Animal Reproduction Science 166 (2016) 28–35

Contents lists available at ScienceDirect

Animal Reproduction Science

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

# Luteal P4 synthesis in early pregnant gilts after induction of estrus with PMSG/hCG

Agnieszka Blitek*, Magdalena Szymanska, Marta Pieczywek, Ewa Morawska-Pucinska

*Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland*

ARTICLE INFO

*Article history:*
Received 26 March 2015
Received in revised form
11 December 2015
Accepted 4 January 2016
Available online 6 January 2016

*Keywords:*
Estrus induction
Progesterone synthesis
Corpus luteum
Pregnancy
Pig

ABSTRACT

The present study was designed to examine whether an estrus induction with gonadotropins could affect luteal P4 synthesis in early pregnant gilts. Sixteen prepubertal gilts received 750 IU of PMSG and 500 IU of hCG 72 h later. Prepubertal gilts in the control group ($n = 17$) were observed daily for estrus behavior. All gilts were inseminated in their first estrus. Corpora lutea (CLs) were collected on days 10, 12 and 15 of pregnancy and analyzed for (1) the mRNA and protein expression of steroidogenic acute regulatory protein (StAR), cytochrome P450 family 11 subfamily A polypeptide 1 (CYP11A1), and 3β-hydroxysteroid dehydrogenase (3βHSD); (2) the tissue concentration of P4; and (3) the mRNA expression of luteinizing hormone receptor (*LHR*) and estrogen receptors (*ESR1* and *ESR2*). Additionally, P4 concentration was analyzed in blood serum of all animals. PMSG/hCG injections to induce estrus decreased mRNA expression of *StAR*, *CYP11A1* and *3βHSD* on day 10 and *CYP11A1* on day 12 of pregnancy compared with the control group, while CYP11A1 and 3βHSD proteins were down-regulated on day 10 in the hormonally-treated gilts. Concentrations of P4 in luteal tissue and blood serum were also lower in animals after gonadotropin-induced estrus. In contrast, *LHR* and *ESR1* mRNA expression was greater in PMSG/hCG-treated than control gilts on day 15 of gestation. In conclusion, induction of estrus with a PMSG/hCG protocol in prepubertal gilts impaired expression of the luteal P4 synthesis system. Low P4 content may, in turn, induce local mechanisms involving *LHR* and *ESR1* expression to support CL function.



## 1. Introduction

The corpus luteum (CL) plays a key role in the regulation of reproductive events associated with pregnancy establishment and maintenance because it is the main source of progesterone (P4). The concentration of P4 in peripheral blood increases with maturation of CLs. P4 is the hormone of pregnancy in all mammals and is required for maternal support of conceptus survival and development (Spencer and Bazer, 2002). Sustained P4 action on the uterus is essential for the attainment of endometrial receptivity for implantation in ruminants and pigs, and determines endometrial expression of several genes encoding proteins participating in embryo-maternal communication (Bazer et al., 2010; Spencer et al., 2004; Ziecik et al., 2011).

In the pig, the most established treatment protocol for the control of ovulation includes using a combination of pregnant mare serum gonadotropin (PMSG) to induce follicular development and human chorionic gonadotropin (hCG) to trigger ovulation (Britt et al., 1989; Estill, 2000). Although the treatment of gilts with exogenous gonadotropins can induce a fast and synchronous

* Corresponding author. Fax: +48 89 5357421.
*E-mail address:* a.blitek@pan.olsztyn.pl (A. Blitek).

http://dx.doi.org/10.1016/j.anireprosci.2016.01.001
0378-4320/

ovulatory response (Britt et al., 1989; Knox et al., 2000), administration of hormones may also have a detrimental effect on reproductive functions. Temporal relationships among serum estrogens, prolactin and luteinizing hormone (LH) concentrations were different in prepubertal gilts induced to ovulate compared with mature gilts (Pinkert et al., 1988). The patterns of morphological and biochemical development of ovarian follicles also differ between gilts exhibiting gonadotropin-stimulated vs. natural estrus (Wiesak et al., 1990). Moreover, a greater proportion of gilts with follicular cysts and poorly formed CLs was observed after hormone treatment (Breen and Knox, 2012). Administration of hormones to induce estrus in domestic animals may cause decreased concentrations of circulating P4, which results from impairment of the luteal P4 metabolic pathway (Kineman et al., 1987a) and/or reduced sensitivity of CLs to luteotropic factors (Kineman et al., 1987b; Skarzynski et al., 2009). Moreover, induced CLs are more sensitive to luteolytic prostaglandin (PG) F2α, resulting in pregnancy failure (Puglisi et al., 1978; Rampacek et al., 1976a). Low concentrations of P4 in blood serum of heifers affected the expression of endometrial genes that potentially contribute to histotroph composition and conceptus elongation. This altered endometrial transcriptome was associated with a reduced capacity of the uterus to support conceptus development after embryo transfer (Forde et al., 2011, 2012).

In the pig, lower concentrations of P4 in blood serum, accompanied by decreased expression of homeobox A10, leukemia inhibitory factor and transforming growth factor β1 genes in the endometrium, were observed on day 12 in pregnant gilts after induction of puberty with PMSG/hCG (Blitek et al., 2010a). Moreover, the expression of PG synthesis enzymes in the endometrium and PG content in the uterine lumen and peripheral blood were negatively affected by the administration of gonadotropins (Blitek et al., 2010b). These changes may be attributed to impaired endometrial preparation for implantation resulting from insufficient P4 synthesis and/or secretion by luteal tissue. Therefore, the current study was conducted to examine the effect of estrus induction with PMSG/hCG in prepubertal gilts on the quality of subsequent CLs with respect to P4 synthesis. Using the same gilts as described previously (Blitek et al., 2010a), we analyzed: (1) the profiles of steroidogenic acute regulatory protein (StAR), cytochrome P450 family 11 subfamily A polypeptide 1 (CYP11A1) and 3β-hydroxysteroid dehydrogenase (3βHSD) mRNA and protein expression in luteal tissue; (2) the concentrations of P4 in luteal tissue and blood serum; and (3) the expression of LH receptor (*LHR*) and estrogen receptors (*ESR1* and *ESR2*) mRNA in the CL. Samples were collected on days 10, 12, and 15 after insemination, because this period is critical for prolonged CL lifespan and pregnancy establishment in response to conceptus signals.

## 2. Materials and methods

### 2.1. Animals and sample collection

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.

In this study, CLs from the same cross-bred gilts (Polish Landrace × Duroc) as described previously by Blitek et al. (2010a) were used. Prepubertal gilts of similar age (165–175 d old), weight (100–110 kg) and genetic background were assigned randomly into two groups. Day of animal separation into groups was designated as the first day of the experiment. Animals from Group I ($n = 17$; control gilts allowed to exhibit natural estrus) were observed daily for estrus behavior. Gilts were considered to be in estrus when standing in response to the back pressure test during boar exposure. Gilts that expressed estrus within 3–4 weeks after initial assignment into groups continued to be used in the study. Day of onset of estrus behavior was designated as day 0, and gilts were inseminated 24 and 48 h after detection of their first estrus. Animals from Group II ($n = 16$; 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 gonadotropin-stimulated animals were inseminated 24 and 48 h after hCG injection. The day of second insemination was designated 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; $n = 4$–6/group), 12 (maternal recognition of pregnancy; $n = 4$–6/group), or 15 (beginning of implantation; $n = 4$–6/group) of pregnancy. Because of the experimental procedures, gilts from the control group were 2–3 weeks older at slaughter than gilts with induced estrus. Each uterine horn was flushed with phosphate-buffered saline (PBS) to obtain conceptuses. Days of pregnancy were confirmed by 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). The CLs were dissected from surrounding ovarian tissue, frozen in liquid nitrogen and stored at −80 °C. Blood samples were taken at slaughter, incubated overnight at 6 °C, centrifuged at 3000 × *g* for 15 min, and stored at −20 °C for further analyses.

### 2.2. Total RNA isolation and real-time PCR

Total RNA was extracted from frozen CLs 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).

Analysis of selected genes was performed using quantitative real-time PCR with the 7300 or 7900 Sequence Detection System (Life Technologies). For *StAR*, *CYP11A1*, *3βHSD* and *ACTB* (*β*-actin; used as a reference gene) mRNA analysis, each sample contained cDNA (36 ng), forward and reverse primers (0.5 μmol/l each; Table 1) and Power SYBR Green PCR master mix (12.5 μl; Life Technologies). For