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Long form of leptin receptor gene and protein expression in the porcine trophoblast and uterine tissues during early pregnancy and the oestrous cycle

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ABSTRACT

Leptin, the product of the OB gene, is a 16-kDa polypeptide of 146 amino acid residues produced mainly by adipocytes that regulates metabolism and reproduction. The actions of leptin are mediated mainly *via* the long form of the leptin receptor (OB-Rb). The identification of leptin and OB-Rb mRNAs and proteins in human and mouse endometrium, and placental trophoblast suggests that leptin may be involved in the implantation process. Thus, the aim of this study was to compare the expression levels of porcine OB-Rb mRNA and protein in the endometrium and myometrium during mid- and late-luteal phases of the oestrous cycle (days 10–12 and 14–16, respectively) as well as during two stages of pregnancy respondent to the beginning of the implantation process (days 14–16) and the post-implantation period (days 30–32), and in trophoblast during both periods of pregnancy. OB-Rb gene expression in endometrium during the examined stages of pregnancy and the mid- and late-luteal phases of the cycle was at the same level. In contrast, in myometrium leptin receptor gene expression decreased on days 14–16 of pregnancy compared to both phases of the cycle, and on days 30–32 of pregnancy in relation to late-luteal phase. OB-Rb protein expression in the tissues was lower during the examined stages of pregnancy in comparison to the mid- and late-luteal phases of the cycle. In trophoblast, OB-Rb mRNA and protein expression was higher on days 30–32 than during days 14–16 of pregnancy.

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In conclusion, our results might suggest that leptin can participate in the control of pig reproduction by exercising its action at the uterine and trophoblast level and have a direct effect on these organ during both the luteal phase of the cycle and early pregnancy. Moreover, changes in OB-Rb gene and protein expression in tissues of pig reproductive tract strongly suggest that their sensitivity to leptin varies throughout luteal phase of the cycle and early pregnancy.

1. Introduction

Leptin is a small pleiotrophic peptide of 146 amino acid residues (16 kDa) secreted mainly by adipocytes that plays an important role in the regulation of metabolism and reproduction (Zhang et al., 1994; Houseknecht and Portocarrero, 1998; Clarke and Henry, 1999; Caprio et al., 2001; Moshos et al., 2002). The actions of leptin are mediated *via* specific transmembrane receptors that belong to the class I cytokine receptor superfamily and exist in six isoforms. The long form (OB-Rb), with the longest intracellular domain, is considered to be the primary signalling isoform, a number of C-terminally truncated forms (OB-Ra, -Rc, -Rd, -Rf) may act as transporters of leptin through physiological barriers, and the soluble isoform (OB-Re), which contains only the extracellular domain is the leptin-binding protein in serum (Tartaglia et al., 1995; Tartaglia, 1997; Bjorbaek et al., 1997).

Expression of leptin and OB-Rb mRNAs and proteins in human and mouse endometrium (Gonzalez et al., 2000; Kawamura et al., 2002; Yoon et al., 2005), and placental trophoblast (Masuzaki et al., 1997; Senaris et al., 1997; Hoggard et al., 1997; Henson et al., 1998) suggests that leptin may be involved in the implantation process. To date, OB-Rb mRNA expression has been identified in porcine uterus (Lin et al., 2000), and the leptin receptor protein expression has been localized in porcine placenta (Ashworth et al., 2000). However, these studies were made based on qualitative RT-PCR (Lin et al., 2000), or qualitative immunolocalization (Ashworth et al., 2000). The expression of OB-Rb gene and protein expression in the porcine trophoblast and uterus of cyclic and pregnant pigs remains poorly understood. Thus, the aim of this study was to compare the expression levels of porcine OB-Rb mRNA by semiquantitative RT-PCR and *in situ* hybridization, as well as the receptor protein by Western blotting in the endometrium and myometrium during mid- and late-luteal phases of the oestrous cycle (days 10–12 and 14–16, respectively) as well as during two stages of pregnancy respondent to the beginning (days 14–16) and the end (days 30–32) of the implantation process, and in trophoblast during both periods of pregnancy.

2. Material and methods

2.1. Experimental animals

The studies were carried out in accordance with the principles and procedures of the Animal Ethics Committee at the University of Warmia and Mazury in Olsztyn. Sixteen Polish Large White gilts were assigned to one of four experimental groups ($n = 4$ per group) as follows: the mid-luteal group (days 10–12 of the cycle connected with the period of fully active corpora lutea), the late-luteal group (days 14–16 of the cycle connected with period of luteal regression), early-implantation group (days 14–16 of gestation), post-implantation (placentation) group (days 30–32 of gestation). Within 10 min after slaughter endometrium, myometrium, trophoblast and medial basal hypothalamus (MBH) samples were collected. All tissue samples were frozen in liquid nitrogen and maintained at −80 °C until RNA isolation.

2.2. RNA isolation and semiquantitative reverse transcription-polymerase chain reaction

Total RNA from all collected tissues was isolated, reverse transcribed, amplified and quantified as we have previously described (Smolinska et al., 2007a). Briefly, complementary DNA was amplified