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Pro-inflammatory Cytokines and Their Receptors: Expression and Regulation in the Uterine Endometrium during the Estrous Cycle in Pigs†

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ABSTRACT

Pro-inflammatory cytokines, interleukin-1β (IL1B), IL6, and tumor necrosis factor-alpha (TNF), are known to play important roles in regulating the endometrial function in the uterus during the estrous cycle and pregnancy in several species. However, the expression and function of these cytokines and their receptors in the uterine endometrium during the estrous cycle have not been studied in pigs. Thus, this study determined the expression and regulation of *IL1B*, *IL6*, *TNF* and their respective receptors, *IL1R1*, *IL1RAP*, *IL6R*, *GP130*, *TNFRSF1A*, and *TNFRSF1B* during the estrous cycle in pigs. To analyze levels of each gene expression in the uterine endometrium we obtained from endometrial tissues on Days 0, 3, 6, 9, 12, 15, and 18 of the estrous cycle. Real-time RT-PCR analysis showed that levels of *IL1B*, *IL1RAP*, *IL6R*, *GP130*, *TNF*, *TNFRSF1A*, and *TNFRSF1B* mRNAs were highest on Day 15 or 18 of the estrous cycle, which corresponds to the proestrus period. Levels of *IL1R1* were highest on Day 0, while levels of *IL6* were biphasic with high levels on Day 6 and Day 15. The abundance of *IL1B*, *IL6*, *IL6R*, and *TNF* mRNAs was decreased by progesterone, while levels of *GP130* were increased by progesterone in endometrial tissue explants. These results showed that expression of pro-inflammatory cytokines and their receptors changed stage-specifically during the estrous cycle and regulated by progesterone in the uterine endometrium in pigs, suggesting that these pro-inflammatory cytokines may be involved in the regulation endometrial function during the estrous cycle in pigs.

(Key words: pig, estrous cycle, pro-inflammatory cytokine)

INTRODUCTION

Pro-inflammatory cytokines interleukin-1β (IL1B), IL6, and tumor necrosis factor-α (TNF) play important roles in various physiological processes, including inflammation, immunity, metabolism, hematopoiesis, angiogenesis, and reproduction (Hunt *et al.,,* 1997; Hunter and Jones 2015; Prins *et al.,,* 2012). It has been shown that these cytokines and their receptors are expressed in various cell types, including leukocyte, keratinocyte, fibroblast, and epithelial cell (Hirano *et al.,,* 1990). The expression and function of these pro-inflammatory cytokines in the uterus during the reproductive cycle and pregnancy and in conceptus during pregnancy have been studied in several species.

In primates, IL1B is produced by blastocyst and acts on uterine receptivity (Simon *et al.,* 1997; Simon *et al.,* 1998). Mouse blastocysts express *Il1b* and *Il1r1* during early pregnancy (Choudhuri and Wood 1993). In pigs, the implanting conceptuses express IL1B2 (Mathew *et al.,* 2015), which is suggested to play important roles in regulation of gene expression associated with prostaglandin (PG) synthesis and transport (Seo *et al.,* 2012; Seo *et al.,* 2014). The receptor for IL1B is composed of IL1 receptor type 1 (IL1R1), IL1R2, and IL1 receptor accessory protein (IL1RAP) (Subramaniam *et al.,* 2004). By binding to IL1RAP, IL1R1 transduces the signal intracellularly, while IL1R2 is a decoy receptor which is not involved in signal transduction (Subramaniam *et al.,* 2004). Although the expression and function of IL1B and its receptors in the uterus during pregnancy have been studied in pigs (Ross *et al.,* 2003; Seo *et al.,* 2012; Seo *et al.,* 2014), the expression and function of IL1B and its receptor family in the uterine endometrium during the estrous cycle have not been well determined in pigs.

IL6 and its receptors IL6R and glycoprotein 130 (GP130)

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are expressed in the uterine endometrium and placental tissues in several species (De *et al.,* 1992; Diehl and Rincon 2002; Hondo *et al.,* 2005; Lim *et al.,* 1998; Tabibzadeh *et al.,* 1995). In the human uterine endometrium decreased levels of IL6 are associated with infertility, suggesting the importance of IL6 during pregnancy (Laird *et al.,* 2000). In mice, Il6 is detected in non-pregnant uterus and its expression is regulated by steroid hormones (Jacobs *et al.,* 1992). In cows, secretion of IL6 has been shown in endometrial epithelial cells (Healy *et al.,* 2015). Expression of IL6 and its receptors is detected in uterine endometrium and conceptus during early pregnancy in pigs (Anegon *et al.,* 1994; Blitek *et al.,* 2012; Mathialagan *et al.,* 1992). It has been suggested that IL6 affects trophoblast attachment (Anegon *et al.,* 1994; Modric *et al.,* 2000), and that IL6 and IL1B stimulate secretion of estrogen from the uterine endometrium during the estrous cycle and early pregnancy in pigs (Franczak *et al.,* 2013).

The expression of TNF and its receptors, TNF receptor superfamily 1A (TNFRSF1A) and TNFRSF1B, in the uterine endometrium and placentas has also been shown in some species (Correia-Alvarez *et al.,* 2015; Tabibzadeh *et al.,* 1995; Woodward *et al.,* 2013). In humans and rats, TNF is expressed in glandular epithelia and decidualized stromal cells (Chen *et al.,* 1991; Yelavarthi *et al.,* 1991). Uterine neutrophils produce IL1B and TNF in response to lipopolysaccharide treatment, resulting in placental hemorrhage and fetal death in mice (Zhao *et al.,* 2015). In pigs, TNF and other pro-inflammatory cytokines including IL1B and IL6 stimulate secretion of $PGF_2\alpha$ and PGE_2 from chorioamniotic membrane during mid-pregnancy (Jana *et al.,* 2008), indicating that pro-inflammatory cytokines may have a role in placentation and fetal development. However, the role of TNF and its receptors in the uterine endometrium during the estrous cycle is not clearly understood in pigs.

The expression and function of IL1B, IL6, and TNF in the uterine endometrium under the normal and disease conditions have been reported in many species, but the precise role of these cytokines in the uterine endometrium during the estrous cycle has not been well understood. Thus, to initiate the study on the role of IL1B, IL6, and TNF in the uterine endometrium during the estrous cycle in pigs, this study determined the expression of pro-inflammatory cytokine IL1B, IL6, TNF and their receptors in the uterine endometrium during the estrous cycle and the effects of ovarian steroid hormones on endometrial expression of these molecules in pigs.

MATERIALS and METHODS

1. Animals and Tissue Preparation

All the experimental procedures involving animals were conducted in accordance with the Guide for Care and Use of Research Animals in Teaching and Research and approved by the Institutional Animal Care and Use Committee of National Institute of Animal Science. Sexually mature crossbred female gilts were assigned randomly to cyclic status. Endometrial tissues were obtained immediately after slaughter on Days 0, 3, 6, 9, 12, 15, or 18 (n = 3-4/day) of the estrous cycle. Endometrium, dissected free of myometrium, was collected from two different areas of the middle portion of each uterine horn, snap-frozen in liquid nitrogen, and stored at - 80°C for RNA extraction.

2. Explant Cultures

Endometrium of sexually immature female gilts from the local slaughterhouse was dissected from the myometrium and placed into warm phenol red-free Dulbecco modified Eagle medium/F-12 culture medium (DMEM/F-12; Sigma, St. Louis, MO) containing penicillin G (100 IU/ml) and streptomycin (0.1 mg/ml) as described previously (Ka *et al.,* 2001), with some modifications. The endometrium was minced with scalpel blades into small pieces (2-3 mm^3), and aliquots of 500 mg were placed into T25 flasks with serum-free modified DMEM/F-12 containing 10 μg/ml insulin (Sigma), 10 ng/ml transferrin (Sigma), and 10 ng/ml hydrocortisone (Sigma). To determine the effects of steroid hormones on expression of endometrial genes, explant tissues were treated with 0, 5, 50, 500, or 5000 pg/ml estradiol-17β (E_2; Sigma) or 0, 0.3, 3, 30 or 300 ng/ml progesterone (P_4; Sigma) for 24 h with rocking in an atmosphere of 5% CO_2 in air at 37°C. Explant tissues were then harvested and total RNA was extracted for real-time RT-PCR analysis to determine expression levels for *IL1B, IL6, TNF* and their receptor mRNAs. These experiments were conducted using endometria from eight immature gilts.

3. Total RNA Extraction and RT-PCR for IL1B, IL6, TNF and Their Receptors cDNAs

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

Four micrograms of total RNA from endometrial tissues were treated with DNase I (Promega, Madison, WI) and reverse transcribed using SuperScript II Reverse Transcriptase (Invitrogen) to obtain cDNAs. The cDNA templates were then diluted 1:4 with nuclease-free water and amplified by PCR using Taq polymerase (Takara Bio, Shiga, Japan), and specific primers based on porcine *IL1B, IL6, TNF* and their receptor mRNA sequences. The PCR conditions, sequences of primer pairs for *IL1B, IL6, TNF* and their receptors and expected product sizes 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).

4. Quantitative Real-time RT-PCR

To analyze levels of *IL1B*, *IL6*, *TNF* and their receptor mRNAs in the uterine endometrial tissues, real-time RT-PCR was performed using the Applied Biosystems StepOnePlus System (Applied Biosystems, Foster City, CA) using the SYBR Green method. Complementary DNAs were synthesized from 4 μg total RNA isolated from different uterine endometrial tissues, and newly synthesized cDNAs (total volume of 21 μl) were diluted 1:4 with nuclease-free water and then used for PCR. The Power SYBR Green PCR Master Mix (Applied Biosystems) was used for PCR reactions. The final reaction volume of 20 μl included 2 μl of cDNA, 10 μl of 2X Master mix, 2 μl of each primer (100 nM), and 4 μl of dH_2O. PCR conditions and sequences

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

Primer	Sequence of forward (F) and reverse (R) primers (5' → 3')	Annealing Temperature (°C)	Product size (bp)	No. of cycles	GenBank accession no.
IL1B	F: CAG CCA TGG CCA TAG TAC CT	60	216	40	NM_214055.1
	R: CCA CGA TGA CAG ACA CCA TC				
IL1R1	F: AAT GCA CTT CCT AGG CTT TCT G	60	65	40	XM_013995919.1
	R: GGA ACA GGA TGT GGT GAC AA				
IL1RAP	F: AAA TGC CAA AGG GGA GGT T	60	66	40	XM_005670066.2
	R: TGC TGT GTG CAT CCA TTA CC				
IL6	F: AGC AAG GAG GTA CTG GCA GA	60	222	40	NM_214399.1
	R: CAG GGT CTG GAT CAG TGC TT				
IL6R	F: AAG GCC GTG TTA CTG GTG AG	60	240	40	NM_214403.1
	R: GAC CGT GAT GTT GAC AGG TG				
GP130	F: TTG GAA CCA GAT TCC TCC TG	60	197	40	EF151500.1
	R: ACC AGA AAC TTG GTG CCT TG				
TNF	F: ATC ATC GTC TCA AAC CTC AGA TAA G	60	391	40	JF831365.1
	R: ACT GAG TCG ATC ATC CTT CTC C				
TNFRSF1A	F: AGA GAT AAG GAG TGT GTC TCC TGT G	60	215	40	U19994.1
	R: ATA ATG GAG TAG AGC TTT GGT TTC C				
TNFRSF1B	F: GCC CCT GAA AGA ATA CTA TGA CAC	60	214	40	NM_001097441.2
	R: AGT GCA GGC TTG AGT TTC TAC C				
RPL7	F: AAG CCA AGC ACT ATC ACA AGG AAT ACA	60	172	40	NM_001113217
	R: TGC AAC ACC TTT CTG ACC TTT GG				
UBB	F: GCA TTG TTG GCG GTT TCG	60	65	40	NM_001105309.1
	R: AGA CGC TGT GAA GCC AAT CA				
TBP	F: AAC AGT TCA GTA GTT ATG AGC CAG A	60	153	40	XM_013991786.1
	R: AGA TGT TCT CAA ACG CTT CG				

of primer pairs are listed in Table 1. The results are reported as expression relative to the level detected on Day 12 of the estrous cycle after normalization of the transcript amount to the endogenous *RPL7*, *UBB* and *TBP* control by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

5. Statistical Analysis

Data from real-time RT-PCR for *IL1B*, *IL6*, *TNF* and their receptor expression during the estrous cycle and from explant cultures were subjected to regression analysis using the General Linear Models procedures of SAS (Cary, NC). As sources of variation, the model included day to evaluate steady-state levels of *IL1B*, *IL6*, *TNF* and their receptor mRNAs during the estrous cycle and dose to evaluate effects of steroid hormones in explant cultures. Data are presented as means with SEM. A P value of 0.05 or less was considered significant, whereas a P value of 0.05 to 0.10 was considered a trend toward significance.

RESULTS

1. Expression of IL1B, IL6, TNF and Their Receptors mRNA in the Uterine Endometrium during the Estrous Cycle in Pigs

To determine the expression levels of mRNAs for the *IL1B*, *IL6*, *TNF* and their receptors in the porcine uterine endometrium, we performed real-time RT-PCR analysis. As shown in Fig. 1, the expression of *IL1B* (quadratic effect of day, $P < 0.01$) and *IL1RAP* (linear effect of day, $P < 0.05$) mRNAs, but not *IL1R1* mRNA was changed during the estrous cycle with highest levels at proestrus stage. The expression of *IL6* was not changed during the estrous cycle, but the expression of IL6 receptors *IL6R* and *GP130*

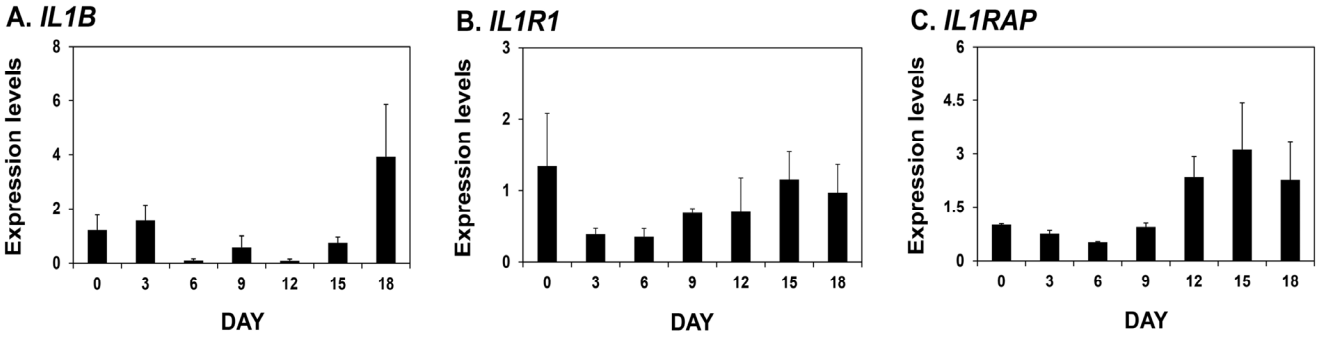

Fig. 1. Expression of *IL1B* (A), *IL1R1* (B) and *IL1RAP* (C) mRNAs in porcine uterine endometria during the estrous cycle. Endometrial tissue samples from cyclic gilts were analyzed by real-time RT-PCR and data are reported as expression relative to that detected on Day 0 of the estrous cycle after normalization of the transcript amount to the endogenous *RPL7*, *UBB* and *TBP* control. Data are presented as means with standard error.

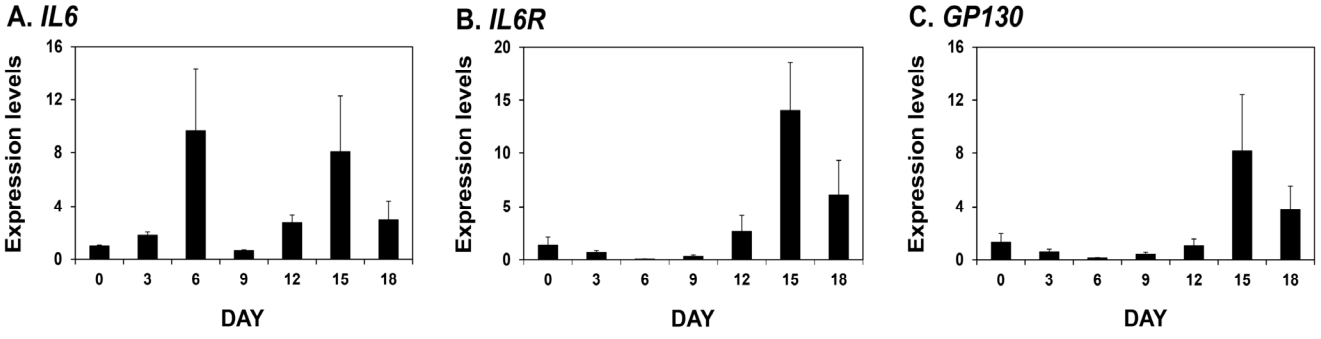

Fig. 2. Expression of *IL6* (A), *IL6R* (B) and *GP130* (C) mRNAs in porcine uterine endometria during the estrous cycle. Endometrial tissue samples from cyclic gilts were analyzed by real-time RT-PCR and data are reported as expression relative to that detected on Day 0 of the estrous cycle after normalization of the transcript amount to the endogenous *RPL7*, *UBB* and *TBP* control. Data are presented as means with standard error.

mRNAs was changed with highest levels on Day 15 of the estrous cycle (linear effect of day, $P < 0.01$ for *IL6R*; $P = 0.051$ for *GP130*) (Fig. 2). Levels of all *TNF* (quadratic effect of day, $P < 0.01$), *TNFRSF1A* (linear effect of day, $P < 0.01$), and *TNFRSF1B* mRNAs (linear effect of day, $P = 0.0647$) changed with the highest levels at proestrus stage of the estrous cycle (Fig. 3).

2. Effects of E_2 and P_4 on IL1B, IL6, TNF and Their Receptors Expression in the Uterine Endometria from Sexually Immature Gilts

Because the expression of most *IL1B*, *IL6*, *TNF* and their receptor mRNAs increased at proestrus of the estrous cycle, we wanted to determine if the steroid hormones, E_2 or P_4, affected the expression of *IL1B*, *IL6*, *TNF* and their receptors in the uterine endometrium. Since the uterine endometrium of mature cycling gilts is under the influence of steroid hormones during the estrous cycle, which cause the changes in the expression patterns of estrogen and progesterone receptors, we took advantage of the explant cultures using uterine endometrial tissues from immature gilts. As shown in Fig. 4, increasing doses of E_2 did not affect the expression of *IL1B, IL6, TNF* and their receptor mRNAs in explant tissues. Interestingly, however, increasing doses of P_4 decreased the levels of *IL1B*, *IL6*, *IL6R*, and *TNF* mRNAs (linear effect of dose, $P < 0.01$ for *IL1B*; $P < 0.05$ for *IL6*, *IL6R*, and *TNF*), whereas increasing doses of P_4 increased *GP130* in endometrial tissue explants (linear effect of dose, $P < 0.05$) (Fig. 5).

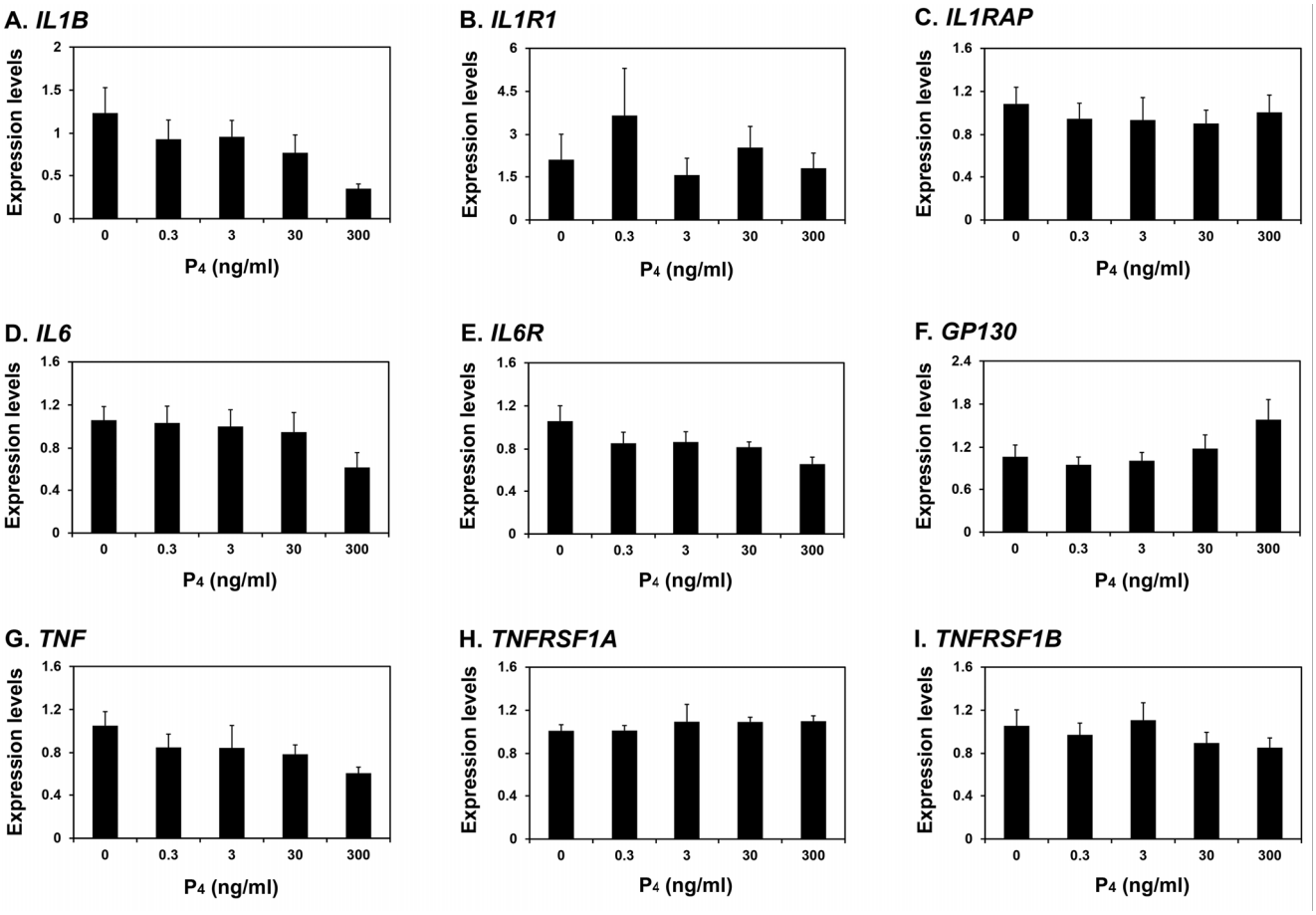

Fig. 5. Effect of progesterone on expression of pro-inflammatory cytokines and their receptors *IL1B* (A), *IL1R1* (B), *IL1RAP* (C), *IL6* (D), *IL6R* (E), *GP130* (F), *TNF* (G), *TNFRSF1A* (H) and *TNFRSF1B* (I) mRNAs in endometrial explant cultures. Endometrial explants from immature gilts were cultured in DMEM/F-12 with increasing doses (0, 0.3, 3, 30, and 300 ng/ml) of at 37°C for 24 h. Experiments were conducted using endometrial tissues from eight immature gilts. Abundance of mRNA expression based on real-time RT-PCR analyses is relative to that for each mRNAs in the control group of endometrial explants after normalization of transcript amounts to *RPL7*, *UBB* and *TBP* mRNA. Data are presented as means with standard errors.

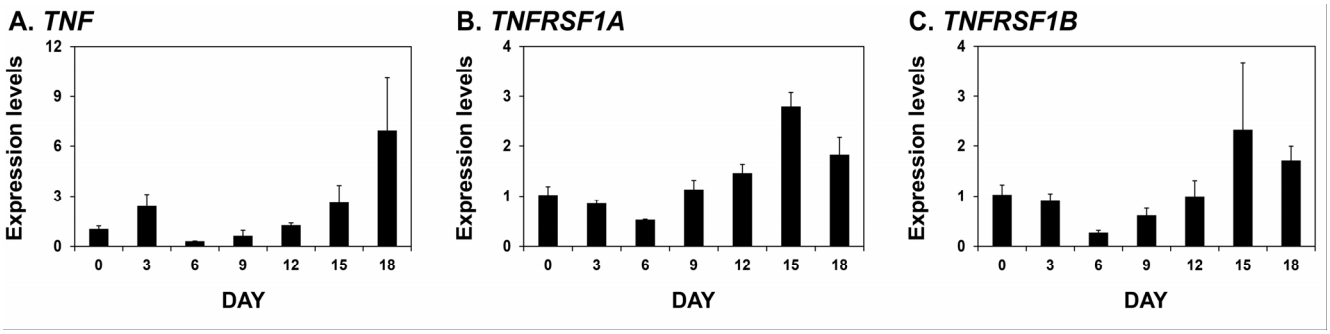

Fig. 3. Expression of *TNF* (A), *TNFRSF1A* (B) and *TNFRSF1B* (C) mRNAs in porcine uterine endometria during the estrous cycle. Endometrial tissue samples from cyclic gilts were analyzed by real-time RT-PCR and data are reported as expression relative to that detected on Day 0 of the estrous cycle after normalization of the transcript amount to the endogenous *RPL7*, *UBB* and *TBP* control. Data are presented as means with standard error.

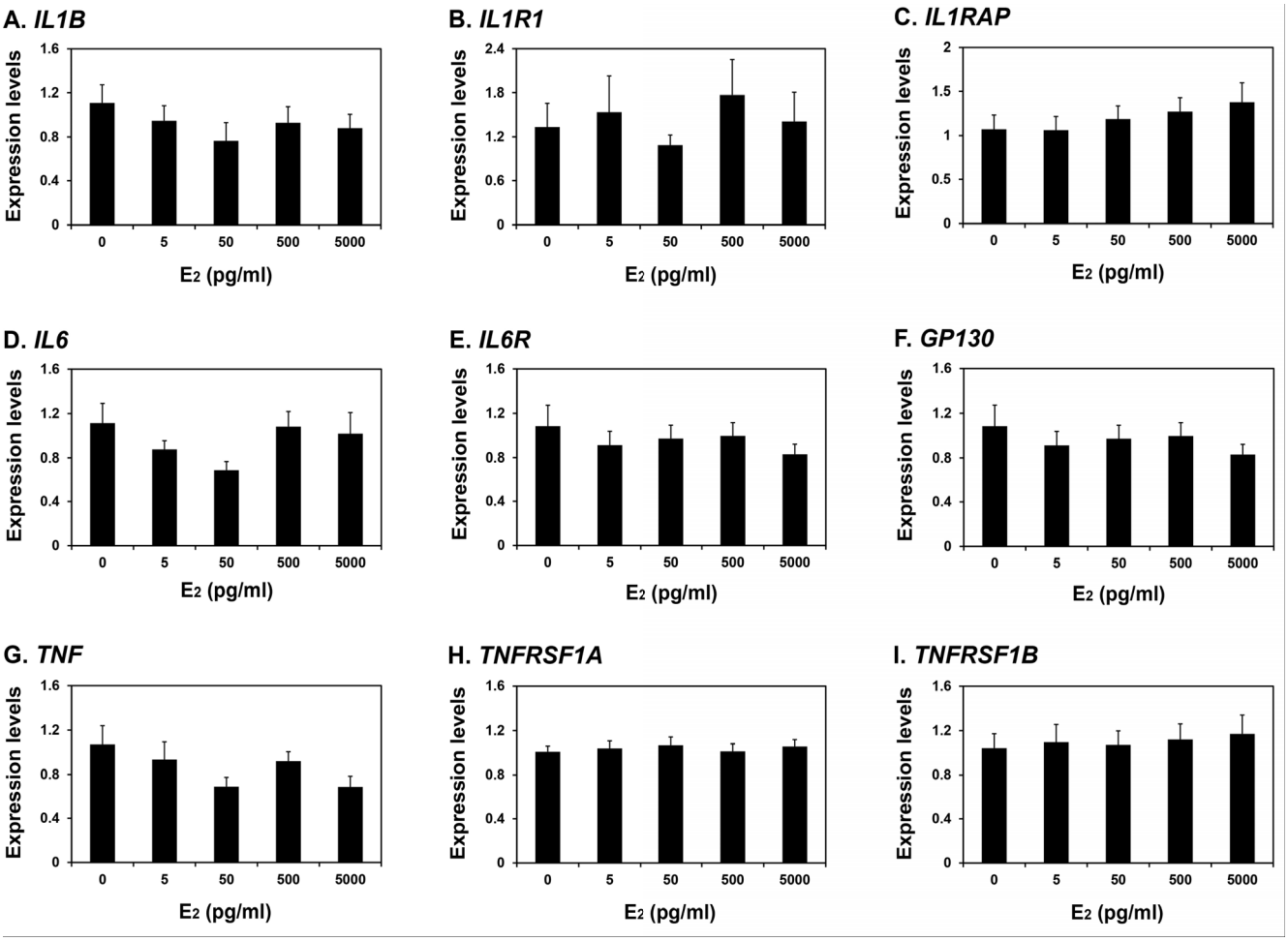

Fig. 4. Effect of estradiol-17β on expression of pro-inflammatory cytokines and their receptors *IL1B* (A), *IL1R1* (B), *IL1RAP* (C), *IL6* (D), *IL6R* (E), *GP130* (F), *TNF* (G), *TNFRSF1A* (H) and *TNFRSF1B* (I) mRNAs in endometrial explant cultures. Endometrial explants from immature gilts were cultured in DMEM/F-12 with increasing doses (0, 5, 50, 500, and 5000 pg/ml) of estradiol-17β (E_2) at 37°C for 24 h. Experiments were conducted using endometrial tissues from eight immature gilts. Abundance of mRNA expression based on real-time RT-PCR analyses is relative to that for each mRNAs in the control group of endometrial explants after normalization of transcript amounts to *RPL7*, *UBB* and *TBP* mRNA. Data are presented as means with standard errors.

DISCUSSION

The significant findings of this study in pigs are that 1) the expression of pro-inflammatory cytokines and their receptors, *IL1B*, *IL1RAP*, *IL6R*, *GP130*, *TNF*, *TNFRSF1A*, and *TNFRSF1B* changes in the uterine endometrium with the highest levels during proestrus of the estrous cycle, and 2) P_4 regulates expression of *IL1B*, *IL6*, *IL6R*, *GP130*, and *TNF* in the uterine endometrial explant tissues.

The uterine endometrium undergoes cyclical change of morphology and function during the estrous cycle, and the change is mediated by various factors including hormones, growth factors, prostaglandins, and cytokines (Clark and Kruger 2016; Gray *et al.,* 2001; Soede *et al.,* 2011). Ovarian hormones regulate various endometrial functions such as gene expression and secretory activity, which, in turn, affect ovarian function. Among many factors known to be involved in regulation of endometrial function during the estrous cycle and pregnancy, pro-inflammatory cytokines IL1B, IL6, and TNF are shown to be expressed in the uterine endometrium in humans (Hunt *et al.,* 1992), mice (Robertson and Seamark 1990), mares (Fumuso *et al.,* 2003; Palm *et al.,* 2008), pigs (Franczak *et al.,* 2013; Jana *et al.,* 2008; Seo *et al.,* 2012). However, the expression and function of these cytokines in the uterine endometrium during the estrous cycle has not been fully understood in pigs.

In this study we evaluated the expression of pro-inflammatory cytokines and their receptors in the uterine endometrium during the estrous cycle in pigs. Result of this study showed that the expression of pro-inflammatory cytokines *IL1B* and *TNF* mRNAs, but not IL6 mRNAs, changed in the uterine endometrium during the estrous cycle with higher levels at proestrus than diestrus. Levels of cytokine receptors *IL1RAP*, *IL6R*, *GP130*, *TNFRSF1A*, and *TNFRSF1B* mRNAs were also highest at proestrus phase during the estrous cycle. Since the endometrial function is regulated by the ovarian steroid hormones during the estrous cycle and there is a transition in steroid hormone dominance between progesterone and estrogen at around Day 15 of pregnancy in pigs (Soede *et al.,* 2011), we postulate that the high levels of pro-inflammatory cytokine expression at proestrus phase are closely related to the levels of steroid hormones. Interestingly, levels of *IL6* mRNAs during the estrous cycle were biphasic with high levels on Day 6 and Day 15 of pregnancy. It has been shown that *IL6* levels in the uterine endometrium are high on Day 16 between Days 10 and 18 of the estrous cycle in pigs (Blitek *et al.,* 2012). This finding suggest that high levels of *IL6* expression is also related to decreased progesterone and increased estrogen levels at proestrus, similar to other pro-inflammatory cytokines and their receptors. The reason why *IL6* levels were increased on Day 6 of the estrous cycle is not clear.

It has been shown that the expression of *IL1B*, *IL6*, *TNF* and their receptors in the uterine endometrium during pregnancy has been shown in several species, including rodents (Hunt *et al.,* 1997; Robertson *et al.,* 1992) and pigs (Blitek *et al.,* 2012; Seo *et al.,* 2012) and indicated that conceptus-derived factors affect the expression of these molecules in the endometrium. It also has been shown that administration of steroid hormone promotes expression of *Il6* in ovariectomized mice (Robertson *et al.,* 1992; Sanford *et al.,* 1992), and TNF mRNA and protein are produced in the murine endometrium by estrogen and progesterone (Roby and Hunt 1994).

In this study, our results showed that the expression of most *IL1B*, *IL6*, *TNF* and their receptors was changed in the uterine endometrium with higher levels at proestrus of the estrous cycle, which corresponds to the follicular phase of the ovarian cycle. The expression pattern of the pro-inflammatory cytokines and their receptors in this study made us to hypothesize that the transition of dominance of steroid hormones estrogen and progesterone during the estrous cycle is a critical factor for expression of the pro-inflammatory cytokines and their receptors. Thus, we took advantage of the explant cultures using uterine endometrial tissues from immature gilts to examine the effect of steroid hormones on cytokine expression and found that increasing doses of P_4 decreased the levels of *IL1B*, *IL6*, *IL6R*, and *TNF* mRNAs and increased *GP130* mRNA levels. However, estrogen did not have any effect on *IL1B*, *IL6*, *TNF* and their receptors in explant cultures. These data suggest that the endometrial expression of *IL1B*, *IL6*, *IL6R*, and *TNF* during the estrous cycle is regulated by progesterone rather than estrogen; during metestrus to diestrus, the progesterone-dominant period, progesterone down-regulates *IL1B*, *IL6*, *IL6R*, and *TNF* expression, while progesterone-mediated down-regulation of *IL1B*, *IL6*, *IL6R*, and *TNF* expression during late diestrus to proestrus is released due to the decreased levels of

progesterone during this period. In pigs, infiltration and distribution of leukocytes in the uterine endometrium change during the estrous cycle depending on the levels of steroid hormones with the high number of lymphocytes and macrophages at diestrus and neutrophils at proestrus (Kaeoket *et al.,* 2002). Since that those immune cells produce pro-inflammatory cytokines or affect cytokine production in other cell types, it is possible that the immune cells may affect endometrial expression of pro-inflammatory cytokines.

Even though endometrial expression of *IL1RAP* were not affected by estrogen, our previous study has shown that estrogen increased *IL1RAP* in the uterine endometrial tissues derived from Day 12 of the estrous cycle (Seo *et al.,* 2012), suggesting that endometrial responsiveness to estrogen may different depending on maturity of gilt and the stage of estrous cycle. In spite that levels of *GP130* mRNA were highest on Day 15 of pregnancy during the estrous cycle, the reason why *GP130* is increased by the increasing doses of progesterone is not clear. Also, the mechanism regulating other cytokine and their receptor expression in the endometrium during the cycle is yet to be clarified.

It has been reported that IL1B and TNF act on the process of implantation and cervical ripening and dilation at term in human (Fumuso *et al.,* 2003; Kelly 2002). In rodents, IL6 induces endometrial production of PGE_2 and $PGF_{2\alpha}$ and myometrial expression of PTGFR ($PGF_{2\alpha}$ receptor) and OXTR (oxytocin receptor) to regulate the timing of parturition by in the uterus (Prins *et al.,* 2012; Robertson *et al.,* 2010). IL6 also induces endometrial E_2 and $PGF_{2\alpha}$ production during early pregnancy and attachment and proliferation of trophoblast cells in vitro in pigs (Blitek *et al.,* 2012; Franczak *et al.,* 2012). TNF family members expressed at the maternal-fetal interface induce apoptosis, syncytialization, and trophoblast proliferation and invasion in normal conditions, and are also involved in recurrent spontaneous abortion, preterm labor, pre-eclampsia, and intrauterine growth restriction in humans (Haider and Knofler 2009).

Although the expression and function of pro-inflammatory cytokines at the maternal-fetal interface have been reported, their function in the uterine endometrium during the estrous cycle is very limited. In this study, the finding that several pro-inflammatory cytokines and their receptor were highly expressed in the uterine endometrium at late diestrus and proestrus indicates that these cytokines may play a critical role in endometrial remodeling during this period. In primates, dramatic endometrial remodeling occurs at menstruation and is associated with TNF. Although the dramatic change as observed in primates is not evident in the uterine endometrium during the cycle in pigs, it is well known that there are morphological and functional alterations of endometrial cells during the estrous cycle in pigs (Spencer *et al.,* 1993; Tarleton *et al.,* 1999), and this process may be related with the expression of pro-inflammatory cytokines. It is also possible that these pro-inflammatory cytokines are directly involved in endometrial production of PG synthesis for luteolysis. However, detailed function of pro-inflammatory cytokines during the estrous cycle in pigs needs to be further investigated.

In conclusion, this study showed that pro-inflammatory cytokine IL1B, IL6, TNF, and their receptors are expressed in the uterine endometrium with high levels during proestrus of the estrous cycle and progesterone decreases the expression of some of cytokines in pigs. These results indicate that the expression of endometrial pro-inflammatory cytokines is regulated depending the stage of the estrous cycle and pro-inflammatory cytokines may play an important role in regulation of endometrial function during the estrous cycle in pigs.

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