Novel insights into the mechanisms of pregnancy establishment: regulation of prostaglandin synthesis and signaling in the pig

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Abstract

Ovarian progesterone induces essential changes leading to a temporary state of uterine receptivity for conceptus implantation. Estrogens secreted by the porcine conceptus on days 11 and 12 of pregnancy provide the initial signal for maternal recognition of pregnancy and maintenance of a functional corpus luteum (CL) for continued production of progesterone. As prostaglandins $F_{2\alpha}$ ($PGF_{2\alpha}$) and E_2 (PGE_2) exert opposing actions on the CL, a tight control over their synthesis and secretion is critical either for the initiation of luteolysis or maintenance of pregnancy. One of the supportive mechanisms by which conceptus inhibits luteolysis is changing PG synthesis in favor of luteoprotective PGE_2 . Conceptus PGE_2 could be amplified by PGE_2 feedback loop in the endometrium. In pigs, as in other species, implantation and establishment of pregnancy is associated with upregulation of expression of proinflammatory factors, which include cytokines, growth factors, and lipid mediators. The conceptus produces inflammatory mediators: interferon γ and interferon δ , interleukins IL1B and IL6, and PGs, which probably activate inflammatory pathways in the endometrium. The endometrium responds to these embryonic signals by enhancing further progesterone-induced uterine receptivity. Understanding the mechanisms of pregnancy establishment is required for translational research to increase reproductive efficiencies and fertility in humans and animals.

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Introduction

Species-specific variations in maternal recognition of pregnancy and implantation processes exist, and these differences preclude the formulation of unifying theories for the molecular basis of these events. This review addresses recognized signaling cascades and new research in mechanisms responsible for conceptus–endometrial interactions during the peri-implantation period in the pig with some comparisons with other species.

Prostaglandins (PGs) play a pivotal role in luteolysis as well as in establishment of pregnancy (Bazer & Thatcher 1977, McCracken *et al.* 1999, Waclawik *et al.* 2009a). Corpus luteum (CL) regression on days 15–16 of the estrous cycle results from an increase in pulsatile endometrial secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in pigs (Moeljono *et al.* 1977). The porcine CL does not display a luteolytic response to exogenous $PGF_{2\alpha}$ until days 12–13 of the estrous cycle. The insensitivity of the early CL to exogenous $PGF_{2\alpha}$ is caused partially by lower luteal $PGF_{2\alpha}$ receptor concentration on day 14 of pregnancy in comparison with the corresponding day of the estrous cycle and a deficiency in post – $PGF_{2\alpha}$ receptor signaling (Gadsby *et al.* 1993, Zorrilla *et al.* 2009).

The role of oxytocin (OXT) in controlling $PGF_{2\alpha}$ secretion is not as well defined in pigs as in ruminants

(Mirando *et al.* 1995, Waclawik *et al.* 2010). In ewes, luteal OXT acts through endometrial OXT receptors to generate a $PGF_{2\alpha}$ pulse which in turn stimulates further secretion of OXT as well as inducing functional regression of the CL through inhibiting progesterone synthesis (reviewed in Jenkin (1992)). In the pig, OXT may not be responsible for the initiation of luteolysis but is more likely involved in the control of pulsatile release of $PGF_{2\alpha}$, especially the height and frequency of the peaks of this hormone during luteolysis (Kotwica *et al.* 1999). Surprisingly, OXT concentration in the uterine lumen significantly increases on days 12–14 of pregnancy compared with the corresponding days of the estrous cycle (Vallet *et al.* 1998). Moreover, OXT is not luteolytic when administered locally to the uterine lumen as it is when administered systemically (Sample *et al.* 2000) and i.u. infusion of OXT decreases plasma concentrations of $PGF_{2\alpha}$ metabolite, PGFM, on day 16 after estrus (Sample *et al.* 2004). In addition, OXT stimulates synthesis and secretion of PGE_2 (PG which has the opposite action to $PGF_{2\alpha}$ on the CL) by endometrial luminal epithelial (LE) cells only during early pregnancy but not on the corresponding days of the estrous cycle (Waclawik *et al.* 2010).

Pregnancy recognition mechanisms

Pregnancy establishment in mammals requires prolongation of luteal life span and progesterone production. Progesterone stimulates secretory functions of the endometrium required for conceptus development and implantation. The pregnancy recognition signals secreted by the conceptus may be luteotrophic, if they directly maintain CL function, or antiluteolytic, if they decrease uterine release of luteolytic $\text{PGF}_{2\alpha}$ (Auletta & Flint 1988, Bazer *et al.* 2010).

Maternal recognition and establishment of pregnancy in pigs requires the biphasic pattern of estrogen secretion (Geisert *et al.* 1990). Conceptuses secrete increased levels of estrogens, mainly 17β -estradiol (E_2), on days 11–12 and between days 15 and 25–30 of pregnancy. Maternal recognition of pregnancy occurs simultaneously with rapid transformation of trophoblast from spherical to tubular, and then filamentous forms between days 10 and 12 before implantation (Geisert *et al.* 1990). The observation that conceptus elongation has not been achieved *in vitro* indicate that this process may not be exclusively controlled by conceptus transcriptional changes and the uterine environment has a pivotal role in the initiation of trophoctodermal remodeling in the pig (Ross *et al.* 2009).

Luteoprotective action of estrogen is complex. It stimulates luteal progesterone secretion directly (Conley & Ford 1989). Indirect action of estrogen involves increasing luteal LH receptor concentration (Garverick *et al.* 1982), decreasing $\text{PGF}_{2\alpha}$ release from uterus into the peripheral circulation (Bazer & Thatcher 1977), and regulating PG synthesis and signaling in the endometrium (Waclawik *et al.* 2009a) and conceptus (A Waclawik, unpublished observations). Estrogen receptor (ESR) expression in LE and glandular epithelium (GE) of the endometrium (Geisert *et al.* 1993) and in the conceptus (Kowalski *et al.* 2002) coincides with estrogen secretion from the conceptus, suggesting both autocrine and paracrine actions. On day 12 of pregnancy, *ESR1* mRNA abundance is elevated in the LE (Ross *et al.* 2010). Both a sufficient amount of conceptus estrogen synthesis and timing of the endometrial exposure to estrogen is crucial for establishment of pregnancy. Premature administration of estrogen on days 9–10 of pregnancy results in embryonic loss by day 15 and altered endometrial expression of many genes (including PG-endoperoxide synthase 2; *PTGS2*), probably causing desynchronization of the uterine environment and conceptus implantation (Geisert *et al.* 2006).

Estrogen has both systemic and local effects in the maternal system (Ford *et al.* 1982). It is likely that the local effect of estrogen on the endometrium is limited to regions in close proximity to the conceptus due to metabolic activity of trophoctoderm. During pregnancy, the endometrium rapidly metabolizes E_2 to estrone sulfate, which is the biologically inactive form and is

present in greater amounts in the uterine lumen of pregnant pigs (Flood 1974). Sulfatase enzyme activity of the trophoctoderm restores the biological activity of estrogen that results in a localized effect of estrogen to regulate expression of some endometrial genes (Johnson *et al.* 2009, Waclawik *et al.* 2009a).

Prostaglandins

Role of PGs during pregnancy establishment

A tight control over PG synthesis and secretion is critical either for the initiation of luteolysis or maintenance of pregnancy in domestic animals (Kennedy *et al.* 2007). Reduced PG synthesis in the human endometrium may lead to poor endometrial receptivity for embryo implantation since PG synthesis appears to be disrupted in women with repeated IVF failure (Achache *et al.* 2010). It was demonstrated that inhibition of PG synthesis causes pregnancy failure before the implantation process in animals with different types of placentation, beginning from non-invasive placenta in the pig (Kraeling *et al.* 1985) and in ruminants (Erdem & Guzeloglu 2010, Dorniak *et al.* 2011) to invasive placenta in rodents (reviewed in Kennedy *et al.* (2007)). Interestingly, blocking of PG synthesis does not affect elongation of trophoblast in pigs (Geisert *et al.* 1986).

Moeljono *et al.* (1977) demonstrated a decrease in peak amplitude and concentration of $\text{PGF}_{2\alpha}$ in the utero-ovarian vein in pregnant gilts compared with cyclic pigs. However, another group reported that this difference in $\text{PGF}_{2\alpha}$ concentration in the ovarian–uterine vein is much less between cyclic and pregnant animals (Hunter & Poyser 1982). Our results indicate that the content of PGF synthase (PGFS) mRNA and/or protein in the conceptus and endometrium is decreased in the first day of maternal recognition of pregnancy, between days 10 and 13 compared with later stages of pregnancy (Waclawik *et al.* 2006). However, after day 14 of pregnancy, content of PGFS mRNA and protein increases in both the endometrium and conceptus (Waclawik *et al.* 2006, Waclawik & Ziecik 2007). Moreover, uterine flushings of pregnant pigs contain higher amounts of $\text{PGF}_{2\alpha}$ than those from cyclic animals (Zavy *et al.* 1980). It is likely that this upregulation of conceptus and endometrial PGFS resulting in an increase of $\text{PGF}_{2\alpha}$ in the uterine lumen during implantation could be involved in angiogenesis because $\text{PGF}_{2\alpha}$ signaling mediates expression of angiogenic genes (Sales *et al.* 2005). Nevertheless, the action of $\text{PGF}_{2\alpha}$ in this period should be restricted only to the uterus and conceptus. Therefore, to avoid luteolytic action of $\text{PGF}_{2\alpha}$ on the CL in ovary, $\text{PGF}_{2\alpha}$ is sequestered in the uterus probably by redirection of $\text{PGF}_{2\alpha}$ secretion from the uterine venous drainage (endocrine) to the uterine lumen (exocrine) by conceptus estrogen (Bazer & Thatcher 1977). In addition, an antiluteolytic mechanism could involve

the retrograde transfer of $\text{PGF}_{2\alpha}$ from the venous blood and uterine lymph into the uterine lumen and ability of uterine veins and arterial walls to accumulate $\text{PGF}_{2\alpha}$ (Krzymowski & Stefanczyk-Krzymowska 2004).

Another potential mechanism by which the conceptus contributes to prevention of luteolysis is by changing PG synthesis in favor of the luteoprotective PGE_2 . The conceptus and endometrium synthesize increased amounts of PGE_2 before implantation (Waclawik *et al.* 2006, Waclawik & Ziecik 2007). A greater PGE_2 : $\text{PGF}_{2\alpha}$ ratio stimulates progesterone and E_2 secretion by luteal cells collected on days 10–12 of the estrous cycle (Gregoraszczyk & Michas 1999). I.u. infusion of PGE_2 delays the decline in plasma progesterone concentrations that normally occurs around day 15 and extends the luteal function in cyclic gilts (Akinlosotu *et al.* 1986). Greater PGE_2 secretion in the gravid uterine horn of unilaterally pregnant pigs is associated with increased luteal weights and progesterone concentrations of ipsilateral CLs (Christenson *et al.* 1994). The infusion of PGE_2 into the ovarian artery elevates the concentration of progesterone in the ovarian venous blood on days 13 and 14 of pregnancy (Stefanczyk-Krzymowska *et al.* 2006).

It should be emphasized that the conceptus and endometrial PGE_2 secretion, similarly to embryo estrogen secretion, increases and begins to exert the luteoprotective action before days 14–15 of pregnancy (which in cycling gilts corresponds to initiation of luteolysis). In contrast to the pattern of PG secretion exhibited by nonpregnant gilts, pregnant animals have an earlier (days 11–13) transient rise in PGE_2 and $\text{PGF}_{2\alpha}$, measured in the utero-ovarian venous plasma, during which PGE_2 is the predominant uterine PG secreted (Christenson *et al.* 1994). Moreover, in unilateral pregnancy PGE_2 concentrations in the utero-ovarian venous blood draining the gravid uterine horn are greater than in the non-gravid uterine horn, whereas $\text{PGF}_{2\alpha}$ concentrations do not differ between these two horns (Christenson *et al.* 1994). A local and systemic effect of E_2 on luteal progesterone secretion was observed in cyclic gilts receiving E_2 unilaterally into an isolated uterine horn on days 11–15 (Ford *et al.* 1982). The local effect of E_2 in stimulating progesterone production by ipsilateral ovary is not due to reduced $\text{PGF}_{2\alpha}$ concentrations in the utero-ovarian blood draining the E_2 -injected horn. Wasielek *et al.* (2008) indicated that in unilateral pregnancy on days 12–14, there is more PGE_2 in CLs ipsilateral to the gravid uterine horn compared with CLs from ovary adjacent to the non-gravid horn. This PGE_2 probably originates from the conceptus and endometrium but not from the CL (Waclawik *et al.* 2008a). The conceptus PGE_2 signal could be amplified by PGE_2 feedback loop only in the gravid horn because of its local regulation (Waclawik *et al.* 2009a).

PGE_2 , after reaching the ovaries, may act through luteal PGE_2 receptors (PTGER), PTGER2 and PTGER4 (Waclawik *et al.* 2010). Other subtypes of PTGER have

not been studied in the CL in this species yet. PTGER2 and PTGER4 are coupled to adenylate cyclase and generate cAMP that in turn activates the protein kinase A signaling pathway. PGE_2 stimulates luteal progesterone secretion through a cAMP-mediated pathway in the CL in humans (Hahlin *et al.* 1988, Harris *et al.* 2001), rabbits (Boiti *et al.* 2000), and ruminants (Fitz *et al.* 1984, Weems *et al.* 2006). Moreover, the luteoprotective action of PGE_2 may be mediated by elevated content of vascular endothelial growth factor (VEGFA) in the luteal cells on days 10–12 of pregnancy (Kowalczyk *et al.* 2008). In addition, downregulation of strong endogenous antagonist of this growth factor, its soluble receptor, on day 12 of pregnancy increases amount of available VEGFA (Kaczmarek *et al.* 2009). Increased content of VEGFA in the CL may maintain progesterone production by increasing luteal capillary permeability, which facilitates transport of PGs from the circulation and delivery of cholesterol to the luteal cells.

Enzymes involved in PG synthesis during early pregnancy

PGE_2 and $\text{PGF}_{2\alpha}$ are synthesized by PTGS and specific terminal PG synthases: PGE synthase and PGFS. PGFS is considered to have both activities of PGH 9,11-endoperoxidase (reduction of the 9,11-endoperoxide group of PGH_2 to two hydroxyl groups of $\text{PGF}_{2\alpha}$) and PGD 11-ketoreductase (reduction of the PGD_2 11-keto group to 9α -, 11β - PGF_2 ; Watanabe 2002). Moreover, PGE_2 can be converted into $\text{PGF}_{2\alpha}$ by PG 9-ketoreductase/carbonyl reductase (CBR1). It was demonstrated that highly inducible forms of PTGS and PGE synthase in the porcine endometrium are PTGS2 (also known as PGHS-2 or COX-2; Ashworth *et al.* 2006) and microsomal PGE synthase-1 (mPGES-1), respectively (Waclawik *et al.* 2006).

Endometrial mPGES-1 expression exhibits a biphasic profile, corresponding with the profile of conceptus estrogen secretion (Waclawik *et al.* 2006). Our recent results indicate that estrogen elevates PGE_2 synthesis and the PGE_2 : $\text{PGF}_{2\alpha}$ ratio by an increase of expression enzymes involved in PGE_2 synthesis (PTGS2 and mPGES-1) and a decrease in the content of enzymes involved in $\text{PGF}_{2\alpha}$ production (PGFS and CBR1) in the endometrium on days 11–12 after estrus (Waclawik *et al.* 2009a). Accordingly, mPGES-1 expression is relatively high, whereas PGFS and CBR1 low in the endometrium between days 10 and 13 of pregnancy compared with the implantation period (Waclawik *et al.* 2006, Waclawik & Ziecik 2007). Similarly, but more pronounced alterations of expression of enzymes involved in PG synthesis were detected in the conceptus. A PGE_2 autoamplification loop can additionally contribute to the increase of the PGE_2 : $\text{PGF}_{2\alpha}$ ratio in the endometrium during the peri-implantation window (Waclawik *et al.* 2009a). PGE_2 acting through endometrial PTGER2 receptor,

activates cAMP signaling pathway and elevates the mRNA and protein expression of enzymes involved in PGE₂ synthesis (PTGS2 and mPGES-1) and secretion of this PG by the endometrium (Waclawik *et al.* 2009a; Fig. 1). This finding is consistent with the reports that PGE₂ itself may induce its own secretion in other cell types (Weems *et al.* 1999, Jabbour *et al.* 2001, Sales *et al.* 2001). Besides PTGER2, presence of PTGER4 in the porcine endometrium has been demonstrated (Waclawik *et al.* 2009a, 2009b) but receptors PTGER1 and PTGER3 have not been studied in this tissue.

The uterine receptivity and implantation period

In pigs, implantation occurs from days 14 to 19 of pregnancy. Uterine LE cells and conceptus trophoblast are involved in an adhesion cascade within a restricted time of the estrous/reproductive cycle defined as the window of the uterine receptivity. This state is induced by progesterone from the ovary and may be further enhanced by additional factors secreted by embryo (Burghardt *et al.* 2002). The hallmark of the uterine receptivity window for conceptus implantation in many species is a loss of progesterone receptors (PGR) from LE and GE as result of sustained stimulation of the endometrium by progesterone. In pigs, loss of PGR occurs by day 10 of the estrous cycle and pregnancy (Geisert *et al.* 2006, Mathew *et al.* 2011). However, PGR are maintained in stroma and myometrium. Therefore,

the effects of ovarian progesterone on expression of many factors in LE may be mediated indirectly either by progesterone-induced progestagens produced by PGR-positive stromal cells or by induction of molecules in LE that causes downregulation of PGR to regulate expression of endometrial genes (Geisert *et al.* 2006, Ka *et al.* 2007). Downregulation of PGR in LE and GE coincides with activation of nuclear factor kappa B (NFKB); however, a role of this transcription factor in inhibition of PGR expression was not confirmed in the pig (Ross *et al.* 2010, Mathew *et al.* 2011). NFKB activation is an important component in opening the implantation window in pigs and is probably involved in the increase in endometrial PTGS2 expression on day 12 after estrus (Geisert *et al.* 2006).

Loss of PGR from LE and GE induced by progesterone results in the decrease in mucin-1 from the apical surface of uterine LE and exposure of integrins for trophoblast attachment (Bowen *et al.* 1996). Pigs have a true epitheliochorial placenta in which LE remains intact throughout pregnancy. It has been suggested that mucin-4 expressed in LE and in the superficial deep GE of the endometrium plays a role in protecting the surface epithelium from invasion (Ferrell *et al.* 2003, Østrup *et al.* 2010). Mucin-4 expression is significantly greater in the pregnant compared with the nonpregnant endometrium and may be responsible for modulating the proteolytic activity of the porcine conceptus (Ferrell *et al.* 2003). On the contrary, in an invasive

Figure 1 Proposed mechanism involved in increasing luteoprotective prostaglandin E₂ (PGE₂) level and the PGE₂:PGF_{2α} ratio by a PGE₂ positive feedback loop during the period of the maternal recognition of pregnancy in the pig. The primary conceptus-derived factor, 17β-estradiol (E₂), and another conceptus factor PGE₂ regulate the protein expression of endometrial PG synthesis pathway enzymes to favor production of luteoprotective PGE₂. E₂ upregulates prostaglandin-endoperoxide synthase 2 (PTGS2) and PGE synthase (mPGES-1) protein expression and downregulates endometrial PGF synthase (PGFS) and prostaglandin 9-ketoreductase/carbonyl reductase (CBR1) protein concentrations increasing PGE₂ secretion and the PGE₂:PGF_{2α} ratio. PGE₂ acts through endometrial PGE₂ receptor (PTGER2), activates cAMP signaling pathway and elevates the mRNA and protein expression of enzymes involved in PGE₂ synthesis (PTGS2 and mPGES-1) and its own secretion in the endometrium. In addition, lysophosphatidic acid (LPA) present in the uterus may contribute to prostaglandin synthesis pathway by elevation of PTGS2 expression via its receptor (LPAR3). LPAR3 is upregulated by estrogen during the maternal recognition of pregnancy. Increased content of PGE₂ in the uterine lumen could directly promote uterine function and conceptus development. Expression of PTGER2 is upregulated on days 11–12 of pregnancy and was shown to be involved in a PGE₂ positive feedback loop during the period of the maternal recognition of pregnancy. PGE₂ originating from the conceptus and endometrium, after reaching the ovaries, may act through luteal PGE₂ receptors (PTGER2 and PTGER4) stimulating vascular endothelial growth factor (VEGFA) and progesterone secretion (P₄; modified from Waclawik *et al.* (2009a)).

placentation type as in rodents, mucins are down-regulated just before the implantation of the blastocyst (McNeer *et al.* 1998). Transforming growth factor β (TGFB1) stimulates expression of extracellular matrix molecules (fibronectin – ‘trophoblastic glue’) and integrins, as well as proteases and protease inhibitors to facilitate conceptus–uterus connections during implantation and to limit trophoblast invasiveness (Burghardt *et al.* 2002, Jaeger *et al.* 2005).

Attachment of trophoblast to LE is facilitated by interactions between integrins and extracellular matrix proteins. Expression of $\alpha 4$, $\alpha 5$, $\beta 1$ integrin subunits increases in LE between days 11 and 15 in both cyclic and pregnant gilts and is regulated by progesterone alone, or together with estrogen (Bowen *et al.* 1996). Conceptus estrogen induces expression of secreted phosphoprotein 1 (SPP1/osteopontin) in LE during the apposition phase of implantation (White *et al.* 2005). SPP1 binds to integrins on trophoblast and LE resulting in activation of integrin receptors and cytoskeletal proteins to form focal adhesions in trophoblast cells (Erikson *et al.* 2009). In trophoblast cells, SPP1 acts through its Arg-Gly-Asp (RGD) sequence to bind $\alpha v \beta 3$ and $\alpha 5 \beta 1$ integrin heterodimers and to induce focal adhesions, as well as MTOR, p38, and ERK1/2 signaling pathways to affect cell adhesion and migration (Kim *et al.* 2010).

Growth factors are other key molecules involved in maternal–conceptus signaling and conceptus

development. Endometrial expression of fibroblast growth factor 7 (FGF7), TGFB1 and uterine lumen content of insulin-like growth factor 1 (IGF1) are elevated during peri-implantation period and may be regulated by estrogen and/or progesterone (for review see, Waclawik *et al.* (2009b); Fig. 2).

Proinflammatory environment during the implantation period

Implantation displays hallmark signs of inflammation (Jabbour *et al.* 2009). However, both pro- and anti-inflammatory pathways are involved in establishment of the receptive endometrium and activation of them is tightly controlled. Overactivation of inflammatory pathways leads to the onset of reproductive disorders or diseases (Jabbour *et al.* 2009). For example, in pigs, highly localized and abundant expression of interferon γ (IFNG), tumor necrosis factor α (TNF), and interleukin 1 β (IL1B) in the endometrium is associated with arrested conceptus development between days 15 and 23 of pregnancy (Tayade *et al.* 2007, Croy *et al.* 2009). Cytokines are involved in the proliferation, differentiation, and cell survival that play a role in conceptus growth and implantation. It seems that in pigs, as in other species, implantation and establishment of pregnancy is associated with upregulation of expression of inflammatory mediators, which include cytokines, growth

Figure 2 Establishment of pregnancy is dependent on various biological molecules produced by conceptus (indicated in orange and yellow boxes), the endometrium (indicated in blue boxes), and both endometrium and conceptus (green boxes). Many factors originating from porcine conceptus are inflammatory mediators: interferon γ (IFNG) and interferon δ (IFND), interleukin 1 β (IL1B), IL6, and prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$, indicating that the embryo induces inflammatory pathways in the endometrium during the peri-implantation period. Endometrium responds to these embryonic signals by enhancing further, progesterone-induced uterine receptivity and establishment of proinflammatory environment during implantation. LE, uterine luminal epithelium; GE, uterine glandular epithelium; E_2 , 17 β -estradiol; TNF, tumor necrosis factor α ; LIF, leukemia inhibitory factor; NK cells, natural killer cells; IL6R, receptor of interleukin 6; LIFR, receptor of leukemia inhibitory factor; IL1R1, interleukin 1 receptor type I; PGR, progesterone receptor; ESR, estrogen receptor; PTGER2, PGE_2 receptor; LPAR3, lysophosphatidic acid receptor; TGFB1, transforming growth factor β ; VEGFA, vascular endothelial growth factor; IGF1, insulin-like growth factor 1; FGF7, fibroblast growth factor 7; HOXA10, Homeobox A10; NFkB, nuclear factor kappa B; SPP1, secreted phosphoprotein 1; SLA I, swine leukocyte antigens class I; B2M, β 2 microglobulin.

factors, and lipid mediators. As in humans and mice, early porcine pregnancy is dominated by pro-inflammatory type I cytokines such as IFNG and TNF (Croy *et al.* 2009).

Before implantation and during the attachment period, the porcine conceptus produces various inflammatory mediators: IFNG and interferon δ (IFND; Joyce *et al.* 2007), IL1B (Ross *et al.* 2003), IL6 (Modrić *et al.* 2000), PGE₂ and PGF_{2 α} (Waclawik & Ziecik 2007), indicating that the embryo induces inflammatory pathways in the endometrium (Fig. 2). Conceptus secreted IL1B stimulates endometrial expression of *PTGS1* and *PTGS2* mRNA, as well as tended to increase expression of IL1 receptor type I (IL1R1) in the endometrium (White *et al.* 2009). A microarray study revealed that the specific cytokine receptors *IL6R*, leukemia inhibitory factor receptor (*LIFR*), and IL11 receptor α (*IL11RA*) are significantly differentially expressed in the endometrium of pregnant pigs compared with nonpregnant animals (Østrup *et al.* 2010). Therefore, it is suggested that endometrial regulation of signaling in cytokines, particularly the IL6 family members, is to a great extent controlled by the expression of their specific receptors in the endometrium.

The endometrium responds to the embryonic signals during the implantation window by enhancing expression of genes involved in communications between the conceptus and uterus. IFND and IFNG exert an effect in the endometrium by cooperative induction of cytokine-specific transcription factors, such as STAT1 (Joyce *et al.* 2007). Interferon response genes, swine leukocyte antigens (MHC class I molecules in pigs; SLA) class I and β 2 microglobulin (B2M), which are involved in the discrimination of self from non-self by the immune system, are cell type-specifically regulated in the endometrium (Joyce *et al.* 2008). Downregulation of SLA class I and B2M expression in uterine LE and lack of expression of these genes in trophoblast (Ramsoondar *et al.* 1999), may be important for preventing fetal allograft rejection.

During early pregnancy the endometrium also produces another proinflammatory cytokine, LIF (Modrić *et al.* 2000). LIF is involved in controlling the uterine receptivity in other species and is essential for implantation in mice (Paria *et al.* 2002). Interestingly, content of *LIF* mRNA gradually increases in the porcine endometrium between days 10 and 15 of pregnancy (Blitek *et al.* 2010a). It needs to be determined if estrogen of conceptus origin can stimulate LIF expression in the pig similarly as ovarian estrogen does in mice and other species. Another cytokine, expressed in the porcine endometrium during early pregnancy is TNF (Yu *et al.* 1998). The source of TNF may be macrophages, which are present in the endometrium during the estrous cycle and early pregnancy, as well as endometrial cells and conceptus (Hunt *et al.* 1996). Recent studies indicate that TNF stimulates PGE₂ synthesis and secretion through upregulation of *PTGS2* mRNA, as well as

mPGES-1 mRNA and protein content in porcine LE cells collected from gilts on days 11–12 after estrus (Waclawik *et al.* 2010).

Similarly to inflammation, vascular changes, increased uterine vascular permeability, and endometrial edema are observed during conceptus implantation in pigs (Keys *et al.* 1986, Laforest & King 1992). Elevated vascular permeability and blood flow in the uterus are suggested to enhance transport of nutrients toward the conceptus and allow access of embryo-induced products to the maternal circulation (Keys *et al.* 1986). One important molecule that is involved in remodeling of endometrial vasculature is VEGFA. During the maternal recognition of pregnancy, both IGF1 and PGE₂ stimulate VEGFA expression in stromal cells (Kaczmarek *et al.* 2008). In addition, the conceptus contributes to synthesis of VEGFA since its expression increases gradually until day 16 of pregnancy (Kaczmarek *et al.* 2009). Transcription profiles for endometrial lymphocytes indicate that lymphocytes contribute to and regulate angiogenesis through transcription of VEGFA, hypoxia inducible factor 1 α , and IFNG (Tayade *et al.* 2007). During implantation period, number of uterine natural killer (NK) cells increases in the porcine endometrium. These cells are scattered through the stroma, beneath LE, around blood vessels, and uterine glands (Engelhardt *et al.* 2002). In contrast to the recruitment of NK cells in humans and mice, which is driven by decidual cells, porcine NK cells require conceptus-derived signals.

During the implantation period, PG synthesis pathway is altered in the endometrium and the conceptus (Waclawik *et al.* 2006, Waclawik & Ziecik 2007). On days 16–21 of pregnancy, content of PGFS is upregulated in the endometrium (Waclawik *et al.* 2006). Similarly, content of mPGES-1 protein is increased in the endometrium on days 18–19 of pregnancy comparing with days 18–19 of the estrous cycle. In later stage of implantation and early placentation, a twofold upregulation of mPGES-1 protein expression is detected in the endometrium in the implantation sites compared with inter-implantation sites (Waclawik *et al.* 2009a). After initiation of implantation, expression of *PTGS1*, *PGFS*, and *CBR1* mRNA rapidly increase in conceptus/trophoblast (Waclawik & Ziecik 2007).

PG signaling during the implantation period

The localization of PGE₂ synthesis corresponds highly to expression of PGE₂ receptor in the porcine endometrium (Waclawik *et al.* 2006, 2009a). PTGER2 protein is highly expressed in LE and GE and in uterine blood vessels, and moderately in myometrium. Endometrial expression of PTGER2 is greater during implantation and can be upregulated by estrogen and PGE₂ itself (Waclawik *et al.* 2009a).

Identification of the PGE₂ receptors PTGER2 and PTGER4 in the porcine endometrium (Waclawik *et al.* 2009a) and in the conceptus (Waclawik *et al.* 2008b) indicates that PGE₂ could act directly to promote uterine function, as well as conceptus development and migration in the uterus (Pope *et al.* 1982, Geisert *et al.* 1990, Giguère *et al.* 2000). PGE₂ is implicated in the local increase in endometrial vascular permeability and preparation for angiogenesis and implantation (Hamilton & Kennedy 1994, Kennedy *et al.* 2007). PGE₂ could stimulate angiogenesis in the porcine endometrium through upregulating VEGFA synthesis and secretion by endometrial cells (Kaczmarek *et al.* 2008).

It is possible that PG species other than PGE₂ and PGF_{2α} play an important role in the establishment of pregnancy. For example, in rodents and ruminants, prostacyclin is considered to be involved in implantation and signaling pathways between the uterus and the conceptus during peri-implantation period, respectively (Cammis *et al.* 2006, Kennedy *et al.* 2007, Ulbrich *et al.* 2009). However, in the pig, the role of prostacyclin in the early pregnancy still remains to be elucidated.

Uterine receptivity markers involved in PG synthesis

Lysophosphatidic acid (LPA) is a phospholipid-derived mediator increasing endometrial PTGS2 expression (Seo *et al.* 2008; Fig. 1). Content and type of LPA in the porcine uterine lumen differs between day 12 of the estrous cycle and pregnancy. LPA acts through specific G-protein coupled receptors LPAR1–LPAR4. Among them, LPAR3 is considered to be an uterine receptivity marker critical for embryo migration and spacing in mice (Ye *et al.* 2005). In women displaying recurrent implantation failure expression of LPAR3 is decreased (Achache *et al.* 2010). In the pig, endometrial LPAR3 expression is upregulated during early pregnancy with the highest levels on days 11–12 (Kaminska *et al.* 2008). LPAR3 mRNA content is greater in the porcine endometrium from the gravid compared with the non-gravid uterine horn in unilateral pregnancy (Kaminska *et al.* 2008). This receptor is localized to LE, GE, and the conceptus and its expression is stimulated by estrogen (Seo *et al.* 2008).

Another uterine receptivity marker linked to PG synthesis pathway is homeobox A10 (HOXA10). Although the *HOXA10* gene plays a role in uterine development, it is also expressed in adult uteri in many species, including pigs (Blitek *et al.* 2010a). In mice, targeted disruption of *HOXA10* expression leads to implantation failure. This transcription factor may mediate steroid action in endometrial tissue and regulate PTGS2 expression and PG synthesis in murine uterus (Paria *et al.* 2002). Our recent studies indicate that both *PTGS2* and *HOXA10* genes may be simultaneously regulated by steroids in the porcine endometrium (Blitek *et al.* 2010b). Endometrial *HOXA10* expression is

stimulated by E₂ (Blitek *et al.* 2010b) and its mRNA content is increased during implantation in the pig (Blitek *et al.* 2010a).

Some new insights on PG synthesis during early pregnancy in other species

Studying interactions of human endometrium and trophoblast during the peri-implantation period is difficult *in vitro* and impossible *in vivo*. Studies using stromal cells of the human endometrium decidualized with progesterone and treated with the conditioned media from trophoblasts indicated that human trophoblast signals the decidua, which in turn amplifies conceptus signals, especially related to immune modulation and angiogenesis, resulting in an enriched cytokine environment (Hess *et al.* 2007). Immune response is one of the most highly represented gene ontology categories of upregulated genes in response to trophoblast-secreted products in decidual stromal cells. *In vitro* models such as decidualized stromal cells treated with conditioned media from trophoblasts and an *in vitro* co-culture system of endometrial stromal cells with the first-trimester trophoblast explants revealed upregulation of mPGES-1 (PTGES) in human endometrial cells (Popovici *et al.* 2006, Hess *et al.* 2007). Moreover, a recent study showed that the human embryonic signal, chorionic gonadotropin, regulates the mPGES-1 promoter and induces mPGES-1 synthesis in endometrial epithelial cells via the PI3K–ERK1/2 pathway (Banerjee *et al.* 2009). Interestingly, in rats, mPGES-1 mRNA and protein were only detected in the subluminal stroma surrounding the implanting blastocyst at the implantation site on day 6 of pregnancy, but were not observed in the inter-implantation site on day 6 of pregnancy and on day 6 of pseudopregnancy (Cong *et al.* 2006). Thus, it is suggested that the presence of an active blastocyst is required for mPGES-1 expression at the implantation site in this species. The strong mPGES-1 expression in the implantation site and decidual cells indicates that mPGES-1 play an important role during implantation and decidualization in mice (Ni *et al.* 2002). Moreover, the conceptus signal in ruminants, IFNT, increases PGE₂ secretion in epithelial cells of bovine endometrium (Asselin & Fortier 2000) and in the ovine endometrium (Dorniak *et al.* 2011). IFNT increases endometrial PTGS activity and the amount of PGs in the ovine uterine lumen. When PG synthesis is inhibited, IFNT stimulation of many genes (*FGF2*, *ISG15*, *RSAD2*, *CST3*, *CTSL*, *GRP*, *LGALS15*, *SLC2A1*, *SLC5A1*, and *SLC7A2*) is reduced (Dorniak *et al.* 2011). Thus, PGs are suggested to be mediators of endometrial responses to progesterone and conceptus signal in the uterus, as well as being important regulators of ovine conceptus elongation. Although embryonic pregnancy recognition signals and type of placentation differ across species, it seems that the

common mechanism occurring in early pregnancy is that the conceptus targets PG synthesis, especially PGE₂ synthase mPGES-1 in the endometrium.

Conclusions and future directions

This review summarizes recognized signaling cascades and new research in mechanisms responsible for conceptus–endometrial interactions during the peri-implantation period in the pig with comparison with other species. One of the most intriguing challenges in understanding mechanisms of pregnancy establishment is to establish a hierarchy and timing of molecular relationships during the maternal–conceptus crosstalk. The porcine conceptus signal, estrogen, may prevent luteal regression through modification of expression of the enzymes involved in PG synthesis, reducing endometrial release of PGF_{2α} and favoring PGE₂ release on days 10–13 of pregnancy. Conceptus PGE₂ could be amplified by a PGE₂ feedback loop in the endometrium. A balanced interplay among the enzymes and receptors of PGs inside the uterus during the early pregnancy is required to provide a suitable environment for the embryo, which first inhibits luteolysis to initiate the maternal recognition of pregnancy, and then proceed for implantation. It seems that in pigs, as in other species, implantation and establishment of pregnancy are associated with upregulation of expression of proinflammatory factors that include cytokines, growth factors, and lipid mediators. During the peri-implantation period, the porcine conceptus produces inflammatory mediators: IFNG, IFND, IL1B, IL6, and PGs, which probably activate inflammatory pathways in the endometrium. The endometrium responds to these embryonic signals by enhancing further progesterone-induced uterine receptivity. In comparative studies, the pig may be a useful model for an initial step of implantation. This knowledge is required for translational research to increase reproductive efficiencies and reproductive health in humans and animals. The possibility of isolating specific key parameters in the endometrium may help to identify risk factors for implantation failure and, thus, hopefully lead to the development of treatments that would improve pregnancy rate.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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