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The effect of interleukin-1β, interleukin-6, and tumor necrosis factor-α on estradiol-17β release in the myometrium: The *in vitro* study on the pig model

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ABSTRACT

Estradiol-17β (E_2) is a potent regulator of early pregnancy and the estrous cycle in pigs. Production of E_2 occurs in the porcine myometrium, but the factors involved in its regulation are unknown. In this *in vitro* study, it was investigated whether interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α affect the release of E_2 from the porcine myometrium on Days 10 to 11, 12 to 13, and 15 to 16 of pregnancy and the estrous cycle. The expression of the cytochrome P450 family 19 (*CYP19*) gene and the presence of the aromatase cytochrome P450 protein in the myometrium confirmed the ability of the tissue to produce E_2. In gravid pigs, the expression of *IL1RI* mRNA and *IL6R* mRNA was markedly increased on Days 15 to 16 of gestation, whereas *TNFRI* mRNA was increased on Days 10 to 11 of gestation. In cyclic pigs, the expression of myometrial *IL1RI* mRNA did not differ among the studied days, although the expression of *IL6R* and *TNFRI* mRNAs was increased on Days 15 to 16. In gravid pigs, IL-1β, IL-6, and TNF-α increased myometrial E_2 secretion on Days 15 to 16 but did not affect E_2 release on Days 10 to 11 and 12 to 13 of pregnancy. In cyclic pigs, IL-1β, IL-6, and TNF-α did not increase myometrial E_2 release. In conclusion, IL-1β, IL-6, and TNF-α affected myometrial E_2 release in a manner that is dependent on the physiologic status of the female. The porcine myometrium expresses *IL1RI*, *IL6R*, and *TNFRI* genes and is the target tissue for IL-1β, IL-6, and TNF-α. In gravid pigs, IL-1β, IL-6, and TNF-α may increase myometrial release of E_2 *in vitro* specifically on Days 15 to 16 of pregnancy. These findings may be of interest to researchers using pigs as an animal model for fetal programming.

1. Introduction

Previously, we have demonstrated that the porcine uterus produces steroids, including estradiol-17β (E_2), during early pregnancy and luteolysis (i.e., on Days 14–16) [1,2,3]. We have determined that the myometrium secretes approximately 35% and 59% of the total uterine amount of E_2 production during the days of early pregnancy and luteolysis, respectively [2]. Thus, in addition to the embryos [4] and the cyclic and pregnant endometrium [2], the myometrium may be a significant source of E_2, an important and unique regulator of early pregnancy in pigs.

Estradiol-17β is synthesized in a reaction catalyzed by a microsomal member of the cytochrome P450 superfamily, aromatase cytochrome P450 (P450arom), which is the product of the cytochrome P450 family 19 (*CYP19*) gene [5]. In a previous study, we showed that the P450arom transcript gene was present in the myometrium on Days 14 to 16 of pregnancy and the estrous cycle [3]. Based on additional data related to the steroidogenic activity of the uterus on Days 14 to 16 of pregnancy, we hypothesized that *CYP19* and P450arom are present in the myometrium

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during important periods of early pregnancy (i.e., on Days 10 to 11, 12 to 13, and 15 to 16) to support uterine production of E_2. However, the pattern of the *CYP19* gene expression and the localization of the P450arom protein in the myometrium during these important periods of early pregnancy in the pigs are unknown.

On Days 14 to 16 of pregnancy and the estrous cycle, progesterone (P_4) serves as a substrate for E_2 synthesis in the porcine myometrium [2,3]. However, other factors that are involved in the regulation of myometrial E_2 synthesis remain unknown. Interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α are involved in the regulation of steroidogenesis in the various steroidogenic organs of different species [6,7,8,9]. Furthermore, the specific stimulatory effect of IL-1β and IL-6 on E_2 secretion was observed in pregnant porcine endometrium harvested on Days 15 to 16 of gestation [10]. For these reasons, we hypothesized that cytokines could act as regulators of steroidogenesis in the porcine myometrium. In pigs, the influence of cytokines on steroid hormone production was also demonstrated in the ovarian follicles [11,12,13] and CLs [7,14,15]. Whether IL-1β, IL-6, and TNF-α regulate myometrial E_2 release in early pregnant or cyclic pigs was unknown.

Important sources of cytokines acting in the uterus include endometrial cells, embryos, and leukocytes that infiltrate the uterus mainly during early pregnancy [16,17,18,19]. The presence and distribution of immune cells was well-established in the endometria of both pregnant and nonpregnant pigs [20,21,22,23,24], but the distribution of cells that specifically secrete cytokines in porcine myometrium was not studied. However, macrophages and cells of the natural killer lineage were found in myometria of peripartum and cyclic mice, respectively [25,26], and leukocytes were present in the perinatal myometria of women [27]. Thus, the porcine myometrium may produce cytokines or may respond to cytokine action. Cytokines act in the target cells through specific receptors. Previously, the expression of IL-1β receptor type I (*IL1RI*) mRNA was determined in the endometrium [19] and CL of gravid and cycling pigs [28]. Interleukin 6 receptor (*IL6R*) mRNA was localized in different types of endometrial cells of early pregnant and cycling pigs [17,18]. The present study focused on the expression of *IL1RI*, *IL6R*, and TNF-α receptor *(TNFRI)* mRNAs in the porcine myometrium.

Previously, the study of the effect of cytokines in the porcine myometrium focused on the synthesis and secretion of prostaglandin $F_{2}\alpha$ ($PGF_{2}\alpha$), prostaglandin E_2 (PGE_2), and estrone (E_1) [29,30,31]. It was found that IL-1β stimulates synthesis and secretion of $PGF_{2}\alpha$ in the myometrium on Days 10 to 13 of both pregnancy and the estrous cycle in pigs [29]. During the first third of gestation in pigs, IL-1β, IL-6, and TNF-α increased myometrial synthesis and secretion of $PGF_{2}\alpha$ and PGE_2 in a day-of-pregnancy–dependent manner [30]. A previous study showed that IL-1β and IL-6, acting during maternal recognition of pregnancy in pigs (i.e., on Days 12–13 of gestation), increased release of E_1 by the myometrium *in vitro* [31]. Because IL-1β, IL-6, and TNF-α are involved in the successful establishment of pregnancy in pigs [7,10,14,17,18,19,28,29,30,31], we hypothesized that these cytokines can also affect myometrial secretion of E_2. To demonstrate the responsiveness of the myometrium to IL-1β, IL-6, and TNF-α, we have investigated whether the porcine myometrium expresses *IL1RI*, *IL6R*, and *TNFRI* mRNAs.

To confirm the ability of the porcine myometrium to produce E_2, we measured the expression of the *CYP19* mRNA and detected the presence of the P450arom protein in myometrial explants harvested from pigs on Days 10 to 16 of pregnancy. We have examined whether the porcine myometrium produces E_2 in the presence of IL-1β, IL-6, and TNF-α during three important periods of early pregnancy: (1) before the time of maternal recognition of pregnancy and initiation of the embryo–maternal communication (Days 10–11 of pregnancy), (2) during the time of maternal recognition of pregnancy and the first surge of estrogens produced by porcine embryos (Days 12–13), and (3) during the time of CL maintenance, the protection against luteal regression and the beginning of the implantation (Days 15–16 of pregnancy). The effect of cytokines in myometrial tissue harvested from pregnant pigs on Days 10 to 11, 12 to 13, and 15 to 16 were compared with the effect of cytokines in the myometrium harvested from pigs during the respective days of the estrous cycle.

To demonstrate the ability of the myometrium to produce and release E_2 *in vitro*, we first investigated if the *CYP19* mRNA and P450arom protein are present in porcine myometrial explants. Second, we determined whether IL-1β, IL-6, and TNF-α can affect myometrial E_2 release *in vitro* in pigs on Days 10 to 11, 12 to 13, and 15 to 16 of pregnancy and the estrous cycle.

2. Material and methods

2.1. Animals and collection of myometrial tissue

All experiments were approved by the Animal Ethics Committee of the University of Warmia and Mazury in Olsztyn, Poland. Pigs were used during early pregnancy or the estrous cycle. The animals on Days 10 to 11 ($n = 5$), 12 to 13 ($n = 5$), and 15 to 16 ($n = 5$) of pregnancy or the estrous cycle were slaughtered in a commercial slaughterhouse. Sections of the middle part of the uterine horns were opened longitudinally on the mesometrial surface, and the myometrium was separated from the endometrium and the perimetrium by careful scraping using a tweezers and a scalpel blade. Small fragments of the myometrium were then minced, snap frozen in liquid nitrogen, and stored at −80 °C for visualization of P450arom. The remaining part of the myometrium was sliced thinly (200–210 mg, 3 mm thick) and washed twice in PBS supplemented with antibiotics using a modification of the technique of Flowers, et al. [32]. Precision of separation of the myometrium was verified under a dissecting microscope and histologically.

2.2. In vitro incubation of myometrial slices

Individual fresh myometrial slices were placed in culture vials containing 2 mL of Medium 199 (Sigma, Steinheim, Germany) supplemented with 0.1% BSA fraction V (ICN, Solon, OH) and 20 μg gentamycin (Sigma, Steinheim, Germany). These tissue cultures were pre-incubated in a water bath for 18 hours at 37 °C in an atmosphere of 95% O_2