development. Using gilts with PMSG/hCG-induced estrus, we examined the effect of exogenous P_4 treatment on: (1) the expression of genes encoding proteins important for endometrial receptivity or conceptus development or both, and (2) total amounts of estradiol-17β (E_2) and selected PG and cytokines in the uterine lumen.

2. Materials and methods

All procedures involving the use of animals were conducted in accordance with the national guidelines for agricultural animal care and were approved by the Animal Ethics Committee, University of Warmia and Mazury in Olsztyn, Poland.

2.1. Animals and sample collection

A total of fourteen crossbred gilts (Polish Landrace x Duroc) of similar age (165–175 day old), weight (100 ± 10 kg), and genetic background from one commercial herd were used in this experiment. Under general anesthesia, a silastic catheter (o.d. 1.4 mm, i.d. 1.0 mm) was inserted into the jugular vein to collect samples of systemic blood. The catheters were flushed with heparinized saline solution twice a day. Gilts were kept in separate pens, and fed with a commercial grain mixture with water *ad libitum*. To induce estrus, all gilts received i.m. injections of 750 IU PMSG (Folligon; Intervet, Boxmeer, The Netherlands) and 500 IU hCG (Chorulon; Intervet) 72 h later. All gilts were inseminated 24 and 48 h after the hCG injection. The day of the second insemination was designated as the first day of pregnancy in all animals. Gilts were assigned randomly to receive daily i.m. injections from day 3 to day 10 of either corn oil vehicle (CO; $n = 7$) or P_4 (Sigma-Aldrich, St Louis, MO, USA; $n = 7$). The dose of P_4 was 25 mg/100 kg BW on day 3 and day 4 after insemination, then increased to 50 mg/100 kg BW on days 5 to 10. Blood samples (10 ml) were collected daily from CO- and P_4-treated gilts via the jugular vein beginning on day 0 until day 10 of gestation. Plasma was harvested immediately by centrifugation (3000 x *g* for 15 min at 4 °C) of blood samples mixed with 0.5 M EDTA (200 μl per 10 ml of blood sample) and stored at −20 °C for later analyses. All gilts were slaughtered on day 12 of pregnancy. The number of corpora lutea (CL) on both ovaries was counted. Each uterine horn was flushed with 20 ml of sterile PBS to recover conceptuses. The morphology of conceptuses was examined by light microscopy. After that, conceptuses were snap frozen in liquid nitrogen. The volume of the uterine luminal flushing (ULF) was recorded, clarified by centrifugation, aliquoted and frozen at −80 °C. The protein content in ULF was determined by the method of Bradford (1976). Immediately after ULF collection, uterine horns were opened longitudinally on the antimesometrial surface, and sections of endometrium were physically dissected from the myometrium, frozen in liquid nitrogen, and stored at −80 °C.

2.2. Total RNA isolation and real-time PCR

Total RNA was extracted from frozen endometrial tissue and conceptuses using a Total RNA Prep Plus kit (A&A Biotechnology, Gdansk, Poland) and treated with DNase I (Invitrogen Life Technologies Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Samples were reverse transcribed using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) as previously described (Blitek et al., 2010a). Diluted cDNA from RT-PCR was used to analyze quantitative gene expression with an ABI Viia7 Sequence Detection System (Life Technologies). All gene names, abbreviations and tissues analyzed are listed in Table 1.

For *PTGS2, PGIS, CYP19A1, HOXA10, LIF, TGFβ1, IL6, IL1β, PPARγ* and *GAPDH* gene expression analysis, each sample contained cDNA (36 or 42 ng), forward and reverse primers (0.5 μmol/l each; Table 2) and Power SYBR Green PCR master mix (12.5 μl; Life Technologies). Each PCR reaction (25 μl) was performed in duplicates in 96-well plates using the following conditions: initial denaturation for 10 min at 95 °C, followed by 37 (for *HOXA10, LIF, TGFβ1, IL6* and *GAPDH*) or 40 (for *PTGS2, PGIS, CYP19A1, IL1β* and *PPARγ*) cycles of 15 s of denaturation at 95 °C and 30 s of annealing at 57 °C (for *IL1β*) or 59 °C (for *PGIS, HOXA10, PPARγ, LIF, IL6, TGFβ1* and *GAPDH*) or 60 °C (for *PTGS2* and *CYP19A1*), followed by 45 s of elongation at 72 °C. After these stages, melting curves were obtained by stepwise increases in the temperature from 60 to 95 °C to ensure single product amplification.

To evaluate *mPGES1, VEGFA, HPRT* and *β-actin* gene expression, 15 ng of complementary cDNA was amplified using *PTGES* (Ss03392129_m1), *VEGFA* (Ss03393993_m1), *HPRT* (Ss03388274_m1) and β-*actin* (Ss03376081_u1) TaqMan Gene Expression assays (Life Technologies). Each PCR reaction (10 μl) was performed in duplicates in 384-well plate using the following conditions: initial denaturation for 10 min at 95 °C, followed by 40 cycles of 15 s of denaturation at 95 °C and 60 s of annealing at 60 °C.

Data obtained by real-time PCR were analyzed using the Miner method (Zhao and Fernald, 2005). Briefly, raw fluorescence values were exported from the Viia7 software into Data Miner software. Once efficiency and Ct have been computed, these values were used for quantification. Control reactions in the absence of reverse transcriptase were performed to test for genomic DNA contamination. NormFinder software (Andersen et al., 2004) was used to select the most stable reference gene. The stability values for *GAPDH, HPRT* and β-*actin* were, respectively, 0.268, 0.208 and 0.165 in the endometrium and 0.361, 0.445 and 0.252 in conceptuses. All expression data for each target gene were divided by geometric averaging of *HPRT* and *GAPDH* (the stability value was 0.042 for endometrium and 0.043 for conceptuses).