Maternal-Conceptus Interactions: Mediators Regulating the Implantation Process in Pigs

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ABSTRACT

For successful embryo implantation, the communication of the maternal endometrium with the conceptus trophectoderm is required essentially. In pigs, conceptuses undergo morphological change in length to enlarge the physical contact area with the maternal endometrium and secrete estrogen to induce the maternal recognition of pregnancy during the peri-implantation period. Conceptus-derived estrogen prevents luteolysis by conversion in direction of $PGF_{2\alpha}$ secretion from the uterine vasculature to the uterine lumen as well as it affects on expression of the uterine endometrial genes. In addition to estrogen, conceptuses release various signaling molecules, including cytokines, growth factors, and proteases, and, in response to these signaling molecules, the maternal uterine endometrium also synthesizes many signaling molecules, including hormones, cytokines, growth factors, lipid molecules, and utilizes ions such as calcium ion by calcium regulatory molecules. These reciprocal interactions of the conceptus trophectoderm with the maternal uterine endometrium make development and successful implantation of embryos possible. Thus, signaling molecules at the maternal-conceptus interface may play an important role in the implantation process. This review summarized syntheses and functions of signaling molecules at the maternal-conceptus interface to further understand mechanisms of the embryo implantation process in pigs.
(Key words : Pig, Uterus, Conceptus, Implantation, Endometrium)

INTRODUCTION

In pigs, approximately 30~40% of conceptuses undergo embryonic mortality between days 12~30 of pregnancy, indicating that implantation process is important for establishment and maintenance of pregnancy (Pope, 1988). Thus, it is essential to understand the process of implantation and regulatory mechanisms that govern the process for successful establishment and maintenance of pregnancy.

Implantation process is affected by various signaling molecules at the maternal-conceptus interface. The signaling molecules derived from the embryo and the maternal uterus mediate maternal recognition of pregnancy and uterine receptivity to the conceptus for successful implantation. In pigs, the implantation process initiates on day 14 of pregnancy, and biochemical interactions between the maternal endometrium and the conceptus trophectoderm are initiated by day 11 of pregnancy (Bazer, 1982). Morphological transition of bl-

astocysts from spherical (3~10 mm), to ovoid, to tubular (10~50 mm), and finally to filamentous (100~800 mm) forms between days 10 and 12 of pregnancy, which allows a large contact surface area between the conceptus trophectoderm and the uterine endometrium. Elongation of conceptus depends on molecules derived from the uterine endometrium and conceptuses, and these molecules are referred to as histotroph (Spencer *et al.*, 2004). During this period of rapid elongation, the trophectoderm secretes estrogens (catecholestrogens, estrone, estradiol, and estriol). Estrogen acts as the signal for maternal recognition of pregnancy in pigs. It induces redirection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secretion from the uterine endometrium into uterine lumen (exocrine secretion) in pregnant pigs, while endometrial $PGF_{2\alpha}$ is secreted into uterine vasculature (endocrine secretion) in cyclic pigs. Estrogen sequesters $PGF_{2\alpha}$ within the uterus to be metabolized and prevents its luteolytic effect on the corpus luteum (CL). Conceptus estrogen also regulates expression of the uterine endometrial genes responsible for endometrial remodeling

for uterine receptivity to implantation between days 13 and 25 of pregnancy (Joyce *et al.*, 2007).

The purpose of these reciprocal communications between the maternal endometrium and the conceptus trophectoderm is the synchronization of blastocyst elongation with the receptivity of the uterine endometrium for implantation. Elongating conceptuses secrete estrogens, cytokines, growth factors, and proteases. In response to these molecules the uterine endometrium undergoes the structural changes and also releases a variety of signaling molecules, such as hormones, protease inhibitors, growth factors, transport proteins, and extracellular matrix proteins (Geisert and Yelich, 1997). These molecules play an important role in dialogue between the conceptus trophectoderm and the maternal uterine endometrium for successful implantation in pigs. This review focuses on various signaling molecules for the communication between the maternal uterus and the conceptus to understand the mechanism regulating the implantation process in pigs.

MEDIATORS REGULATING THE IMPLANTATION PROCESS AT MATERNAL-CONCEPTUS INTERFACE DURING PREGNANCY IN PIGS

In pigs, biochemical interactions between the maternal endometrium and the conceptus trophectoderm are initiated by day 11 of pregnancy. During this period, embryonic signaling is required to induce maternal recognition of pregnancy and to prepare for the uterine receptivity to implantation.

Hormones

The mechanisms for the regulation of the estrous cycle and establishment of pregnancy in pigs are well studied. Synthesis and secretion of progesterone from CL after ovulation during the estrus phase trigger endometrial secretion and the uterine receptivity for early conceptus development and implantation (Geisert and Yelich, 1997). When conceptuses are absent, progesterone stimulates synthesis and pulsatile release of $PGF_{2\alpha}$ from the uterine endometrium into the uterine vasculature after day 12 of the estrous cycle to induce regression of CL between days 15 and 16 and re-initiation of estrus between days 18 and 21 in pigs. During pregnancy, the elongating conceptuses suppress regression of CL in the sow on day 12 of pregnancy (Geisert *et al.*, 1982). Estrogen secreted from the elongating conceptuses redirects endometrial $PGF_{2\alpha}$ secretion into the

uterine lumen, and $PGF_{2\alpha}$ is sequestered and metabolized to inhibit luteolysis (Bazer and Thatcher, 1977).

Progesterone

Uterine environment is spatiotemporally changed by the rapid and sustained increase in plasma progesterone (P_4), the hormone of pregnancy, from the CL of the ovary after ovulation in pigs. Treatment of P_4 to gilts on days 2 and 3 of pregnancy advances both uterine secretion and conceptus development (Vallet *et al.*, 1998; Vallet and Christenson, 2004), and treatment of mifepristone, the antiprogestin, induces delayed conceptus development (Vallet and Christenson, 2004), indicating that P_4 plays a pivotal role in the establishment and maintenance of pregnancy in mammals. P_4 regulates directly a number of the uterine endometrial genes by activation of PGR expressed in the uterine endometrial epithelium and stroma during the early luteal phase, but consistent exposure of P_4 to the endometrium down-regulates PGR expression in the endometrial epithelium. This cessation of PGR expression in the endometrial epithelium immediately before implantation is common to domestic animals including sheep, cattle, and pigs (Geisert *et al.*, 1994; Kimmins and MacLaren, 2001; Spencer and Bazer, 1995). Thus, it is postulated that regulation of endometrial epithelial function in response to P_4 during the peri-implantation period when endometrial epithelial cells lose PGR is affected by specific factors (progestamedins) from PGR-positive stromal cells (Cunha *et al.*, 1985). P_4-stimulated genes expressed in endometrial epithelia include *UF* (Basha *et al.*, 1980), *UPTI* (Fazleabas *et al.*, 1982), *RBP* (Adams *et al.*, 1981), and *FGF7* (Ka *et al.*, 2007).

Estrogen

Porcine conceptuses produce estrogen from day 11 of pregnancy (Geisert *et al.*, 1982). Secretion of estrogen shows a biphasic pattern with increased release on day 12 and day 23~30 of pregnancy (Bazer *et al.*, 1982). Estrogen secreted from the elongating conceptuses is the signal for maternal recognition of pregnancy that changes direction of secretion of luteolysin, $PGF_{2\alpha}$, from the uterine vasculature to the uterine lumen for preventing luteolysis and that induce uterine changes in secretion and morphology required for implantation process in pigs (Bazer *et al.*, 1982). Estrogen also induces maintenance of luteinizing hormone (LH) receptor levels both in the CL (Garverick *et al.*, 1982) and the uterus (Ziecik *et al*, 1992). Endometrial ESR1 is expressed in the luminal and glandular epithelium on day 12 of pregnancy, but its level is reduced on day 15 of pregnancy. This change in endometrial ESR1 expression is consistent with a physiological role for es-

trogen secreted from conceptuses at the time of maternal recognition of pregnancy in pigs. Conceptus estrogen affects expression of many uterine endometrial genes including *FGF7* (Ka *et al.*, 2007), *SPP1* (White *et al.*, 2005), *LPAR3* (Seo *et al.*, 2008), *STAT1* (Joyce *et al.*, 2007), and *TRPV6* (Choi *et al.*, 2009).

Oxytocin

The porcine CL synthesizes oxytocin (OT) (Choy and Watkins, 1988). Circulating OT concentration increases during luteolysis and then this increase of OT is associated with an elevation in the uterine secretion of $PGF_{2\alpha}$ (Kotwica *et al.*, 1990). OT binds to its endometrial receptor (OTR) and utilizes the phosphoinositide pathway to initiate luteolytic secretion of $PGF_{2\alpha}$ in pigs. Low levels of OTRs in the endometrium of early prengnancy in pigs (Okano *et al.*, 1996) could indicate that this suppression is an important component of the mechanism of the recognition of pregnancy in pigs. The endometrial responsiveness to OT is regulated by the levels of OTRs coupling to G protein and phospholipase C pathway (Ludwig *et al.*, 1998).

Luteinizing Hormone

The porcine uterus expresses luteinizing hormone (LH) receptors (LHCGRs) (Ziecik *et al.*, 1986). The appearance of relatively high amounts of LHCGRs in the endometrium coincides with the increase of $PGF_{2\alpha}$ secretion and perhaps with the down-regulation of PGRs. After luteolysis, LHCGRs decline in the endometrium. LHCGR up-regulates cyclooxygenase-2 (COX2) protein expression and $PGF_{2\alpha}$ secretion from the endometrium. The uterine LHCGRs are involved in the maintenance of early pregnancy in pigs, since LH induces PGE_2 release from the endometrium on days $14 \sim 16$ of the estrous cycle or early pregnancy (Blitek and Ziecik, 2005; Ziecik *et al.*, 2000). Moreover, LH affects secretion of the known angiogenic factor, vascular endothelial growth factor (VEGF), from endometrial cells.

Proalctin

The function of proalctin (PRL) in the maintenance of pregnancy in the pig has not been fully determined (Dusza and Tilton, 1990). There is no difference in circulating concentrations of PRL between the estrous cycle and early pregnancy (Dusza and Krzymowska, 1981). PRL aids estrogen action in the exocrine secretion of $PGF_{2\alpha}$ during the establishment of pregnancy in pigs (Gross *et al.*, 1990), and estrogen up-regulates endometrial PRL receptors (PRLRs) on day 12 of pregnancy (Young *et al.*, 1990), suggesting that PRL may cooperate with estrogen for the maternal recognition of pregnancy.

Cytokines

Interleukin 1-Beta

A number of mammals express an intriguing pro-inflammatory cytokine, interleukin 1-beta (IL1B), during the implantation period (Takacs and Kauma, 1996; Kruessel *et al.*, 1997; Schafer-Somi *et al.*, 2008). IL1B is considered as a mediator the acute-phase inflammatory response, and the ability of IL1B to induce inflammation needs expression of members of the IL1 signaling system. The IL1 signaling system consists of two ligands, IL1A and IL1B, two receptors, IL1R1 and IL1R2, functional and pseudo-receptors, respectively, converting enzymes, receptor accessary proteins (IL1RAP), and multiple isoforms of receptor antagonists (Mantovani *et al.*, 1998). It has been known that IL1B plays an important role in the implantation process by regulating the immunotolerance mechanism at the maternal-fetal interface (Paulesu *et al.*, 2008). In pigs, conceptuses with rapid elongation between days 11 and 12 of pregnancy secrete IL1B into the uterine lumen temporally and spatially (Ross *et al.*, 2003). IL1B participates in remodeling of the trophectoderm during rapid elongation of trophectoderm and prostaglandins (PGs) by the release of arachidonic acid from the plasma membrane. Activation and secretion of IL1B require cleavage by caspase-1 (CASP1), an intracellular cysteine protease which converts IL1B to its biologically active form. Conceptus also expresses CASP1 coincidently with IL1B secretion between days 12 and 13 of pregnancy.

Conceptus-derived IL1B in pigs plays a key role in implantation for the establishment and maintenance of pregnancy. IL1B receptors, IL1R1 and IL1RAP, are expressed in the uterine endometrium in response to IL1B and estrogen from conceptus during the implantation period in pigs (Seo *et al.*, 2012), suggesting that components of the IL1 system are expressed in the uterus during this period in pigs. The IL1 signaling system regulates expression of a number of endometrial genes including PG synthesis-involved enzymes, *PTGS1* and *PTGS2* (Seo *et al.*, 2012), and *SAL1* (Seo *et al.*, 2011) at the maternal-fetal interface.

Interferons

Porcine conceptuses secrete both type I and type II interferons (IFNs) during the peri-implantation period. The major IFN species is type II IFN-gamma (IFNG) and the other is the type I IFN-delta (IFND) (La Bonnardiere *et al.*, 1991, Lefevre *et al.*, 1998). Although synthesis and secretion of IFNs by conceptuses occurs between days 12 and 20 of pregnancy, the peak of synthesis and secretion of IFNs is detected on between days 15 and 16 of pregnancy in pigs. IFNs are not in-

volved in the maternal recognition of pregnancy in pigs, however, they influence secretion of PGE_2 (Harney and Bazer, 1989), expression of several IFN-responsive genes in the uterine endometrium (Hicks *et al*., 2003; Joyce *et al*., 2007a; 2007b; 2008), and expression of genes in endometrial stroma and glandular epithelium in a paracrine manner (Joyce *et al*., 2007a; 2007b; 2008).

Expression of signal transducer and activator of transcription (STAT1) is differentially expressed in the uterine endometrium in a cell-type specific manner and regulated by conceptus signals, estrogen and IFNs. Down-regulation of swine leukocyte antigen (SLA) class I and beta 2 microglobulin (B2M) expression in the uterine luminal epithelium may be important for preventing fetal allograft rejection. In contrast to down-regulation of these genes in luminal epithelial cells, expression of SLA class I and B2M increases in stromal cells on day 15 of pregnancy by conceptus IFNs and remains detectable through day 40 of pregnancy (Joyce *et al*., 2008). In addition, levels of *SLA-DQA* and *SLC-DQB* in the uterine endometrium are up-regulated by IFNG treatment in the presence of progesterone and estrogen to the endometrial explant tissues from day 12 of the estrous cycle (Kim *et al*, 2012). These suggest that conceptus-derived IFNs may control immune regulatory molecules in the uterine endometrium to provide the immunologically tolerant environment for development of semi-allograft fetus during pregnancy.

Growth Factors

Epidermal Growth Factors

The epidermal growth factor (EGF) family includes EGF itself, transforming growth factor-α (TGFα), heparin binding EGF-like factor (HB-EGF), and amphiregulin. All of these molecules bind to the cell surface tyrosine kinase receptor (Prigent and Lemoine, 1992). The possible function of EGFs during pregnancy is to stimulate embryonic growth and development. The porcine conceptuses possess EGF receptors during both pre- (days 7∼12) and post-elongation (days 15∼22) of blastocyst (Corps *et al*., 1990). However, *EGF* mRNA expression is limited to post-elongation of blastocyst. EGF is predominantly expressed in the embryo and amnion (Vaughan *et al*., 1992). EGF may be involved in PGE_2 synthesis by the amnion, and PGE_2 concentration in amniotic fluid increases as pregnancy progresses. TGFα is detected only in the developing blastocyst on days 8∼12. Because the porcine blastocyst begins to elongate from day 10.5 of pregnancy, TGFα may be involved in the complex developmental reorganization of the conceptuses. EGF receptors in the porcine uterus are detected on day 13 of pregnancy and the binding ca-

pacity is higher for stroma than for glandular epithelial cells (Zhang *et al*., 1992).

Fibroblast Growth Factors

Fibroblast growth factors (FGFs) are structurally related proteins to stimulate fibroblast proliferation, and also to affect cell differentiation, matrix formation, and cell movement. FGFs affect ECM deposition for embryonic development, suggesting that FGFs could be critical for embryogenesis (Baird and Bohlen, 1991). Both FGF1 and FGF2 are expressed in the uterine endometrium and conceptuses between days 10 and 14 of pregnancy, with differential expression patterns by pregnancy status (Gupta *et al*., 1997). FGF2 is localized to luminal and glandular epithelium and stroma with stronger levels from day 12 of pregnancy, suggesting that FGF2 expression may be affected by conceptus-derived E_2, and also detected in cells of the embryonic disc and visceral endoderm on days 10 and 11 of pregnancy. Mesoderm cells were positive stained for FGF2 on days 11 and 12 of pregnancy. FGF1 is localized only to stromal cells of porcine endometrium. These indicate that FGF2, but not FGF1, may directly influence the development and/or differentiation of porcine conceptuses (Gupta *et al*, 1997). FGF7 (also known as keratinocyte growth factor) mediates epithelial-mesenchymal interactions in the female reproductive tract in a paracrine manner. FGF7 is predominantly expressed in the uterine endometrial epithelium throughout pregnancy with highest levels between days 12∼15 of pregnancy (Ka *et al*., 2000), and its abundance on day 12 of pregnancy is higher than that on day 12 of the estrous cycle in pigs. Receptors for FGF7 are localized only to LE and GE in the uterine endometrium. Also, FGF7 receptors are expressed in the porcine trophectoderm cells, suggesting that in pigs, FGF7 may play a role in paracrine epithelial-epithelial interactions between conceptus and uterus during the early stage of pregnancy in pigs (Ka *et al*., 2000). Moreover, expression of urokinase plasminogen activator (uPA, a marker of differentiation) in the porcine conceptus is stimulated by FGF7, indicating that FGF7 affects trophectoderm cell differentiation (Ka *et al*, 2001; 2007).

Insulin-Like Growth Factors

The insulin-like growth factors (IGFs) are implicated in the control of proliferation and differentiation of the uterus in preparation for blastocyst implantation and during later feto-placental development. *IGF1* mRNA is expressed in the uterine endometrium between days 8 and 14 of pregnancy (Letcher *et al*., 1989) and *IGF2* mRNA after implantation (Simmen *et al*., 1992). Thus, in the porcine uterus IGF1, rather than IGF2, appears to

dominate in early pregnancy. The expression and secretion of endometrial transcripts and proteins of IGF1 peaks on day 12 of pregnancy, concomitant with maximal E_2 production by the conceptuses (Simmen *et al.*, 1989; 1995). In addition, IGF1 participates in blastocyst development by mediating IGF1 receptors in blastocyst during the peri-implantation period in pigs (Green *et al.*, 1995).

Vascular Endothelial Growth Factor

Dramatic growth and remodeling of endometrial vasculature are prerequisite for the close apposition between fetal and maternal blood supplies during the implantation period in pigs (Lee and DeMayo, 2004). Endometrial vascular endothelial growth factor (VEGF) expression has been investigated in many species, and VEGF may be closely involved in remodeling of the uterine endometrial vasculature during pregnancy. VEGF receptors are localized in the luminal and glandular epithelium of either gravid or non-gravid uterus in pigs (Winther *et al.*, 1999). In addition, VEGF protein levels increase in the uterine endometrium before ovulation and early pregnancy, suggesting that VEGF plays a key role in the development and remodeling of the uterine vasculature during the implantation period in pigs (Kaczmarek *et al.*, 2004). Expression of VEGF in the uterine endometrium is regulated by both IGF1 and relaxin (RLX), indicating that IGF1 and RLX play a role in angiogenesis and the maintenance of vascular function during the implantation and the placentation processes in pigs (Kaczmarek *et al.*, 2008).

Other Lipid Mediators

Prostaglandins

Prostaglandins (PGs) are converted from arachidonic acid by cyclooxygenase-1 and -2 (PTGS1 and PTGS2). The first product is PGH_2, the common precursor of various forms of PGs, including PGE_2 and $PGF_{2\alpha}$. PGE synthases (PTGES, PTGES2, and PTGES3) and PGF synthase (AKR1B1) convert PGH to PGE_2 and $PGF_{2\alpha}$, respectively (Smith and Dewitt, 1996). PGE_2-9-oxoreductase (CBR1) can catalyze the transformation of PGE_2 to $PGF_{2\alpha}$. These molecules are critical for the establishment of pregnancy in pigs, since inhibition of PG synthesis results in pregnancy failure (Kraeling *et al.*, 1985). $PGF_{2\alpha}$ is the major luteolysin in pigs.

PGE_2 possesses luteotrophic and antiluteolytic effects in pigs (Ziecik, 2002). Pulsatile secretion of $PGF_{2\alpha}$ during the estrous cycle increases significantly on day 13 and continues to increase through days 16 and 18 (Cristernson *et al.* 1994; Kotwica *et al.*, 1999). PG secretion in mated gilts peaks early (day $11\sim12$) with PGE_2 be-

ing the predominant eicosanoid. PGE_2 concentration is higher in the utero-ovarian venous blood draining the gravid than the non-gravid uterine horn, and ratio of secreted PGE_2 and $PGF_{2\alpha}$ increases in harvested stromal cells from endometrium of pregnant pigs compared to cyclic gilts (Zhang and Davis, 1991).

PTGS1 and PTGS2 are localized in the uterine stromal and epithelial cells in pigs, and PTGS2 levels in the uterine endometrium are coincident with the time of luteolysis (Dubois *et al.*, 1993; Ashworth *et al.*, 2006; Seo *et al.*, 2008). In pregnant gilts, abundance of endometrial PTGS2 transcript and protein increases at the time of implantation process, indicating that PTGS2 may be involved in elevated PG production during luteolysis and implantation in pregnant pigs (Franczak *et al.*, 2010; Seo *et al.*, 2012). Expression of PTGS2 is also detected in conceptuses and regulated during elongation of conceptuses in pigs (Wilson *et al*, 2002). PTGS2 expression is not expressed in spherical/tubular conceptuses, but is up-regulated by the time a conceptus reaches a filamentous form (Franczak *et al.*, 2010).

PTGES and AKR1B1 are expressed in the uterine endometrium in pigs (Ross *et al.*, 2007; Franczak *et al.*, 2010). High levels of PTGES expression in the uterine endometrium before implantation may be involved in the change of PGE_2:$PGF_{2\alpha}$ ratio necessary for maternal recognition of pregnancy. Porcine conceptuses also express PTGES and AKR1B1 (Ziecik *et al.*, 2005), and alterations in amount of mRNA and protein of these enzymes in conceptuses correlated with changes of these enzymes in the uterine endometrium in pigs. (Waclawik *et al.*, 2005).

Lysophosphatidic Acids

Lysophosphatidic acid (LPA) is a lysophospholipid composed of a glycerol or sphingoid backbone with a fatty acid of various length and saturation (Ishii *et al.*, 2004), and generated from lysophosphatidylcholine by removing of the choline group through the action of ectonucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2; also called autotaxin) (Stracke *et al.*, 1992). LPA is found in various body fluids, including serum, saliva, seminal plamsa, and follicular fluids (Aoki, 2004; Sugiura *et al.*, 2002; Hama *et al.*, 2002; Tokumura *et al.*, 1999) and also present in the fluids of the uterine lumen in pigs and sheep (Seo *et al.*, 2008; Liszewska *et al.*, 2009).

There are at least six specific receptors of LPA receptors, LPAR1-6. These mediate biological LPA functions which show many growth factor-like biological effects, such as cell proliferation, survival, migration, differentiation, and aggregation in various cell types (Gardell *et al.*, 2006). Increasing evidence suggests that ma-

ny reproductive processes in vertebrates are affected by LPA (Ye and Chun, 2010). LPAR3 knockout mice show abnormal embryo spacing and delayed implantation (Ye *et al.*, 2005). In ewes, expression of LPA, ENPP2, LPAR1, and LPAR3 is detected in the uterus and conceptus during the early stage of pregnancy, and bioactive LPA induces cell proliferation and PGE_2 and $PGF_{2\alpha}$ in the trophectoderm cells (Liszewska *et al.*, 2009). LPAR1-3 are expressed in the uterine endometrium, and LPAR3 is expressed stage-specifically with highest expression level at the time of implantation. LPA increases endometrial *PTGS2* mRNA expression during

the implantation period (Seo *et al.*, 2008). It also has been shown that ENPP2, which acts on production of LPA, is expressed in the uterine endometrium, and that ENPP2 protein is detected in uterine flushings on D12 of the estrous cycle and pregnancy, with higher levels on D12 of pregnancy. Furthermore, lysophospholipase D activity was detected in uterine flushings on D12 of the estrous cycle and pregnancy, with higher levels on D12 of pregnancy. (Seo *et al.*, 2012).

Calcium Ions

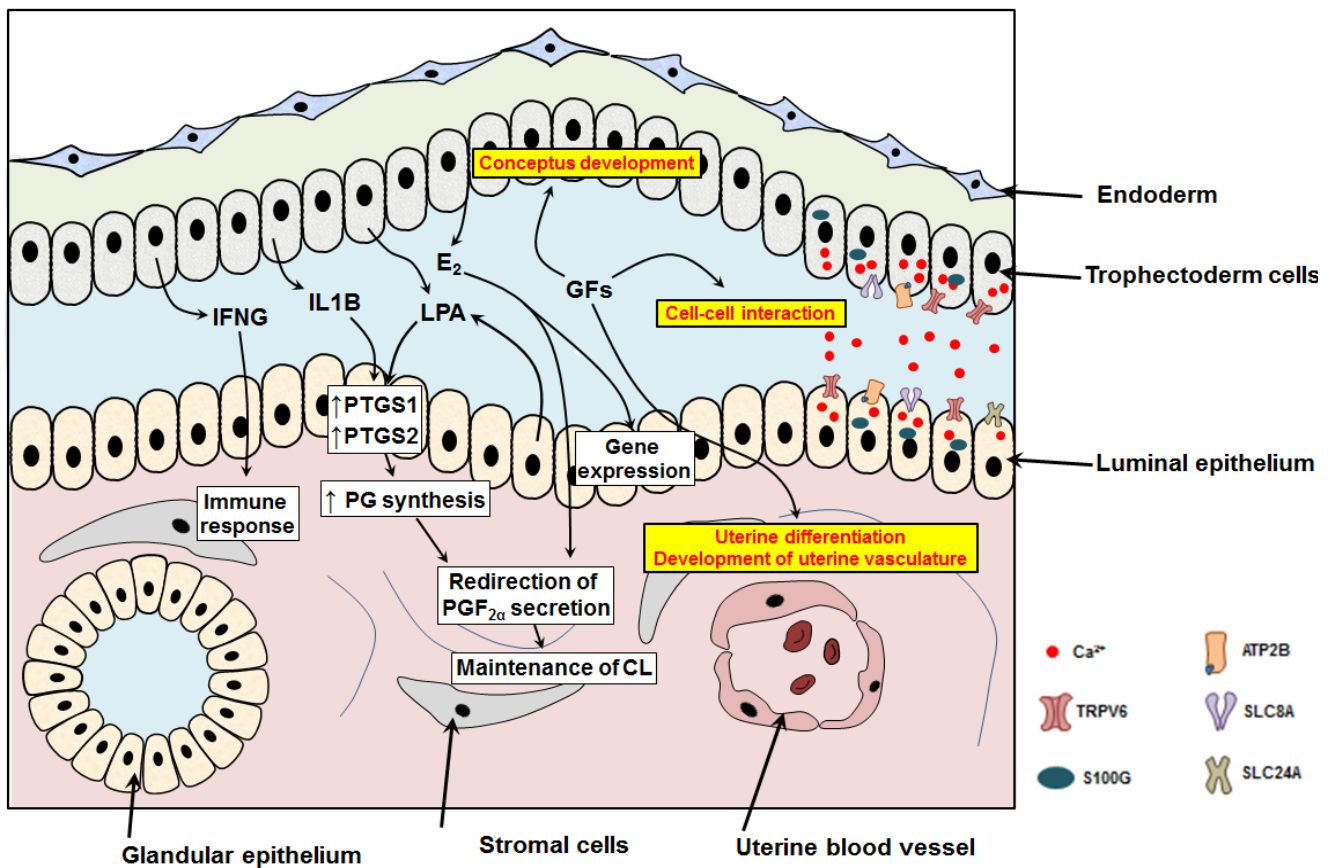Calcium is a highly versatile intracellular signaling

Fig. 1. A working model illustrating the signaling molecules and their roles at the maternal-conceptus interface during the implantation period in pigs. During the implantation period in pigs, the conceptus trophectoderm undergoes dramatic morphological change in length and secretes several signaling molecules, including estrogen, cytokines (interleukin and interferons), and growth factors. Conceptus-derived estrogen induces the maternal recognition of pregnancy by redirection of $PGF_{2\alpha}$ secretion from the uterine vasculature to the uterine lumen. These conceptus-derived molecules regulate gene expression patterns of the uterine endometrial genes involved in many physiological processes such as immune response, cellular signal transduction, cell-cell interaction, and calcium homeostasis. Various growth factors are synthesized from the uterine endometrium and the conceptus trophectoderm and affect to conceptus development, uterine differentiation and vascular development, and trophectoderm-uterine epithelial cell interaction during this period. Lysophosphatidic acid regulates synthesis of prostaglandin in the uterine endometrium by binding with its receptor on the uterine epithelial cells. Levels of calcium in the uterus during the implantation period are regulated by calcium regulatory molecules including TRPV6, S100G, ATP2Bs, SLC8As, and SLC24As. E2, estrogen; IL1B, interleukin 1-beta; IFNG, interferon gamma; GF, growth factor; LPA, lysophosphatidic acid.

mediator that can regulate a number of cellular processes (Berridge *et al*., 2003; Clapham, 2007). Adhesion of the trophectoderm with the maternal endometrium by cell adhesion molecules (CAMs) including integrins, cadherins, and selectins is dependent on calcium ions (Reddy and Mangale, 2003; Singh and Aplin, 2009). During the implantation period in pigs, calcium level significantly increases in the uterine lumen, and this increase coincides with elongation of conceptus and estrogen secretion (Geisert *et al*., 1982).

Recently, we have determined expression of calcium regulatory molecules in the uterine endometrium during the estrous cycle and pregnancy in pigs (Choi *et al*., 2009; 2012; 2014). Calcium regulatory molecules are involved in the maintenance of calcium concentration and transcellular calcium transport in the intestine, kidney, and placenta (Hoenderop *et al*., 2002). Transient receptor potential vanilloid type 5 (TRPV5) and 6 (TRPV6) are involved in extracellular calcium influx into the cell. Calbindin-d9k (S100G) and calbindin-d28k participate in buffering the intracellular calcium concentration and transport of cytoplasmic calcium from the apical side to the basolateral side of the cell (Hoenderop *et al*., 2002; 2005). Calcium extrusion regulatory molecules, including plasma membrane calcium ATPases (ATP2Bs) and sodium/calcium exchangers (SLC8As), mediate the extrusion of calcium to outside of the cell (Hoenderop *et al*., 2002; 2005). In addition, potassium-dependent sodium/calcium exchangers (SLC24As) also regulate calcium extrusion indirectly by interacting with calcium regulatory molecules (Altimimi and Schnetkamp, 2007). These molecules are expressed in the uterine endometrium during the estrous cycle and pregnancy in a pregnancy status- and stage-specific manner in pigs, and endometrial expression of *TRPV6* and *S100G* is affected by conceptus-derived estrogen during the implantation period (Choi *et al*., 2009; 2012). These suggest that the level of calcium ions in the uterine endometrium and lumen is tightly regulated by calcium regulatory molecules during the implantation period in pigs. Collectively, calcium regulatory molecules may play an important role in the implantation process by regulating calcium levels in the uterus in pigs. Interestingly, it has been shown that expression of calcium extrusion regulatory molecules, ATP2Bs, SLC8As, and SLC24As, is not affected by conceptus-derived steroid hormone or cytokines during the implantation period (Choi *et al*., 2014), suggesting that there may be another pathways involved in calcium transport in the uterus during the implantation period in pigs.

CONCLUSION

This review summaries various signaling molecules at the maternal-conceptus interface during the implantation period in pigs. In pigs, the conceptus produces various signaling molecules, including estrogen, IL1B, IFND, and IFND, for cellular and molecular changes of the uterine endometrium. In response to these factors, the uterine endometrium changes the pattern of gene expression and histo-architecture for development and implantation of embryos. As shown in Fig. 1, signaling molecules and their actions at the maternal-conceptus interface make successful implantation process possible. Although many signaling molecules responsible for the maternal-conceptus communications and their functions are well studied, it is still not completely understood on maternal-conceptus interaction for the successful establishment of pregnancy. Thus, further investigation to clarify signaling molecules at the maternal-conceptus interface and their function are required to understand the implantation process and to increase the implantation rate in pigs.

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