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Effect of conceptus secretions on *HOXA10* and *PTGS2* gene expression, and PGE_2 release in co-cultured luminal epithelial and stromal cells of the porcine endometrium at the time of early implantation

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Abstract

Homeobox A10 (*HOXA10*) gene expression was demonstrated in the endometrium of adult porcine uteri, however there is little information concerning the role of this gene in the pig. Objectives of the present study were to examine: 1) the expression of *HOXA10* in the endometrium of cyclic and early pregnant gilts; 2) the effect of estradiol (E_2) and progesterone (P_4) on *HOXA10* expression in porcine luminal epithelial (LE) and stromal (ST) cells *in vitro*; 3) the effect of E_2 and conceptus-exposed medium (CEM) on *HOXA10* and prostaglandin endoperoxide synthase (*PTGS2*) gene expression and prostaglandin (PG) E_2 secretion from LE and ST cells in a co-culture model. The abundance of *HOXA10* mRNA was increased on day 15 of pregnancy in comparison to day 15 of the estrous cycle. Moreover, increased *HOXA10* mRNA level was detected in ST cells after E_2 and P_4 treatment. E_2 stimulated the expression of *HOXA10* in LE cells cultured on collagen and pre-treated with steroids, but not in LE on plastic surfaces. Addition of CEM to LE cells cultured in collagen-coated inserts of the co-culture system resulted in elevated *HOXA10* and *PTGS2* gene expression and PGE_2 secretion in these cells, but not in ST cells cultured in basal compartments. ST cells directly treated with E_2 or CEM showed higher levels of *HOXA10* and *PTGS2* expression. Blocking of estrogen receptors with ICI-182,780 did not influence the stimulatory effect of CEM. We conclude that *HOXA10* expression in the porcine endometrium is closely related to the implantation process and stimulated by conceptus products. Moreover, the co-culture system of LE and ST cells is a promising model for the study of endometrial response to conceptus-derived factors.

Keywords: Pig; Endometrium; Pregnancy; Conceptus; HOXA10; Steroids; Prostaglandins

1. Introduction

In early pregnancy, the establishment of a uterine environment suitable for successful implantation is crucial and remains under strict regulation by ovarian steroids. The uterine endometrium undergoes morphological and physiological changes during each estrous cycle in order to prepare the uterus for embryo implantation. Disorders in endometrial development may lead to lack of or improper implantation, pregnancy failure, or infertility. It became obvious that the action of progesterone (P_4) in priming the uterus is absolutely essential to attain uterine receptivity in many species, including the pig [1–4]. The requirement of ovarian estrogen for implantation is species-specific. While estrogen is essential for preparation of the P_4-primed uterus in mice and rats, ovarian P_4 alone is sufficient to

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support implantation in species, such as the hamster, guinea pig, rabbit and pig (see [1] for a review). However, porcine preimplantation embryos are an important source of estrogens [5], which influence the implantation process to a considerable degree [6]. As a result of P_4 and E_2 action, the expression of several biological molecules such as cytokines, growth factors and integrins, is altered in the endometrium. These changes lead to a temporary state of uterine receptivity for implantation and for further maintenance of pregnancy.

Although the necessity of ovarian and conceptus signals in the preparation of endometrium for pregnancy establishment is well documented, molecular mechanisms by which uterine receptivity is achieved are largely unknown. Since development of endometrial receptivity to conceptus signals involves both down- or up-regulation of several genes, it is likely that transcription factors whose expression in the endometrium are under the regulation of ovarian P_4 and/or embryonic stimuli would be critical for implantation. One such factor that is reported to play an important role in uterine physiology is the homeobox A10 (HOXA10) protein. *HOX* genes are vertebrate homologs of *Drosophila melanogaster* homeotic genes that determine the identity of specific body segments [7]. Products of these genes function as transcription factors that play essential roles in the regulation of morphogenesis and tissue differentiation during embryogenesis [7,8]. The *HOXA* family of genes is responsible for segmental development of the female reproductive tract [9]. However, *HOXA10* has been shown to be expressed in adult murine [10], canine [11], non-human primate [12], human [10,13], and more recently porcine [14,15] uteri. *HOXA10* was shown to be essential not only for uterine development, but also for implantation [13]. Targeted mutation of the *Hoxa10* gene in mice resulted in failure of implantation [16,17]. In the human endometrium, *HOXA10* demonstrates a spatiotemporal pattern of expression throughout the menstrual cycle with increased transcripts detected in the mid- and late-secretory phases [13]. Moreover, *HOXA10* expression is regulated by P_4 and/or E_2 in human [13], non-human primate [12], murine [18], and canine [11] uteri. Recently, E_2-stimulated expression of *HOXA10* was observed in uteri of postnatal day 14 piglets [15].

Prostaglandins (PGs) produced by the uterus play an important role in regulation of the estrous cycle and during early pregnancy in many species, including the pig [4,19]. In the porcine endometrium, luteoprotective PGE_2 and luteolytic $PGF_{2\alpha}$ are the main PGs produced [20] and the proper ratio between PGE_2 and $PGF_{2\alpha}$ is responsible for successful pregnancy establishment [21,22]. In pigs, inhibition of PG synthesis results in pregnancy failure [23]. The rate-limiting enzyme in PG production is prostaglandin endoperoxide synthase (PTGS; also known as prostaglandin G/H synthase or cyclooxygenase), which catalyzes the conversion of arachidonic acid to PGH_2 [24], the common substrate for various PGs. The expression of *PTGS2* in the porcine endometrium during the estrous cycle and early pregnancy was previously reported [25,26]. Since then, it was demonstrated that E_2, as well as PGE_2 may up-regulate *PTGS2* expression and increase PGE_2 secretion in endometrial explants [27]. Moreover, P_4, E_2, oxytocin, tumor necrosis factor α, and LH were shown to modulate PGE_2 and $PGF_{2\alpha}$ production and release in porcine endometrial cells *in vitro* [28–30]. Interestingly, *Hoxa10(−/−)* mice lack *Ptgs2* expression in the stromal cells of the endometrium. In contrast, the *Hoxa10* gene is expressed in *Ptgs2(−/−)* mice. These results indicate that *Hoxa10* is functionally upstream of *Ptgs2* in uterine stroma [31,32].

The data obtained for primate, murine and canine uteri indicate that *HOXA10* gene expression is essential for uterine receptivity and embryo implantation. Although, HOXA10 mRNA content and protein localization was detected in adult porcine uterus during early pregnancy [14], there is little information on the hormonal regulation of *HOXA10* expression in the pig. Therefore, the current study was performed to: (1) examine the expression of *HOXA10* gene in the porcine endometrium on days 9, 12, and 15 of early pregnancy and the estrous cycle; (2) study the effect of E_2 and P_4 on *HOXA10* expression in luminal epithelial (LE) and stromal cells (ST) *in vitro* and (3) investigate the effect of E_2 and conceptus-exposed medium (CEM) on *HOXA10* and *PTGS2* gene expression and PGE_2 release in a co-culture system of porcine endometrial LE and ST cells in the presence or absence of estrogen receptor antagonist.

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. Thirty pubertal, crossbred gilts (*Sus scrofa domesticus*) of similar age (8–8.5 mo), weight (140–150 kg), and genetic background from