



# Expression and regulation of interleukin 6 and its receptor at the maternal-conceptus interface during pregnancy in pigs

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## ABSTRACT

It has been well established that interleukin 6 (IL6), a pleiotropic cytokine with multiple functional roles, is widely expressed in the female reproductive tract and mediates blastocyst implantation and placental development in many species. Uterine expression of IL6 during early pregnancy has been studied in pigs, but expression and function of IL6 at the maternal-placental interface throughout pregnancy have not been determined. Thus, we examined expression of IL6 and its receptors, IL6 receptor (IL6R) and GP130, in the uterine endometrium on Days 12 and 15 of the estrous cycle, and Days 12, 15, 30, 60, 90, and 114 of pregnancy, conceptus on Days 12 and 15, and chorioallantoic tissues on Days 30, 60, 90, and 114 of pregnancy in pigs. The expression of *IL6*, *IL6R*, and *GP130* mRNA in the endometrial tissues increased dramatically during mid-to late-pregnancy and decreased at term. *IL6*, *IL6R*, and *GP130* mRNAs were also expressed in conceptus and chorioallantoic tissues. Expression of *IL6* mRNA was mainly localized to endometrial epithelial and stromal cells and chorioallantoic tissues, while *IL6R* and *GP130* mRNAs were localized to glandular epithelial cells during pregnancy. The expression of *IL6* mRNA was decreased by estrogen and progesterone treatment, whereas increasing doses of IL1 $\beta$  induced the expression of *IL6* mRNA, but not *IL6R* and *GP130* mRNAs, in endometrial tissue explants. These results indicate that expression of *IL6* and its receptors at the feto-maternal interface is regulated in a stage- and cell-type-specific manner during pregnancy, suggesting that IL6 and its receptor signaling system may play an important role in the maintenance of pregnancy in pigs.

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## 1. Introduction

Interleukin 6 (IL6) is a multifunctional cytokine that plays important roles in the inflammatory response by recruiting neutrophils and other leukocytes into the inflammatory sites and in adaptive immunity by stimulating proliferation and activation of T cells and differentiation of B cells. It also affects many other biological processes such as metabolism, hematopoiesis, angiogenesis, and reproduction [1,2]. Expression of IL6 has been detected in various cell types, including lymphocyte, macrophage, dendritic cell, keratinocyte, fibroblast, and epithelial cells [3]. The IL6 receptor is composed of two subunits, a ligand-binding IL6 receptor (IL6R) and signal transducing subunit gp130 (GP130) [1]. IL6 activates the JAK (janus kinase) and STAT (signal transducers and

activators of transcription) signaling pathways in the cells by binding to IL6R and GP130 [1].

Expression and function of the IL6 and IL6 receptor system in the female reproductive tract and in pregnancy have been reported in many species [4]. In mice, *Il6* is expressed in the uterus during the estrous cycle and pregnancy, and its expression is mainly localized to endometrial luminal (LE) and glandular epithelial (GE) cells and stromal fibroblast cells [5,6]. *Il6r* is expressed primarily in endometrial LE and GE cells during the estrous cycle, while its expression is expanded to decidual stroma by Day 5 of pregnancy in mice [7]. In humans, IL6 is expressed in the uterine endometrium during the menstrual cycle and pregnancy, and endometrial IL6 expression in epithelial cells and stroma increases during menstruation and at the time of implantation [8,9]. IL6R and GP130 proteins are detected mainly in endometrial GE cells and weakly in the stroma during the menstrual cycle [8]. IL6 expression in pre-implantation embryos has also been shown in a number of species,

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including humans, cows, sheep, and mice [10–12]. The expression of *IL6* in placenta is highest on Day 13 of pregnancy in mice [5].

In mice, *IL6* expression is highest during the proestrus phase of the estrous cycle and is induced by administration of ovarian steroid hormones, estrogen and progesterone, in ovariectomized mice [6,13]. Additionally, it has been shown that estrogen inhibits *IL6* production in endometrial epithelial and stromal cells in mice, while *IL1A* stimulates stromal *IL6* production [14]. In humans, *IL6* is expressed in the uterine LE cells and stroma, with the highest level during the peri-implantation period [8,9,15]. Soluble gp130 (sgp130) increases significantly in the endometrium during the mid-secretory phase, while *IL6R* expression does not change during the menstrual cycle [16].

*IL6* regulates proliferation and differentiation of T cells and their production of cytokines [1]. *IL6* is important in pregnancy because *IL6* expression is elevated in unexplained infertility, recurrent miscarriage, preeclampsia, and preterm delivery in humans [2]. *IL6*-null mice develop normally, but they fail to demonstrate immune and acute phase responses after tissue damage or infections to virus and bacteria [17]. In addition, *IL6*-null mice demonstrate elevated fetal resorption and delayed parturition [18]. However, the mechanism of action of *IL6* in pregnancy is not fully understood.

In pigs, expression of *IL6* and its receptors *IL6R* and *GP130* has been reported in the uterine endometrium and peri-implantation conceptuses during early pregnancy [11,19–21]. *IL6* increases endometrial estradiol and prostaglandin (PG)  $F_{2\alpha}$  secretion in vitro [22,23], suggesting that *IL6* has a role in establishing pregnancy in pigs. However, the steady-state levels of *IL6* and its receptor and the mechanism regulating their expression in the uterine endometrium during the estrous cycle and pregnancy are not fully understood.

Therefore, this study evaluated 1) the expression of *IL6* mRNA and its receptors *IL6R* and *GP130* mRNAs in the uterine endometrium during the estrous cycle and pregnancy and in early stage conceptus and chorioallantoic tissues during pregnancy; 2) localization of mRNAs for *IL6* and its receptors in the uterine endometrium; and 3) the effects of sex steroids and *IL1B* on expression of *IL6* and its receptors in the uterine endometria of pigs.

## 2. Materials and methods

### 2.1. Animals and tissue preparation

All experimental procedures involving animals were conducted in accordance with the Guide for Care and Use of Research Animals in Teaching and Research and were approved by the Institutional

Animal Care and Use Committee of Yonsei University. Sexually mature crossbred female gilts were assigned randomly to either a cyclic or pregnant status. Gilts were observed daily for estrous behavior and were artificially inseminated with fresh boar semen at the onset of estrus (Day 0) and 24 h later. The reproductive tracts of gilts were obtained immediately after they were sacrificed at a local slaughterhouse on either Day 12 or 15 of the estrous cycle or on Days 12, 15, 30, 60, 90, or 114 of pregnancy ( $n = 3–6$  gilts/day/status). Pregnancy was confirmed by the presence of apparently normal filamentous conceptuses in uterine flushings on Days 12 and 15 and presence of embryos and placenta in later days of pregnancy. Chorioallantoic tissues were obtained at Days 30, 60, 90, and 114 of pregnancy ( $n = 3–4$ /day).

Endometrium, dissected free of myometrium, was collected from the middle portion of each uterine horn, snap-frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  prior to RNA extraction. For in situ hybridization, cross-sections of endometrium were fixed in 4% paraformaldehyde in PBS (pH 7.4) for 24 h and then embedded in paraffin as previously described [24].

### 2.2. Total RNA extraction and cloning of porcine *IL6*, *IL6R*, and *GP130* cDNAs

Total RNA was extracted from endometrial, conceptus, and chorioallantoic tissues using TRIzol reagent (Invitrogen Life Technology, Carlsbad, CA) according to the manufacturer's recommendations. The quantity of RNA was assessed spectrophotometrically, and the integrity of RNA was validated following electrophoresis in 1% agarose gel.

Four micrograms of total RNA were treated with DNase I (Promega, Madison, WI) and reverse transcribed using SuperScript II Reverse Transcriptase (Invitrogen) to obtain cDNA. The cDNA templates were then diluted 1:4 with sterile water and amplified by PCR using Taq polymerase (Takara Bio, Shiga, Japan). The PCR conditions for *IL6*, *IL6R*, and *GP130* and the sequences of the primer pairs are listed in Table 1. The PCR products were separated on 2% agarose gel and visualized by ethidium bromide staining. The identity of each amplified PCR product was verified by sequence analysis after cloning into the pCRII vector (Invitrogen).

### 2.3. Quantitative real-time RT-PCR

To analyze the expression of mRNAs for *IL6*, *IL6R*, and *GP130* in the uterine endometrium and chorioallantoic tissues, real-time RT-PCR was performed using the Applied Biosystems StepOnePlus System (Applied Biosystems, Foster City, CA) using the SYBR Green

**Table 1**  
Summary of PCR primer sequences and expected product sizes.

Primer	Sequence of forward (F) and reverse (R) primers (5' → 3')	Annealing temperature ( $^{\circ}\text{C}$ )	Product size (bp)	No. of cycles	GenBank accession no.
For In situ Hybridization					
<i>IL6</i>	F: AGC AAG GAG GTA CTG GCA GA R: ATT ATC CGA ATG GCC CTC AG	60	395	40	NM_214399.1
<i>IL6R</i>	F: AAG GCC GTG TTA CTG GTG AG R: TGG TCT GTG AAA CCA GCA AC	60	659	40	NM_214403.1
<i>GP130</i>	F: GGA ATC CGA ATC ATT TCA GG R: ACC AGA AAC TTG GTG CCT TG	60	652	40	EF151500.1
For RT-PCR and real-time RT-PCR					
<i>IL6</i>	F: AGC AAG GAG GTA CTG GCA GA R: CAG GGT CTG GAT CAG TGC TT	60	222	40	NM_214399.1
<i>IL6R</i>	F: AAG GCC GTG TTA CTG GTG AG R: GAC CGT GAT GTT GAC AGG TG	60	240	40	NM_214403.1
<i>GP130</i>	F: TTG GAA CCA GAT TCC TCC TG R: ACC AGA AAC TTG GTG CCT TG	60	197	40	EF151500.1
<i>RPL7</i>	F: AAG CCA AGC ACT ATC ACA AGG AAT ACA R: TGC AAC ACC TTT CTG ACC TTT GG	60	172	40	NM_001113217

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