Domestic Animal Endocrinology 53 (2015) 17–25

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Ovarian stimulation with human chorionic gonadotropin and equine chorionic gonadotropin affects prostacyclin and its receptor expression in the porcine oviduct

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ARTICLE INFO

Article history:
Received 8 February 2015
Received in revised form 14 March 2015
Accepted 3 April 2015

Keywords:
Pig
Oviduct
Prostacyclin
hCG
eCG

ABSTRACT

Prostaglandins are well-known mediators of crucial events in the female reproductive tract, eg, early embryo development and implantation. Prostacyclin (PGI_2) is the most synthesized prostaglandin in the human oviduct during the postovulatory period, indicating its important role in supporting and regulating the oviductal environment. The present study was undertaken to determine the influence of insemination and ovarian stimulation with human chorionic gonadotropin (hCG)/equine chorionic gonadotropin (eCG) on PGI_2 synthesis in the porcine oviduct on day 3 post coitus. Mature gilts (n = 25) were assigned into 2 experiments. In experiment I, gilts were divided into cyclic (control; n = 5) and inseminated (control; n = 5) groups. In experiment II, there were 3 groups of animals: inseminated (n = 5), induced ovulation/inseminated (750 IU eCG, 500 IU hCG; n = 5), and superovulated/inseminated (1,500 IU eCG, 1,000 IU hCG; n = 5) gilts. Parts of oviducts (isthmus and ampulla) were collected 3 days after phosphate-buffered saline treatment (cyclic gilts of experiment I) or insemination (all other groups). Expression of messenger RNA for PGI_2 synthase (PGIS) and its receptor (IP) was measured by real-time reverse transcription polymerase chain reaction (real-time RT PCR) and protein levels using Western blots. Concentrations of the PGI_2 metabolite 6-keto $PGF1_\alpha$ were evaluated by enzyme immunoassay and localization of PGIS and IP in the oviductal tissues using immunohistochemical staining. Insemination by itself increased PGIS protein levels in the oviductal isthmus ($P < 0.05$) and IP protein expression in the ampulla ($P < 0.05$). The concentration of 6-keto $PGF1_\alpha$ increased significantly in the oviductal ampulla after insemination ($P < 0.05$). Induction of ovulation decreased IP protein levels in the oviductal ampulla ($P < 0.05$), whereas superovulation reduced IP levels in both parts of the oviduct ($P < 0.01$). Synthesis of 6-keto $PGF1_\alpha$ was reduced by induction of ovulation and by superovulation in the oviductal ampulla ($P < 0.05$). Immunohistochemical staining confirmed the presence of PGIS in the muscular layer of the isthmus and both mucosa and muscular layers of the ampulla. IP-positive cells were observed in both mucosal and muscular layers of the isthmus and ampulla. This study showed for the first time that PGI_2 synthesis and IP expression are insemination dependent. Moreover, ovarian stimulation with hCG/eCG decreases IP expression and 6-keto $PGF1_\alpha$ concentrations in porcine oviducts. Therefore, disturbances in PGI_2/IP expression and synthesis may lead to disruption of the oviductal environment and, in turn, perturbed development of embryos and their transport to the uterus.

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http://dx.doi.org/10.1016/j.domaniend.2015.04.002

1. Introduction

Prostaglandins (PGs) are well-known proinflammatory mediators, which exert many actions in the body. The biosynthesis of PGs requires conversion of arachidonic acid to PGG_2 and then the unstable endoperoxide PGH_2, catalyzed by PGH synthase, also called cyclooxygenase (COX-1 and COX-2). Subsequently, PGH_2 is catalyzed by individual PGs synthases to: prostacyclin (PGI_2) by PGI synthase (PGIS), PGE by PGE synthase, and $PGF_{2\alpha}$ by PGF synthase. Different cells of all parts of the body synthesize PGs on a variety of stimuli [1,2]. It is well documented that, in the female reproductive tract, PGs are crucial factors in key processes such as fertilization, embryo development, and implantation [3,4]. A multiplicity of PGI_2 actions result from binding with the IP receptor, which belongs to a group of receptors coupled with G proteins [5]. Predominantly, PGI_2 binding to IP induces the G_s-dependent signaling pathway, leading to an increase in cAMP [6]. Nevertheless, various cells seem to activate different G-type signaling pathways via binding of PGI_2 with IP. For example, in cultured preadipocytes and human erythroleukemia cell line cells, incubation with PGI_2 stimulates the G_g-dependent PGI_2 signaling pathway leading to increased Ca^{2+} concentrations in these cells [7,8]. Although it was revealed that 40% to 50% of the PGs synthesized in human oviducts was PGI_2 [9], PGE_2 and $PGF_{2\alpha}$ are still the most studied PGs in this part of the female reproductive tract [4]. The influence of oviduct-derived PGI_2 on mouse embryos in vitro is well established [10,11]. Supplementing culture media with PGI_2 increased the frequency of mouse embryo hatching as well as implantation and live births [12,13]. However, in the porcine oviduct there is still very little information about PGI_2 synthesis. Since Kim et al [14] documented that iloprost, a PGI_2 analogue, stimulates oocyte meiotic maturation and early development in pigs, it could be assumed that PGI_2 synthesis in the oviduct is crucial for ensuring an environment beneficial for early embryo development. Moreover, PGI_2 is also considered as a relaxant factor, which may regulate contractions of oviductal smooth muscle and in this way indirectly participate in gamete and embryo transport [15]. Therefore, PGI_2 seems to be a crucial factor determining the function of the oviduct.

Although there is evidence that PGI_2 synthesis and release in the porcine uterus during the estrous cycle is influenced by many factors, such as steroid hormones [16], knowledge about the regulation of PGI_2 synthesis in the porcine oviduct is still inadequate. Our previous study revealed that ovarian stimulation with equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) to induce ovulation and evoke superovulation completely changed the synthesis of PGE_2 and $PGF_{2\alpha}$ [17]. Therefore, we propose that disturbances of proper embryo development and viability, which were documented after administering hCG/eCG [18] may be connected with alterations in PGI_2 production and expression of its receptor in porcine oviducts. The present study was undertaken to examine, for the first time, whether insemination by itself and with hCG/eCG affect PGI_2 synthesis in the porcine oviduct on day 3 post coitus (p.c.).

2. Materials and methods

2.1. Experimental scheme

All experimental procedures involving use of animals were approved by the Animal Ethics Committee, University of Warmia and Mazury in Olsztyn (No. 69/2008/N). Twenty-five crossbreed gilts of similar age (5–5.5 mo), weight (100–110 kg), and genetic background were observed daily for the onset of estrus. After exhibiting one natural cycle, gilts were divided into 2 experiments.

2.1.1. Experiment 1: effect of insemination on PGIS and IP expression and 6-keto $PGF_{1\alpha}$ concentration in the porcine oviduct

Ten mature gilts were assigned to the cyclic (control; n = 5) or inseminated (n = 5) groups. The onset of estrus (day 0) was determined as the day of occurrence of a standing reflex in the presence of a boar. Gilts in the cyclic (control) group received a 100 mL intrauterine infusion of phosphate-buffered saline (PBS; pH 7.4), whereas the other group was inseminated twice via a transcervical catheter with 100 mL of semen (containing 2.5×10^9 spermatozoa), diluted in Safe Cell Plus commercial extender (IMV Technologies, Szczecinek, Poland), 12 and 24h after detection of their third estrus. The ratio of neat semen-to-semen extender was determined according to the concentration and motility of the spermatozoa.

2.1.2. Experiment 2: influence of hCG and eCG treatment on PGIS and IP expression and 6-keto $PGF_{1\alpha}$ concentration in porcine oviducts

Gilts (n = 15) were divided into 3 groups: inseminated (control group; n = 5), induced ovulation/inseminated (n = 5), and superovulated/inseminated (n = 5). Day 0 was determined as the day of occurrence of a standing reflex in the presence of a boar. Gilts that were only inseminated (control group) received 100 mL of diluted semen, via a transcervical catheter, 12 and again 24 h after detection of their third estrus. Between days 12 and 16 of their second estrous cycle, gilts of the induced ovulation group were injected with a single dose of 750 IU eCG (Folligon; Intervet, Boxmeer, The Netherlands), followed by 500 IU hCG (Chorulon; Intervet) 72 h later. For superovulation, gilts received 1,500 IU eCG followed by 1,000 IU hCG 72 h later between days 12 and 16 of their second estrus cycle. Gilts of both hormonally treated groups received 100-mL intrauterine infusions of diluted semen via transcervical catheter 24 and again 48 h after hCG administration (as described by Małysz-Cymborska et al [2013, 2014]).

2.2. Sample collection

Oviducts (isthmus and ampullary parts) were collected immediately after slaughter, 3 days after the second insemination, and frozen in liquid nitrogen for total RNA and protein extraction or fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin for immunohistochemical staining. Each oviduct was flushed with 2 mL of PBS to obtain oocytes/embryos for verifying the effectiveness of insemination. Corpora lutea were counted to evaluate the superovulation yield (as described by Małysz-Cymborska et al [2014]).