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Expression of adiponectin and adiponectin receptors 1 and 2 in the porcine uterus, conceptus, and trophoblast during early pregnancy

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ABSTRACT

Adiponectin, one of the several adipocytokines secreted mainly by the adipose tissue, plays an important role in regulating energy homeostasis and controls female fertility. Female reproductive functions are closely associated with nutritional status, and adiponectin seems to be an important factor linking the regulation of metabolic homeostasis with reproductive processes. The biological activity of adiponectin is mediated by two distinct receptors, adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2). The objective of this study was to determine the presence of and changes in the gene and protein expression pattern of adiponectin and its receptors in the porcine uterus during early pregnancy and on Days 10 to 11 of the estrous cycle and in the conceptus and trophoblast. The highest level of adiponectin transcript was observed on Days 15 to 16 of gestation, Days 10 to 11 of the cycle in the endometrium, and Days 15 to 16 of gestation in the myometrium. The highest expression of AdipoR1 and AdipoR2 genes was detected on Days 10 to 11 of gestation in the endometrium, and Days 12 to 13 in the myometrium. The highest content of adiponectin protein was noted on Days 12 to 13 and 30 to 32 of gestation in the endometrium and Days 10 to 11 of the cycle in the myometrium. The expression of adiponectin protein was higher on Days 27 to 28 and 30 to 32 in the conceptuses. AdipoR1 protein content in the myometrium was highest on Days 12 to 13 and 30 to 32. In contrast, in the endometrium, it was more constant. The highest content of AdipoR2 protein was detected on Days 15 to 16 and 30 to 32 of gestation, Days 10 to 11 of the cycle in the endometrium, and Days 10 to 11 of gestation in the myometrium. In the conceptuses, the highest AdipoR1 protein content was observed on Days 15 to 16, and the highest AdipoR2 protein expression was determined on Days 15 to 16 and 27 to 28. In the trophoblasts, AdipoR1 protein content was higher on Days 27 to 28 than on Days 30 to 32, whereas the expression of AdipoR2 was higher on Days 30 to 32. This study demonstrated the presence of adiponectin and its receptors in the uteri, conceptuses, and trophoblasts of pregnant pigs and that the local adiponectin system is dependent on the stage of pregnancy.

1. Introduction

Adiponectin, the most abundant secretory product of white adipose tissue, is a 30-kDa protein with structural features of the collagen superfamily [1]. Circulating

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adiponectin is composed of trimers (low molecular weight), combinations formed by a dimer of trimers (middle molecular weight), and complexes of six trimers (high molecular weight) [2]. Adiponectin also occurs in a smaller globular form, but plasma adiponectin is nearly always found in full-length form [3]. Adiponectin regulates energy homeostasis by fatty acid oxidation, glucose uptake, and inhibition of gluconeogenesis. These lead to intensified thermogenesis and weight loss [4]. Adiponectin acts as an insulin-sensitizing agent by reducing hepatic glucose production and enhancing insulin action in the liver. Adiponectin levels are low in insulin-resistant subjects regardless of body weight, and they increase in insulin-sensitive states [5,6]. In contrast to other adipokines, adiponectin levels are reduced in obesity. Circulating adiponectin concentrations in plasma range from 2 to 10 μg/mL and reveal a sexual dimorphism, with females having higher levels of adiponectin than males [7,8]. The biological actions of adiponectin are mediated by 2 seven-transmembrane receptors, AdipoR1 and AdipoR2. AdipoR1 demonstrates higher affinity for adiponectin in the form of a trimer, and it is found in the highest concentrations in skeletal muscles, whereas AdipoR2, which is found mainly in the liver, shows greater affinity for middle molecular weight and high molecular weight forms [9]. AdipoR1 activates AMP kinase and mitogen-activated kinase pathways, whereas AdipoR2 acts mainly through the PPARα pathway [10].

Reproduction is dependent on energy availability. Adiponectin may serve as a signal-linking metabolic status with endocrine control of reproduction. It affects the reproductive system by exerting central effects on the hypothalamus and pituitary, peripheral effects on the ovaries and the uterus, and direct effects on the oocytes and embryos [11,12]. The presence of the adiponectin system (adiponectin, AdipoR1, and AdipoR2) has been confirmed in human, rat, and porcine hypothalami [13,14], pituitary glands [15,16], and ovaries [17–20] and in human, mouse, and porcine uteri [19,21–23]. High circulating adiponectin levels appear to be associated with *in vitro* fertilization success [24], and serum adiponectin levels are reduced in women with endometriosis [25], polycystic ovarian syndrome [26], and endometrial cancer [27]. Changes in the expression of the adiponectin system in the porcine uterus and variations in plasma adiponectin levels in cyclic pigs further suggest that adiponectin expression and secretion are regulated by gonadal steroids or other hormones that control the estrous cycle [20,23].

The expression of adiponectin and its receptors in the porcine uteri, conceptuses, and trophoblasts during pregnancy and the possible effect of the phase of pregnancy on adiponectin, AdipoR1, and AdipoR2 concentrations have not been previously reported. The aim of the present study was to compare the expression levels of (1) adiponectin, AdipoR1, and AdipoR2 genes by quantitative real-time polymerase chain reaction (PCR) and (2) adiponectin, AdipoR1, and AdipoR2 proteins determined by Western blotting in the endometrium and myometrium on Days 10 to 11 of the estrous cycle and Days 10 to 11, 12 to 13, 15 to 16, 27 to 28, and 30 to 32 of gestation, in the conceptuses on Days 15 to 16, 27 to 28, and 30 to 32 of gestation and in the trophoblasts (chorioallantois) on Days 27 to 28 and 30 to 32 of gestation. Adiponectin system proteins in the porcine endometrium and myometrium were localized by fluorescent immunohistochemistry.

2. Materials and methods

2.1. Experimental animals

The experiments were carried out in accordance with the ethical standards of the Animal Ethics Committee at the University of Warmia and Mazury in Olsztyn. Thirty mature gilts (Large White x Polish Landrace; 7–8 months of age, body weight of 120–130 kg) descended from private breeding were used in the study. The gilts were assigned to 1 of 6 experimental groups (n = 5 per group) as follows: Days 10 to 11 of the estrous cycle (the midluteal phase connected with period of fully active corpora lutea corresponding to the activity of corpora lutea during pregnancy), Days 10 to 11 (after insemination), 12 to 13 (just before implantation, the maternal recognition of pregnancy), 15 to 16 (implantation), 27 to 28 (the end of implantation), and 30 to 32 (placentation) of pregnancy. Females were monitored daily for estrus behavior in the presence of an intact boar. The day of onset of the second estrus was designated as Day 0 of the estrous cycle. The phase of the estrous cycle was also confirmed on the basis of the morphology of the ovaries. Insemination was performed on Days 1 to 2 of the estrous cycle. The endometrium (from implantation site) and myometrium was collected from the uterus. Pregnancy was confirmed by the presence of conceptuses. On Days 10 to 11 and 12 to 13 of pregnancy, the uterine horns were flushed with 20 mL of sterile PBS to recover conceptuses. On Days 15 to 16 of pregnancy, whole conceptus connected with fragments of trophoblast was dissected from the endometrium. Dissection of trophoblast tissues from conceptuses was done only on Days 27 to 28 and 30 to 32 of pregnancy; therefore, the expression of adiponectin and its receptors genes and proteins were analyzed in 15- to 16-day conceptuses connected with fragments of trophoblast, 27- to 32-day conceptuses, and 27- to 32-day trophoblast tissues (chorioallantois). All samples harvested from the endometrium, myometrium, conceptuses, trophoblast, subcutaneous adipose tissue, muscle, and liver were collected within several minutes after slaughter and snap frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.2. Total RNA isolation and cDNA synthesis

Total RNA was extracted from all the tissue samples with the Absolutely RNA Miniprep Kit (Stratagene). RNA concentration and quality were checked spectrophotometrically (NanoDrop ND-1000, NanoDrop Technologies Inc.). One microgram of RNA was reverse transcribed into cDNA in a total volume of 20 μl with 0.5-μg oligo$(dT)_{15}$ primer (Roche, Germany) using the Omniscript RT Kit (Qiagen) at 37 °C for 1 hour. The process was terminated by incubation at 93 °C for 5 minutes.

2.3. Quantitative real-time PCR

Specific primer pairs used to amplify parts of adiponectin, AdipoR1, AdipoR2, cyclophilin, and GAPDH genes