Embryo-maternal relationships during the peri-implantation period – new and old players

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This review attempts to integrate available data on embryo-maternal communication during maternal recognition of pregnancy and implantation in the pig. Progesterone (P₄) is an essential hormone that makes the uterus receptive to accept conceptuses for implantation and subsequent placentation. As well as P₄, the receptive stage of the endometrium is further primed by paracrine factors secreted by the conceptus prior to and during implantation. Oestrogen secretion by elongating conceptuses on days 11 to 12 is necessary for the maternal recognition of pregnancy to protect the corpus luteum from regression. Oestrogens increase the expression of various uterine secretory proteins that are components of the histotroph, which in turn support conceptus growth and implantation. The PGE₂ positive feedback loop in the endometrium contributes to an increased PGE₂/PGF₂α ratio during the peri-implantation period. Greater synthesis of prostacyclin by the uterus, along with activation of the VEGF-receptor system, may be involved in regulating the uterine vascular bed and blood flow, both being prerequisite for sufficient embryo nutrition. HOXA10 expressed by the endometrium may be involved in tissue remodelling and proliferation during conceptus implantation. LIF, IL6 and their receptors are present at the maternal-conceptus interface, and both cytokines increase the attachment and proliferation of trophoblast cells. TGFβ1 stimulates the synthesis of fibronectin, which is necessary for firm adhesion between trophoblast and maternal tissue. Moreover, cytokines regulate a maternal immune response that is permissive for successful pregnancy establishment.

Introduction

The endometrium undergoes morphological and physiological changes during each oestrous cycle in order to prepare this tissue for embryo implantation. The action of progesterone (P₄) in priming the uterus is essential to attain uterine receptivity for implantation in many species, including the pig (Spencer et al. 2004, Ziecik et al. 2011). Under the influence of P₄, the endometrium is transformed into a secretory tissue to create an environment permissive to conceptus development, implantation and placentation (Spencer et al. 2004). P₄ stimulates the expression of vascular endothelial growth factor (VEGF; Welter et al. 2003), swine leukocyte antigens (SLA; MHC class I molecules in pigs) and β₂-microglobulin (β₂m; Joyce et al. 2008), as well as homeobox (HOX) A10, prostaglandin endoperoxide synthase 2 (PTGS2; Blitek et al. 2010b) and prostacyclin synthase (PGIS; Morawska et al. 2012) levels in the porcine
endometrium. P₄ alone is able to increase vascular development in the uterus (Bailey et al. 2010). Interestingly, sustained P₄ action on endometrial tissue results in down-regulation of progesterone receptors (PGR) in luminal and glandular epithelium (LE and GE) beginning from day 10 after ovulation (Geisert et al. 1994, Ross et al. 2010). Mechanisms regulating PGR expression in the uterine epithelium are P₄-dependent and occur before day 6 of pregnancy (Mathew et al. 2011). Loss of PGR is negatively correlated with nuclear factor κβ (NFκβ) expression, which is a critical component of implantation window opening in the pig (Ross et al. 2010). However, the activation of NFκβ is involved in elevation of endometrial PTGS2 expression on day 12 after oestrus (Geisert et al. 2012), rather than in PGR down-regulation (Ross et al. 2010, Mathew et al. 2011).

In pigs, P₄ action is a prerequisite for the attainment of uterine receptivity, but developing conceptuses further modulate the endometrial milieu, making it competent for implantation. Pregnancy recognition is the result of oestrogen synthesis and secretion by conceptuses on days 11 to 12 after fertilization (Geisert et al. 1990). Oestrogen actions in the porcine uterus are both antiluteolytic and luteotropic. Oestradiol-17β (E₂) decreases the amount of luteolytic PGF₂α reaching the CL (Bazer & Thatcher 1977, Krzymowski & Stefanczyk-Krzymowska 2004). Moreover, E₂ stimulates luteal P₄ secretion directly (Conley & Ford 1989), and also increases the concentration of luteinizing hormone receptors in the CL (Garverick et al. 1982). Effects of conceptus E₂ in the uterus include regulation of PG synthesis to favour luteoprotective PGE₂ (Waclawik et al. 2009), stimulation of nitric oxide production (Andronowska & Chrusciel 2008) and increased uterine blood flow (Ford et al. 1982). Second, a more sustained peak of oestrogen release occurs between days 15 and 25-30 of gestation and is required to complete pregnancy establishment in the pig (Geisert et al. 1990).

Components of conceptus-maternal communication during early implantation

Implantation involves a complex series of events that establish the connection between uterine and conceptus tissues. The absence of functional PGR in LE and GE coincides with decreased expression of mucin-1 (Muc-1) on the apical surface of the uterine epithelium. Muc-1 is considered as a barrier to implantation, because reduction of its expression due to cell surface protease activity results in increased accessibility of integrin receptors to their ligands (for a review see: Bazer et al. 2009).

Reciprocal embryo-maternal interactions modify the local environment, enabling conceptus development and implantation. These interactions may occur either by cell-to-cell contact or through soluble mediators. Among them PGs, cytokines, growth factors and their receptors are involved (Fig. 1). Besides P₄, E₂, and PGE₂, which role in pregnancy establishment is well described, some new factors e.g. prostacyclin and HOXA10 seem to be important for embryo-maternal dialogue in pigs.

Prostaglandin E₂ (PGE₂)

The proper ratio between luteoprotective PGE₂ and luteolytic PGF₂α synthesized by the endometrium and the conceptus is essential for successful establishment of pregnancy in the pig (Waclawik 2011). Differential expression profiles for PTGS2, mPGES-1 (the microsomal isoform of PGE synthase) and PGFS (PGF synthase) were demonstrated in the endometrium of cyclic and early pregnant gilts (Blitek et al. 2006, Waclawik et al. 2006). The content of PGE₂ and PGF₂α in the uterine lumen increases progressively during early pregnancy (Table 1; Ashworth et al. 2006). Moreover, greater concentrations of both PGs were found in gravid
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Fig. 1 Endometrial receptivity for conceptus implantation in pigs is established by the action of ovarian progesterone; nonetheless, conceptus oestradiol-17β (E₂) further modulates the production of PGs, cytokines, growth factors, transcription regulators and integrins. All these factors acting through autocrine, paracrine and juxtacrine signaling pathways modify the local intrauterine environment, enabling conceptus development and implantation. Abbreviations: IFNγ, interferon γ; IFNβ, interferon δ; PGR, progesterone receptor; TGFβ1, transforming growth factor β1; VEGF, vascular endothelial growth factor; FGF7, fibroblast growth factor 7; IL1β, interleukin 1β; IL6, interleukin 6; LIF, leukemia inhibitory factor; PGE₂, prostaglandin E₂; PGF₂α, prostaglandin F₂α; PGI₂, prostacyclin; ESR, estrogen receptor; TGFβ receptors; VEGF receptors; IL6R, IL6 receptor; LIFR, LIF receptor; IL1βR, IL1β receptor; PTGER2, PGE₂ receptor; PPARs, peroxisome proliferator activated receptors; HOXA10, homeobox A10; STAT1, signal transducer and activator of transcription 1; NFκB, nuclear factor κB; LE, luminal epithelium; GE, glandular epithelium.

than in non-gravid uterine horns on day 14 of pregnancy (Wasielak et al. 2009). Interestingly, E₂ up-regulates endometrial PGE₂ synthesis and the PGE₂/PGF₂α ratio by increasing PTGS2 and mPGES-1 expression, and decreasing PGFS expression (Waclawik et al. 2009). PGE₂ is considered to be involved in protecting the CL against the luteolytic effect of PGF₂α during early pregnancy. Additionally, elongating conceptuses are an important source of PGE₂ (Waclawik & Ziecik 2007), and PGE₂ acting through the endometrial PGE₂ receptor (PTGER2) activates cAMP signaling pathway and elevates its own synthesis (Waclawik et al. 2009). Moreover, greater endometrial expression of PTGER2 during conceptus attachment (Waclawik et al. 2009) indicates an important local effect of PGE₂ in establishing the proper environment for embryo implantation.

Prostacyclin (prostaglandin I₂; PGI₂)

Besides PGE₂, another PTGS-derived prostaglandin, PGI₂, seems to be involved in pregnancy establishment. Similar to PGE₂, PGI₂ is an important regulator of vascular function. Moreover, PGI₂ is critical for blastocyst implantation in mice (Lim et al. 1999). Recently, we demonstrated different profiles of PGIS expression and 6-keto PGF₁α (a PGI₂ metabolite) concentration in the endometrium of cyclic and pregnant gilts (Morawska et al. 2012). A higher PGIS protein level and PGI₂ concentration in the endometrium observed on day 12 in pregnant gilts, followed by a greater concentration of PGI₂ in the endometrium and uterine lumen, hints at a conceptus-dependent regulation of PGI₂ synthesis. Consistent with this idea, incubation of endometrial explants with conceptus-conditioned medium stimulated PGIS protein expression.
Furthermore, using an *in vivo* model of gilts with unilateral pregnancy, we demonstrated that conceptus presence up-regulated PGI₂ synthesis in the endometrium on day 14 of gestation (Morawska et al. 2012). Greater synthesis of PGI₂ on day 18 in pregnant gilts may be associated with regulation of blood flow, which is essential for sufficient foetal nutrition. PGI₂ is also synthesized by pig conceptuses (Morawska et al. 2012). PGI₂ not only elicits its effect on target cells via a cell surface PGI receptor, but may also act via nuclear peroxisome proliferator activated receptors (PPARs). The expression of PPARs has been demonstrated in porcine endometrium and trophoblast tissue (Lord et al. 2006). Thus, an effect of PGI₂ on the permeability of maternal and conceptus vasculature is very likely. Moreover, treating porcine blastocyst with PGI₂ resulted in higher numbers of inner cell mass and trophectoderm cells compared with nontreated blastocysts (Kim et al. 2010).

**Homeobox A10 (HOXA10)**

Transcription factors in the endometrium are regulated by ovarian P₄ and/or embryonic stimuli and thus may be critical for implantation. One such factor that plays an important role in uterine physiology is HOXA10. In the porcine uterus, HOXA10 protein was localized in LE, GE, stromal cells and blood vessels. Endometrial expression of HOXA10 increases progressively during early pregnancy, and day 15 pregnant gilts express a 2-fold higher level of HOXA10 transcripts than their cyclic counterparts (Blitek et al. 2010a, 2011). These results indicate that conceptus presence may induce HOXA10 gene expression in the porcine endometrium, but also confirm a role of HOXA10 during the implantation period, rather than at the time of maternal recognition of pregnancy. It seems likely that HOXA10 is involved in uterine remodelling to support placentation. Ovarian steroids, P₄ and E₂, as well as conceptus-derived products, stimulate the expression of HOXA10 in porcine endometrial cells (Blitek et al. 2010b, 2011), thereby probably promoting proliferation and differentiation of endometrial cells during implantation. A role of HOXA10 in a local immunomodulation within the uterus has also been
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postulated (Yao et al. 2003). Interestingly, increased HOXA10 mRNA levels in response to E₂ and conceptus products were accompanied by greater PTGS2 gene expression and PGE₂ secretion from endometrial cells (Blitek et al. 2011).

Leukemia inhibitory factor (LIF) and interleukin 6 (IL6)

LIF and IL6 are present within the uterine microenvironment during early pregnancy. Endometrial expression of LIF mRNA changes during peri-implantation period, with the lowest level observed on day 10 followed by a sharp increase on day 12. Moreover, in vitro experiments revealed that the addition of conceptus-exposed medium to endometrial explants results in greater LIF mRNA content in the tissue (Blitek et al. 2012). Nevertheless, endometrial LIF expression increases also in cyclic gilts, suggesting that besides a conceptus effect, maternal regulation of LIF synthesis occurs in the pig uterus. A dramatic increase in LIF protein content in the uterine lumen is observed between days 10 and 12 of pregnancy (Table 1; Anegon et al. 1994, Blitek et al. 2012). The main source of high LIF concentrations in the uterine lumen is the endometrial tissue, because no (Modrić et al. 2000) or very low (Blitek et al. 2010a) LIF mRNA expression was detected in conceptuses. Greater expression of IL6 protein in the endometrium (Blitek et al. 2012) as well as IL6 concentration in the uterine lumen (Anegon et al. 1994) was observed in pregnant than in cyclic gilts. Peak expression of IL6 mRNA in the endometrium was detected on day 12 of pregnancy and was accompanied by higher IL6 protein content. Similar to LIF, IL6 synthesis in the porcine endometrium seems to be stimulated by products of conceptus origin (Blitek et al. 2012). In contrast to LIF, pig conceptuses are an important source of IL6 (Modrić et al. 2000). Moreover, specific cytokine receptors, LIFR and IL6R, have been detected in the endometrium (Modrić et al. 2000) and conceptuses (Blitek et al. 2012). The attachment and proliferation of trophoblast cells are both stimulated by LIF and IL6 (Blitek et al. 2012). Additionally, IL6 activates endometrial PGF₂α release and metabolism in a manner that is consistent with protection of the CL against regression (Franczak et al. 2012).

Vascular endothelial growth factor (VEGF) and the vascular system

Development of the placental vascular architecture is of considerable importance in the exchange of nutrients, oxygen, and carbon dioxide between the mother and fetus. In pigs, increased uterine arterial blood flow was observed on days 12-13 of gestation close to the embryonic disc. It was accompanied by elevated vascular permeability, leading to edema of the endometrium (Krzymowski & Stefanczyk-Krzymska 2004, Ziecik et al. 2011). Most of the vascular changes are initiated after the first embryonic signal, however, VEGF is thought to be an effective component of this system. Interestingly, increased expression of VEGF164 protein was observed on days 10-15 of pregnancy, coincident with the time of maternal recognition of pregnancy and major vascular events of the peri-implantation period in pigs (Kaczmarek et al. 2008b). Both insulin-like growth factor I and relaxin seem to be potent inducers of VEGF in the porcine uterus. Endometrial synthesis of VEGF164 by day 15 of pregnancy can be also stimulated by PGE₂ (Kaczmarek et al. 2008a). Identification of VEGF164 expression in conceptuses on days 12 and 15-16 (Kaczmarek et al. 2009) suggests that attachment-associated changes in the vascular compartment and in endometrial morphology can be directed by this factor acting in a local manner.

Other factors involved in conceptus-endometrial interactions

Interleukin 1β (IL1β) and its signalling system are expressed in the endometrium and conceptuses of the pig, and IL1β may be important for immunotolerance at the maternal-placental interface.
Conceptus synthesis and secretion of IL1β occurs within a short time window associated with trophoblast elongation between days 11 and 12 of pregnancy (Ross et al. 2003). It is supposed that IL1β acting in an autocrine manner may induce the morphological transformation of trophectoderm during the process of elongation (Geisert et al. 2012). In the endometrium, IL1β stimulates PTGS2 and mPGES-1 expression and PGE$_2$ content (Franczak et al. 2010). Thus, conceptus IL1β may participate in blocking luteolysis by increasing PGE$_2$ production, as well as in establishment of a pro-inflammatory environment in the uterus.

Pig conceptuses produce interferon γ (IFNγ) and IFNδ during the peri-implantation period (Joyce et al. 2007), and IFNs activity in the uterine lumen increases significantly between days 12 and 16 of pregnancy (La Bonnardière et al. 1991). Neither of the IFNs produced by pig conceptuses has antiluteolytic activity, but their paracrine action in the endometrium has been demonstrated (Joyce et al. 2007, 2008). Interestingly, E$_2$ stimulates expression of the signal transducer and activator of transcription (STAT) 1 in LE cells, while stromal expression of STAT1 is up-regulated by IFNs (Joyce et al. 2007). Moreover, down-regulation of SLA molecules and β$_2$m observed in endometrial LE during implantation may be crucial for preventing foetal allograft rejection. In contrast to LE, expression of SLAs and β$_2$m in the stroma increases in response to conceptus IFNs (Joyce et al. 2008). Thus, IFNs seem to be important components of the immune system involved in preparation of an environment suitable for conceptus implantation.

Transforming growth factor β (TGFβ) participates in angiogenesis, apoptosis, proliferation, immunotolerance, embryogenesis and tissue remodelling (Godkin & Dore 1998), and all these processes are important during the peri-implantation period. TGFβ isoforms and its type I and II receptors were localized at the maternal–conceptus interface in the pig. Moreover, the amount of biologically active TGFβs in the uterine lumen increases significantly during the peri-implantation period (Gupta et al. 1998). Localization of phosphorylated SMAD2/3 proteins in conceptus and uterine tissues indicates activation of TGFβ-dependent intracellular signalling, possibly leading to conceptus development and attachment (Massuto et al. 2010). Importantly, TGFβ1 enhances the expression of fibronectin and activates integrin receptors (Jaeger et al. 2005), but also stimulates proliferation of porcine trophoblast cells (A. Blitek, unpublished).

**Conclusion**

Loss of PGR in the uterine epithelium during the peri-implantation period is associated with changes in endometrial gene expression leading to achievement of uterine receptivity for conceptus implantation. In response to conceptus signals, the uterus secretes large amounts of a wide variety of biological factors, and these secretions establish a unique embryotrophic environment that plays an important role in the survival of embryos as well as in implantation and placentation. However, detailed mechanisms involved in reciprocal embryo-maternal relationships are yet to be elucidated. Identification of specific downstream genes/proteins/signalling pathways regulated by components of the histotroph might be helpful in better understanding of embryo-maternal interactions, not only in the pig but also in other species with diffuse type of placenta. Identification of genetic markers of embryo signalling and endometrial receptivity during the peri-implantation period would be of great benefit for increasing reproductive efficiency, and also for optimizing transgenesis, cloning and other biotechniques.

**Declaration of interest**

Authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.
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References

Andronowska A & Chrusciel M 2008 Influence of estradiol-17β and progesterone on nitric oxide production in the porcine endometrium during the first half of pregnancy. Reproductive Biology 8 43-55.

Anegon I, Cuturi MC, Godard A, Moreau M, Terqui M, Martinat-Botté F & Soulillou JP 1994 Presence of leukaemia inhibitory factor and interleukin 6 in porcine uterine secretions prior to conceptus attachment. Cytokine 6 493-499.

Ashworth MD, Ross JW, Hu J, White FJ, Stein DR, DeSilva U, Johnson GA, Spencer TE & Geisert RD 2006 Expression of porcine endometrial prostaglandin synthase during the estrous cycle and early pregnancy, and following endocrine disruption of pregnancy. Biology of Reproduction 74 1007-1015.

Bailey DW, Dunlap KA, Frank JW, Erikson DW, White BG, Bazer FW, Burghardt RC & Johnson GA 2010 Effects of long-term progesterone on developmental and functional aspects of porcine uterine epithelia and vasculature: progesterone alone does not support development of uterine glands comparable to that of pregnancy. Reproduction 140 583-594.

Bazer FW & Thatcher WW 1977 Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F₁₆₅ by the uterine endometrium. Prostaglandins 14 397-401.

Bazer FW, Spencer TE, Johnson GA, Burghardt RC & Wu G 2009 Comparative aspects of implantation. Reproduction 138 199-209.

Blitek A, Waclawik A, Kaczmarek MM, Stadejek T, Pejsak B & Kotwica G 2010 The effect of tumor necrosis factor α (TNFα), interleukin 1β (IL1β) and interleukin 6 (IL6) on endometrial PGE₂ synthesis, metabolism and release in early-pregnant pigs. Theriogenology 77 155-165.

Garverick HA, Polge C & Flint AP 1982 Oestradiol administration raises luteal LH receptor levels in intact and hysterectomized pigs. Journal of Reproduction and Fertility 66 371-377.

Geisert RD, Zavy MT, Moffatt RJ, Blair RM & Yellin T 1990 Embryonic steroids and the establishment of pregnancy in pigs. Journal of Reproduction and Fertility Supplement 40 293-305.

Geisert RD, Pratt TN, Bazer FW, Mayes JS & Watson GH 1994 Immunocytochemical localization and changes in endometrial progesterin receptor protein during the porcine oestrous cycle and early pregnancy. Reproduction, Fertility, Development 6 749-760.

Geisert RD, Fazleabas A, Lucy M & Mathew D 2012 Interaction of the conceptus and endometrium to establish pregnancy in mammals: role of interleukin 1β. Cell and Tissue Research 349 825-838.

Gupta A, Dekaney CM, Bazer FW, Madrigal MM & Jaeger LA 1998 Beta transforming growth factor b (TGFβ) at the porcine conceptus-maternal interface. Part II: Uterine TGFβ bioactivity and expression of immunoreactive TGFβs (TGFβ1, TGFβ2, and TGFβ3) and their receptors (type I and type II). Biology of Reproductive Endocrinology 67 1-19.

Godkin JD & Dore JJ 1998 Transforming growth factor β and the endometrium. Reviews of Reproduction 3 1-6.

Godkin JD & Dore JJ 1998 Transforming growth factor β and the endometrium. Reviews of Reproduction 3 1-6.

Gupta A, Dekaney CM, Bazer FW, Madrigal MM & Jaeger LA 1998 Beta transforming growth factors (TGFβ) at the porcine conceptus-maternal interface. Part II: Uterine TGFβ bioactivity and expression of immunoreactive TGFβs (TGFβ1, TGFβ2, and TGFβ3) and their receptors (type I and type II). Biology of...
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Reproduction 59 911-917.

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

Joyce MM, Burghardt RD, Geisert RD, Burghardt JR, Hooper RN, Ross JW, Ashworth MD & 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.

Joyce MM, Burghardt JR, Burghardt RC, Hooper RN, Bazer FW & Johnson GA 2008 Uterine MHC class I molecules and β2-microglobulin are regulated by progesterone and conceptus interferons during pig pregnancy. The Journal of Immunology 181 2494-2505.

Kaczmarek MM, Blitek A, Kaminska K, Bodek G, Zygmunt M, Schams D & Ziecik AJ 2008a Assessment of VEGF-receptor system expression in the porcine endometrial stromal cells in response to insulin-like growth factor-1, relaxin, oxytocin and prostaglandin E2. Molecular and Cellular Endocrinology 291 33-41.

Kaczmarek MM, Waclawik A, Blitek A, Kowalczyk AE, Schams D & Ziecik AJ 2008b Expression of the vascular endothelial growth factor-receptor system in the porcine endometrium throughout the estrous cycle and early pregnancy. Molecular Reproduction and Development 75 362-372.

Kaczmarek MM, Kiewicz J, Schams D & Ziecik AJ 2009 Expression of VEGF-receptor system in conceptus during peri-implantation period and endometrial and luteal expression of soluble VEGFR-1 in the pig. Theriogenology 71 1298-1306.

Kim J-S, Chae J-I, Song B-S, Lee K-S, Choo Y-K, Chang K-T, Park H, Koo D-B 2010 Iliprost, a prostacyclin analogue, stimulates meiotic maturation and early embryonic development in pigs. Reproduction, Fertility and Development 22 437-447.

Krzymowski T & Stefanczyk-Krzywinska S 2004 The oestrous cycle and early pregnancy – a new concept of local endocrine regulation. The Veterinary Journal 168 285-296.

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

Lim H, Gupta RA, Ma W, Paria BC, Moller DE, Morrow JD, DuBois RN, Trzaskos JM, Dey SK 1999 Cyclooxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARδ. Genes & Development 13 1561-1574.

Lord E, Murphy BD, Desmarais JA, Ledoux S, Beaudry D & Palù M-F 2006 Modulation of peroxisome proliferator-activated receptor δ and γ transcripts in swine endometrial tissue during early gestation. Reproduction 131 929-942.

Mathew DJ, Sellner EM, Green JC, Okamura CS, Anderson LL, Lucy MC & Geisert RD 2011 Uterine progesterone receptor expression, conceptus development, and ovarian function in pigs treated with RU 486 during early pregnancy. Biology of Reproduction 84 130-139.

Massuto DA, Kneese EC, Johnson GA, Burghardt RC, Hooper RN, Ing NH & Jaeger LA 2010 Transforming growth factor beta (TGFβ) signaling is activated during porcine implantation: proposed role for latency-associated peptide interactions with integrins at the conceptus-maternal interface. Reproduction 139 465-478.

Modrić T, Kowalski AA, Green ML, Simmen RCM, Simmen FA 2000 Pregnancy-dependent expression of leukaemia inhibitory factor (LIF), LIF receptor-β and interleukin-6 (IL-6) messenger ribonucleic acids in the porcine female reproductive tract. Placenta 21 345-353.

Morawska E, Kaczmarek MM & Blitek A 2012 Regulation of prostacyclin synthase expression and prostacyclin content in the pig endometrium. Theriogenology 78 2071-2086.

Ross JW, Malayer JR, Ritchey JW & Geisert RD 2003 Characterization of the interleukin-1β system during porcine trophoblastic elongation and early placental attachment. Biology of Reproduction 69 1251-1259.

Ross JW, Ashworth MD, Mathew D, Reagan P, Ritchey JW, Hayashi K, Spencer TE, Lucy M & Geisert RD 2010 Activation of the transcription factor, nuclear factor kappa-B, during the estrous cycle and early pregnancy in the pig. Reproductive Biology and Endocrinology 8 39-55.

Spencer TE, Johnson GA, Burghardt RC, Bazer FW 2004 Progesterone and placental hormone actions on the uterus: insights from domestic animals. Biology of Reproduction 71 2-10.

Stone BA & 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. Journal of Reproduction and Fertility 75 209-221.

Waclawik A 2011 Novel insights into the mechanisms of pregnancy establishment: regulation of prostaglandin synthesis and signaling in the pig. Reproduction 142 389-399.

Waclawik A & Ziecik AJ 2007 Differential expression of prostaglandin (PG) synthesis enzymes in conceptus during peri-implantation period and endometrial expression of carbonyl reductase/PG 9-ketoreductase in the pig. Journal of Endocrinology 194 1-13.

Waclawik A, Rivero-Muller A, Blitek A, Kaczmarek MM, Brokken LJS, Watanabe K, Rahman NA & Ziecik AJ 2006 Molecular cloning and spatiotemporal expression of prostaglandin F synthase and microsomal prostaglandin E synthase-1 in porcine endometrium. Endocrinology 147 210-221.

Waclawik A, Jabbour HN, Blitek A & Ziecik AJ 2009 Estradiol-17β, prostaglandin E	extsubscript{2} (PGE	extsubscript{2}), and the PGE	extsubscript{2} receptor are involved in PGE	extsubscript{2} positive feedback loop in the porcine endometrium. Endocrinology 150 3823-3832.

Wasielek M, Kaminska K & Bogacki M 2009 Effect of the conceptus on uterine prostaglandin-F2α and
prostaglandin-E2 release and synthesis during the periimplantation period in the pig. *Reproduction, Fertility and Development* 21 1-9.

**Welter H, Wollenhaupt K, Tiemann U, Einspanier R** 2003 Regulation of the VEGF-system in the endometrium during steroid-replacement and early pregnancy of pigs. *Experimental and Clinical Endocrinology and Diabetes* 111 33-40.

**Yao MW, Lim H, Schust DJ, Choe SE, Farago A, Ding Y, Michaud S, Church GM & Maas RL** 2003 Gene expression profiling reveals progesterone-mediated cell cycle and immunoregulatory roles of Hoxa-10 in the preimplantation uterus. *Molecular Endocrinology* 17 610-627.

**Ziecik AJ, Waclawik A, Kaczmarek MM, Blitek A, Moza Jalali B, Andronowska A** 2011 Mechanisms for the establishment of pregnancy in the pig. *Reproduction in Domestic Animals* 46 31-41.
