Analysis of Lysophosphatidic Acid Receptor 1 Expression in the Uterus during the Estrous Cycle and Pregnancy in Pigs

Heewon Seo, Mingoo Kim, Yohan Choi and Hakhyun Ka†

Division of Biological Science and Technology, and Institute of Biomaterials, Yonsei University, Wonju 220-710, Korea

ABSTRACT

Lysophosphatidic acid (LPA), a simple phospholipid-derived mediator implicated in diverse biological actions, acts through the specific G-protein coupled receptors, LPA receptor (LPAR) 1~5. Our previous study showed that LPAR3 is expressed in the uterine endometrium in a cell type- and stage-specific manner and LPA via LPAR3 increases *PTGS2* expression in the uterine endometrium during the period of implantation. Although LPAR3 is considered to be predominant LPA receptor in the uterine endometrium, other LPA receptors might play a role to mediate LPA functions in the uterine endometrium during pregnancy. Among LPARs, we investigated expression of LPAR1 during the estrous cycle and pregnancy in this study. Uterine endometrial tissue samples were collected from day (D) 12 and D15 of the estrous cycle and from D12, D15, D30, D60, D90 and D114 of pregnancy. Northern blot analysis determined that *LPAR1* mRNA was constitutively expressed in the uterine endometrial tissues during the estrous cycle and pregnancy of all stages. Analysis by immunoblotting revealed that LPAR1 proteins were present in the porcine uterine endometrium during the estrous cycle and pregnancy. Immunohistochemical experiments demonstrated that LPAR1 protein was localized to endometrial epithelium and stromal cell, specifically to nuclei of these cell types. Results in this study show that LPAR1 is constitutively expressed in the uterine endometrium during the estrous cycle and pregnancy. These results suggest that LPA via LPAR1 may play a role in the uterine endometrial function throughout pregnancy in pigs.

(Key words : Pig, Uterus, Pregnancy, Lysophosphatidic acid, LPAR1)

INTRODUCTION

Implantation for the establishment of pregnancy is a highly coordinated interaction between the apical plasma membranes of the conceptus trophectoderm and the uterine luminal epithelium to form placenta of an epitheliochoral type in pigs (Bowen and Burghardt, 2000). The process is regulated by the steroid hormones, progesterone (P4) and estrogen (E), as well as many other factors such as adhesion molecules, growth factors and cytokines (Bazer *et al.*, 1998; Burghardt *et al.*, 1997). Importance of steroid hormones and prostaglandins in the process of implantation and maternal recognition of pregnancy has been well studied, but the involvement of other lipid mediators in the processes of implantation and maternal recognition of pregnancy is poorly understood. Lysophospholipids (LPs), including lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), has recently been identified as a potentially important factor during pregnancy (Ye, 2008).

LPA is a simple phospholipid composed of a glycerol backbone with a fatty acyl chain and a free phosphate group. There are at least five specific receptors activated by LPA: the G protein-coupled receptors (GPCRs) designated as LPAR1-5. Through these receptors, LPA elicits many growth factor-like biological effects, such as cell proliferation, survival, migration, differentiation, and aggregation in various cell types. Our previous study indicated that LPAR3 is the predominant LPA receptor in the uterine endometrium and plays an important role in implantation, while LPAR1, LPAR2, and LPAR4 are not critical for embryo implantation because of their low levels of expression in the uterine endometrium (Seo *et al.*, 2008).

LPA levels were shown to increase during pregnancy in human (Tokumura *et al.*, 2002). Our preliminary observations indicated that ENPP2, an enzyme with lysophospholipase D (lysoPLD) activity, is expressed in the uterine endometrial tissues throughout pregnancy in pigs (Seo and Ka, unpublished data). These suggest that LPA might present and function in the uterine endometrium throughout gestation. There are many structurally diverse forms of LPA due to it's a fatty acid of varied length and saturation, and LPA

* This work was supported by the BioGreen 21 Program (#20070301034040, #20080401034003), Rural Development Administration, Republic of Korea.

† Corresponding author : Phone: +82-33-760-2369, E-mail: hka@yonsei.ac.kr

can bind to different receptor according to LPA form (Bandoh *et al.*, 2000). Although LPAR3 is considered as the predominant LPA receptor in the uterine endometrium, it is possible that other LPA receptors, including LPAR1, LPAR2 and LPAR4, may play a role to mediate LPA functions in the uterine endometrium during pregnancy. Among LPAR1, LPAR2, and LPAR4, we determined to study role of LPAR1 in the uterine endometrium in pig. To initiate the study for LPAR1, we investigated expression of LPAR1 in the uterine endometirum during the estrous cycle and pregnancy in pigs.

MATERIALS AND METHODS

Animals and Tissue Collection

All experimental procedures involving animals were conducted in accordance with the National Research Council publication *Guide for the Care and Use of Laboratory Animals* (National Academy of Science, 1996). Sexually mature crossbred female pigs were assigned randomly to either cyclic or pregnant status. Twenty-four gilts were hysterectomized on day (D) 12 and D15 of the estrous cycle and D12, D15, D30, D60, D90, or D114 of pregnancy (n=3 gilts/day/status). Pregnancy was confirmed by the presence of apparently normal conceptuses in uterine flushings or fetuses. Endometrium dissected from myometrium was collected from the middle portion of the uterine horn. Endometrial tissues were snap-frozen in liquid nitrogen and stored at −80℃ for RNA and protein extraction. For *in situ* hybridization and immunohistochemistry, cross-sections of endometrium and conceptuses were fixed in 4% paraformaldehyde in PBS (pH 7.4) for 24 h and then embedded in paraffin, as previously described (Ka *et al.*, 2000).

Total RNA Extraction and Northern Blot Analysis

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

Total RNA (20 μg) was loaded in each lane and electrophoresed on 1% 3-(N-morpholino) propanesulfonic acid-formaldehyde agarose gel. RNA was transferred overnight onto a nylon membrane in 20X SSC. The RNA probes for *LPAR1* were labeled with digoxigenin (DIG)-UTP using a DIG RNA Labeling kit (Roche, Indianapolis, IN). After transfer, the RNA was fixed to the blot by UV-cross linking (120 mJ). Prehybridization and hybridization was carried out at 68 ℃ using DIG Easy Hyb hybridization reagent (Roche). The blot was washed twice in low stringency buffer (2X SSC and 0.1% SDS) for 5 min each at room temperature. The blot was then washed twice in high stringency buffer (0.1X SSC and 0.1% SDS) for 15 min each at 68℃. After all washes, mRNA species were detected by the alkaline phosphatase reaction (Roche) and exposure to X-ray film (Agfa-Gevaert, Mortsel, Belgium). The optical density of the *LPAR1* and 18S rRNA bands was quantified by scanning densitometry using HP1210 (HP, Seoul, Korea) and GelPro Analyzer software (Media Cybernetics, Silver Spring, MD). Values are presented as the ratio of the *LPAR1*-integrated optical density to the corresponding 18S rRNA-integrated optical density.

Protein Isolation and Western Blot Analysis

Endometrial tissues were homogenized in lysis buffer (1% Triton X-100, 0.5% Nonidet P-40, 150 mM NaCl, 10 mM Tris, 1 mM EDTA, 0.2 mM Na_3VO_3, 0.2 M PMSF, and 0.5 μg/ml NaF) at a ratio of 100 mg tissue per 1 ml buffer, and the cellular debris was removed by centrifugation (16,500×g for 5 min). The concentration of proteins in lysates was determined using a Bradford protein assay (Bio-Rad Laboratories, Richmond, CA) with BSA as the standard. Proteins (20 μg) were loaded in each lane and electrophoresed on 12% SDS-PAGE gels followed by electrotransfer onto nitrocellulose membranes. Nonspecific binding was blocked with 5% (w/v) fat-free milk in TBST buffer (Tris buffered saline with 0.1% Tween-20) for 1 h at room temperature. The blot was incubated overnight at 4℃ with 0.2 μg/ml rabbit polyclonal anti-LPAR1 antibody (GeneTex, San Antonio, TX; Catalog no. GTX16217) diluted with 2% milk/TBST. The blot was washed three times for 10 min in TBST at room temperature, incubated with peroxidase-conjugated goat anti-rabbit secondary antibody (1:20,000; Jackson Laboratories, West Grove, PA) for 1 h at room temperature, and rinsed again for 30 min at room temperature with TBST. Immunoreactive proteins were detected by chemiluminescence (SuperSignal West Pico, Pierce Chemical Co, Rockford, IL) according to the manufacturer's recommendations using X-ray films (Agfa-Gevaert). Blot was reblotted with rabbit polyclonal anti-ACTB antibody (1:5,000; Sigma) to assess consistent loading.

Immunohistochemical Analysis

To determine which type of cells in porcine endometrium express LPAR1 proteins, immunohistochemistry was applied. Sections (5 μm) were deparaffinized and rehydrated in an alcohol gradient. For antigen retrieval, tissue sections were boiled in citrate buffer (pH 6.0) for 10 min. Tissue sections were washed with PBST (PBS with 0.1% Tween-20) three times and the peroxidase block was performed with 0.5% H_2O_2 in methanol for 30 min. Tissue sections were then blocked with 10% normal goat serum for 30 min at room temperature. One microgram per milliliter of

rabbit polyclonal anti-LPAR1 antibody (GeneTex; catalog no. GTX13027) was added and incubated overnight at 4℃ in a humidified chamber. For each tissue tested, normal rabbit IgG was substituted for primary antibody and served as a negative control. Tissue sections were washed with PBST three times. The biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA) was added and incubated for 1 h at room temperature. Following washes with PBST, the steptavidin peroxidase conjugate (Zymed, San Francisco, CA) was added to the tissue sections and tissue sections were incubated for 10 min at room temperature. The sections were washed with PBST and the 3-amino-9-ethylcarbozole in N,N-dimethylformamide (AEC) color development substrate (Zymed, San Francisco, CA) was added to the tissue sections, which were then incubated for 10 min at room temperature. The tissue sections were washed in water, counterstained with Mayer's hematoxylin, and coverslipped.

Statistical Analysis

Densitometry data from Northern blot for *LPAR1* mRNA expression were subjected to least squares ANOVA using the General Linear Models procedures of SAS (Cary, NC). As sources of variation, the model included day, pregnancy status (cyclic or pregnant), and their interactions to evaluate the steady-state level of *LPAR1* mRNA. Data are presented as least squares means with standard error.

RESULTS

Levels of *LPAR1* mRNA in the Uterine Endometrium during the Estrous Cycle and Pregnancy in Pigs

To determine whether *LPAR1* is expressed by the porcine uterine endometrium, we cloned a 496 bp partial *LPAR1* cDNA by RT-PCR (Seo *et al.*, 2008). Northern blot hybridization analysis using this partial cDNA detected *LPAR1* mRNA as a single transcript in total endometrial RNA from cyclic and pregnant pigs (Fig. 1A). Steady-state levels of *LPAR1* mRNA were not significantly changed by pregnancy status ($p>0.05$). During pregnancy, steady-state levels of *LPAR1* mRNA were changed by day of pregnancy ($p<0.05$), with high levels from D12 to D30 of pregnancy, and low thereafter.

Levels of LPAR1 Proteins in the Uterine Endometrium during the Estrous Cycle and Pregnancy in Pigs

Having determined that *LPAR1* mRNA was detected in the uterine endometrium in a stage-specific manner,

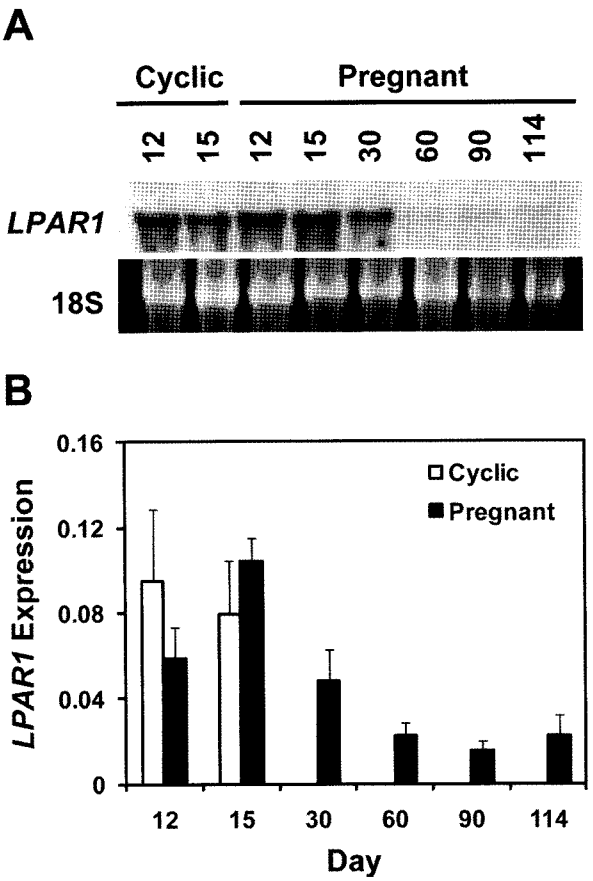

Fig. 1. Analysis of *LPAR1* mRNA levels in the uterine endometrium by Northern blot (A) and densitometric analyses (B) during the estrous cycle and pregnancy in pigs. Endometrial tissue samples from cyclic and pregnant gilts (n=3 per day) were tested. During pregnancy, steady-state levels of *LPAR1* mRNA were high from D12 to D30, and then remained low from D60 to D114. Data are presented as least squares means relative units with standard error.

we then examined protein expression. Immunoblot analysis detected a single band of LPAR1 protein, with a relative molecular mass of 40,000, which is similar in size to that in sheep (Liszewska *et al.*, 2009), during the estrous cycle and pregnancy (Fig. 2). Similarly to levels of *LPAR1* mRNA, LPAR1 protein was constitutively expressed in the uterine endometria from the estrous cycle and pregnancy of all stages.

Localization of LPAR1 Protein in the Porcine Uterine Endometrium during the Estrous Cycle and Pregnancy and Conceptus

Localization of LPAR1 protein in the porcine uterine endometrium during the estrous cycle and pregnancy was determined by immunohistochemistry (Fig. 3). On D12 and D15 of both the estrous cycle and pregnancy, LPAR1 protein was detected in the endometrial epithelia and stromal cells. Interestingly, signals for LPAR1 were detectable in the nuclei of these cell types.

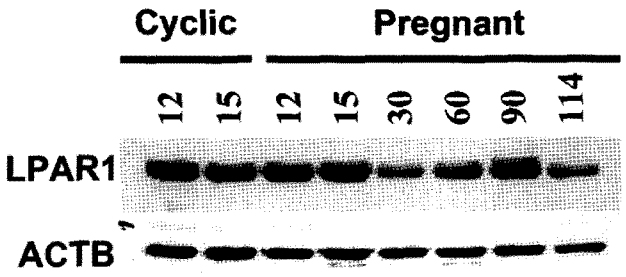

Fig. 2. Analysis of LPAR1 protein levels in the uterine endometrium by immunoblot analyses during the estrous cycle and pregnancy in pigs. Endometrial tissues from cyclic and pregnant gilts (n=3 per day) were tested. ACTB was used as a loading control. Data are presented as least squares means relative units with standard error.

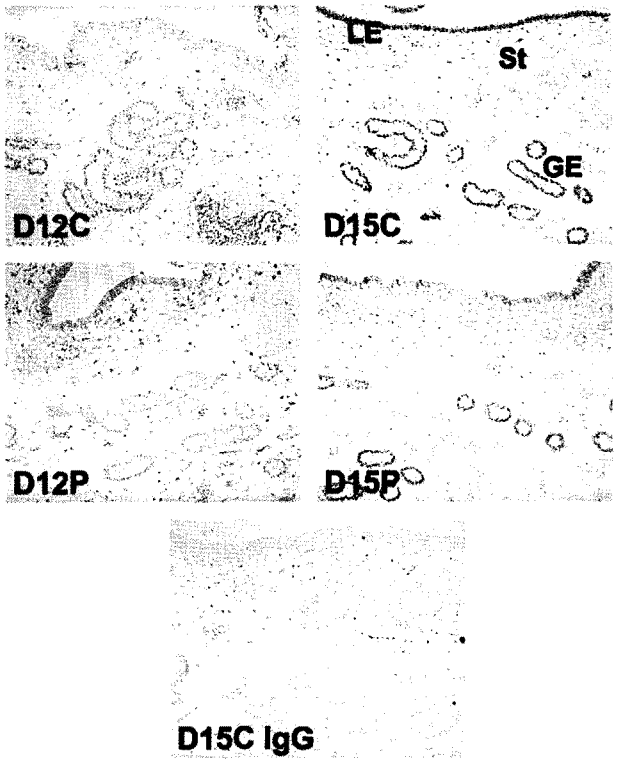

Fig. 3. Immunohistochemical analysis of LPAR1 protein in the uterine endometrium during the estrous cycle and pregnancy in pigs. Immunoreactive LPAR1 protein was detected in the epithelial cells and stroma. D, Day; C, estrous cycle; P, pregnancy; LE, luminal epithelium; GE, glandular epthelium; St, stroma. Original magnification: ×260.

In conceptus from D15 of pregnancy, LPAR1 was also detected (Fig. 4).

DISCUSSION

Since the first report that LPA induces cell proli-

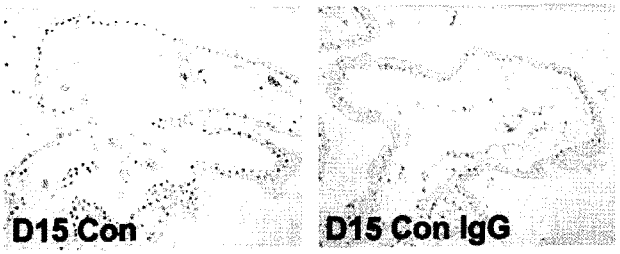

Fig. 4. Immunohistochemical analysis of LPAR1 protein in conceptuses on day 15 of pregnancy in pigs. Immunoreactive LPAR1 protein was detected in cocneptuses. D, day; Con, conceptus. Original magnification: ×260.

feration and differentiation like a growth factor via G-protein coupled receptor (Van Corven *et al.*, 1989), many studies have shown that LPA acts in the diverse physiological functions such as angiogenesis, neuronal and cardiovascular development, immunomodulation, smooth muscle contraction and relaxation and tumorigenesis (Ishii *et al.*, 2004). In uterus, not much has been known about function of LPA until the report of Ye *et al.* (2005), that the LPA-LPAR3 signaling plays an important role in embryo implantation process in mice: deletion of *LPAR3* gene in mice causes delayed implantation, aberrant embryo spacing, hypertrophic placentas, embryonic death, reduction of PTGS-2 (prostaglandin-endoperoxide synthase 2, also called prostaglandin G/H synthase 2 and cyclooxygenase 2) expression, and decreased level of secretion of prostaglandin (PG) E_2 and PGI_2. Recently, our and other reports suggest the importance of LPA during pregnancy in many species including pig (Seo *et al.*, 2008), cow (Woclawek-Potocka *et al.*, 2009) and sheep (Liszewska *et al.*, 2009).

At least five receptors are known to be activated by LPA. LPAR1, 2 and 3 are members of the structurally related endothelial differentiation (EDG) gene family, and LPAR4 and 5 are more distantly related receptors activated by LPA (Meyer zu Heringdorf and Jakobs, 2007). Our previous study showed that LPAR3 expression in porcine uterine endometrium was highest on D12 of pregnancy, the period of implantation in pigs (Seo *et al.*, 2008). Highest expression of LPAR3 on implantation period in pigs and mice suggests that LPAR3 is the predominant LPA receptor in the uterine endometrium and plays an important role in implantation during pregnancy. However, evidence indicating LPA function during pregnancy beyond implantation has been suggested. LPA levels increase as pregnancy progresses in human (Tokumura *et al.*, 2002). Also, ENPP2, a secretory LPA-producing enzyme, is expressed in the uterine endometrial tissues throughout pregnancy in pigs (Seo and Ka, unpublished data). Our present study showed that LPAR1 was constitutively expressed in the uterine endometrium dur-

ing the estrous cycle and all stages of pregnancy. These indicate that both LPAR1 and LPAR3 are expressed in the uterus, at least in pigs, as in many other tissues including brain, heart, lung, kidney which co-express LPA receptor subtypes (Anliker and Chun, 2004). These suggest that LPA may use LPAR1 to play a role in other than implantation during pregnancy in pigs, and LPAR1 and LPAR3 may cooperatively transduce LPA signals in the uterine endometrial cells throughout gestation to maintain pregnancy.

In this study, we determined that the expression of LPAR1 was localized primarily to the endometrial epithelium and moderately to the stroma in the uterine endometrium. It is interesting to note that the LPAR1 expression was localized intracellularly to nuclei. Growing evidence indicated that LPAR1 was constitutively localized in the nucleus of mammalian cells (Gobeil *et al.*, 2003; Waters *et al.*, 2006; Liszewska *et al.*, 2009), and LPA can stimulate translocation of LPAR1 from cell membrane to the nucleus (Waters *et al.*, 2006). Nuclear localization of LPAR1 suggests that LPAR1 may be responsible for intranuclear LPA signaling in contrast to LPAR3 which is located in plasma membrane and responsible for extracellular LPA signals. It has been demonstrated that expression of many uterine genes was affected by steroid hormones such as estrogen and progesterone. Estrogen secreted by conceptus during the period of implantation affects expression of uterine endometrial genes such as fibroblast growth factor 7 (*FGF7*) (Ka *et al.*, 2007), *LPAR3* (Seo *et al.*, 2008), and transient receptor potential vanilloid type 6 (*TRPV6*) (Choi *et al.*, 2009). Present study showed that LPAR1 expression was not affected by status of pregnancy but affected by day of pregnancy, and thus conceptus estrogen may not influence on *LPAR1* expression during the period of implantation. Studies using the explant culture system or ovariectomized pigs will be helpful to further characterize the effect of steroid hormones on *LPAR1* expression.

There are many structurally diverse forms of LPA due to its a fatty acid of varied length and saturation, and LPA receptors can be differentially activated according to LPA species (Bandoh *et al.*, 2000). LPAR3 showed a preference for unsaturated LPA species such as LPA 18:1, LPA 18:2, and LPA 18:3, whereas LPAR1 showed broad ligand specificities. We previously reported that various LPA species are present in uterine fluid in pig (Seo *et al.*, 2008). LPA 16:0, LPA 18:0 and LPA 18:2 were the major LPA species on D12 of pregnancy, whereas LPA 18:0 was the major LPA species on D12 of the estrous cycle. Accordingly, we speculate that different LPA species could bind to and activate LPAR1 or LPAR3 in the uterus depending on pregnancy status or stage.

PGs, especially $PGF_{2\alpha}$ and PGE_2, are lipid mediator that play critical roles in the process of luteolysis, implantation and maternal recognition of pregnancy (Bazer *et al.*, 1998; Spencer and Bazer, 2004; Ziecik, 2002) and are produced by the uterine endometrium during the estrous cycle and early pregnancy and by conceptuses (Watson and Patek, 1979; Geisert *et al.*, 1982; Lewis and Waterman, 1983). We previously showed that LPA induces expression of PTGS2, a rate-limiting enzyme for PG synthesis, in the uterine endometrium from gilts on D12 of pregnancy, the period showing highest expression of LPAR3 (Seo *et al.*, 2008). Thus, we have hypothesized that LPAR3 may act as predominant LPA receptor to induce PTGS2 expression and, possibly, PGs synthesis in the uterine endometrium and conceptuses. Because in present study LPAR1 shows similar expression pattern to LPAR3 in the uterine endometrium and conceptus in pigs, but with different localization in cellular levels, we speculate that LPA-LPAR1 signalling might participate in an intracellular LPA signaling pathway to produce PG in the uterine endometrium and conceptus. However, further studies are needed to understand the function of intracellular LPA-LPAR1 signaling.

In summary, we observed that: 1) LPAR1 was expressed in the uterine endometrium during the estrous cycle and all stages of pregnancy; 2) LPAR1 expression was localized to endometrial epithelia and stromal cells in the porcine uterus. Although LPA function mediated LPAR1 in the uterine endometrium is not known, our present study suggest that LPA-LPAR1 signaling system may play a role in the maintenance of pregnancy in pigs.

ACKNOWLEDGMENT

The authors thank the members of the Animal Research Team, Gyeonggido Veterinary Service for assistance and for management of animals.

REFERENCES

1. Anliker B, Chun J (2004): Cell surface receptors in lysophospholipid signaling. Semin Cell Dev Biol 15: 457-465.
2. Bandoh K, Aoki J, Taira A, Tsujimoto M, Arai H, Inoue K (2000): Lysophosphatidic acid (LPA) receptors of the EDG family are differentially activated by LPA species. Structure-activity relationship of cloned LPA receptors. FEBS Lett 478:159-165.
3. Bazer FW, Ott TL, Spencer, TE (1998): Endocrinology of the transition from recurring estrous cycles to establishment of pregnancy in subprimate ma-

mmals. In: Bazer FW, eds, The Endocrinology of Pregnancy. Totowa: Humana Press, pp 1-34.
4. Burghardt RC, Bowen JA, Newton GR, Bazer FW (1997): Extracellular matrix and the implantation cascade in pigs. J Reprod Fertil Suppl 52:151-164.
5. Choi Y, Seo H, Kim M, Ka H (2009): Dynamic expression of calcium-regulatory molecules, TRPV-6 and S100G, in the uterine endometrium during pregnancy in pigs. Biol Reprod [Epub ahead of print].
6. Geisert RD, Renegar RH, Thatcher WW, Roberts RM, Bazer FW (1982): Establishment of pregnancy in the pig: I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biol Reprod 27:925-939.
7. Gobeil F Jr, Bernier SG, Vazquez-Tello A, Brault S, Beauchamp MH, Quiniou C, Marrache AM, Checchin D, Sennlaub F, Hou X, Nader M, Bkaily G, Ribeiro-da-Silva A, Goetzl EJ, Chemtob S (2003): Modulation of pro-inflammatory gene expression by nuclear lysophosphatidic acid receptor type-1. J Biol Chem 278:38875-38883.
8. Ishii I, Fukushima N, Ye X, Chun J (2004): Lysophospholipid receptors: signaling and biology. Annu Rev Biochem 73:321-354.
9. Ka H, Al-Ramadan S, Erikson DW, Johnson GA, Burghardt RC, Spencer TE, Jaeger LA, Bazer FW (2007): Regulation of expression of fibroblast growth factor 7 in the pig uterus by progesterone and estradiol. Biol Reprod 77:172-180.
10. Ka H, Spencer TE, Johnson GA, Bazer FW (2000): Keratinocyte growth factor: expression by endometrial epithelia of the porcine uterus. Biol Reprod 62:1772-1778.
11. Lewis GS, Waterman RA (1983): Metabolism of arachidonic acid *in vitro* by porcine blastocysts and endometrium. Prostaglandins 25:871-880.
12. Liszewska E, Reinaud P, Billon-Denis E, Dubois O, Robin P, Charpigny G (2009): Lysophosphatidic acid signaling during embryo development in sheep: involvement in prostaglandin synthesis. Endocrinology 150:422-434.
13. Meyer zu Heringdorf D, Jakobs KH (2007): Lysophospholipid receptors: signalling, pharmacology and regulation by lysophospholipid metabolism. Biochim Biophys Acta 1768:923-940.
14. Seo H, Kim M, Choi Y, Lee CK, Ka H (2008): Analysis of lysophosphatidic acid (LPA) receptor and LPA-induced endometrial prostaglandin-endoperoxide synthase 2 expression in the porcine uterus. Endocrinology 149:6166-6175.
15. Spencer TE, Bazer FW (2004): Conceptus signals for establishment and maintenance of pregnancy. Reprod Biol Endocrinol 2:49.
16. Tokumura A, Kanaya Y, Miyake M, Yamano S, Irahara M, Fukuzawa K (2002): Increased production of bioactive lysophosphatidic acid by serum lysophospholipase D in human pregnancy. Biol Reprod 67:1386-1392.
17. van Corven EJ, Groenink A, Jalink K, Eichholtz T, Moolenaar WH (1989): Lysophosphatidate-induced cell proliferation: identification and dissection of signaling pathways mediated by G proteins. Cell 59:45-54.
18. Waters CM, Saatian B, Moughal NA, Zhao Y, Tigyi G, Natarajan V, Pyne S, Pyne NJ (2006): Integrin signalling regulates the nuclear localization and function of the lysophosphatidic acid receptor-1 (LPA1) in mammalian cells. Biochem J 398: 55-62.
19. Watson J, Patek CE (1979): Steroid and prostaglandin secretion by the corpus luteum, endometrium and embryos of cyclic and pregnant pigs. J Endocrinol 82:425-428.
20. Woclawek-Potocka I, Brzezicka E, Skarzynski DJ (2009): Lysophosphatic acid modulates prostaglandin secretion in the bovine endometrial cells differently on days 8~10 of the estrous cycle and early pregnancy. J Reprod Dev doi:10.1262/jrd. 20-206.
21. Ye X (2008): Lysophospholipid signaling in the function and pathology of the reproductive system. Hum Reprod Update 14:519-536.
22. Ye X, Hama K, Contos JJ, Anliker B, Inoue A, Skinner MK, Suzuki H, Amano T, Kennedy G, Arai H, Aoki J, Chun J (2005): LPA_3-mediated lysophosphatidic acid signalling in embryo implantation and spacing. Nature 435:104-108.
23. Ziecik AJ (2002): Old, new and the newest concepts of inhibition of luteolysis during early pregnancy in pig. Domest Anim Endocrinol 23:265-275.

(Received: 28 August 2009 / Accepted: 2 September 2009)