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

Agnieszka Waclawik

Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Tuwima 10, 10-747 Olsztyn, Poland

Correspondence should be addressed to A Waclawik; Email: waclawik@pan.olsztyn.pl

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 F2α (PGF2α) and E2 (PGE2) 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 PGE2. Conceptus PGE2 could be amplified by PGE2 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.

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 F2α (PGF2α) in pigs (Moeljono et al. 1977). The porcine CL does not display a luteolytic response to exogenous PGF2α until days 12–13 of the estrous cycle. The insensitivity of the early CL to exogenous PGF2α is caused partially by lower luteal PGF2α receptor concentration on day 14 of pregnancy in comparison with the corresponding day of the estrous cycle (Gadsby et al. 1993, Zorrilla et al. 2009).

The role of oxytocin (OXT) in controlling PGF2α 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 PGF2α 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 PGF2α, 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 PGF2α metabolite, PGFM, on day 16 after estrus (Sample et al. 2004). In addition, OXT stimulates synthesis and secretion of PGE2 (PG which has the opposite action to PGF2α 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 PGF2α (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 (E2), 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 trophoblastic 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 PGF2α 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 trophectoderm. During pregnancy, the endometrium rapidly metabolizes E2 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 trophectoderm 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 PGF2α in the utero-ovarian vein in pregnant gilts compared with cyclic pigs. However, another group reported that this difference in PGF2α concentration in the ovarian–uterine vein is much less between cyclic and pregnant animals (Hunter & Pousier 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 PGF2α 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 PGF2α in the uterine lumen during implantation could be involved in angiogenesis because PGF2α signaling mediates expression of angiogenic genes (Sales et al. 2005). Nevertheless, the action of PGF2α in this period should be restricted only to the uterus and conceptus. Therefore, to avoid luteolytic action of PGF2α on the CL in ovary, PGF2α is sequestered in the uterus probably by redirection of PGF2α 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 PGF$_{2\alpha}$ from the venous blood and uterine lymph into the uterine lumen and ability of uterine veins and arterial walls to accumulate PGF$_{2\alpha}$ (Krzymowski & Stefanczyk-Krzybowska 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 & Zieciak 2007). A greater PGE$_2$:PGF$_{2\alpha}$ ratio stimulates progesterone and E$_2$ secretion by luteal cells collected on days 10–12 of the estrous cycle (Gregoraszczuk & 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-Krzybowska 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 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 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 PGF$_{2\alpha}$ concentrations in the utero-ovarian blood draining the E$_2$-injected horn. Wasielak 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 oocytes, 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 PGF$_{2\alpha}$ are synthesized by PTGS and specific terminal PG syntheses: PG 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 PGF$_{2\alpha}$) and PGD 11-ketoreductase (reduction of the PGD 11-keto group to 9$\alpha$-PGF$_2$; Watanabe 2002). Moreover, PGE$_2$ can be converted into 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 PG synthase-1 (mPGS-1), respectively (Waclawik et al. 2006).

Endometrial mPGS-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$_2$:PGF$_{2\alpha}$ ratio by an increase of expression enzymes involved in PGE$_2$ synthesis (PTGS2 and mPGS-1) and a decrease in the content of enzymes involved in PGF$_{2\alpha}$ production (PGFS and CBR1) in the endometrium on days 11–12 after estrus (Waclawik et al. 2009a). Accordingly, mPGS-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 & Zieciak 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$_2$: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 trophoderm 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 progametadins 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:PGF2α 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 PGE2 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:PGF2α 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 PTG2 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).
placentaion type as in rodents, mucins are down-regulated just before the implantation of the blastocyst (McNeer et al. 1998). Transforming growth factor β (TGFβ1) 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 α4, α5, β1 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 αβ3 and α5β1 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), TGFβ1 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 1β (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 factors:

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**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 1β (IL1B), IL6, and prostaglandin E2 (PGE2) and PGF2α, 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; E2, 17β-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, PGE2 receptor; LPAR3, lysophosphatidic acid receptor; TGFβ1, 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.

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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 (Modrič et al. 2000), PGE2 and PGF2α (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 1 (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 (Ostrup 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 (Ramoondar et al. 1999), may be important for preventing fetal allograft rejection.

During early pregnancy the endometrium also produces another proinflammatory cytokine, LIF (Modrič 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 PGF₂α 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 (Cammas 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 placentaion 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 PGF2α 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

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References


Weems YS, Lammoglia MA, Lewis AW, Randel RD, Sasser RG, Morita I & Weems CW 1999 PGE2 induces its own secretion in vitro by bovine 270-
day placenta but not by 200-day placenta. Prostaglandins & Other Lipid Mediators 57 189–205. (doi:10.1016/S0090-6980(99)00003-9)


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