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Secretion of estradiol-17β by porcine endometrium and myometrium during early pregnancy and luteolysis

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Abstract

Past studies of the source of estrogens secreted during maternal recognition of pregnancy in pigs have focused on embryonic rather than uterine origin of these steroids. The present study documents: (1) the expression of the gene CYP 17, encoding cytochrome P450 17α-hydroxylase/$C_{17–20}$ lyase and (2) the synthesis and secretion of estradiol-17 β (E_2) in endometrial and myometrial tissues in gilts. The expression of CYP 17 gene was shown in porcine endometrium and myometrium. Basal endometrial secretion of E_2 was higher in pregnant gilts than in cyclic gilts (days 14–16). The myometrium secreted more E_2 during the expected time of luteolysis compared to early pregnancy. Basal secretion of E_2 during pregnancy was higher from the endometrium than from the myometrium. Conversely, during luteolysis E_2 secretion was higher from the myometrium and lower from the endometrium. In pregnant and cyclic gilts (days 14–16), progesterone (P_4, 10^{-5} M) in vitro significantly increased E_2 secretion regardless of reproductive status. Oxytocin (OT, 10^{-7} M) had no influence on E_2 secretion and did not change the stimulatory effect of P_4 in both tissues examined. In conclusions: (1) the CYP 17 gene transcript is present in porcine endometrium and myometrium; (2) porcine endometrium and myometrium release E_2 in vitro; (3) the endometrium releases more E_2 than the myometrium during early pregnancy; (4) the myometrium releases E_2 mainly during luteolysis; (5) the endometrium and myometrium can increase E_2 release in vitro if substrate (P_4) is provided during early pregnancy and luteolysis. These data suggest active estrogen production by the myometrium and endometrium as an alternative source for this signal for recognition of pregnancy in the pig.

Keywords: Endometrium; Estradiol-17β; Luteolysis; Myometrium; Pigs; Pregnancy

1. Introduction

Uterine estrogens present between days 11 and 12 of gestation in the pig are produced in part by spherical blastocysts. Estrone and estradiol-17β (E_2) provide a signal for maternal recognition of pregnancy in this species [1–3]. These estrogens stimulate the release of proteins, ions, carbohydrates and prostaglandins into the uterine lumen during early pregnancy. Estrogens also exert a luteotrophic effect, preventing luteolysis and transforming of the endometrium to support the implantation of blastocysts [3–10].

Estradiol concentrations in porcine concepti are approximately 50-fold higher at day 12 than at day 14 of development and the total content of estrogens in flushings from uterine horns at about day 12 of pregnancy is higher than that at days 14–16 [3]. However, estrogen synthesis by porcine blastocysts declines markedly as they develop from the tubular to

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the filamentous form [11]. Whether conceptuses are the sole source of all these estrogens is unclear especially when implantation begins.

The role of endometrium in the regulation of early pregnancy and luteolysis in pigs is well documented [6,7,12–18]. We previously showed significant production of luteotrophic prostaglandin E_2 and luteolytic $F_2\alpha$ by porcine myometrium, confirming the role of this tissue in the regulation of early pregnancy and luteolysis [19]. Both endometrial and myometrial synthesis and secretion of prostaglandins can be regulated by oxytocin (OT) [14,17,19,20].

The current study was carried out to: (1) confirm the expression of the steroidogenic gene CYP 17 encoding cytochrome P450 17α-hydroxylase/$C_{17–20}$ lyase in porcine endometrium and myometrium; (2) determine whether porcine endometrium and myometrium are involved in the synthesis and secretion of estradiol-17β at the time of corpus luteum maintenance during early pregnancy or corpus luteum regression (days 14–16); (3) examine whether progesterone is a substrate for estrogen synthesis in the uterus; (4) determine if oxytocin, one of the main stimulators of endocrine activity of uterine tissues, regulates uterine estradiol-17β synthesis and secretion.

2. Materials and methods

2.1. Animals and collection of endometrial and myometrial tissue

All experiments were approved by the Animal Ethics Committee, University of Warmia and Mazury in Olsztyn, Poland. Post-pubertal crossbred pigs (Large White × Polish Landrace) weighing 90–110 kg were used during the estrous cycle or early pregnancy. Gilts were observed daily for estrus behavior in the presence of an intact boar. The onset of the second estrus was designated as day 0 of the estrous cycle. Gilts assigned to the early pregnancy group were naturally bred on the second day of estrus. The animals were slaughtered on days 14–16 of pregnancy ($n = 6$), or days 14–16 of the estrous cycle ($n = 6$). Pregnancy was confirmed by the presence of embryos after flushing each uterine horn with 20 ml sterile saline. Blood samples were collected at slaughter for analysis of plasma P_4 and E_2 concentrations. Endometrial and myometrial tissues from cyclic or early-pregnant gilts were placed immediately in ice-cold PBS supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin and transported to the laboratory on ice within 30 min after slaughter.

2.2. Preparation of endometrial and myometrial slices

Slices from the middle part of uterine horns collected from gilts were opened longitudinally on the mesometrial surface and the endometrium and the perimetrium were separated from the myometrium by careful scraping using a scalpel blade. The separation of the endometrium and the myometrium was confirmed under a dissecting microscope and histologically at higher magnification. The endometrium and the myometrium were minced into small slices (200–210 mg, 3 mm thick) and washed twice with PBS using a modification of the technique of Flowers et al. [21].

2.3. Total RNA isolation and reverse transcription

Endometrial and myometrial tissues from pregnant and cyclic gilts were separated, frozen in liquid nitrogen and stored at −70 °C until later analysis. Total RNA extraction was performed using Qiagen RNasy columns (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Total RNA quality was confirmed by spectrophotometry and visualized on 1.5% agarose gel to rule out RNA degradation or DNA contamination. Approximately 1 μg of RNA was reverse-transcribed into cDNA in a total volume of 20 μl with 0.5 μg oligo(dT)15 primer (Roche, Germany) using the Omniscript RT Kit (Qiagen, USA) at 37 °C for 1 h and was terminated by incubation at 93 °C for 5 min.

2.4. Expression of the CYP 17 gene

Expression of the mRNA encoding cytochrome P450 17α-hydroxylase/$C_{17–20}$ lyase in porcine endometrium and myometrium during early pregnancy and luteolysis was investigated by reverse transcription-polymerase-chain reaction (GeneAmp PCR System 2400, Perkin Elmer, USA). Primers for CYP 17 forward-5′TCCAGGATGCTATCGACCAG3′ and CYP 17 reverse-5′GGCGCTCCTTGATCTTCACT3′ were designed according to Tanavde and Maitra [22]. These primers were used to amplify a 495-base pair (bp) fragment of the CYP 17 gene. All PCR amplifications were carried out in a total volume of 25 μl containing 12.5 μl REDTaq ReadyMix PCR Reaction Mix (1.5 units Taq DNA polymerase, 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 0.2 mM dNTP) and 20 pM each of sense and antisense primer. After a series of preliminary studies optimizing conditions for PCR, 40 cycles were used for the current study. Each thermal cycle included denaturation for 1 min at 94 °C, annealing for