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The effect of estrone and estradiol on the expression of the adiponectin system in the porcine uterus during early pregnancy

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ABSTRACT

Adiponectin is secreted by the white adipose tissue and is one of the most important hormones that regulate metabolic homeostasis. The expression of adiponectin and adiponectin receptor genes and proteins in reproductive organs, such as the testes, ovaries, and uterus, suggests that adiponectin is also involved in the regulation of reproductive functions. Changes in the expression of adiponectin and adiponectin receptor genes and proteins in the porcine uterus during the estrous cycle and early pregnancy imply that adiponectin activity may be controlled by the local hormonal milieu. Estrone (E1) and estradiol (E2) are the key steroid hormones that regulate reproductive functions, including the early recognition of pregnancy and implantation. We hypothesize that E1 and E2 may regulate the expression of the adiponectin system in a pregnant uterus. The aim of this study was to investigate the influence of E1 and E2 on the expression of adiponectin and its receptor genes and proteins by porcine endometrial and myometrial explants harvested from gilts ($n = 5$ per group) on Days 10 to 11, 12 to 13, 15 to 16, and 27 to 28 of pregnancy and on Days 10 to 11 of the estrous cycle. The expression of adiponectin and AdipoRs genes was examined with the real-time polymerase chain reaction, adiponectin secretion was evaluated with the ELISA method, and the expression of receptor proteins was determined using the Western blotting method. The results revealed that both E1 and E2 significantly influenced the expression of the adiponectin gene, hormone secretion *in vitro*, and the expression of AdipoRs genes and proteins. The influence of E1 and E2 on the expression of the adiponectin system varied in the early gestation, during the estrous cycle and between different stages of gestation. The examined steroids had a tissue-specific and a dose-dependent effect. This is the first ever study to describe the modulatory effect of E1 and E2 on the expression of the adiponectin system in the porcine uterus during early gestation.

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1. Introduction

A female reproductive success is closely linked with their nutritional status. In this context, adiponectin – an adipokine group hormone which participates in glucose and lipid metabolism – seems to be an important factor linking reproductive functions with the energy balance. Adiponectin, a protein of 244 amino acids, has the molecular weight of 30 kDa. It is produced mainly by the adipose tissue and circulates in the serum in the form of three homomultimers: trimer (low molecular weight), hexamer (medium molecular weight), and multimer (high molecular weight) [1]. Adiponectin may also exist as a smaller globular fragment, but almost whole adiponectin in plasma seems to

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exist as a full-length protein [2]. Its activity is mediated *via* two types of receptors: adiponectin receptor type 1 (AdipoR1) and adiponectin receptor type 2 (AdipoR2). These receptors consist of seven transmembrane domains but are structurally and functionally distinct from the family of G-protein–coupled receptors. AdipoR1 has a greater affinity for the globular domain of adiponectin and is mostly expressed in the skeletal muscles. In turn, AdipoR2 has a higher affinity for the multimeric forms of adiponectin and is highly expressed in the liver [3]. Adiponectin controls energy homeostasis and insulin sensitivity. It also affects inflammatory processes, lipid synthesis, vasodilatation, and atherogenic activity [4,5]. Plasma adiponectin levels are inversely correlated with the body mass index [6]. Physiological concentrations of this adipokine in the plasma are higher in women than in men or prepubertal individuals [7–9]. In pubescent boys, adiponectin concentrations were found to be lower than in girls of the same age, which can probably be attributed to a higher secretion of androgens [8].

Adiponectin may affect reproductive functions by influencing the hypothalamic-pituitary-gonadal axis. The analyzed hormone inhibits gonadoliberin (GnRH) and GnRH-induced LH secretion in rats [10–12]. The expression of the adiponectin system (adiponectin, AdipoR1, and AdipoR2) was also observed in the porcine hypothalamus and pituitary [13,14]. AdipoRs were found in rat CL and ovarian follicles [15]; in porcine CL, theca, and granulosa cells [16]; and in the uteri of pregnant and nonpregnant pigs [17,18]. The expression of adiponectin and its receptors was also confirmed in rat testes [19]. The adiponectin system was found in the preimplantation embryos of mice [20] and in porcine trophoblasts and conceptuses [17]. Experimental data revealed that adiponectin may be involved in the regulation of steroidogenesis in various animal species, including mice, cattle, and pigs [21–23]. Plasma adiponectin levels and tissue expression of adiponectin and AdipoRs are correlated with the phase of the estrous cycle or pregnancy and steroid concentrations [17,18,23]. The above findings suggest that gonadal steroids could participate in the regulation of the adiponectin system.

Estrogens, including estrone (E1) and estradiol (E2), are the major hormones responsible for reproductive functions control. Estrone and estradiol take part in the maternal recognition of pregnancy in pigs [24,25]. They are secreted mainly by the ovaries and conceptuses; however steroidogenesis may also take place in the uterus. When synthesized in the porcine uterus, E1 and E2 act as alternative sources of steroids in the maternal recognition of pregnancy [26,27].

The relationship between the expression of the adiponectin system and steroid hormones has been poorly researched. The aim of the present study was to investigate the influence of E1 and E2 on the expression of adiponectin, AdipoR1, and AdipoR2 in porcine endometrial and myometrial tissue explants and adiponectin secretion *in vitro* in early pregnancy on Days 10 to 11 (embryo migration into the uterus), 12 to 13 (maternal recognition of pregnancy), 15 to 16 (implantation), 27 to 28 (end of implantation) and on Days 10 to 11 of the estrous cycle (midluteal phase, linked with the period when CLs are fully active, corresponding to the activity of the CL during pregnancy).

2. Materials and methods

2.1. Animals and tissue collection

All studies were conducted in accordance with ethical standards of the Animal Ethics Committee at the University of Warmia and Mazury in Olsztyn. Mature gilts (Large White × Polish Landrace) at the age of 7 to 8 months and weight of 120 to 130 kg were obtained from a private breeding farm. Twenty-five gilts were assigned to one of five experimental groups ($n = 5$ per group) as follows: 10 to 11, 12 to 13, 15 to 16, and 27 to 28 days of pregnancy and Days 10 to 11 of the estrous cycle. Cyclic gilts were daily observed for estrus behavior in the presence of an intact boar. The onset of the second estrous was marked as Day 0 of the estrus cycle. The phase of the estrous cycle was also confirmed on the basis of morphology of the ovaries [28]. In the case of pregnant gilts, the day after coition was marked as the first day of pregnancy. Insemination was performed on Days 1 to 2 of the estrous cycle. Pregnancy was confirmed by the presence of conceptuses. Few minutes after slaughter, liver and skeletal muscles samples were removed and frozen in the liquid nitrogen. The removed uterus was placed in ice-cold PBS supplemented with 100 IU/mL penicillin and 100 μg/mL streptomycin and transported to the laboratory on ice within 1 hour for *in vitro* explant tissue culture.

2.2. Tissue cultures

Endometrial and myometrial explants were performed based on the modified technique of Franczak and Kotwica [27]. The endometrial and myometrial tissues from the uterine horns were cut into small slices (100 mg) and then washed three times in medium M199 (Sigma–Aldrich, USA). Endometrial and myometrial slices were placed in the separate sterile culture vials with 2 mL of medium 199 containing 0.1% BSA (MP Biomedicals, USA), 5% dextran/charcoal-stripped newborn calf serum (Sigma–Aldrich), penicillin (100 IU/mL), and streptomycin (100 μg/mL). The cultures were preincubated for 2 hours (37 °C, 95% O_2, 5% CO_2). To determine the influence of E1 and E2 on adiponectin, *AdipoR1* and *AdipoR2* genes expression, adiponectin secretion, and AdipoR1 and AdipoR2 protein expression, endometrial and myometrial slices were treated with E1 or E2 at the concentration of 1 (subphysiological), 10 (physiological), and 100 nM (supraphysiological) and incubated for another 24 hours in the same conditions. The doses of E1 and E2 were chosen according to Blitek et al. [29]. Control probes were incubated without any treatment. Our preliminary studies indicated that the influence of E1 and E2 solvent (ethanol) on gene/protein expression and hormone secretion was negligible. All cultures were prepared in duplicates in five separate experiments for each group ($n = 5$ per group). At the end of the experiment, the media were collected and stored at −20 °C. Endometrial and myometrial slices were frozen and stored at −80 °C for further analyses. The viability of slices was monitored by measuring lactate dehydrogenase (LDH) activity in media after 2 hours of preincubation and at the end of the treatment period. The release of LDH was performed using a

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