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Short Communication

Downregulation of LH and FSH receptors after hCG and eCG treatments in the porcine oviduct

I. Małysz-Cymborska, A. Andronowska*

Department of Hormonal Action Mechanisms, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland

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ABSTRACT

The influence of induction of ovulation and superovulation with eCG and hCG on LH and FSH receptor levels in porcine oviducts on day 3 postcoitum was studied. In experiment I, gilts were assigned into cyclic (control; n = 5) and inseminated (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 (1500 IU eCG, 1000 IU hCG; n = 5) gilts. Oviduct tissues were collected 3 d after insemination or PBS infusion. The messenger RNA (mRNA) expression of FSH receptor (FSHR) and luteinizing hormone/chorionic gonadotropin receptor (LH/CGR) was measured by real-time reverse transcription PCR and protein levels using Western blots. Localization of LH/CGR and FSHR-positive cells was studied by immunohistochemical staining. Insemination by itself did not influence mRNA and protein levels of LH/CGR. However, FSHR mRNA expression in the isthmus and ampulla of the oviduct was affected by insemination ($P < 0.05$). Similarly, insemination decreased FSHR protein level in the isthmus ($P < 0.05$). Stimulation with hCG and eCG did not affect *LH/CGR* and *FSHR* mRNA expression, either in the isthmus or in the ampulla. Nevertheless, superovulation decreased LH/CGR protein level in the oviductal ampulla ($P < 0.05$) in comparison with inseminated gilts. Similarly, protein levels of FSHR in the oviductal ampulla decreased after superovulation ($P < 0.05$). LH/CGR-positive cells were observed in the mucosa as well as in smooth muscle cells of both parts of the oviduct. Follicle-stimulating hormone receptor–positive cells were observed in smooth muscle cells and blood vessels of the isthmus. In the ampulla, FSHR-positive cells were observed in the smooth muscle as well as in the mucosa. Summarizing, the present study revealed for the first time that stimulation with eCG and hCG, especially in high doses, can change LH/CGR and FSHR levels in porcine oviducts. This may in turn alter many signaling pathways, eg, PGs or vascular endothelial growth factor synthesis, and consequently disturb the oviductal environment, with possible detrimental effects on fertilization and/or embryonic development.

1. Introduction

Luteinizing hormone and FSH are glycoprotein polypeptide hormones, produced and secreted by anterior pituitary gland cells. Luteinizing hormone and FSH, commonly known as gonadotropins, control gamete and sex hormone production in the gonads by binding to specific membrane receptors, luteinizing hormone/chorionic gonadotropin receptor (LH/CGR) and FSH receptor (FSHR).

Until the 1980s, LH/CGR was thought to be a tissue-specific receptor, present only in granulosa and luteal cells of the ovary and the Leydig cells of the testis. However, in 1986, Zięcik et al [1] revealed that LH/CGR binding sites are present in the porcine uterus. These days,

* Corresponding author. Tel.: +48 89 523 46 86; fax: +48 89 524.
E-mail address: a.andronowska@pan.olsztyn.pl (A. Andronowska).

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the presence of binding sites for this receptor has been confirmed in many organs and tissues of numerous species [2]. Although extragonadal expression of FSHR is less explored than LH/CGR, nevertheless, its presence was confirmed in oviducts of several species including human [3] and porcine [4]. However, there is still a little information about the role of LH/CG and FSH receptors in the oviducts. Many years of research of our and other groups revealed that in pigs treated with eCG/hCG prostaglandins, vascular endothelial growth factor (VEGF) an Wnt-1 signaling pathways were affected in the whole reproductive tract [5–8]. Nevertheless, it was hard to explain, whether this was a direct effect of hCG/eCG on gonadal tissues or if they were affected indirectly. It is known that exogenously administered hCG and eCG bind to LH/CGR and FSHR [9], and their elimination half-life is much longer than in the natural situation [10]. In pigs, hCG was still present in the blood about 33 to 44 h after intramuscular injection [1]. Therefore, we hypothesize that hCG and eCG may affect LH/CG and FSH receptors and in turn launch a cascade of successive events changing at the same time the feedback loops between the bloodstream, pituitary, hypothalamus, and gonads. In effect, all signaling pathways of PGs and VEGF may be affected that are crucial for proper reproductive tract functions [5–8].

Therefore, the present study was undertaken to investigate, for the first time, whether exogenous hCG and eCG influence LH/CG and FSH receptors in porcine oviducts.

2. Materials and methods

2.1. Experimental scheme

All experiments were approved by the Animal Ethics Committee, University of Warmia and Mazury in Olsztyn (No. 69/2008/N). Twenty-five Large White × Landrace crossbreed gilts of similar age (5–5.5 mo), weight (100–110 kg), and genetic background were used. The onset of estrus (day 0) was determined as the day of occurrence of a standing reflex in the presence of a boar. Gilts were considered to be in estrus when standing in response to the backpressure test during boar exposure. After exhibiting 1 natural cycle, gilts were divided into 2 experiments:

In experiment I, gilts were randomly assigned into cyclic (control; n = 5) and inseminated (n = 5) groups. Gilts from the cyclic group received 100-mL intrauterine infusions of phosphate-buffered saline (PBS; pH 7.4), whereas the other group was infused with 100-mL of semen diluted in Safe Cell Plus commercial extender (2.5×10^9 spermatozoa; IMV Technologies, Szczecinek, Poland).

In experiment II, gilts were randomly divided into 3 groups: inseminated (control group; n = 5); induced ovulation/inseminated (n = 5), and superovulated/inseminated (n = 5). Gilts of the inseminated group received 100 mL of diluted semen, via a transcervical catheter, 12 and 24 h after detection of their third estrus. Gilts assigned to the induced ovulation/inseminated group were injected with 750-IU eCG (Folligon; Intervet, Boxmeer, The Netherlands), followed by 500-IU hCG (Chorulon; Intervet) 72 h later. The superovulated/inseminated group of gilts received 1,500-IU eCG followed by 1,000-IU hCG 72 h later. Then, gilts of both groups (induced ovulation/inseminated and superovulated/inseminated) received 100-mL intrauterine infusions of diluted semen; via transcervical catheter 24 and 48 h after hCG administration.

2.2. Sample collection

Tissues of oviducts were collected immediately after slaughter, 3 d after the insemination procedures and frozen in liquid nitrogen for total RNA and protein extraction or preserved in 4% paraformaldehyde for immunohistochemistry. For examination of the differences between anatomical parts of the oviducts, the tissues were divided into isthmus (from the utero-isthmic junction to the ampulla) and ampulla (from isthmic-ampullary part to the infundibulum). The effectiveness of insemination was verified by counting the numbers of embryos flushed from the oviducts. Corpora lutea were counted to evaluate the yield of superovulation [11].

2.3. Total RNA extraction and reverse transcription

Total RNA was extracted from oviducts using a commercial kit (Total RNA Prep Plus kit A&A Biotechnology, Gdansk, Poland). The RNA concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc, DE, USA) and RNA quality using a Bioanalyzer Agilent 2100 (Agilent Technologies, Waldbronn, Germany). Reverse transcription (RT) reactions were done using DNase I (Invitrogen Life Technologies, Inc, Carlsbad CA, USA) and the Reverse Transcription System Kit (Applied Biosystems, Foster City, CA, USA), according to the protocol described previously (Małysz-Cymborska and Andronowska 2015). Two types of RT controls were made, one without cDNA and another in the absence of reverse transcriptase.

2.4. Real-time quantitative PCR

Real-time quantitative PCR analysis was done using the Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems). Each sample contained 3-μL (15 ng) cDNA, 1.5-μL RNAse-free water (Promega), 5-μL TaqMan Universal MasterMix II, with UNG (Life Technologies), and 0.5 μL TaqMan assays (Life Technologies): *FSHR* (Ss03384581_u1) and *LH/CGR* (Ss03384991_u1). The following PCR conditions were performed: an initial denaturation step (15 min at 95°C), followed by 40 cycles of denaturation (15 s at 95°C) and annealing (60 s at 60°C). All samples for each gene were assayed in duplicate and run on the same plate (384 wells). Each PCR run included a nontemplate control with water added instead of cDNA, as well as an RT negative control for each gene. Stability of the reference genes *β-actin*, *cyclophilin*, and *GAPDH* was assessed using the statistical algorithm Normfinder 2.0. Based on the results of this analysis, the most stably expressed gene was *β-actin*. Therefore, data obtained from the real-time PCR for *LHCGR* and *FSHR* were normalized using the ratio of target messenger RNA (mRNA) to the