J. Emb. Trans. (2012) Vol. 27, No. 4, pp. 211~221
http://dx.doi.org/10.12750/JET.2012.27.4.211

Signaling Molecules at the Conceptus-Uterine Interface during Early Pregnancy in Pigs

Heewon Seo, Yohan Choi, Jangsoo Shim, Mingoo Kim and Hakhyun Ka*

Division of Biological Science and Technology and IPAID, Yonsei University, Wonju 220-710, Republic of Korea

ABSTRACT

The process of embryo implantation requires physical contact and physiological communication between the conceptus trophectoderm and the maternal uterine endometrium. During the peri-implantation period in pigs, the conceptus undergoes significant morphological changes and secretes estrogens, the signal for maternal recognition of pregnancy. Estrogens secreted from the conceptus act on uterine epithelia to redirect $PGF_{2\alpha}$, luteolysin, secretion from the uterine vasculature to the uterine lumen to prevent luteolysis as well as to induce expression of endometrial genes that support implantation and conceptus development. In addition, conceptuses secrete cytokines, interferons, growth factors, and proteases, and in response to these signals, the uterine endometrium produces hormones, protease inhibitors, growth factors, transport proteins, adhesion molecules, lipid molecules, and calcium regulatory molecules. Coordinated interactions of these factors derived from the conceptus and the uterus play important roles in the process of implantation in pigs. To better understand mechanism of implantation process in pigs, this review provides information on signaling molecules at the conceptus-uterine interface during early pregnancy, including recently reported data reported.

(Key words : pig, uterus, conceptus, implantation, endometrium)

INTRODUCTION

Implantation process requires a physical and physiological contact between the conceptus and the uterine endometrium and is accompanied by proper conceptus development, increased uterine receptivity, and reciprocal conceptus-uterine interaction (Tranguch *et al*., 2005). In pigs, 20 to 30% of conceptuses die between days 12 and 30 of pregnancy, suggesting the importance of implantation process for establishment and maintenance of pregnancy (Pope, 1988; Pope *et al*., 1986). Just before implantation, the conceptus signals its presence to the mother to obviate luteolysis and prolong lifespan of the corpus luteum (CL) beyond the estrous cycle, which provides continuous secretion of progesterone by the CL for establishment and maintenance of pregnancy (Niswender *et al*., 2000). In pigs, signal for the maternal recognition of pregnancy is estrogen of conceptus origin which acts on the uterine endometrium to induce directional change of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secretion (Bazer and Thatcher, 1977). In cyclic pigs, endometrial $PGF_{2\alpha}$ is secreted into uterine vasculature, which is transported to the ovary to cause luteolysis. However, in pregnant pigs, uterine endometrium in response to estrogen produced by conceptuses secretes $PGF_{2\alpha}$ into uterine lumen, where it is sequestered to exert its biological actions in the uterus and/or be metabolized to prevent luteolysis (Bazer and Thatcher, 1977; Bazer 1998).

Shortly before implantation, the porcine blastocysts undergo dramatic morphological changes between days 11 and 12. Blastocysts elongate from spherical (3 to 10 mm in diameter), to ovoid, to tubular (10 to 50 mm in length), and then to filamentous forms (100 to 800 mm in length) and become a conceptus (embryo/fetus and associated extraembryonic membranes). During the peri-implantation period, conceptus secretes a variety of molecules such as cytokines, interferons, growth factors, and proteases as well as estrogen (Jaeger *et al*., 2001). Consistent with conceptus changes, the uterine endometrium also undergoes structural changes and secretes hormones, protease inhibitors, growth factors, transport proteins, and extracellular matrix (Geisert and Yelich, 1997). Coordinated interactions of various factors derived from conceptus and the uterus play important roles in the process of implantation in pigs. This

† This study was supported, in part, by the National Research Foundation (NRF-2010-0012304) funded by the Korean Government, and by the BioGreen 21 Program (#PJ007997), Rural Development Administration, Republic of Korea.
* Correspondence : E-mail : hka@yonsei.ac.kr

review focuses on these signaling molecules involved in conceptus-uterine interaction during the implantation period in pigs.

1. Steroid Hormones

Plasma progesterone concentration decreases between 6 and 4 days before estrus and increase rapidly between days 2 and 6 of the estrous cycle, and continue to increase to reach a maximum level (about 30 ng/ml) by day 12. In cyclic pigs, after reaching a peak on day 12, the concentration decreases sharply from day 15 to less than 1 ng/ml on day 18 of the estrous cycle (Guthrie *et al*., 1972; Henricks *et al*., 1972). Progesterone is also detected in uterine lumen (Zavy *et al*., 1980; Stone and Seamark, 1985). Progesterone as well as androgens and other steroid metabolites are a potential precursor for estrogen synthesis by conceptus (Bazer *et al*., 1979; Heap *et al*., 1983).

There is negative correlation between plasma estrogen levels and plasma progesterone levels during the estrous cycle (Guthrie *et al*., 1972). Levels of plasma estrogen are low until day 16 or 17 of the estrous cycle, and then increase with a maximum level prior to estrus (Guthrie *et al*., 1972, Henricks *et al*., 1972). There is no difference in estrogen concentrations for the first 16 days after estrus between cyclic pigs and pregnant pigs (Hanricks *et al*., 1972). Estrogen levels increase greatly on day 18 in cyclic pigs, while the levels remain relatively constant in pregnant pigs (Guthrie *et al*., 1972). Estrogens are also present in uterine lumen of pigs (Zavy *et al*., 1980; Geisert *et al*., 1982). Levels of estrone and estradiol, estriol, and conjugated estrogens in uterine lumen of cyclic pigs are not significantly changed (Geisert *et al*., 1982). In pregnant pigs, levels of estrogens in uterine lumen are affected by the developmental stage of blastocysts (Geisert *et al*., 1982). Levels of estrone, estradiol, and estriol increase from days 10 to 12 with highest level in uterine flushing with filamentous conceptuses on day 12. Levels of estrone sulphate and estradiol sulphate also increase from days 10 to 12 in uterine flushings of pregnant pigs. Catechol estrogens are produced from estradiol by estrogen 2/4-hydrolase activity of conceptus with increased activity on days 10 to 13 (Mondschein *et al*., 1985). It has been suggested that catechol estrogens are associated with prostaglandin synthesis in the rat, rabbit, and human uterus (Kelly and Abel, 1980, 1981; Pakrasi and Dey, 1983), and blastocyst activation during implantation in mice (Paria *et al*., 1998), but function of catechol estrogen is not known in pigs.

Actions of estrogen and progesterone in the uterus are mediated mainly by their nuclear receptors, ESR1 and PGR, respectively. Levels of *ESR1* mRNA are highest in the uterine endometrium on day 10, decrease by day 15, and then increase by day 18 during the estrous cycle and pregnancy, but remain suppressed after day 18 of pregnancy (Geisert *et al*., 1993). ESR1 proteins are localized to luminal epithelium (LE), glandular epithelium (GE), and stroma at estrus in cyclic pigs (Geisert *et al*., 1993). From days 5 to 15 of the estrous cycle and pregnancy, ESR1 is present in LE and GE, but absent in stroma. Levels of PGR in the endometrium are highest on days 0 to 5, decrease by day 10 and then remain low on days 12 to 18 in both cyclic and pregnant pigs (Geisert *et al*., 1994). PGR is strongly expressed in LE, GE and stroma between days 0 to 5 (Geisert *et al*., 1994). After day 5, expression of PGR decreases in LE and GE and is undetectable from days 12 to 18 of both the estrous cycle and pregnancy (Geisert *et al*., 1994). PGR is detectable in stroma throughout the estrous cycle and pregnancy (Geisert *et al*., 1994). Thus, it is suggested that progesterone acts on PGR-positive stromal cells to exert paracrine effects on epithelial cells through progesterone-mediated molecules, called progestamedins.

Uterine arterial blood flow increases between days 11 and 13 of pregnancy, when levels of estrone and estradiol increase (Ford *et al*., 1982). The reduced levels of $PGF_{2\alpha}$ in the uteroovarian vein during the period of maternal recognition of pregnancy may be due to diluted effect of $PGF_{2\alpha}$ by the increased quantities of uterine venous blood associated with increased uterine blood flow (Ford *et al*., 1982). Catechol estrogen converted from estrogen decreases uterine arterial tone by decreasing calcium uptake via potential-sensitive channels, whereas progesterone increases α_1-adrenergic receptor numbers to maintain the phasic contractility of uterine arterial smooth muscle throughout pregnancy (Ford, 1995). Estrogen and progesterone also affect contraction of myometrium (Lye and Porter, 1978; Porter and Lye, 1983). Progesterone decreases myometrial contractions, whereas estrogen increases myometrial contraction. Amplitude of myometrial contraction is low or absent during the luteal phase of the estrous cycle or progesterone treatment after ovariectomy.

Estrogen and progesterone regulate expression of the uterine endometrial genes. Progesterone increases expression of uterine secretory proteins including uteroferrin (Roberts and Bazer, 1988; Fliss *et al*., 1991), plasmin/trypsin inhibitor (Mullins *et al*., 1980; Fazleabas *et al*., 1983), retinol-binding protein (Trout *et al*., 1992; Harney *et al*., 1993), and insulin-like growth factor 1 (IGF1) (Simmen *et al*., 1990) as well as adhesion molecules

such as integrin receptor subunits α_4, α_5, and β_1 (Bowen *et al*., 1996). Progesterone suppresses expression of ESR1, PGR (Geisert *et al*., 1993), and MUC1 (Bowen *et al*., 1996) in the uterine endometrium. During the period of maternal recognition of pregnancy, estrogen affects expression of the uterine endometrial genes aldo-keto reductase family 1, member B1 (*AKR1B1*) (Ross *et al*., 2007), fibroblast growth factor 7 (*FGF7*) (Ka *et al*., 2001), secreted phosphoprotein 1 (*SPP1*) (White *et al*., 2005), lysophosphatidic acid receptor 3 (*LPAR3*) (Seo *et al*., 2008), and transient receptor potential vanilloid type 6 (*TRPV6*) (Choi *et al*., 2009).

2. Cytokines

The IL1B system is composed of two receptors, IL1 receptor type 1 (IL1R1) and IL1R2, a co-receptor (IL1 receptor accessory protein; IL1RAP), and a natural receptor antagonist (IL1 receptor antagonist; IL1RN). IL1R1 is a signaling receptor, whereas IL1R2 is a decoy receptor that does not transduce a signal. IL1R1 does not transduce cell signaling on its own and IL1RAP itself does not bind IL1. A complex composed of IL1B, IL1R1 and IL1RAP is required to initiate IL1B cell signaling. During the peri-implantation period porcine conceptuses express *IL1B* (Tuo *et al*., 1996) and secrete abundant amounts of IL1B into uterine lumen with highest level on day 12 of pregnancy (Ross *et al*., 2003). Uterine endometrium expresses IL1B, IL1R1, IL1RAP, and IL1RN (Ross *et al*., 2003). The levels of IL1B expression is higher in the endometrium on days 0 to 5 compared to those on days 10 to 18 of the estrous cycle and early pregnancy. Thus, IL1B produced by the uterine endometrium has minimal contribution to highest concentration of IL1B in the uterine lumen on day 12 of pregnancy. Expression of IL1R1 and IL1RAP is highest in the uterine endometrium on day 12 of pregnancy, whereas IL1RN is expressed with low level during early pregnancy. The highest levels of IL1B, IL1R1 and IL1RAP, and low level of IL1R1 during the implantation period suggest that IL1B secreted by conceptuses plays a critical role in implantation by binding to IL1R1 and IL1RAP on uterine endometrium. Function of IL1B signaling system in the uterine endometrium plays an important role in embryo implantation in human and mouse by regulating endometrial gene expression. In pigs, IL1B increases expression of PG-endoperoxide synthase 2 (*PTGS2*) and PGE synthase (*PTGES*) during the implantation period in pigs (Franczak *et al*., 2010), suggesting that IL1B enhances prostaglandin synthesis in the uterine endometrium during early pregnancy in pigs. It appears that IL1B regulates many endometrial genes important for the implantation process as well as genes involved in PG action in pigs. IL1B induces salivary lipocalin (*SAL1*) expression in the uterine endometrium on day 12 of pregnancy (Seo *et al*., 2011).

The porcine conceptus trophectoderm uniquely secretes both type I and type II IFNs during the peri-implantation period. IFNG accounts for 75% of antiviral activity of conceptus secretory proteins, and IFND accounts for 25% (La Bonnardiere *et al*., 1991; Lefevre *et al*., 1998). *IFNG* mRNA is abundantly expressed in trophectoderm between days 13 and 20 of pregnancy, whereas *IFND* mRNA is detectable in conceptuses on day 14 only by RT-PCR (Joyce *et al*., 2007). Interferon-τ (IFNT) has an antiluteolytic effect to act as the signal for maternal pregnancy recognition in ruminants (Spencer *et al*., 2007), whereas IFNs do not have antiluteolytic effects that increase inter-estrus intervals or plasma progesterone levels in pigs (Harney and Bazer, 1989; Lefevre *et al*., 1998). Binding site for IFNG is localized to endometrial epithelial cells (D'Andréa and La Bonnardière, 1998) and paracrine action of IFNs results in increased PGE_2 secretion (Harney and Bazer, 1989). Intrauterine infusion of conceptus secretory protein containing IFNG and IFND into estrogen-treated cyclic pigs increases signal transducer and activator of transcription 1 (*STAT1*) expression in stroma (Joyce *et al*., 2007). Several genes are known to be IFN-responsive in the uterine endometrium, including *B2M*, *IRF1*, and *ISG15* (Johnson *et al*., 2009). IFNG increases expression of *SLA-DQA* and *SLA-DQB* in the uterine endometrium (Kim *et al*., 2012).

3. Growth Factors

Insulin-like growth factors (IGF1 and IGF2) are approximately 7 kDa polypeptide hormones with homology to proinsulin and are required for uterine and fetal growth (Simmen *et al*., 1992). IGF1 expression is localized to LE, GE, endothelium and vascular smooth muscle in the endometrium and conceptus trophectoderm (Persson *et al*., 1997). Levels of *IGF1* expression in the uterine endometrium increase during early pregnancy and reach the highest levels on days 12 to 13 (Simmen *et al*., 1990, 1992). Type I receptor for IGF are constitutively expressed in the endometrium during the peri-implantation period (Hofig *et al*., 1991). *IGF2* expression increases from day 10 and is highest during mid-gestation (Simmen *et al*., 1992). IGF1 in uterine flushings is detected with highest level on day 12 of pregnancy (Letcher *et al*., 1989). The biological activity of

IGFs is regulated by IGF-binding proteins (IGFBPs) (Clemmons, 1997). In uterine lumen, several IGFBPs, including IGFBP3 being most abundant, are present on days 10 to 11 and then substantially decrease by day 12 (Lee *et al*., 1998). Early exposure of pregnant gilts to estrogen causes premature loss of IGFBPs in uterus during the period of conceptus elongation (Ashworth *et al*., 2005).

Fibroblast growth factor-7 (FGF7), also known as keratinocyte growth factor (KGF), is a member of the heparin-binding fibroblast growth factor family and stimulates epithelial growth and differentiation (Rubin *et al*., 1995). *FGF7* is usually expressed by cells of mesenchymal origin. However, *FGF7* in the porcine uterus is expressed in the endometrial epithelium (Ka *et al*., 2000). Levels of *FGF7* expression is abundant between days 12 and 15 of the estrous cycle and pregnancy with the highest levels on day 12 in pregnant gilts and day 15 in cyclic gilts (Ka *et al*., 2000). FGF7 protein is present in uterine flushings of both day 12 of cyclic and pregnant gilts. FGF7 receptor, known as FGF receptor 2IIIb (FGF2IIIb), is expressed in both endometrial epithelium and conceptus trophectoderm. Estrogen of conceptus origin increases *FGF7* expression (Ka *et al*., 2001). Further, FGF7 treatment leads to proliferation and differentiation of conceptus trophectoderm in pigs (Ka *et al*., 2001).

Transforming growth factors-β (TGFβs) are growth factors with multiple functions regulating cellular growth, differentiation, adhesion, motility, and death (Zimmermann and Padgett, 2000). Three isoforms of TGFβ including TGFβ1, 2, and 3 and TGFβ receptor type I and II are expressed in the porcine uterine endometrium and conceptuses (Gupta *et al*., 1996, 1998a, b). Levels of all three TGFβ expression increase in the uterine endometrium from days 10 to 14. TGFβs are secreted from cell in latent forms associated with their isoform-specific latency-associated peptides (LAPs). The latent forms of TGFβ-LAP complex are converted into activated forms of TGFβ possibly by plasminogen activator or other protease (Geisert and Yelich, 1997; Rifkin *et al*., 1999). The active TGFβ are present in uterine lumen and TGFβ levels increase between days 11 to 13 of pregnancy (Gupta *et al*., 1998b). Expression of TGFβ receptor type I and II increases in the uterine endometrium from days 12 to 14 of pregnancy and is localized to apical side of endometrial epithelial cells (Gupta *et al*., 1998b). TGFβ stimulates production of extracellular matrix molecules and expression of integrin receptors (Roberts *et al*., 1990). Since LAP has the Arg-Gly-Asp (RGD) sequence, it can bind to integrin receptors. LAP released from TGFβ mediates attachment between conceptus and uterine luminal epithelium by interacting with integrins on trophectoderm and luminal epithelium (Jaeger *et al*., 2005).

4. Integrins and Extracellular Matrix Proteins

Integrins are cation-dependent heterodimeric intrinsic membrane glycoproteins that are formed by non-covalent linkage of α and β subunits. The functions of integrins in cell adhesion, migration, invasion and organization of cytoskeleton result from binding to various extracellular matrix (ECM) proteins and cell adhesion molecules (Burghardt *et al*., 1997). At least, 17 α and 8 β subunits are identified and 23 heterodimer combinations are formed (Luscinskas and Lawler, 1994). Several integrin subunits including α_1, α_3, α_4, α_v, β_1, and β_3 are expressed in uterine endometrial epithelial cells and conceptus trophectoderm in pigs (Bowen *et al*., 1996). Expression of α_4, α_5 and β_1 is highest during the period of maternal recognition of pregnancy, and α_4, α_5, α_v, β_1, and β_3 are detected at the implantation sites (Bowen *et al*., 1996).

The ECM ligands for these integrin receptors including vitronectin and oncofetal fibronectin are expressed in the uterine endometrium and conceptus (Bowen *et al*., 1996; Tuo and Bazer, 1996). SPP1 is an ECM secreted by many tissues including uterus (Johnson *et al*., 2003). In the porcine uterus, expression of *SPP1* mRNA is detected in discrete regions of LE in close proximity to conceptus just before attachment, and expands to entire LE by day 20, and after day 35 of pregnancy, expression of *SPP1* mRNA increases in GE until day 85 (Galow *et al*., 2002). Estrogen of conceptus origin induces *SPP1* expression in LE (White *et al*., 2005). SPP1 proteins with 70 kDa and 45 kDa are detected in the uterine endometrium and only 70 kDa form is present in uterine flushings from days 9 to 15 of the estrous cycle and pregnancy (Garlow *et al*., 2002). SPP1 released from LE binds integrin receptors on trophectoderm and LE to promote trophectodermal cell migration and attachment to LE (Erikson *et al*., 2009).

5. Proteases and Their Inhibitors

A variety of proteases play an important role in endometrial remodeling and trophoblast invasion. Although the porcine conceptuses do not invade the uterine luminal epithelium, they are invasive if they are transplanted to ectopic tissues (Samuel, 1971; Samuel and Perry, 1972). The pig endometrium secretes protease inhibitors such as plasmin/trypsin inhibitor to block trophoblastic invasion by inhibiting action of protease secreted

by conceptus origin (Fazleabas *et al*., 1983). Proteases degrading ECM are classified into three categories: serine, cysteine and metalloproteinases (Barrett, 1994). During the peri-implantation period, the porcine blastocyst secretes various proteases including urokinase type plasminogen activator (uPA), matrix metalloproteinase-2 (MMP-2) and MMP-9 (Mullins *et al*., 1980; Fazleabas *et al*., 1983; Menino *et al*., 1997). uPA, a serine protease, cleaves plasminogen which is present in uterine lumen with highest levels on day 12 and release plasmin (Fazleabas *et al*., 1983). Plasmin, in turn, activates other proteolytic enzymes, such as collagenase, that are secreted as latent form to degrade cell basement membrane and ECM (Werb *et al*., 1980). Cathepsins B (CTSB) and CTSL1, cysteine proteases, and their inhibitor, cystatin C (CST3) are expressed in the endometrial epithelium and their expression increases in response to progesterone during pregnancy in pigs (Song *et al*., 2010). CTSL1 is also expressed in the placental areolae and neonatal intestine (Song *et al*., 2010). Expression of legumain (LGMN), a cysteine protease, and its inhibitor, CST6, is detected in the uterine endometrium and placental areolae during pregnancy (Shim and Ka, unpublished data). These suggest that interactions between CTSs, such as CTSL1, CTSB, and LGMN, and their inhibitors, such as CST3 and CTS6, may be involved in remodeling of endometrial and placental tissues and transplacental transport of nutrients and macromolecules (Song *et al*., 2010).

6. Transport Proteins

Uterine endometrium synthesizes and secretes proteins, carbohydrates, lipids, and ions. The uterine secretions, termed histotroph, are essential to development of the conceptus during the peri-implantation period (Spencer and Bazer, 2004). In pigs, a species that has a relatively long pre-implantation period and forms a true epitheliochorial type placenta, uterine secretions play a critical role in the process of maternal recognition of pregnancy, implantation, and placentation (Roberts *et al*., 1987). The protein components of uterine secretions include enzymes, growth factors, hormones, extracellular proteins and transport proteins (Roberts and Bazer, 1988; Johnson *et al*., 2001; Spencer and Bazer, 2004). Among the many uterine secretory proteins released from the porcine endometrium, the best characterized are uteroferrin (Roberts *et al*., 1986), retinol binding protein (RBP) (Clawitter *et al*., 1990) and folate binding protein (FBP) (Vallet *et al*., 1998), which transfer iron, retinol and folic acid to the developing conceptus, respectively.

Uteroferrin is a most abundant protein in the uterine secretions and an iron-binding protein with a deep purple color and acid phosphatase activity (Zavy *et al*., 1984; Roberts *et al*., 1986). Uterine glandular epithelium begins to secrete uteroferrin on day 10 of the estrous cycle and pregnancy. The level of uteroferrin secretion increases after day 30 of pregnancy and reaches highest level on day 60 (Basha *et al*., 1979). The secreted uteroferrin is taken up by the placental areolae (Chen *et al*., 1975; Renegar *et al*., 1982; Raub *et al*., 1985) and ultimately enters the fetal circulation to be distributed to iron metabolizing sites such as liver and spleen (Renegar *et al*., 1982). Excess uteroferrin are cleared by fetal kidney and iron is temporally stored in the allantoic sac (Renegar *et al*., 1982; Roberts and Bazer *et al*., 1988).

The lipocalin protein family is composed of small extracellular proteins that have a common structural β-barrel feature. This property has been shown to allow these proteins to bind hydrophobic molecules and act as transporters. A subset of the lipocalin family known to be expressed in the uterine endometrium in pigs is RBP. RBP is one of major components in the uterine secretions. RBP can bind to retinol, a major serum transport form of vitamin A. In the uterus, retinol bound to RBP is taken up by the developing conceptus through cellular RBP (Napoli *et al*., 1991) and is metabolized retinal and retinoic acid, the most biologically active metabolites (Ross, 1991). Expression of RBP in the uterine endometrium increases from days 10 to 15 and decrease on day 18 of the estrous cycle. In pregnancy, expression of RBP increases from days 10 to 30, declines until day 75, and increases again from day 90. RBP proteins are detected in LE, GE, and placental areolae (Harney *et al*., 1993 and 1994; Johansson *et al*., 2001). Expression of RBP decreased in the uterine endometrium of pigs carrying cloned fetuses compared to the endometrium with fetuses resulting from natural mating. This indicates that decreased cloned fetuses may suffer retinol deficiency due to decreased expression of RBP by the uterine endometrium that affects development of fetuses (Kim *et al*., 2009).

Salivary lipocalin (SAL1) is a member of the lipocalin family that is comprised of a large group of small extracellular proteins, which act as transporters of hydrophobic compounds in aqueous biological fluids (Flower, 1996). Even though SAL1 is originally identified as boar specific sex pheromone-binding protein (Marchese *et al*., 1998; Loebel *et al*., 2000), SAL1 is also a component of uterine secretions (Kayser *et al*., 2006). SAL1 is expressed in the GE with highest levels on day 12

of pregnancy (Seo *et al*., 2011). Endometrial SAL1 protein is secreted into the uterine lumen and transported to conceptuses. IL1B of conceptus origin induces *SAL1* expression in the uterine endometrium on day 12 of pregnancy. In addition, the abundance of *SAL1* mRNA significantly increases in the endometrium with cloned embryos by somatic cell nuclear transfer compared to those in the endometrium with normal embryos on day 30 of pregnancy (Ka *et al*., 2008). These suggest that proper expression of *SAL1* is required for the establishment of pregnancy in pigs. Although identity of the ligand(s) and role of SAL1 are not known at the maternal-fetal interface during the implantation period, it is suggested that SAL1 is newly identified transport protein and may play a critical role in the establishment of pregnancy in pigs.

7. Lysophosphatidic Acids

Membrane phospholipids generate numerous lipid signaling molecules, such as prostaglandins and lysophosphatidic acids (LPAs). LPA is a simple phospholipid composed of a glycerol backbone with a fatty acyl chain and a free phosphate group. Many structurally diverse forms of LPA are present due to varying length and saturation of the fatty acyl side chain (Tigyi and Miledi, 1992; Gerrard *et al*., 1989). LPA production and release into extracellular body fluids involve hydrolysis of lysophospholipids, mainly lysophosphatidylcholine (LPC), by at least two enzymes: phospholipase A1/A2 (PLA1/PLA2) and lysophospholipase D (lysoPLD) (Aoki *et al*., 2008). Several LPA species are present in the uterine lumen in pigs (Seo *et al*., 2008). ENPP2 (ectonucleotide pyrophosphatase /phosphodiesterase 2), an enzyme with lysoPLD activity, is expressed in the uterine endometrium and conceptus and secreted into uterine lumen during early pregnancy in pigs (Seo *et al*., 2012). Higher levels of ENPP2 protein and higher lysoPLD activity in the uterine lumen on day 12 of pregnancy than on day 12 of the estrous cycle are associated with increased production of some LPA species in the uterine luminal fluids (Seo *et al*., 2008; Seo *et al*., 2012).

Biological functions of LPA are mediated by at least six specific receptors, LPAR1-6. Through these receptors, LPA elicits many growth factor-like biological effects, such as cell proliferation, survival, migration, differentiation, and aggregation in various cell types. LPA signaling plays an important role in the establishment and maintenance of pregnancy (Ye *et al*., 2005; Seo *et al*., 2008; Liszewska *et al*., 2009). In mice, deleting the *Lpar3* gene causes uneven embryo spacing and delayed implantation, which is associated with suppressed PG production (Ye et el., 2005). In sheep, LPA has been found in uterine flushings, and LPA increases cell proliferation and production of PGE_2 and $PGF_{2\alpha}$ in trophectoderm cells (Liszewska *et al*., 2009). In pigs, LPAR1, LPAR2, and LPAR3 are expressed in the uterine endometrium and expression of LPAR3 increases on day 12 of pregnancy, when the conceptus begins to implant, and LPA increases endometrial *PTGS2* expression (Seo *et al*., 2008).

8. Calcium Regulatory Molecules

Calcium ions are implicated in the embryo implantation process. It has been shown that uterine luminal calcium concentration increases on about day 12 of pregnancy and that calcium secretion is increased by estrogen in the uterine endometrium in pigs (Geisert *et al*., 1982). Several calcium-regulatory molecules are involved in calcium homeostasis. Stanniocalcin 1 (STC1) is phosphoglycoprotein that regulates intracellular calcium and phosphate levels (Wagner *et al*., 1986; Madsen *et al*., 1998). *STC1* is expressed in the uterine LE and secreted into uterine lumen during the implantation period in pigs (Song *et al*., 2009). Progesterone from CL induces *STC1* expression in LE and estrogen of conceptus origin further stimulates *STC1* expression. It seems that estrogen of conceptus origin increases calcium transport from LE to lumen by inducing *STC1* expression and secretion during the implantation period. TRPV6 is a calcium ion channel responsible for calcium uptake. S100G (also known as calbindin-d9k) functions in transport of cytoplasmic calcium ions from the apical to the basolateral side of cell (Hoenderop *et al*., 2002). Expression of *TRPV6* and *S100G* adds to the uterine LE and conceptus during the implantation period in pigs (Choi *et al*., 2009). Calcium content in the uterine flushings increases in association with increased estrogen of conceptus on days 11 to 12 and then declined by day 14. The rapid decline of calcium content in the uterine flushings by day 14 may be due to calcium reabsorption through TRPV6 expressed by uterine LE and conceptus. Collectively, coordination of theses calcium regulatory molecules may regulate calcium ion concentration in the uterine epithelial cells for the establishment and maintenance of pregnancy in pigs.

CONCLUSION

This review summarizes signaling molecules responsible for

conceptus-uterine interactions during the implantation period in pigs. During the implantation period, conceptus secretes estrogen, IL1B, IFNG, and IFND, whereas the uterine endometrium responds to these molecules secreted by conceptuses and arranges for the establishment of pregnancy by producing growth factors, protease inhibitors, transporters, adhesion molecules, lipids, and calcium regulatory molecules. Coordinate interactions among those molecules mediate successful establishment and maintenance of pregnancy. However, knowledge on signaling molecules at the conceptus-uterine interface and their function on the implantation process is still far from complete. Thus, further identification and analysis of those signaling molecules will help understand mechanism of implantation process and improve pregnancy rate in pigs.

REFERENCES

Aoki J, Inoue A and Okudaira S. 2008. Two pathways for lysophosphatidic acid production. Biochim. Biophys. Acta. 1781:513-518.

Ashworth MD, Ross JW, Stein DR, Allen DT, Spicer LJ and Geisert RD. 2005. Endocrine disruption of uterine insulin-like growth factor expression in the pregnant gilt. Reproduction 130:545-51.

Barrett AJ. 1994. Classification of peptidases. Methods. Enzymol. 244:1-15.

Basha SMM, Bazer FW and Roberts RM. 1979. The secretion of a uterine specific, purple phosphatase by cultured explants of porcine endometrium: Dependency upon the state of pregnancy of the donor animal. Biol. Reprod. 20:431-441.

Bazer FW and Thatcher WW. 1977. Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F_2alpha by the uterine endometrium. Prostaglandins 14: 397-400.

Bazer FW, Ott TL and Spencer TE. 1998. Endocrinology of the transition from recurring estrous cycles to establishment of pregnancy in subprimate mammals. In: Bazer FW, ed. Endocrinology of Pregnancy. Totowa, NJ: Humana Press 1-34.

Bazer FW, Roberts RM and Thatcher WW. 1979. Actions of hormones on the uterus and effect on conceptus development. J. Anim. Sci. 49:35-45.

Bowen JA, Bazer FW and Burghardt RC. 1996. Spatial and temporal analyses of integrin and Muc-1 expression in porcine uterine epithelium and trophectoderm *in vivo*. Biol. Reprod. 55:1098-1106.

Burghardt RC, Bowen JA, Newton GR and Bazer FW. 1997. Extracellular matrix and the implantation cascade in pigs. J. Reprod. Fertil. Suppl. 52:151-164.

Chen TT, Bazer FW, Gebhardt BM and Roberts RM. 1975. Uterine secretion in mammals: synthesis and placental transport of a purple acid phosphatase in pigs. Biol. Reprod. 13:304-313.

Choi Y, Seo H, Kim M and Ka H. 2009. Dynamic expression of calcium-regulatory molecules, TRPV6 and S100G, in the uterine endometrium during pregnancy in pigs. Biol. Reprod. 81:1122-1130.

Clawitter J, Trout WE, Burke MG, Araghi S and Roberts RM. 1990. A novel family of progesterone-induced, retinol-binding proteins from uterine secretions of the pig. J. Biol. Chem. 265:3248-3255.

Clemmons DR. 1997. Insulin-like growth factor binding proteins and their role in controlling IGF actions. Cytokine. Growth. Factor. Rev. 8:45-62.

D'Andréa S and La Bonnardière C. 1998.: Cloning of the porcine interferon-gamma receptor and its foeto-endometrial expression in early pregnancy. Mol. Reprod. Dev. 51:225-234.

Erikson DW, Burghardt RC, Bayless KJ and Johnson GA. 2009. Secreted phosphoprotein 1 (SPP1, osteopontin) binds to integrin alpha v beta 6 on porcine trophectoderm cells and integrin alpha v beta 3 on uterine luminal epithelial cells, and promotes trophectoderm cell adhesion and migration. Biol. Reprod. 81:814-825.

Fazleabas AT, Geisert RD, Bazer FW and Roberts RM. 1983. Relationship between release of plasminogen activator and estrogen by blastocysts and secretion of plasmin inhibitor by uterine endometrium in the pregnant pig. Biol. Reprod. 29:225-238.

Finch PW, *et al*. 1995. Keratinocyte growth factor. Cell. Biol. Int. 19:399-411.

Fliss AE, Michel FJ, Chen CL, Hofig A, Bazer FW, Chou JY and Simmen RC. 1991. Regulation of the uteroferrin gene promoter in endometrial cells: interactions among estrogen, progesterone, and prolactin. Endocrinology 129:697-704.

Flower DR. 1996. The lipocalin protein family: structure and function. Biochem. J. 318:1-14.

Ford SP, Christenson RK and Ford JJ. 1982. Uterine blood flow

and uterine arterial, venous and luminal concentrations of oestrogens on days 11, 13 and 15 after oestrus in pregnant and non-pregnant sows. J. Reprod. Fertil. 64:185-190.

Ford SP. 1995. Control of blood flow to the gravid uterus of domestic livestock species. J. Anim. Sci. 73:1852-160.

Franczak A, Zmijewska A, Kurowicka B, Wojciechowicz B and Kotwica G. 2010. Interleukin 1β-induced synthesis and secretion of prostaglandin E_2 in the porcine uterus during various periods of pregnancy and the estrous cycle. J. Physiol. Pharmacol. 61:733-742.

Garlow JE, Ka H, Johnson GA, Burghardt RC, Jaeger LA and Bazer FW. 2002. Analysis of osteopontin at the maternal-placental interface in pigs. Biol. Reprod. 66:718-725.

Geisert RD and Yelich JV. 1997. Regulation of conceptus development and attachment in pigs. J. Reprod. Fertil. Suppl. 52:133-149.

Geisert RD, Brenner RM, Moffatt RJ, Harney JP, Yellin T and Bazer FW. 1993. Changes in oestrogen receptor protein, mRNA expression and localization in the endometrium of cyclic and pregnant gilts. Reprod. Fertil. Dev. 5:247-260.

Geisert RD, Pratt TN, Bazer FW, Mayes JS and Watson GH. 1994. Immunocytochemical localization and changes in endometrial progestin receptor protein during the porcine oestrous cycle and early pregnancy. Reprod. Fertil. Dev. 6: 749-760.

Geisert RD, Renegar RH, Thatcher WW, Roberts RM and 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.

Gerrard JM and Robinson P. 1989. Identification of the molecular species of lysophosphatidic acid produced when platelets are stimulated by thrombin. Biochim. Biophys. Acta. 1001:282-285.

Gupta A, Bazer FW and Jaeger LA. 1996. Differential expression of beta transforming growth factors (TGF beta 1, TGF beta 2, and TGF beta 3) and their receptors (type I and type II) in peri-implantation porcine conceptuses. Biol. Reprod. 55:796-802.

Gupta A, Dekaney CM, Bazer FW, Madrigal MM and Jaeger LA. 1998b. Beta transforming growth factors (TGFbeta) at the porcine conceptus-maternal interface. Part II: uterine TGFbeta bioactivity and expression of immunoreactive TGF-betas (TGFbeta1, TGFbeta2, and TGFbeta3) and their receptors (type I and type II). Biol. Reprod. 59:911-917.

Gupta A, Ing NH, Bazer FW, Bustamante LS and Jaeger LA. 1998a. Beta transforming growth factors (TGFss) at the porcine conceptus-maternal interface. Part I: expression of TGFbeta1, TGFbeta2, and TGFbeta3 messenger ribonucleic acids. Biol. Reprod. 59:905-910.

Guthrie HD, Henricks DM and Handlin DL. 1972. Plasma estrogen, progesterone, and luteinizing hormone prior to estrus and during pregnancy in pigs. Endocrinology 91:675-679.

Harney JP and Bazer FW. 1989. Effect of porcine conceptus secretory proteins on interestrous interval and uterine secretion of prostaglandins. Biol. Reprod. 41:277-284.

Harney JP, Ott TL, Geisert RD and Bazer FW. 1993. Retinol-binding protein gene expression in cyclic and pregnant endometrium of pigs, sheep, and cattle. Biol. Reprod. 49: 1066-1073.

Heap RB, Flint AFP and Staple LD. 1983. Endocrinology of trophoblast in farm animals. In: Loke YW, Whyte A (eds) Biology of Trophoblast. Elsevier, New York. 353–400.

Henricks DM, Guthrie HD and Handlin DL. 1972. Plasma estrogen, progesterone and luteinizing hormone levels during the estrous cycle in pigs. Biol. Reprod. 6:210-218.

Hoenderop JG, Nilius B and Bindels RJ. 2002. Molecular mechanism of active Ca^{2+} reabsorption in the distal nephron. Annu. Rev. Physiol. 64:529-549.

Hofig A, Michel FJ, Simmen FA and Simmen RC. 1991. Constitutive expression of uterine receptors for insulin-like growth factor-I during the peri-implantation period in the pig. Biol. Reprod. 45:533-539.

Jaeger LA, Johnson GA, Ka H, Garlow JG, Burghardt RC, Spencer TE and Bazer FW. 2001 Functional analysis of autocrine and paracrine signalling at the uterine-conceptus interface in pigs. Reprod. Suppl. 58:191-207.

Jaeger LA, Spiegel AK, Ing NH, Johnson GA, Bazer FW and Burghardt RC. 2005. Functional effects of transforming growth factor beta on adhesive properties of porcine trophectoderm. Endocrinology 146:3933-3942.

Johansson S, Dencker L and Dantzer V. 2001. Immunohistochemical localization of retinoid binding proteins at the materno-fetal interface of the porcine epitheliochorial placenta. Biol. Reprod. 64:60-68.

Johnson GA, Bazer FW, Burghardt RC, Spencer TE, Wu G and Bayless KJ. 2009. Conceptus-uterus interactions in pigs: endometrial gene expression in response to estrogens and interferons from conceptuses. Soc. Reprod. Fertil. Suppl. 66:321-332.

Johnson GA, Bazer FW, Jaeger LA, Ka H, Garlow JE, Pfarrer C, Spencer TE and Burghardt RC. 2001. Muc-1, integrin, and osteopontin expression during the implantation cascade in sheep. Biol. Reprod. 65:820-828.

Johnson GA, Burghardt RC, Bazer FW and Spencer TE. 2003. Osteopontin: roles in implantation and placentation. Biol. Reprod. 69:1458-1471.

Joyce MM, Burghardt RC, Geisert RD, Burghardt JR, Hooper RN, Ross JW, Ashworth MD and Johnson GA. 2007. Pig conceptuses secrete estrogen and interferons to differentially regulate uterine STAT1 in a temporal and cell type-specific manner. Endocrinology 148:4420-4431.

Ka H, Jaeger LA, Johnson GA, Spencer TE and Bazer FW. 2001. Keratinocyte growth factor is up-regulated by estrogen in the porcine uterine endometrium and functions in trophectoderm cell proliferation and differentiation. Endocrinology 142:2303-2310.

Ka H, Seo H, Kim M, Moon S, Kim H and Lee CK. 2008. Gene expression profiling of the uterus with embryos cloned by somatic cell nuclear transfer on day 30 of pregnancy. Anim. Reprod. Sci. 108:79-91.

Ka H, Spencer TE, Johnson GA and Bazer FW. 2000. Keratinocyte growth factor: expression by endometrial epithelia of the porcine uterus. Biol. Reprod. 62:1772-1778.

Kayser JP, Kim JG, Cerny RL and Vallet JL. 2006. Global characterization of porcine intrauterine proteins during early pregnancy. Reproduction 131:379-388.

Kelly RW and Abel MH. 1980. Catechol oestrogens stimulate and direct prostaglandin synthesis. Prostaglandins 20:613-626.

Kelly RW and Abel MH. 1981. A comparison of the effects of 4-catechol oestrogens and 2-pyrogallol oestrogens on prostaglandin synthesis by the rat and human uterus. J. Steroid. Biochem. 14:787-791.

Kim M, Seo H, Choi Y, Hwang W, Lee CK and Ka H. 2009. Aberrant expression of retinol-binding protein, osteopontin and fibroblast growth factor 7 in the porcine uterine endometrium of pregnant recipients carrying embryos produced by somatic cell nuclear transfer. Anim. Reprod. Sci. 112: 172-181.

Kim M, Seo H, Choi Y, Shim J, Bazer FW and Ka H. 2012. Swine leukocyte antigen-DQ expression and its regulation by interferon-gamma at the maternal-fetal interface in pigs. Biol. Reprod. 86:43.

La Bonnardière C, Martinat-Botte F, Terqui M, Lefèvre F, Zouari K, Martal J and Bazer FW. 1991. Production of two species of interferon by Large White and Meishan pig conceptuses during the peri-attachment period. J. Reprod. Fertil. 91:469-478.

Lee CY, Green ML, Simmen RC and Simmen FA. 1998. Proteolysis of insulin-like growth factor-binding proteins (IGFBPs) within the pig uterine lumen associated with peri-implantation conceptus development. J. Reprod. Fertil. 112:369-377.

Lefèvre F, Guillomot M, D'Andréa S, Battegay S and La Bonnardière C. 1998. Interferon-delta: the first member of a novel type I interferon family. Biochimie 80:779-788.

Letcher R, Simmen RC, Bazer FW and Simmen FA. 1989. Insulin-like growth factor-I expression during early conceptus development in the pig. Biol. Reprod. 41:1143-1151.

Liszewska E, Reinaud P, Billon-Denis E, Dubois O, Robin P and Charpigny G. 2009. Lysophosphatidic acid signaling during embryo development in sheep: involvement in prostaglandin synthesis. Endocrinology 150:422-434.

Loebel D, Scaloni A, Paolini S, Fini C, Ferrara L, Breer H and Pelosi P. 2000. Cloning, post-translational modifications, heterologous expression and ligand-binding of boar salivary lipocalin. Biochem. J. 350:369-379.

Luscinskas FW and Lawler J. 1994. Integrins as dynamic regulators of vascular function. FASEB. J. 8:929-938.

Lye SJ and Porter DG. 1978. Demonstration that progesterone 'blocks' uterine activity in the ewe *in vivo* by a direct action on the myometrium. J. Reprod. Fertil. 52:87-94.

Madsen KL, Tavernini MM, Yachimec C, Mendrick DL, Alfonso PJ, Buergin M, Olsen HS, Antonaccio MJ, Thomson AB and Fedorak RN. 1998. Stanniocalcin: a novel protein regulating calcium and phosphate transport across mammalian intestine. Am. J. Physiol. 274:G96–G102.

Marchese S, Pes D, Scaloni A, Carbone V and Pelosi P. 1998. Lipocalins of boar salivary glands binding odours and pheromones. Eur. J. Biochem. 252:563-568.

Menino AR Jr, Hogan A, Schultz GA, Novak S, Dixon W and Foxcroft GH. 1997. Expression of proteinases and proteinase inhibitors during embryo-uterine contact in the pig. Dev. Genet. 21:68-74.

Mondschein JS, Hersey RM, Dey SK, Davis DL and Weisz J. 1985. Catechol estrogen formation by pig blastocysts during the preimplantation period: biochemical characterization of estrogen-2/4-hydroxylase and correlation with aromatase activity. Endocrinology 117:2339-2346.

Mullins DE, Bazer FW and Roberts RM. 1980. Secretion of a progesterone-induced inhibitor of plasminogen activator by the porcine uterus. Cell 20:865-872.

Napoli JL, Posch KP, Fiorella PD and Boerman MH. 1991. Physiological occurrence, biosynthesis and metabolism of retinoic acid: evidence for roles of cellular retinol-binding protein (CRBP) and cellular retinoic acid-binding protein (CRABP) in the pathway of retinoic acid homeostasis. Biomed. Pharmacother. 45:131-143.

Niswender GD, Juengel JL, Silva PJ, Rollyson MK and McIntush EW. 2000. Mechanisms controlling the function and life span of the corpus luteum. Physiol. Rev. 80:1-29.

Pakrasi PL and Dey SK. 1983. Catechol estrogens stimulate synthesis of prostaglandins in the preimplantation rabbit blastocyst and endometrium. Biol. Reprod. 29:347-354.

Paria BC, Lim H, Wang XN, Liehr J, Das SK and Dey SK. 1998. Coordination of differential effects of primary estrogen and catecholestrogen on two distinct targets mediates embryo implantation in the mouse. Endocrinology 139:5235-5246.

Persson E, Sahlin L, Masironi B, Dantzer V, Eriksson H and Rodriguez-Martinez H. 1997. Insulin-like growth factor-I in the porcine endometrium and placenta: localization and concentration in relation to steroid influence during early pregnancy. Anim. Reprod. Sci. 46:261-281.

Pope WF, Lawyer MS, Nara BS and First NL. 1986. Effect of asynchronous superinduction on embryo survival and range of blastocyst development in swine. Biol. Reprod. 35:133-137.

Pope WF. 1988. Uterine asynchrony: a cause of embryonic loss. Biol. Reprod. 39:999-1003.

Porter DG and Lye SJ. 1983. Partial reversal of the myometrial progesterone 'block' in the non-pregnant ewe *in vivo* by oestradiol-17 beta. J. Reprod. Fertil. 67:227-234.

Raub TJ, Bazer FW and Roberts RM. 1985. Localization of the iron transport glycoprotein, uteroferrin, in the porcine endometrium and placenta by using immunocolloidal gold. Anat. Embryol. (Berl). 171:253-258.

Renegar RH, Bazer FW and Roberts RM. 1982. Placental transport and distribution of uteroferrin in the fetal pig. Biol. Reprod. 27:1247-1260.

Roberts AB, Heine UI, Flanders KC and Sporn MB. 1990. Transforming growth factor-beta. Major role in regulation of extracellular matrix. Ann. N. Y. Acad. Sci. 580:225-232.

Roberts RM and Bazer FW. 1988. The functions of uterine secretions. J. Reprod. Fertil. 82:875-892.

Roberts RM, Murray MK, Burke MG, Ketcham CM and Bazer FW. 1987. Hormonal control and function of secretory proteins. Adv. Exp. Med. Biol. 230:137-150.

Roberts RM, Raub TJ and Bazer FW. 1986. Role of uteroferrin in transplacental iron transport in the pig. Fed. Proc. 45: 2513-2518.

Ross AC. 1991. Vitamin A: Current understanding of the mechanisms of action. Nutrition. today. 26:6-12.

Ross JW, Ashworth MD, White FJ, Johnson GA, Ayoubi PJ, DeSilva U, Whitworth KM, Prather RS and Geisert RD. 2007. Premature estrogen exposure alters endometrial gene expression to disrupt pregnancy in the pig. Endocrinology 148:4761-4773.

Ross JW, Malayer JR, Ritchey JW and Geisert RD. 2003. Characterization of the interleukin-1beta system during porcine trophoblastic elongation and early placental attachment. Biol. Reprod. 69:1251-1259.

Rubin JS, Bottaro DP, Chedid M, Miki T, Ron D, Cheon G, Taylor WG, Fortney E, Sakata H, Samuel CA and Perry JS. 1972. The ultrastructure of pig trophoblast transplanted to an ectopic site in the uterine wall. J. Anat. 113:139-149.

Samuel CA. 1971. The development of pig trophoblast in ectopic sites. J. Reprod. Fertil. 27:494-495.

Seo H, Choi Y, Shim J, Kim M and Ka H. 2012. Analysis of ENPP2, a lysophosphatidic acid-generating enzyme, in the uterus during pregnancy in pigs. Biol. Reprod. 87:77.

Seo H, Kim M, Choi Y and Ka H. 2011. Salivary lipocalin is uniquely expressed in the uterine endometrial glands at the time of conceptus implantation and induced by interleukin 1beta in pigs. Biol. Reprod. 84:279-287.

Seo H, Kim M, Choi Y, Lee CK and 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.

Simmen FA, Simmen RC, Geisert RD, Martinat-Botte F, Bazer FW and Terqui M. 1992. Differential expression, during the estrous cycle and pre- and postimplantation conceptus development, of messenger ribonucleic acids encoding components of the pig uterine insulin-like growth factor system. Endocrinology 130:1547-1556.

Simmen RC, Simmen FA, Hofig A, Farmer SJ and Bazer FW. 1990. Hormonal regulation of insulin-like growth factor gene expression in pig uterus. Endocrinology 127:2166-2174.

Song G, Bailey DW, Dunlap KA, Burghardt RC, Spencer TE, Bazer FW and Johnson GA. 2010. Cathepsin B, cathepsin

L, and cystatin C in the porcine uterus and placenta: potential roles in endometrial/placental remodeling and in fluid-phase transport of proteins secreted by uterine epithelia across placental areolae. Biol. Reprod. 82:854-864.

Song G, Dunlap KA, Kim J, Bailey DW, Spencer TE, Burghardt RC, Wagner GF, Johnson GA and Bazer FW. 2009. Stanniocalcin 1 is a luminal epithelial marker for implantation in pigs regulated by progesterone and estradiol. Endocrinology 150:936-945.

Spencer TE and Bazer FW. 2004. Uterine and placental factors regulating conceptus growth in domestic animals. J. Anim. Sci. 82 E-Suppl:E4-13.

Spencer TE, Johnson GA, Bazer FW, Burghardt RC and Palmarini M. 2007. Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. Reprod. Fertil. Dev. 19:65-78.

Stone BA and Seamark RF. 1985. Steroid hormones in uterine washings and in plasma of gilts between days 9 and 15 after oestrus and between days 9 and 15 after coitus. J. Reprod. Fertil. 75:209-221.

Tigyi G and Miledi R. 1992. Lysophosphatidates bound to serum albumin activate membrane currents in Xenopus oocytes and neurite retraction in PC12 pheochromocytoma cells. J. Biol. Chem. 267:21360-21367.

Tranguch S, Daikoku T, Guo Y, Wang H and Dey SK. 2005. Molecular complexity in establishing uterine receptivity and implantation. Cell. Mol. Life. Sci. 62:1964-1973.

Trout WE, Hall JA, Stallings-Mann ML, Galvin JM, Anthony RV and Roberts RM. 1992. Steroid regulation of the synthesis and secretion of retinol-binding protein by the uterus of the pig. Endocrinology 130:2557-2564.

Tuo W and Bazer FW. 1996. Expression of oncofetal fibronectin in porcine conceptuses and uterus throughout gestation. Reprod. Fertil. Dev. 8:1207-1213.

Tuo W, Harney JP and Bazer FW. 1996. Developmentally regulated expression of interleukin-1 beta by peri-implantation conceptuses in swine. J. Reprod. Immunol. 31:185-198.

Vallet JL, Christenson RK and Klemcke HG. 1998. Purification and characterization of intrauterine folate-binding proteins from swine. Biol. Reprod. 59:176-181.

Wagner GF, Hampong M, Park CM and Copp DH. 1986. Purification, characterization, and bioassay of teleocalcin, a glycoprotein from salmon corpuscles of Stannius. Gen. Comp. Endocrinol. 63:481–491.

Werb Z, Banda MJ and Jones PA. 1980. Degradation of connective tissue matrices by macrophages. I. Proteolysis of elastin, glycoproteins, and collagen by proteinases isolated from macrophages. J. Exp. Med. 152:1340-1357.

White FJ, Ross JW, Joyce MM, Geisert RD, Burghardt RC and Johnson GA. 2005. Steroid regulation of cell specific secreted phosphoprotein 1 (osteopontin) expression in the pregnant porcine uterus. Biol. Reprod. 73:1294-1301.

Ye X, Hama K, Contos JJ, Anliker B, Inoue A, Skinner MK, Suzuki H, Amano T, Kennedy G, Arai H, Aoki J and Chun J. 2005. LPA3-mediated lysophosphatidic acid signalling in embryo implantation and spacing. Nature 435:104-108.

Zavy MT, Bazer FW, Thatcher WW and Wilcox CJ. 1980. A study of prostaglandin F_2 alpha as the luteolysin in swine: V. Comparison of prostaglandin F, progestins, estrone and estradiol in uterine flushings from pregnant and nonpregnant gilts. Prostaglandins 20:837-851.

Zavy MT, Roberts RM and Bazer FW. 1984. Acid phosphatase and leucine aminopeptidase activity in the uterine flushings of non-pregnant and pregnant gilts. J. Reprod. Fertil. 72:503-507.

Zimmerman CM and Padgett RW. 2000. Transforming growth factor beta signaling mediators and modulators. Gene 249: 17-30.

(received: 2012. 9. 4 / revised: 2012. 9. 5 / accepted: 2012. 10. 4)