# The Early Stages of Implantation and Placentation in the Pig

**Gregory A. Johnson, Fuller W. Bazer, and Heewon Seo**

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**Abstract** Pregnancy in pigs includes the events of conceptus (embryo/fetus and placental membranes) elongation, implantation, and placentation. Placentation in pigs is defined microscopically as epitheliochorial and macroscopically as diffuse. In general, placentation can be defined as the juxtapositioning of the endometrial/uterine microvasculature to the chorioallantoic/placental microvasculature to facilitate the transport of nutrients from the mother to the fetus to support fetal development and growth. Establishment of epitheliochorial placentation in the pig is achieved by: (1) the secretions of uterine glands prior to conceptus attachment to the uterus; (2) the development of extensive folding of the uterine–placental interface to maximize the surface area for movement of nutrients across this surface; (3) increased angiogenesis of the vasculature that delivers both uterine and placental blood and, with it, nutrients to this interface; (4) the minimization of connective tissue that lies between these blood vessels and the uterine and placental epithelia; (5) interdigitation of microvilli between the uterine and placental epithelia; and

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(6) the secretions of the uterine glands, called histotroph, that accumulate in areolae for transport though the placenta to the fetus. Placentation in pigs is not achieved by invasive growth of the placenta into the uterus. In this chapter, we summarize current knowledge about the major events that occur during the early stages of implantation and placentation in the pig. We will focus on the microanatomy of porcine placentation that builds off the excellent histological work of Amoroso and others and provide a brief review of some of the key physiological, cellular, and molecular events that accompany the development of "implantation" in pigs.

**Keywords** Pig/Porcine · Pregnancy · Conceptus · Uterus · Implantation · Placentation

## 1 Introduction: Basic Definition for Epitheliochorial Placentation

Placentation is epitheliochorial in pigs, as uterine endometrial luminal epithelium (LE) remains intact throughout pregnancy. Porcine placental trophectoderm/chorion cells directly attach to the uterine LE, and these two epithelia serve as the conduit for maternal hemotrophic support for conceptus growth and development (Bjorkmann 1973). Amoroso stated that "In its histological structure the epitheliochorial placenta is exceeding simple. . . .with the pig as exhibiting structurally the simplest type of placenta" (Amoroso 1952). Epitheliochorial placentation is less intimate than other types of placentation because both the uterine LE and the chorionic epithelium remain intact. The result is a significant barrier to the transfer of nutrients from the uterine vasculature to the placental vasculature. A nutrient in a subepithelial uterine capillary must pass through the cytoplasm and basement membrane of the endothelial cell of the uterine capillary. It must then pass across the remaining stromal connective tissue. The nutrient then must pass through the basement membrane and cytoplasm of the uterine LE cell, through the cytoplasm and basement membrane of the chorionic epithelial cell, across the remaining allantoic connective tissue, and through the basement membrane and cytoplasm of the endothelial cell of the allantois (see Fig. 1). Although simplistic in microscopic structure, epitheliochorial placentation and the macroscopic classification of "diffuse" characteristic of pigs are not primitive and have evolved from a more invasive placental type, hemochorial/endotheliochorial, and discoid (Wildeman et al. 2006). Indeed, although it could be inferred that the noninvasive types of placentation are the least efficient because there are more physical barriers to limit the movement of nutrients from mother to fetus, the newborn pig is relatively mature compared to the newborn of a mouse or human. Epitheliochorial placentation has arisen evolutionarily in three distinct mammalian lineages and is present in pigs, horses/donkeys, camels, some whales and primates, and in multiple other species across different genera (Amoroso 1952; Wildeman et al. 2006).

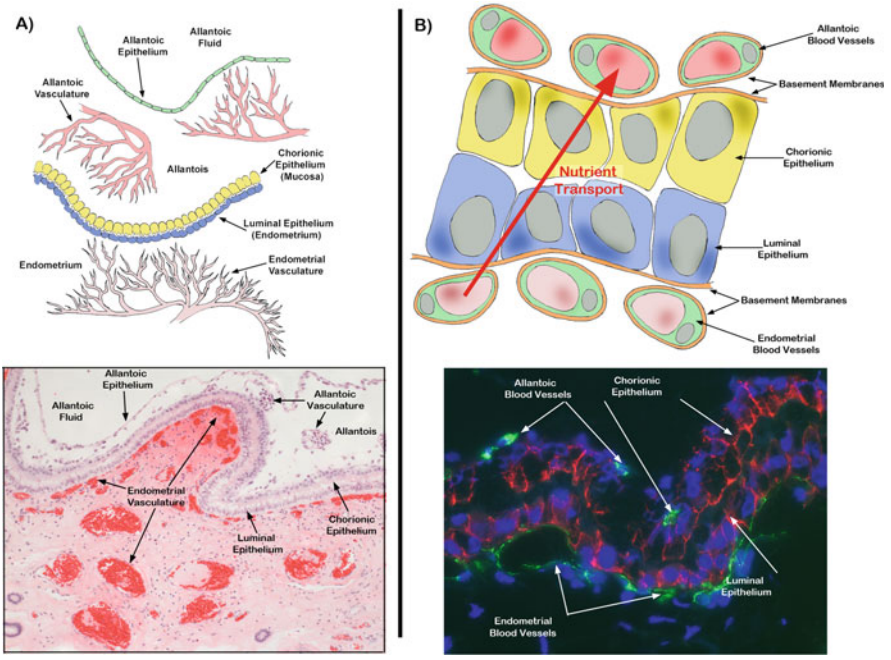


**Fig. 1** Epitheliochorial placentation in the pig. (**a**) The upper panel is a cartoon depiction of porcine placentation at lower magnification, and the lower panel represents an H&E-stained thin section depicting porcine placentation on day 20 of gestation. (**b**) The upper panel is a cartoon depiction of porcine placentation at higher magnification, and the lower panel is a thin section depicting immunofluorescence-staining for epithelial cadherin (red color, width of field is 230 μm) and platelet endothelial cell adhesion molecule (green color). (Panels **a** and **b** are original drawings by Gregory A. Johnson. Panel **a** is adapted with permission from Bazer FW, Kim J, Song G, Ka H, Wu G, Johnson GA, and Vallet JL. 2013. Roles of selected nutrients in development of the porcine conceptus during pregnancy. In: Control of Pig Reproduction IX. A. Rodriquez-Martinez, N.M. Soede and W.L. Flowers Eds., Context Products Ltd., British Library Cataloguing in Publication Data, Leicestershire, United Kingdom, pp. 159–174)

## 2 Uterine Histoarchitecture and Early Conceptus Development

The pig uterine wall differentiates embryologically from the Mullerian ducts into the endometrial and myometrial compartments (Mossman 1937). The myometrium is composed primarily of smooth muscle cells and vascular elements, and bundles of smooth muscle fibers are organized into inner circular and outer longitudinal layers. In contrast, the endometrium is a complex epitheliomesenchymal organ consisting of a simple columnar LE that overlays a thick lamina propria subdivided into upper stratum compactum and lower stratum spongiosum regions composed of stromal cells, immune cells, and vasculature. Invaginating downward from the LE are long branched and coiled tubular glands that traverse the lamina propria and are referred

to as the glandular epithelium (GE) (Cooke et al. 1998) (*see* Fig. 2a). These various cell types communicate in an autocrine, paracrine and/or, endocrine manner to coordinate responses to hormones and cytokines (Geisert et al. 1982a). Examples of these complex responses include increasing uterine blood flow (Ford et al. 1982), facilitating water and electrolyte movement (Veldius et al. 1980), maternal recognition of pregnancy (Bazer and Thatcher 1977), receptivity to conceptus attachment for implantation (Keys and King 1980; Burghardt et al. 1997), and uterine secretory activity (Bazer 1975).

By 26 h after fertilization within the oviduct, the one-cell porcine ovum/zygote cleaves to form a two-cell embryo that, 22–30 h later, enters the uterus at the 4- to 8-cell stage. The blastocyst forms when early embryonic cells differentiate into the embryonic disc, trophectoderm, and extraembryonic endoderm. The blastocoel forms and there is continued development to a conceptus (Bazer and Johnson 2014) (Fig. 2b). Conceptuses "hatch" from the zona pellucida and begin to elongate. At hatching, the conceptuses are 0.5–1 mm diameter spheres, then increase in size to 2–6 mm by day 10 of pregnancy. They then undergo a morphological transition to large spheres of 10–15 mm diameter, then to 15 mm by 50 mm tubular forms, and then to 1 mm by 100–200 mm filamentous forms on day 11 (*see* Fig. 2b). Pig conceptuses elongate at 30–45 mm/h during the transition from tubular to filamentous forms, primarily by proliferation, morphological remodeling, and migration of the trophectoderm and endoderm. However, the subsequent growth and elongation of the conceptus by day 15 into 800–1000 mm length filaments is supported by the hyperplasia of trophectoderm cells. An elongation zone of densely packed endoderm and trophectoderm cells extends from the inner cell mass (ICM) to the tip of the blastocyst on day 10. Then, through alterations in microfilaments and junctional complexes of trophectoderm cells and formation of filopodia by endodermal cells, there is further rapid elongation of the 100–200 mm long conceptus to a conceptus of 800–1000 mm in length by day 16 of pregnancy. Each conceptus within the litter eventually achieves maximum surface area for contact between the trophectoderm and uterine LE to facilitate uptake of nutrients from uterine LE and uterine GE, the numbers of which increase, coincidentally, with elongation of the conceptuses (Bazer and Johnson 2014) (*see* Fig. 2c).

## 3 Select Hormones and Cytokines That Prepare the Uterus for Placentation

Much is known about the localization of genes and their regulation by hormones within the uterus and conceptus during implantation and early placentation in pigs (Johnson et al. 2009; Bazer and Johnson 2014; Waclawik et al. 2017). Progesterone dominates the uterine environment during the establishment of pregnancy in pigs, but other factors are required to maintain a successful pregnancy. These include the secretion of estrogens from the conceptus (Bazer and Thatcher 1977; Geisert et al.

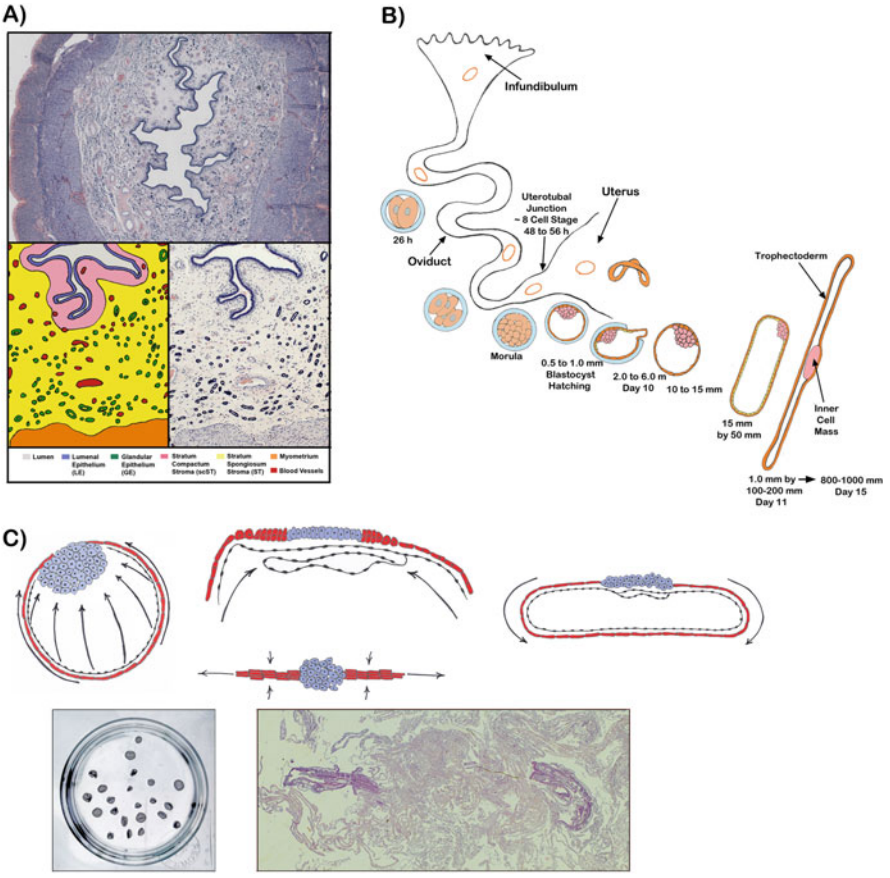


**Fig. 2** (**a**) The top and bottom right panels show H&E-stained thin sections of the wall of the porcine uterus. Bottom left panel is a cartoon in which the different tissues and cell types within the uterus are color coded for identification. (**b**) Shows a cartoon depicting early development of porcine conceptuses. The embryos enter the oviduct at bout the eight-cell stage 48–56 h postfertilization, hatch from the zona pellucida, and increase in size to 10–15 mm disks by day 10, then transform to tubular and filamentous conceptuses by day 11 of gestation. (**c**) Top panel shows a cartoon depicting the trophectoderm cells (red color) of the round blastocyst (left panel) migrating inward towards the ICM to form the elongation zone (middle panels), then trophectoderm cells migrate outwards (right panel) to form the filamentous conceptus. Bottom panels show a uterine flushing from Day 10 of gestation (left panel) containing 22 round blastocysts, and H&E staining of two pig conceptuses (right panel) from a uterine flushing obtained on Day 15 of gestation. The cartoon in Panel **c** is an interpretation of data published by Geisert et al. (1982b). (Panels **b** and **c** are original drawings by Gregory A. Johnson. Panel **b** is adapted with permission from Bazer FW, Kim J, Song G, Ka H, Wu G, Johnson GA, and Vallet JL. 2013. Roles of selected nutrients in development of the porcine conceptus during pregnancy. In: Control of Pig Reproduction IX. A. Rodriquez-Martinez, N.M. Soede and W.L. Flowers Eds., Context Products Ltd., British Library Cataloguing in Publication Data, Leicestershire, United Kingdom, pp. 159–174. Panel **c** is adapted with permission from Bazer FW, Johnson GA (2014) Pig blastocyst-uterine interactions. Differentiation 87:52–65. Published on behalf of the International Society of Differentiation by Elsevier)

2005; Johnson et al. 2009), secretions from the uterine LE and GE, i.e., histotroph (Bazer 1975; Johnson et al. 2009), and cellular remodeling at the interface between the uterine LE and trophectoderm during implantation and early placentation (Burghardt et al. 2002; Johnson et al. 2014). These complex events are orchestrated through, among others, five important cell signaling pathways for endocrine and paracrine communication between the ovary, conceptus, and uterus. These five hormones and cytokines are progesterone, estrogen, prostaglandins, interleukin-1 beta (IL1B), and the interferons [interferon gamma (IFNG) and interferon delta (IFND)].

Progesterone is considered to be the hormone of pregnancy, and it profoundly influences the uterus and placenta throughout pregnancy (Bailey et al. 2010a). Progesterone binds its nuclear progesterone receptor (PGR) to exert its actions on tissues. The PGR is encoded by a single gene and has three isoforms (A, B, and C) that differ in their activities (Wetendorf and DeMayo 2014; Mulac-Jericevic and Conneely 2004). PRB is expressed in the uterine LE, GE, stromal fibroblasts, and myometrium through day 7 of the estrous cycle and pregnancy; however, prolonged exposure to progesterone downregulates PGR in the uterine LE by day 10 and in the majority of the uterine GE by day 12 of both the estrous cycle and pregnancy. PGR protein expression in uterine LE returns by day 17 of the estrous cycle, but is not expressed by the uterine LE between days 25 and 85 of gestation. Expression of PGR is maintained in the deep GE throughout the estrous cycle and early pregnancy and continues to decrease, with almost no expression in GE by day 50 of pregnancy. PGR is, however, expressed in the necks of the uterine GE that empty into the uterine lumen proximal to areolae, suggesting that these regions of GE are excretory ducts that are not involved in the synthesis of histotroph, but serve as conduits for the passage of histotroph into the lumens of areolae. In contrast to the epithelia, PGR protein expression is maintained in the uterine stroma and myometrium through day 85 of gestation (*see* Fig. 3a) (Geisert et al. 1994; Sukjumlong et al. 2005; Steinhauser et al. 2017). One of the major roles of progesterone is to stimulate the production and secretion of histotroph, a complex mixture of hormones, growth factors, nutrients, and other substances that are required for growth and development of the conceptus (Knight et al. 1974; Roberts and Bazer 1988; Bailey et al. 2010b). The consensus is that the role of progesterone in producing histotroph is mediated via PGR; however, PGR is not expressed in uterine LE or GE that secrete histotroph during the peri-implantation period (Geisert et al. 1994; Sukjumlong et al. 2005; Steinhauser et al. 2017). It is clear that progesterone regulation of gene expression in the endometrium during the peri-implantation period is complex. Induction of genes in uterine GE may require that progesterone downregulates PGR, thereby eliminating PGR-dependent inhibition of expression of progesterone-regulated genes. However, another explanation is that progesterone induces the expression of genes in uterine via regulation of one or more paracrine-acting factors (progestamedins) produced by the PGR-positive stromal cells (White et al. 2005).

Pig conceptuses secrete estrogens on days 11 and 12 of gestation, and the consensus since 1977 has been that pregnancy recognition in the pig is the result of secretion of estrogens by conceptuses. Evidence to support this idea includes:

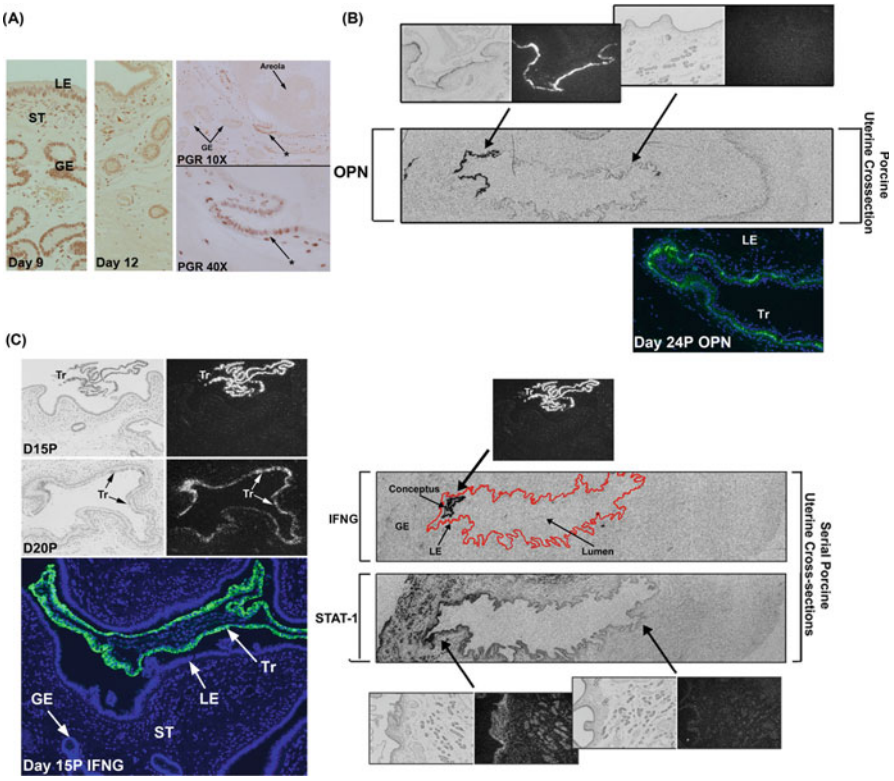


**Fig. 3** (**a**) Progesterone (P4) downregulates progesterone receptor (PGR) in the uterine epithelia, but maintains expression in the necks of uterine glands. Immunohistochemistry for PGR in the endometrium of cyclic (left two panels) and pregnant (right two panels) pigs. (**b**) Conceptus estrogens (E2) induce osteopontin (OPN) in the uterine luminal epithelium (LE), during the peri-implantation period of pregnancy. Top panels show corresponding brightfield and darkfield images of in situ hybridization from a day 15 pregnant uterus probed with *OPN* cRNA. Middle panel shows in situ hybridization analysis of *OPN* mRNA in autoradiographic images (Biomax-MR; Kodak) showing an entire cross-section of the uterine wall from day 15 of pregnancy. Bottom panel shows immunofluorescence staining for OPN in the uterine LE on day 24 of pregnancy. (**c**) Interferon gamma (IFNG) upregulates the interferon-responsive gene (ISG) signal transducer and activator of transcription one (STAT1) in the uterine stroma (ST) and glandular epithelium (GE). Left panels show corresponding brightfield and darkfield images of in situ hybridization from day 15 and day 20 pregnant uteri probed with *IFNG* cRNA, and immunofluorescence staining for IFNG in the conceptus trophectoderm on day 15 of pregnancy. Right panels show in situ hybridization analysis of *IFNG* and *STAT1* mRNAs in autoradiographic images (Biomax-MR; Kodak) showing entire serial cross-sections of the uterine wall from day 15 of pregnancy. The luminal epithelium of tissue probed for *IFNG* mRNA has been outlined in red for histological reference. Corresponding brightfield and darkfield images from the same uterus probed with *IFNG* and *STAT1* cRNAs are also shown. Width of each field of autoradiographic images is 20 mm. Width of each field of brightfield and darkfield images is 940 μm. Width of each field of immunohistochemistry is 890 μm. (Panel **a** is adapted with permission from Steinhauser CB, Bazer FB, Burghardt RC, and Johnson GA (2017) Expression of Progesterone Receptor in the Porcine Uterus and Placenta throughout Gestation: Correlation with Expression of Uteroferrin and Osteopontin. Domestic Anim Endocrinol 58:1–11. Published by Elsevier. Panel **b** is adapted with permission from White FJ, Ross JW, Joyce MM, Geisert RD, Burghardt RC, Johnson GA (2005) Steroid regulation of cell specific secreted phosphoprotein 1 (osteopontin) expression in the pregnant porcine uterus. Biol Reprod

(1) the uterine endometrium secretes PGF during both the estrous cycle and pregnancy; (2) pig conceptuses secrete estrogens which appear to be antiluteolytic; (3) PGF is secreted toward the uterine vasculature in an endocrine manner in cyclic gilts to induce luteolysis; and (4) in pregnant gilts PGF is into the uterine lumen in an exocrine manner where it is sequestered from the corpora lutea (CL) and/or metabolized to prevent luteolysis (Bazer and Thatcher 1977). It should be noted that prostaglandin E2 (PGE2), as well as lysophosphatic acid (LPA), are also proposed to have roles in pregnancy recognition signaling. Expression of PGE2 synthase by the trophectoderm and the endometrium decreases the production of PGF in favor of PGE2, and this supports maintenance of CL (Ziecik et al. 2008; Waclawik et al. 2017). However, when estrogen synthesis by the conceptus was ablated by targeting the aromatase (CYP19A1) gene utilizing CRISPR/Cas9 genome editing technology, estrogen was observed to not be essential for preimplantation conceptus development, conceptus elongation, or early CL maintenance. Estrogen was essential for maintenance of pregnancy beyond 30 days and its ablation disrupted a number of biological pathways (Meyer et al. 2019). Conceptus estrogens also modulate uterine gene expression (Geisert et al. 1982c; Johnson et al. 2009; Waclawik et al. 2017). The importance of estrogen to porcine pregnancy is underscored by the fact that exposure of the pregnant uterus to estrogen on days 9 and 10, prior to when pig conceptuses normally secrete estrogens, results in degeneration of all pig conceptuses by day 15 (Ross et al. 2007). Figure 3b illustrates that the timing of estrogen secretion by the conceptus correlates with the induction of osteopontin [OPN, also known as secreted phosphoprotein 1 (SPP1)] expression in the uterine LE, and administration of exogenous estradiol to ovariectomized pigs induces OPN in the uterine LE (White et al. 2005). The upregulation of OPN within uterine LE in close proximity to the implanting conceptus implies paracrine regulation of genes by conceptus estrogens. It is likely that effects of estrogen on the uterus are restricted to regions near the conceptus due to the metabolic activity of trophectoderm. The endometrium of pigs can convert estradiol to estrone and then converts estrone to estrone sulfate, which is biologically inactive and present in high concentrations within the uterine lumen of pregnant pigs (Flood 1974). In contrast, the trophectoderm has sulfatase that can restore the biological activity of estrogen, allowing for estrogen to upregulate genes such as OPN in the LE immediately juxtaposed to the implanting conceptus. To date, only a limited number of estrogen-stimulated genes have been localized in the pig endometrium (reviewed in Johnson et al. 2009), but the number increases each year.

**Fig. 3** (continued) 73:1294–1301. Published on behalf of the Society for the Study of Reproduction by Oxford University Press. Panel **c** is adapted with permission from Joyce MM, Burghardt RC, 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. Published on behalf of the Endocrine Society by Oxford University Press)

In addition to the important role in maintaining the lifespan of the CL, prostaglandins function as autocrine and paracrine regulators of conceptus development and endometrial functions during the peri-implantation period in pigs. Pig conceptuses and endometrium actively secrete PGE2 and PGF (Geisert et al. 1982a; Guthrie and Lewis 1986) and express their receptors (Waclawik et al. 2017). PGF, acting through its receptor (PTGFR), induces endometrial expression of vascular endothelial growth factor-A (VEGFA), biglycan, matrix metalloprotease 9 (MMP9), IL1A, and transforming growth factor B3 (TGFB3), suggesting that PGF is involved in angiogenesis and tissue remodeling during early pregnancy (Kaczynski et al. 2016). Elevated quantities of PGE2 in the uterine lumen during the peri-implantation period stimulate conceptus PTGER2 expression, which increases the synthesis and secretion of estrogen and enhances integrin-dependent trophoblast adhesion via an estrogen receptor-dependent mechanism and MEK/MAPK signaling (Waclawik et al. 2017). Several studies have proposed that PGE2 is involved in maternal recognition of pregnancy in the pig (Christenson et al. 1994; Ford and Christenson 1991; Gregoraszczuk and Michas 1999). Intrauterine infusion of PGE2 into cyclic gilts from days 7 to 23 delays CL regression and extends the length of the estrous cycle (Akinlosotu et al. 1986). PGE2, produced by the conceptuses and endometrium, can be directly luteoprotective due to an increase in the PGE2/PGF ratio. The conceptus produces about twice as much PGE2 as PGF to successfully protect luteal cells from regression (Gregoraszczuk and Michas 1999). Indeed, Meyer et al. (2019) demonstrated that PGE can protect the CL through day 24 in the absence of conceptus estrogen synthesis. However, when CRISPR/Cas9 gene editing was used to knock out prostaglandin-endoperoxide synthase 2 (PTGS2) during the period of conceptus elongation in pigs, the conceptuses elongated and pregnancy was maintained through day 35 of gestation (Pfeiffer et al. 2020). It is possible that conceptus production of estrogen and IL1B may compensate for the lack of PTGS2-derived prostaglandins as estrogen and IL1B2 increase endometrial PTGS2 expression and production of PGE2 (Waclawik et al. 2009a, b; Franczak et al. 2010).

As porcine conceptuses elongate and begin to secrete estrogens, they also secret large amounts of IL1B (Tuo et al. 1996; Ross et al. 2003). There is an initial and acute rise in IL1B on day 12 of pregnancy followed by a precipitous decline in the expression and secretion of IL1B by pig conceptuses by days 15–18 of gestation as the conceptuses become fully elongated filaments. The expression of receptors for IL1B increases in the uterus and conceptus in tandem with this transient expression of IL1B by pig conceptuses, between days 12 and 15 of pregnancy (Ross et al. 2003). This suggests that IL1B from the conceptus may bind IL1B receptors on conceptus and/or uterine tissues to modify interactions between the uterus and conceptus in an autocrine and/or paracrine fashion. It should be noted that there are two transcripts for IL1B expressed in the pig trophectoderm, with both genes in close proximity on chromosome 3 (Mathew et al. 2015). The classical IL1B is expressed in macrophages and endometrial tissue, but another form of IL1B, IL1B2, appears to be unique to the elongating pig conceptus and is expressed just prior to attachment of the conceptus to the uterine LE. The specific roles of conceptus IL1B in trophoblast elongation and uterine receptivity have not been

determined, but when CRISPR/Cas9 gene editing was used to knock out IL1B2 during the period of conceptus elongation in pigs, the conceptuses failed to elongate. The expression of the majority of conceptus developmental genes was not altered in these conceptuses, but the conceptuses expressed less aromatase and secreted less estrogen than normal (Whyte et al. 2018).

Antiviral activity in the uterine flushings of pigs peaks on days 14 and 25 of gestation, and the media from cultured porcine conceptuses exhibits significant antiviral activity (Mirando et al. 1990). The cause for this phenomenon is that the trophectoderm of pigs secretes a Type II IFN gamma (IFNG, 75% of antiviral activity in pig conceptus secretory proteins) and a Type I IFN delta (IFND, 25% of antiviral activity in pig conceptus secretory proteins) during the peri-implantation period (La Bonnardière et al. 1991; Lefèvre et al. 1998a). There is a 567-fold increase in the abundance of IFNG mRNA in porcine conceptuses between days 13 and 20 of gestation, during the transition of conceptuses from spherical to day 14 filamentous forms (Cencič and LaBonnardière 2002; Joyce et al. 2007a) (*see* Fig. 3c). In contrast, RT-PCR analysis is required to detect IFND mRNA in the day 14 conceptuses of pigs (Joyce et al. 2007a, b). Although IFN tau (IFNT), a Type I IFN, is the pregnancy recognition signal in ruminants (Spencer et al. 2007a), IFNG and IFND do not appear to be antiluteolytic in pigs (Harney and Bazer 1989; Lefèvre et al. 1998b). However, paracrine effects for porcine conceptus IFNs are suggested by: (1) localization of IFN receptors on endometrial epithelial cells (Lefèvre et al. 1998b); (2) increased secretion of PGE2 (Harney and Bazer 1989); (3) endometrial expression of several known IFN-responsive genes (reviewed in Johnson et al. 2009); and (4) modulation of uterine stromal and GE gene expression by the IFNs in conceptus secretory protein preparations (Joyce et al. 2007a, b, 2008). Figure 3c illustrates that stromal induction of mRNA for *STAT1,* an IFN responsive gene, correlates with IFNG and IFND secretion by the conceptus. Indeed, intrauterine infusion of conceptus secretory proteins, which contain IFNG and IFND, into pseudopregnant pigs, increased *STAT1* as compared to intrauterine infusion of control proteins (Joyce et al. 2007a). Upregulation of *STAT1* within the uterine stroma and GE in close proximity to the implanting conceptus implies paracrine regulation of genes by conceptus IFNs. It is somewhat surprising that initial increases in stromal *STAT1* are restricted to sites of intimate association between the conceptus and uterus in pigs because the magnitude of IFNG production by pig conceptuses appears to be similar to IFNT synthesis and secretion by ovine conceptuses (Joyce et al. 2007a). *STAT1* increases universally in the stroma and GE of pregnant sheep without regard to conceptus location within the lumen, presumably due to the high levels of secretion of IFNT by conceptuses (Pontzer et al. 1988; Joyce et al. 2005a). One explanation for the more limited expression of *STAT1* in the pig uterus is that IFNG and IFND act synergistically to upregulate interferon responsive genes. Interactions between Type I and Type II IFNs to enhance their activities have been demonstrated previously (Decker et al. 1989). To date, only a limited number of IFN responsive genes have been localized in the pig endometrium and specific roles for these IFN responsive genes remain elusive (reviewed in Johnson et al. 2009).

## 4 Attachment of the Conceptus Trophectoderm to the Uterine LE (Implantation)

In strict terms, porcine conceptuses do not penetrate the uterine LE and invade into the uterine stroma; therefore, for pigs, the term "implantation" is a misnomer. Instead, pig conceptuses undergo a central type of implantation. Nevertheless, elongation and implantation are used to describe the initial stages of placental development in pigs. Although the duration of the preimplantation period is prolonged in the pig, and type of implantation differs from many other species, the initial stages of implantation/placentation are common across species and are characterized as the "Adhesion Cascade for Implantation" (Dantzer 1985; Guillomot 1995; Burghardt et al. 2002). This adhesion cascade begins with shedding of the zona pellucida, followed by elongation of the conceptus trophectoderm. The conceptus trophectoderm then orients itself to the uterine LE in a phase called "apposition," followed by adhesion of the apical surface of trophectoderm to the apical surface of uterine LE, and development of interdigitating microvilli between trophectoderm and uterine LE (see Fig. 4a). As this cascade concludes, adhesion seamlessly transitions to the progressive formation of epitheliochorial placentation that supports fetal-placental development throughout pregnancy (Bazer and Johnson 2014).

During the peri-implantation period of pregnancy in eutherian mammals, the conceptus trophectoderm and uterine LE develop adhesion competency in synchrony to initiate an adhesion cascade within a restricted period of pregnancy termed the "Window of Receptivity" (Fazleabas et al. 2004; Spencer et al. 2007b; Bazer et al. 2011). This window is defined by the actions of progesterone and estrogen to regulate locally produced cytokines and growth factors, cell surface glycoproteins and adhesion molecules, and extracellular matrix (ECM) proteins (Johnson et al. 2009). Progesterone initiates the adhesion cascade for implantation in pigs. Immediately prior to when the endometrium becomes receptive to implantation, just after day 10, progesterone downregulates the expression of PGR in the uterine LE (Geisert et al. 1994; Steinhauser et al. 2017). This downregulation of PGR is associated with a downregulation in the expression of the mucin 1 (MUC1), a component of the apical surface glycocalyx of the uterine LE that physically inhibits attachment of the conceptus trophectoderm to the uterine LE due to its extended carbohydrate configuration (Brayman et al. 2004). Indeed, when cyclic gilts are administered intramuscular injections of progesterone, there is a loss of MUC1 from the apical surface of the uterine LE (Bowen et al. 1996). It is accepted that in all mammals, firm attachment of the conceptus trophectoderm to the uterine LE requires a temporal loss of MUC1 at the apical surface of uterine LE cells (Brayman et al. 2004) that exposes low-affinity carbohydrate ligand-binding molecules including selectins and galectins and, perhaps heparan sulfate proteoglycan, heparin-binding EGF-like growth factors, cadherins, and CD44. These molecules are proposed to then contribute to the initial, if fragile, attachment of the conceptus trophectoderm to the uterine LE (Kimber et al. 1995; Kimber and Spanswick 2000; Spencer et al.

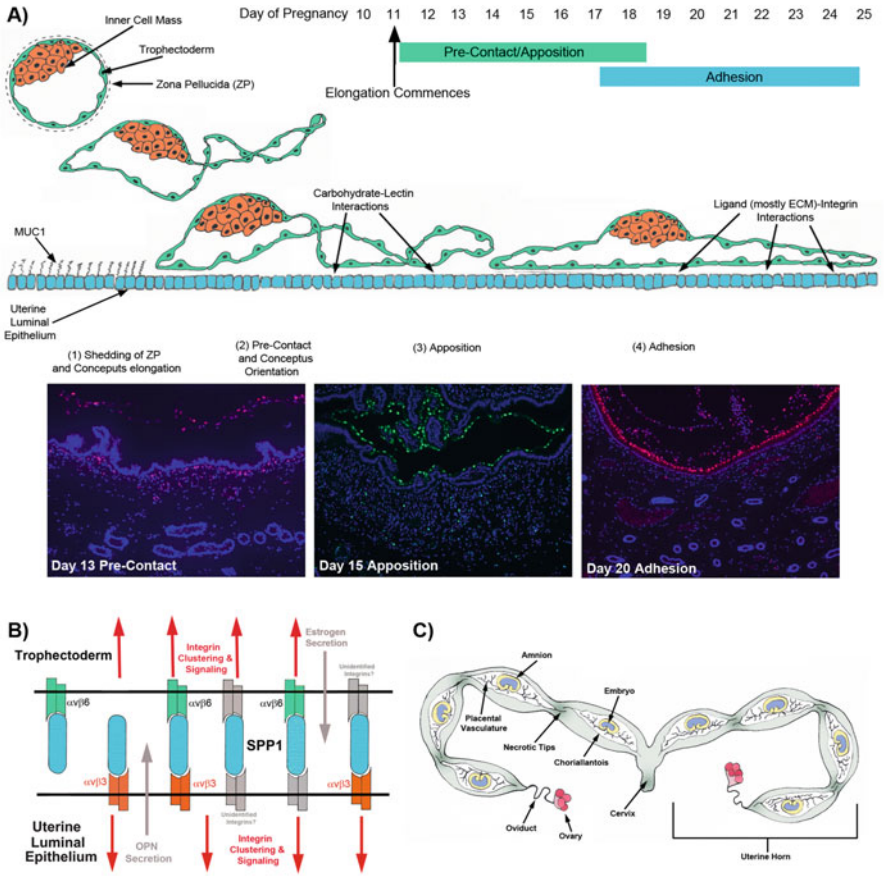


**Fig. 4** (**a**) The initial stages of implantation are common across species and are characterized as the "Adhesion Cascade for Implantation." The phases of this adhesion cascade in pigs include: (1) elongation of the conceptus trophectoderm and shedding of the zona pellucida; (2) downregulation of MUC1 at the apical surface of uterine LE to expose potential, but not yet identified, low-affinity carbohydrate-lectin binding molecules to mediate precontact and conceptus trophectoderm orientation to the uterine LE; (3) low-affinity contacts are then replaced by a more stable and extensive repertoire of adhesive interactions between integrins and maternal ECM to mediate apposition of trophectoderm to LE; (4) integrin receptors expressed at the apical surface of uterine LE cells bind to Arg-Gly-Asp (RGD) and non RGD amino acid sequence-containing ECM molecules and bridge to another complement of potential integrin receptors expressed at the apical surface of conceptus trophectoderm cells to mediate conceptus trophectoderm adhesion; and (5) development of interdigitating microvilli between LE and trophectoderm (not illustrated in figure). Immunofluorescence staining for PCNA illustrates that the conceptus trophectoderm (Tr) proliferates, but the uterine luminal epithelium (LE) does not proliferate during the peri-implantation period of pigs. The left and right panels were immunolabeled with a goat anti-mouse IgG Alexa 594 secondary antibody. The middle panel was immunolabeled with a goat anti-mouse IgG Alexa 488 secondary antibody. (**b**) Shows a cartoon depicting results of in vitro experiments that have identified the αvβ6 integrin receptor on conceptus trophectoderm, and the αvβ3 integrin receptor on uterine LE as binding partners for OPN. OPN may bind individually to these receptors to act as a bridging ligand between these receptors. Alternatively, OPN may serve as a bridging ligand between one of these receptors and an as yet unidentified integrin receptor expressed on the opposing tissue. (**c**) Shows a cartoon depicting conceptus spacing within the

2004). Interactions between carbohydrates and lectins during the adhesion cascade of pigs have not been systematically investigated. However, it is likely that these carbohydrate ligands and their lectin receptors, expressed at the apical surfaces of the conceptus trophectoderm and uterine LE of pigs, undergo a series of attach-and-release events. This results in maximal apposition of the conceptus trophectoderm to the uterine LE, similar to the "rolling and tethering" that occurs when leukocytes adhere to the endothelium for extravasation out of the vasculature and into connective tissues (Kling et al. 1992). Indeed, rolling and tethering has been proposed for the initial attachment of human blastocysts to the uterine wall (Red-Horse et al. 2004). In support of this idea, other domestic farm species, including goats and sheep, express H-type-1 antigens and glycosylation-dependent glycam 1, respectively, at the interface between the conceptus trophectoderm and uterine LE during the attachment phase of implantation (Powell et al. 2000; Spencer et al. 1999). These low-affinity contacts are then stabilized by adhesion between an extensive repertoire of integrins and extracellular matrix (ECM) proteins. The binding of integrins to ECM proteins appears to be the major contributor to firm attachment of the conceptus trophectoderm to the uterine LE during implantation (Hynes 1987; Ruoslahti and Pierschbacher 1987; Aplin et al. 1994; Burghardt et al. 1997, 2002; Lessey 2002; Johnson et al. 2003, 2014). Integrins have been implicated in many cell adhesion cascades (Kling et al. 1992). They are transmembrane glycoprotein receptors composed of noncovalently linked α and β subunits. Integrin-ligand binding promotes cell-cell and cell-ECM adhesion, causes the cytoskeleton within the cells to reorganize and stabilize that adhesion, and transduces numerous intracellular signaling pathways (Giancotti and Ruoslahti 1999; Albelda and Buck 1990). There are 18 α- and 8 β-subunits that can dimerize to form 24 heterodimer combinations that then bind to numerous extracellular ligands including a variety of ECM proteins (Albelda and Buck 1990; Humphries et al. 2006; Gallant et al. 2005). Integrin receptors expressed at the apical surface of the uterine LE can bind to Gly-Arg-Gly-Asp-Ser (GRGDS) amino acid sequence containing ECM molecules and bridge to another complement of integrin receptors expressed at the apical surface of the conceptus trophectoderm (Johnson et al. 2014).

Seven integrin subunits have been shown to be expressed at the apical surface of both the conceptus trophectoderm and the uterine LE of pigs. These include alpha

**Fig. 4** (continued) uterine horns and development of the allantoic and amniotic membranes (Panels **a**, **b**, and **c** are original drawings by Gregory A. Johnson). Panel **a** is adapted with permission from Geisert RD, Johnson GA and Burghardt RC. Implantation and establishment of pregnancy in the pig. In: Regulation of implantation and establishment of pregnancy in mammals: Tribute to 45 year anniversary of Roger V. Short's "Maternal Recognition of Pregnancy". Ed's R.D. Geisert and F.W. Bazer. 2015, pp. 137–164. Springer Sham. Panel **b** is adapted with permission from Erikson DW, Burghardt RC, Bayless KJ, Johnson GA (2009) Secreted phosphoprotein 1 (SPP1, osteopontin) binds to integrin alphavbeta6 on porcine trophectoderm cells and integrin alphavbeta3 on uterine luminal epithelial cells, and promotes trophectoderm cell adhesion and migration. Biol Reprod 81:814–825. Published on behalf of the Society for the Study of Reproduction by Oxford University Press

1 (α1), α3, α4, α5, αv, beta 1 (β1), β3, and β5. The expression of α4, α5, and β1 on the uterine LE increases during the peri-implantation period, and treatment with progesterone increases the expression of these integrins at the apical surface of uterine LE cells of cyclic pigs. Further, α4, α5, αv, β1, β3, and β5 are present at porcine implantation sites on days 12 through 15 of gestation (Bowen et al. 1996). These subunits have the potential to assemble into the integrin heterodimer receptors αvβ1, αvβ3, αvβ5, α4β1, and α5β1, and these integrin receptors may function in the adhesion cascade that adheres the conceptus trophectoderm to the uterine LE (Burghardt et al. 1997). Integrin activation through binding to a ligand results in dynamic macromolecular complexes, termed integrin adhesion complexes (IACs) that are composed of the heterodimeric transmembrane integrin receptors that connect ECM proteins to the actin cytoskeleton, along with a diverse array of cell signaling intermediates (Sastry and Burridge 2000; Wozniak et al. 2004; Larsen et al. 2006). Immunofluorescence staining has revealed that αv and β3 integrin subunits co-localize with an altered distribution of an intracellular signaling intermediate within IACs called talin within large aggregates at sites of attachment between the conceptus trophectoderm and uterine LE on Days 20 and 25 of gestation (Erikson et al. 2009; Frank et al. 2017). The size and nature of these aggregates are similar to the well-characterized IACs that form at the base of cultured cells when they attach to the ECM (Sastry and Burridge 2000; Burghardt et al. 2009). To date, five ligands capable of engaging integrin receptors to induce assembly of IACs have been characterized at sites of implantation in pigs. The inter-α-trypsin inhibitor heavy chain-like (IαIH4) protein contains a von Willebrand type A domain that is a recognition site for the αvβ3 integrin receptor (Geisert et al. 1998). The Latency Associated Peptide (LAP) of transforming growth factor beta (TGFB) binds to αvβ1, αvβ3, and αvβ5 (Massuto et al. 2009a). Fibronectin is capable of binding α4β1, α5β1, and αvβ3 (Bowen et al. 1996). Vitronectin is a major ligand for αvβ3 (Bowen et al. 1996). Finally, OPN is the most promiscuous of the ligands and interacts with αvβ1, αvβ3, αvβ5, and α4β1 (Johnson et al. 2003, 2014).

The IαIH4 is part of the kallikrein-kininogen-kinin protease system. Both IαIH4 protein expression and kallikrein enzymatic activity increase within the uterine environment during the peri-implantation period of pregnancy in pigs (Geisert et al. 1998; Vonnahme et al. 1999). The IαIH4 can not only bind to the αvβ3 integrin receptor but also interact with hyaluronic acid within the ECM, both of which could be involved in conceptus implantation. However, the primary role for IαIH4 during implantation in pigs may be to act in concert with bikunin to stabilize the uterine LE surface glycocalyx during conceptus attachment for implantation (Hettinger et al. 2001). The LAP associates with TGFB to form an inactive homodimer, called the small latent complex. The small latent complex remains in the cell until it is bound by latent TGFB-binding protein to form the large latent complex (LLC) that is then secreted into the ECM (Lawrence 1996). The secreted LAP is then cleaved from the LLC by proteases to release active TGFB (Jenkins et al. 2006). In pigs, TGFB1, TGFB2, and TGFB3, and their receptors, TGFBRI and TGFBRII, are expressed by the conceptus trophectoderm and by the uterine LE between days 10 and 14 of gestation. It has been demonstrated that TGFB acts through LAP to increase

fibronectin synthesis and cell adhesion to fibronectin, and LAP was shown to initiate the formation of IACs in a porcine trophectoderm cell line (Jaeger et al. 2005). In addition, conceptuses failed to implant when LAP was infused into the uteri of pregnant pigs, suggesting that the infused LAP competed with the endogenous LLC for binding to integrins expressed on the conceptus trophectoderm (Massuto et al. 2009b). In support of this idea, IACs containing LAP, β1, β3, and β5 are present at the interface between the conceptus trophectoderm and uterine LE that may support attachment of the conceptus trophectoderm to the uterine LE during porcine implantation (Massuto et al. 2009a). Both fibronectin and vitronectin are present at sites of attachment of conceptus trophectoderm to uterine LE in pigs (Bowen et al. 1996), and oncofetal fibronectin (oFN), a glycosylation variant of fibronectin, is constitutively expressed by the porcine conceptus trophectoderm as well as the uterine LE throughout gestation (Tuo and Bazer 1996). Fibronectin and vitronectin are prototype cell adhesion proteins, and fibronectin recognizes 10 integrin receptors to generate different signals depending upon the specific integrin receptor involved (Johansson et al. 1997; Humphries et al. 2006). It is noteworthy that the human conceptus trophoblast produces oFN so precisely at sites of trophoblast contact with the endometrium that it has been referred to as "trophoblast glue" (Feinberg et al. 1994), and fibronectin is a strong candidate for mediating conceptus adhesion to the uterus in rodents (Armant 2005).

Of the ECM adhesion proteins expressed at the interface between the conceptus trophectoderm and the uterine LE during the peri-implantation period of pregnancy in pigs, OPN is the most extensively studied (Johnson et al. 2003, 2014) (see Fig. 4b). OPN is a member of the small integrin-binding ligand *N*-linked glycoprotein (SIBLING) family of ECM proteins that has multiple physiological functions (Denhardt and Guo 1993; Butler et al. 1996; Sodek et al. 2000), but with regard to implantation, focus has been on OPNs ability to bind integrins to mediate cell adhesion and cell migration (Senger et al. 1994). OPN is an abundant component of the intrauterine environment of pregnant humans, mice, rabbits, sheep, cattle, goats, and pigs (Johnson et al. 1999a, b; Garlow et al. 2002; Apparao et al. 2003; Kimmins et al. 2004; Mirkin et al. 2005; Joyce et al. 2005b; White et al. 2006). In pigs, conceptus estrogens induce OPN expression just prior to the initiation of implantation, beginning on day 13, in discrete regions of the uterine LE next to the conceptus trophectoderm. By day 20, OPN expression extends along the entire uterine LE when stable adhesion of the conceptus trophectoderm to uterine LE occurs, and OPN expression remains high at the uterine–placental interface throughout gestation (Garlow et al. 2002; White et al. 2005). In vitro affinity chromatography and immunoprecipitation experiments have shown that OPN binds the αvβ6 integrin heterodimer on porcine trophectoderm cells, and the αvβ3 integrin heterodimer on porcine uterine LE cells (Erikson et al. 2009). OPN binding, particularly to the αv integrin, promotes dose-dependent attachment of porcine trophectoderm and uterine LE cells and stimulates haptotactic trophectoderm cell migration, meaning that the cells migrated along a physical gradient of nonsoluble OPN (Erikson et al. 2009; Frank et al. 2017). Immunofluorescence staining for integrins at implantation sites of pigs revealed IACs containing these same integrins

distributed in a pattern similar to that suggested by in vitro binding to OPN (Erikson et al. 2009; Frank et al. 2017). Immunofluorescent staining for the αv integrin subunit revealed large IACs at the junction between the apical surfaces of conceptus trophectoderm and uterine LE cells, suggesting these IACs facilitate conceptus trophectoderm attachment to the uterine LE for implantation. The β3 subunit, however, appeared in aggregates at the apical surface of uterine LE cells only, agreeing with affinity chromatography data indicating in vitro binding of αvβ3 to OPN on uterine LE cells (Erikson et al. 2009). Finally, OPN-coated microspheres were used to demonstrate co-localization of OPN, the αv integrin subunit, and talin to IACs at the apical domain of porcine trophectoderm cells (Erikson et al. 2009). Collectively, results indicate that OPN binds integrins to stimulate integrin-mediated IAC assembly within conceptus trophectoderm and uterine LE cells, attachment of conceptus trophectoderm to uterine LE cells, and migration of conceptus trophectoderm cells to promote conceptus implantation in pigs.

The physical changes that occur to the interface between the conceptus trophectoderm and uterine LE during the initial stages of epitheliochorial placentation in pigs have been eloquently described by Dantzer (1985). On all days examined, the glycocalyx at the surface of the uterine LE is thicker than the glycocalyx at the surface of the conceptus trophectoderm. On days 13 and 14, the uterine LE develops protrusions that become enclosed by caps of conceptus trophectoderm cells, and this unique configuration physically immobilizes the conceptus at the uterine LE surface. By day 14, there is close apposition between the apical membranes of conceptus trophectoderm and uterine LE cells, and microvilli form and interdigitate between these plasma membranes through days 15 and 16. The interface between the conceptus trophectoderm and uterine LE cells becomes increasingly complex as it functionally transitions from histotrophic to hemotrophic nutrient transport between days 15 and 20 of pregnancy. It is characterized by apical domes on the uterine LE cells that are closely apposed to the conceptus trophectoderm cells and provide long cytoplasmic extensions into a luminal space between the apical domes. Finally, adhesion transitions into placentation through ever-increasing development of interdigitating microvilli between the conceptus trophectoderm and uterine LE cells that extends into the peripheral zone by day 26 of gestation.

## 5 Folding of the Uterine–Placental Interface to Facilitate Hemotrophic Support of the Fetus

As pregnancy progresses, the uterine–placental interface, composes of the uterine (maternal) placenta and the fetal placenta, undergoes considerable morphological changes to progressively develop more complex folds that increase the surface area of contact between uterine LE and placental chorionic epithelium (CE) for exchange of nutrients, gases and waste products (Vallet and Freking 2007; Vallet et al. 2009). The interface between the maternal (uterine) placenta and fetal placenta of pigs

begins to fold by day 25 of pregnancy, and these early folds increase in length through day 35, and again increase in length between days 50 and 60 of gestation (Seo et al. 2020) (see Figs. 5a and 6a, c). Placental and uterine capillaries lie immediately beneath these epithelia, minimizing the distance between maternal and fetal blood vessels (Dantzer and Leiser 1994). Therefore, the increase in folding at the maternal placental (uterine)–fetal placental (chorioallantoic) interface directly increases the surface area of contact between maternal and placental microvasculatures to optimize the potential for transport of nutrients from maternal to placental blood vessels for eventual utilization by the embryo/fetus. The small intestine utilizes a similar strategy to maximize exposure of the microvasculature for micronutrient transport (Hilton 1902; Simons 2013). Friess et al. (1980) described the microarchitecture of these folds at Days 30 and 58 of gestation. By day 30 of pregnancy, the chorioallantoic and endometrial surfaces interlock into folds composed of endometrial ridges and chorioallantoic troughs. The trophectoderm cells at the bottom of the chorioallantoic troughs are columnar and 40 μm in height, and the trophectoderm cells at the sides and tops of the troughs are 20 μm in height. The height of the uterine LE cells does not vary along the interface of the folds. The apical surfaces of the trophectoderm cells possess long microvilli which interdigitate with microvilli on the apical surfaces of the uterine LE cells. The subepithelial capillaries of both the chorioallantois and endometrium remain separated from the epithelial basal laminae by a limited, but easily visualized, layer of connective tissue (Friess et al. 1980). By day 58 of pregnancy, the height of the trophectoderm cells is 35 μm at the bottom of the chorioallantoic troughs, and these cells are high columnar and narrow in width. Along the sides and tops of the chorioallantoic troughs, the trophectoderm cells are 15 μm in height. The uterine LE cells opposite the base of the chorioallantoic troughs are 25 μm, whereas the uterine LE cells along the sides and at the top of the chorioallantoic troughs are 15 μm. The subepithelial capillaries indent into the trophectoderm cells on the sides and tops of the chorioallantoic troughs, and where the capillaries protrude into the trophectoderm cells, the trophectoderm cell height is reduced to 2 μm. In summary, the lateral sides and tops of the chorioallantoic ridges are designed for gaseous exchange, and the chorioallantoic barrier can often be less than 2 μm, whereas the base of the chorioallantoic troughs is designed for the transport of blood-borne nutrients, i.e., hemotroph (Friess et al. 1980).

An emerging concept is that tissues respond to mechanical forces that coordinate morphogenesis, and it is hypothesized that mechanotransduction and mechanosensation at the interface between the uterus and placenta drives the morphological development of folding characteristic of the epitheliochorial placentation of pigs (Seo et al. 2020). Changes in the length of the placental folds, expression of mechanotransduction-implicated molecules in uterine and placental tissues, and changes in the size of subepithelial blood vessels were examined for days 20 through 60 of gestation in pigs (see Fig. 5). It was observed that: (1) the length of folds increased (2) OPN, talin, and focal adhesion kinase co-localized into aggregates at the uterine–placental interface; (3) filamin, actin-related protein 2, and F-actin were enriched at the tops of uterine folds extending into placental tissue (the bottom of the chorioallantoic troughs described in the preceding paragraph); (4) uterine stromal

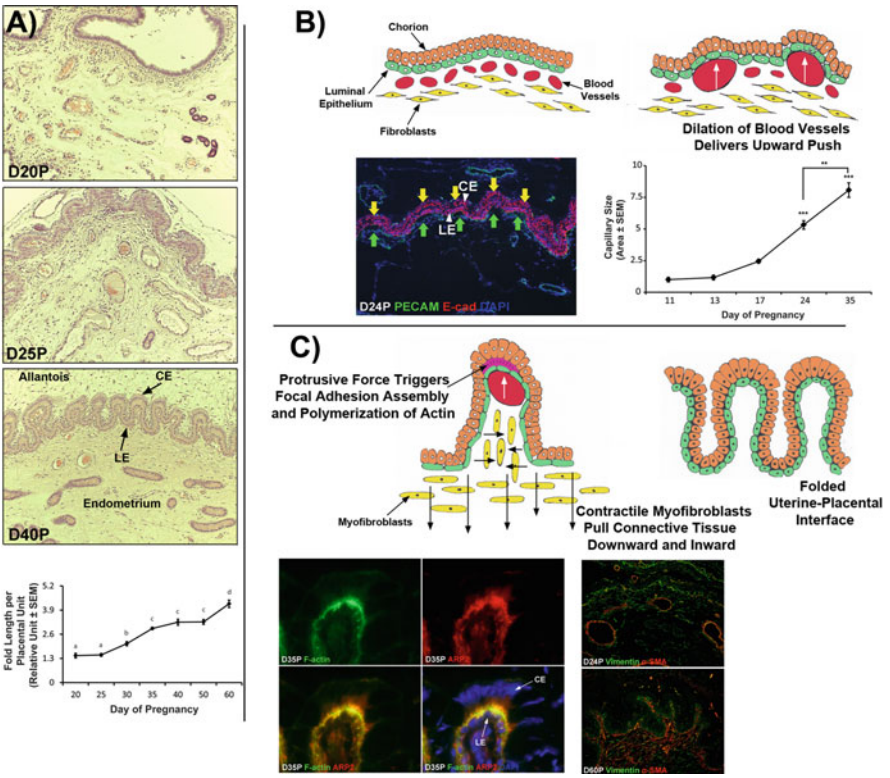


**Fig. 5** (**a**) Representative paraffin-embedded H&E-stained thin sections of the porcine uterine–placental interface on days (D) 20, 25, and 40 of pregnancy (P). The interface between the maternal placenta (endometrium) and fetal placenta (chorioallantois) begins to fold between day 20 and day 25 of pregnancy. The bottom panel is a graphic illustration of morphometric analyses to quantify the length of placental folds on days 20 through 60 of pregnancy. Folding of the maternal placental-fetal placental epithelial bilayer significantly increases from day 30 through 60 of gestation. LE, luminal epithelium; CE, chorionic epithelium; Data are expressed as mean ± standard error of the mean (SEM). *, $P < 0.05$; ***, $P < 0.0001$. Width of field for microscopic images is 890 μm. (**b**) Upper panels show a cartoon depicting the dilation of subepithelial maternal placental (endometrial) blood vessels that increases blood flow to push upward on the interface between the endometrial LE and the chorioallantoic CE. The bottom left panel shows immunofluorescence microscopy for platelet/endothelial cell adhesion molecule 1 (PECAM-1), in uterine-placental tissues from day 24 of pregnancy. Subepithelial blood vessels are dilated from day 24 and day 60 of pregnancy. The right panel shows measurements of the mean area of subepithelial capillaries per unit endometrial tissue from day 11 to day 35 of pregnancy. The mean capillary area significantly increased between day 17 and day 24, and again between day 24 and day 35 of pregnancy. Data are expressed as mean ± standard error of the mean (SEM). **, $P < 0.01$; ***, $P < 0.0001$; E-cad, E-cadherin. Width of field for the microscopic image is 230 μm. (**c**) Upper panels depict protrusive forces at the sites of growing uterine capillaries that trigger IAC assembly and polymerization of actin between the LE and CE. These IACs serve to anchor the interface at the tops of the maternal placental folds and hold the maternal placenta and fetal placenta together. Maternal placental (endometrial) fibroblasts differentiate into contractile myofibroblasts. Because protrusive force continues to be applied to the placental interface at the tops of the maternal placental folds, and the tops of these folds are stabilized by the formation of IACs, contraction of the myofibroblasts does not pull the tops of the folds downward. Instead, the myofibroblast contraction pulls the sides of the folds inward and the tops of the fetal placental folds downward. The result is both a narrowing and a

fibroblasts acquired alpha smooth muscle actin; and (5) uterine blood vessels increased in size. These observations indicate that the lengthening of the folds is associated with polymerization of actin that coincides with IAC assembly, endometrial fibroblasts differentiate into myofibroblasts, and dilation of subepithelial blood vessels correlates with the development of the folds. It is proposed that dilation of subepithelial uterine blood vessels delivers increased blood flow that pushes upward on the interface between the uterine LE and the placental chorioallantois, protrusive forces from growing uterine blood vessels trigger IAC assembly and actin polymerization between the uterine LE and chorionic epithelium at the bottoms of the chorioallantoic troughs, and uterine fibroblasts differentiate into contractile myofibroblasts that pull the connective tissue downward and inward to sculpt folds at the uterine–placental interface (see Fig. 5b, c).

## 6 Areolae Provide for Histotrophic Support of the Fetus

In addition to having chorionic epithelium closely apposed to the uterine LE, there are specialized epithelial cells of the chorionic areolae at the openings of the mouths of uterine glands (see Fig. 6). These are tall columnar cells that have numerous vacuoles containing the secretions of uterine glands (histotroph or uterine milk). Indeed, the open space between the chorion and LE is filled with uterine milk (Dempsey et al. 1955). The blood vessels that supply the folds of the wall of the areolae form a ring towards the periphery and the areolar capillaries converge into one or two stem veins indicating facilitated external inflow of blood into the areola and outflow in a manner different from that of the inter-areolar regions of the placenta (Dantzer and Leiser 1993). This anatomy allows areolae to transport glandular secretions such as macromolecules, particularly proteins, by fluid-phase pinocytosis across the placenta and into the fetal-placental circulation.

After the apposition of the conceptus trophectoderm to the uterine LE at days 13–15 of gestation, by days 15–17, the chorioallantois immediately around each of

**Fig. 5** (continued) lengthening of the folds at the interface between the maternal (uterine) placenta and fetal placenta of pigs. The lower left panels show co-staining for F-actin and actin-related protein 2 (ARP2) in uterine-placental tissues on day 35 of pregnancy. F-actin and ARP2 colocalized to the top of the maternal placental folds. The lower right panels show uterine-placental tissues from day 24 and day 60 of gestation that are double immunostained for vimentin and alpha smooth muscle actin (α-SMA). Vimentin, but not α-SMA, was evident in the stratum compactum of the endometrium of the maternal placenta on day 24, however immunostaining for α-SMA also increased between days 24 and 35 of gestation. Width of fields for microscopic images on the left is 230 μm. Width of fields for microscopic images on the right is 890 μm. (Panel **b** is an original drawing by Gregory A. Johnson. Figure is adapted with permission from Seo H, Li X, Wu G, Bazer FW, Burghardt RC, Bayless KJ and Johnson GA (2020) Mechanotransduction drives morphogenesis to develop folding at the uterine-placental interface of pigs. Placenta 90:62–70. Published on behalf of the International Federation of Placenta Associations by Elsevier)

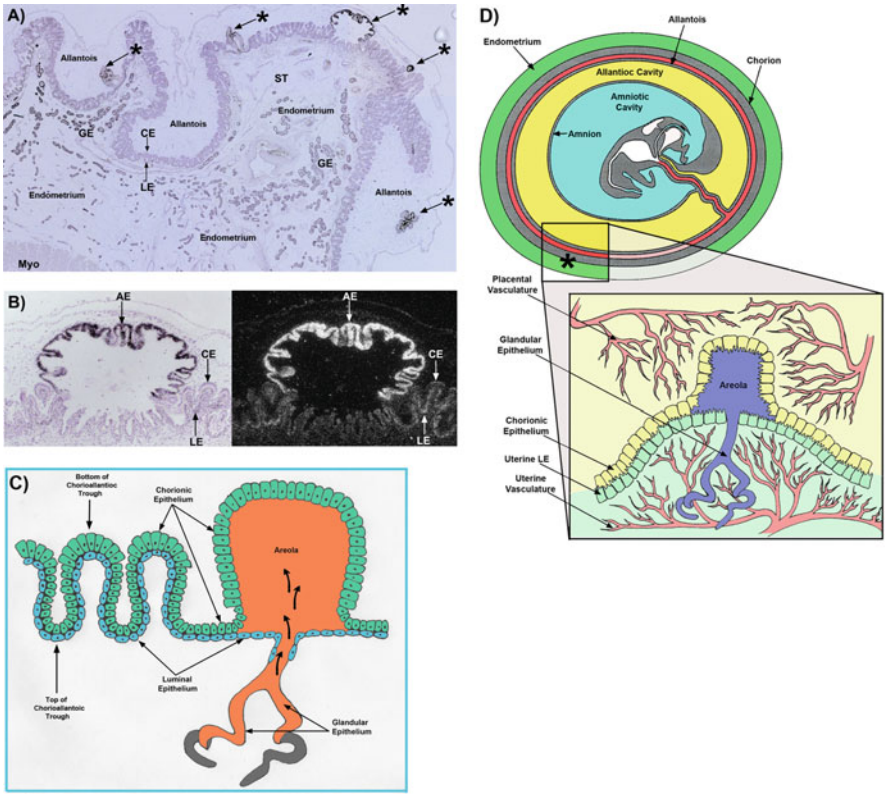


**Fig. 6** (**a**) H&E-stained brightfield image of in situ hybridization analysis of Cathepsin L (*CTSL1*) mRNA, a gene that is expressed in the chorionic epithelium (CE) of areolae, within the chorioallantoic and endometrial tissues of a day-60 pregnant pig (the top-most areola in this panel and the areola in (**b**) are the same) (Song et al. 2010). Note the extensive folding interface between the chorioallantois and the endometrium and the intermittent areolae at this interface denoted by the astericks *. (**b**) Corresponding brightfield and darkfield images of a higher magnification of a placental areola from panel **a**. Width of field of panel **a** is 7.6 mm. *LE* luminal epithelium, *AE* areolar epithelium, *GE* glandular epithelium, *ST* stroma, *CE* chorionic epithelium, *Myo* myometrium. (**c**) Shows an illustration of the uterine placental interface of mature placentation in the pig illustrating folding for hemotrophic support of the fetus and an areola for histotrophic support of the fetus. The orange indicates the synthesis, secretion, and transport of histotroph by the glands and into the lumen of the areola. Note that the epithelial cells of the areola and the chorionic epithelial cells at the bottom of the chorioallantoic troughs are tall columnar. (**d**) Illustration depicting the uterine-placental microenvironment at a placental areola in relationship to the fetus. Nutrients and gases are transported from the maternal capillaries into the placental capillaries and then to the heart via the umbilical vein for distribution to all tissue of the fetal-placental unit. Macromolecules transported across the areolae also go to the heart via the umbilical circulation for utilization by various tissues and cells. (Panels **c** and **d** are original drawings by Gregory A. Johnson. Panels **a**, **b**, and **d** are adapted with permission from Song G, Bailey DW, Dunlap KA, Burghardt RC, Spencer TE, Bazer FW, Johnson GA (2010) Cathepsin B, Cathepsin L and Cystatin C in the Porcine Uterus and Placenta: Potential Roles in Endometrial/Placental Remodeling and in Fluid-Phase Transport of Proteins Secreted by Uterine Epithelia Across Placental Areolae and Neonatal Gut. Biol Reprod 82:854–864. Published on behalf of the Society for the Study of Reproduction by Oxford University Press)

the openings of the uterine glands begins to reach over the mouth of the gland(s) to develop a cavity that separates the uterine LE from the chorioallantois. These structures are termed the areolae. They are initially observed as small white circular discs with a prominent peripheral thickening of 1 mm in diameter (Friess et al. 1981), but quickly develop to cover the openings of the uterine gland(s). The cavity that forms receives the secretions of the uterine glands, and the columnar chorionic epithelial cells that line the placental border of this cavity form a seal between the uterine LE and the walls of the placental areola to prevent dissipation of histotroph into inter-areolar regions of the placenta. The allantoic vasculature that receives the histotroph is clearly discernable from the vasculature that supplies inter-areolar regions of the placenta (Leiser and Dantzer 1994). The endometrial vasculature that supplies the areola develops more slowly than the endometrial vasculature of inter-areolar regions presumably due a less intimate association with the trophectoderm. This prevents direct physical interaction between the trophectoderm and endometrium and decreases the influence of paracrine products that are secreted by the trophectoderm. As the placenta grows, areolar diameter increases and a stretching of the areolar capillary network leads to a progressively widening size. During the early stages of placentation, the placental surface of the areolae is flat, but as placentation progresses the flat surface becomes more complex with formation of ridges and papilla-like structures lined by a columnar chorionic epithelium (Amoroso 1952). The balloon shape of the areola implies that there is an interior pressure against the chorioallantoic surface of the areola delivered by the continuous accumulation of histotroph from the uterine glands. Indeed, the cavity of an areola is a small reservoir for the histotroph that is potentially secreted by the much larger uterine glands (Leiser and Dantzer 1994). There are some 2500 areolae distributed over the entire chorioallantois, and their number is correlated with fetal weight ($r = 0.65$). There are approximately 6 areolae per square centimeter of chorioallantois at mid-pregnancy, but approximately 2.5 areolae per square centimeter of chorioallantois by the end of gestation (Knight et al. 1977). The microanatomy of areolae facilitate the transport of glandular secretions such as macromolecules and proteins, including those for the transport of iron and vitamins, by fluid-phase pinocytosis across the placenta and into the fetal-placental circulation. Macromolecules and proteins are then transported to the heart via the fetal venous system for distribution to all fetal-placental tissues (Renegar et al. 1982; Ducsay et al. 1984; Roberts et al. 1986; Bazer et al. 1991). All nutrients transferred across the placenta may be cleared via the kidney and into the bladder from which they can enter the allantoic sac via the urachus for metabolism, degradation, or reuptake into the placental circulation for redistribution to affect development and function of fetal-placental tissues (Bazer 1989).

## 7 Conclusion

Figure 7 summarizes some of the major events during implantation and placentation in the pig. A primary function of the placenta is the transplacental exchange of gases, micronutrients (amino acids, glucose), and macromolecules (proteins), as well as the production of hormones, cytokines, and other regulatory molecules that affect growth and development of the conceptus throughout gestation. In all mammals, placentation initiates with implantation that includes specialized cell adhesion and cell migration leading to attachment of conceptus trophectoderm to the uterine LE. Once attached, maternal and fetal blood must be brought into close apposition to allow for transplacental exchange of molecules while maintaining separation of the maternal and fetal circulatory systems. Endometrial and placental tissues are remodeled to achieve areas of reduced interhaemal distance regardless of whether the placenta is epitheliochorial, synepitheliochorial, endotheliochorial, or hemochorial. Extensive remodeling to form chorionic (placental) ridges and corresponding endometrial invaginations results in folding that further increases the area of uterine-placental association. Indentation of the uterine LE and conceptus trophectoderm by underlying capillaries further reduces the distance between maternal and fetal blood, providing a short diffusion distance across the placenta.

Placentation is epitheliochorial in pigs as uterine LE remains intact throughout pregnancy. Porcine conceptus trophectoderm directly attaches to the uterine LE, and

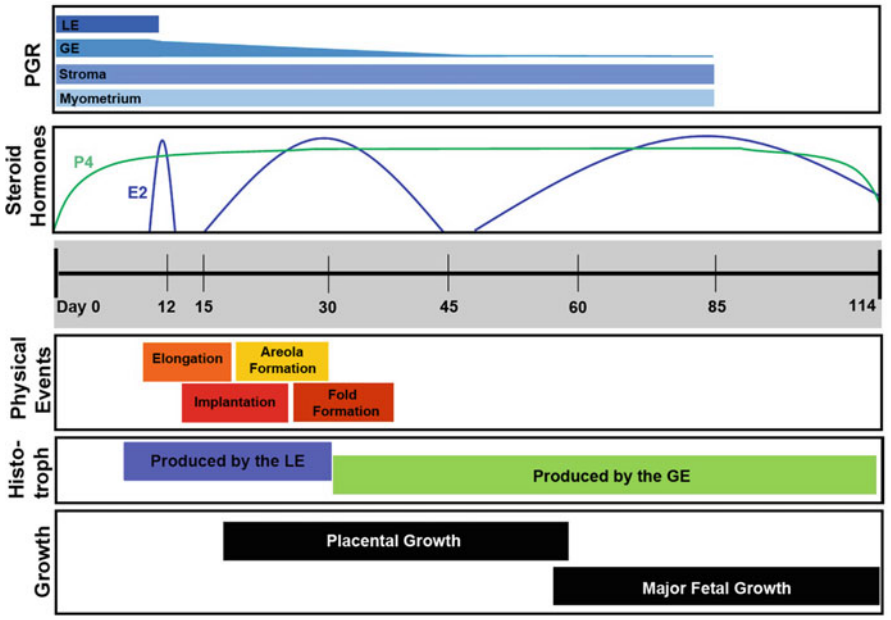


**Fig. 7** Major events during implantation and placentation in pigs. (Figure concept by Dr. Chelsie Steinhauser)

these epithelia serve as the conduit for maternal hematotrophic and histotrophic support for conceptus growth and development. There is progressive interdigitation of microvilli on conceptus trophectoderm and uterine LE. The interface between the maternal (uterine) placenta and fetal placenta of pigs begins to fold by day 25 of pregnancy, and these early folds increase in length through day 35, and again increase in length between days 50 and 60 of gestation to eventually cover the entire placenta, except at the openings of uterine glands. Here the conceptus trophectoderm never fuses with uterine LE, rather it forms a pocket referred to as an areola. Secretions from superficial GE and deep uterine glandular GE, as well as molecules representing selective transudation from maternal serum into uterine GE and LE, are absorbed and transported across the chorioallantoic placenta by fluid-phase pinocytosis for release into the fetal circulation.

## References

Akinlosotu BA, Diehl JR, Gimenez T (1986) Sparing effects of intrauterine treatment with prostaglandin E2 on luteal function in cycling gilts. Prostaglandins 32:291–299

Albelda SM, Buck CA (1990) Integrins and other cell adhesion molecules. FASEB J 4:2868–2880

Amoroso E (1952) Placentation. In: Parkes A (ed) Marshall's physiology of reproduction. Little Brown & Co.Amoroso reference, Boston, MA, pp 127–311

Aplin JD, Seif MW, Graham RA, Hey NA, Behzad F, Campbell S (1994) The endometrial cell surface and implantation. Expression of the polymorphic mucin MUC-1 and adhesion molecules during the endometrial cycle. Ann N Y Acad Sci 30:103–121

Apparao KBC, Illera MJ, Beyler SA, Olson GE, Osteen KG, Corjay MH, Boggess K, Lessey BA (2003) Regulated expression of osteopontin in the peri-implantation rabbit uterus. Biol Reprod 68:1484–1490

Armant DR (2005) Blastocysts don't go it alone. Extrinsic signals fine-tune the intrinsic developmental program of trophoblast cells. Dev Biol 280:260–280

Bailey DW, Dunlap KA, Frank JW, Erikson DW, White BG, Bazer FW, Burghardt RC, Johnson GA (2010a) 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

Bailey DW, Dunlap KA, Erikson DW, Patel AK, Bazer FW, Burghardt RC, Johnson GA (2010b) Effects of long-term progesterone exposure on porcine uterine gene expression: progesterone alone does not induce secreted phosphoprotein 1 (osteopontin) in glandular epithelium. Reproduction 140:595–604

Bazer FW (1975) Uterine protein secretions: relationship to development of the conceptus. J Anim Sci 41:1376–1382

Bazer FW (1989) Allantoic fluid: regulation of volume and composition. In: Brace RA (ed) Fetal and neonatal body fluids. Perinatology Press, Cornell, NY, pp 135–157

Bazer FW, Johnson GA (2014) Pig blastocyst-uterine interactions. Differentiation 87:52–65

Bazer FW, Thatcher WW (1977) Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F2α by the uterine endometrium. Prostaglandins 14:397–400

Bazer FW, Worthington-White D, Fliss MFV, Gross S (1991) Uteroferrin: a progesterone-induced hematopoietic growth factor of uterine origin. J Exp Hematol 19:910–915

Bazer FW, Spencer TE, Johnson GA, Burghardt RC (2011) Uterine receptivity to implantation of blastocysts in mammals. Front Biosci S3:745–767

Bazer FW, Kim J, Song G, Ka H, Wu G, Johnson GA, Vallet JL (2013) Roles of selected nutrients in development of the porcine conceptus during pregnancy. In: Rodriquez-Martinez A, Soede NM, Flowers WL (eds) Control of pig reproduction IX. Context Products Ltd, British Library Cataloguing in Publication Data, Leicestershire, pp 159–174

Bjorkmann N (1973) Fine structure of the fetal-maternal area of exchange in the epitheliochorial and endotheliochorial types of placentation. Acat Anat 61(Suppl):1–22

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

Brayman M, Thathiah A, Carson DD (2004) MUC1: a multifunctional cell surface component of reproductive tissue epithelia. Reprod Biol Endocrinol 2:4–12

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

Burghardt RC, Johnson GA, Jaeger LA, Ka H, Garlow JE, Spencer TE, Bazer FW (2002) Integrins and extracellular matrix proteins at the maternal/fetal interface in domestic animals. Cells Tissues Organs 172:202–217

Burghardt RC, Burghardt JR, Taylor JD II, Reeder AT, Nguyen BT, Spencer TE, Johnson GA (2009) Enhanced focal adhesion assembly reflects increased mechanosensation and mechanotransduction along the maternal/conceptus interface during pregnancy in sheep. Reproduction 137:583–593

Butler WT, Ridall AL, McKee MD (1996) Osteopontin. In: Principles of bone biology. Academic Press, Inc, New York, pp 167–181

Cencič A, LaBonnardière C (2002) Trophoblastic interferon-gamma: current knowledge and possible role(s) in early pig pregnancy. Vet Res 33:139–157

Christenson LK, Farley DB, Anderson LH, Ford SP (1994) Luteal maintenance during early pregnancy in the pig: role for prostaglandin E2. Prostaglandins 47:61–75

Cooke PS, Buchanan DL, Kurita T, Lubahn DB, Cunha GR (1998) Stromal-epithelial cell communication in the female reproductive tract (Chap. 17). In: Bazer FW (ed) The endocrinology of pregnancy. Ahumana Press, Totowa, NJ, pp 491–506

Dantzer V (1985) Electron microscopy of the initial stages of placentation in the pig. Anat Embryol 172:281–293

Dantzer V, Leiser R (1993) Microvascularization of regular and irregular areolae of the areola-gland submunit of the porcine placenta: structural and functional aspects. Anat Embryol (Berl) 188:257–267

Dantzer V, Leiser R (1994) Initial vascularisation in the pig placenta: I. Demonstration of nonglandular areas by histology and corrosion casts. Anat Rec 238:177–190

Decker T, Lew DJ, Cheng YS, Levy DE, Darnell JEJ (1989) Interactions of alpha- and gamma-interferon in the transcriptional regulation of the gene encoding a guanylate-binding protein. EMBO J 8:2009–2014

Dempsey FW, Wislocki B, Amoroso EC (1955) Electron microscopy of the pig's placenta, with especial reference to the cell membranes of the endometrium and chorion. Am J Anat 91:65–101

Denhardt DT, Guo X (1993) Osteopontin: a protein with diverse functions. FASEB J 7:1475–1482

Ducsay CA, Buhi WC, Bazer FW, Roberts RM, Combs GE (1984) Role of uteroferrin in placental iron transport: effect of maternal iron treatment on fetal iron and uteroferrin content and neonatal hemoglobin. J Anim Sci 62:1303–1308

Erikson DW, Burghardt RC, Bayless KJ, Johnson GA (2009) Secreted phosphoprotein 1 (SPP1, osteopontin) binds to integrin alphavbeta6 on porcine trophectoderm cells and integrin alphavbeta3 on uterine luminal epithelial cells, and promotes trophectoderm cell adhesion and migration. Biol Reprod 81:814–825

Fazleabas AT, Kim JJ, Strakova Z (2004) Implantation: embryonic signals and the modulation of the uterine environment—a review. Placenta 25(Suppl A):S26–S31

Feinberg RF, Kliman HJ, Wang CL (1994) Transforming growth factor-beta stimulates trophoblast oncofetal fibronectin synthesis in vitro: implications for trophoblast implantation in vivo. J Clin Endocrinol Metab 78:1241–1248

Flood PF (1974) Steroid-metabolizing enzymes in the early pig conceptus and in the related endometrium. J Endocrinol 63:413–414

Ford SP, Christenson LK (1991) Direct effects of oestradiol-17 beta and prostaglandin E-2 in protecting pig corpora lutea from a luteolytic dose of prostaglandin F-2 alpha. J Reprod Fertil 93:203–209

Ford SP, Christenson RK, Ford JJ (1982) Uterine blood flow and uterine arterial, venous and luminal concentrations of oestrogens on days 11, 13, and 15 after oestrus in pregnant and non-pregnant sows. J Repord Fertil 64:185–190

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

Frank JW, Seo H, Burghardt RC, Bayless KJ, Johnson GA (2017) ITGAV (alpha V integrins) bind SPP1 (osteopontin) to support trophoblast cell adhesion. Reproduction 153:695–706

Friess AE, Sinowatz F, Skolek-Winnisch R, Trautner W (1980) The placenta of the pig. I. Finestructural changes of the placental barrier during pregnancy. Anat Embryol 158:179–191

Friess AE, Sinowatz F, Skolek-Winnisch R, Trautner W (1981) The placenta of the pig. II. Finestructural changes of the placental barrier during pregnancy. Anat Embryol 163:43–53

Gallant ND, Michael KE, García AJ (2005) Cell adhesion strengthening: contributions of adhesive area, integrin binding, and focal adhesion assembly. Mol Biol Cell 16:4329–4340

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

Geisert RD, Renegar RH, Thatcher WW, Roberts RM, Bazer FW (1982a) Establishment of pregnancy in the pig: I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biol Reprod 27:925–939

Geisert RD, Brookbank JW, Roberts RM, Bazer FW (1982b) Establishment of pregnancy in the pig. II. Cellular remodelling of the porcine blastocysts during elongation of day 12 of pregnancy. Biol Reprod 27:941–955

Geisert RD, Thatcher WW, Roberts RM, Bazer FW (1982c) Establishment of pregnancy in the pig: III. Endometrial secretory response to estradiol valerate administered on day 11 of the estrous cycle. Biol Reprod 27:957–965

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

Geisert RD, Yelich JV, Pratt T, Pomp D (1998) Expression of an inter-α-trypsin inhibitor heavy chain-like protein in the pig endometrium during the oestrous cycle and early pregnancy. J Reprod Fert 114:35–43

Geisert RD, Ross JW, Ashworth MD, White FJ, Johnson GA, Hu J, DeSilva U (2005) Maternal recognition of pregnancy signal or endocrine disruptor: the two faces of oestrogen during establishment of pregnancy in the pig. In: Kraeling R, Ashworth C (eds) Control of pig reproduction VII. Nottingham University Press, Nottingham, pp 131–145

Giancotti FG, Ruoslahti E (1999) Integrin signaling. Science 285:1028–1032

Gregoraszczuk EL, Michas N (1999) Progesterone and estradiol secretion by porcine luteal cells is influenced by individual and combined treatment with prostaglandins E2 and F2alpha throughout the estrus cycle. Prostaglandins Other Lipid Mediat 57:231–241

Guillomot M (1995) Cellular interactions during implantation in domestic ruminants. J Reprod Fertil Suppl 49:39–51

Guthrie HD, Lewis GS (1986) Production of prostaglandin F2α and estrogen by embryonal membranes and endometrium and metabolism of prostaglandin F2α by embryonal membranes, endometrium and lung from gilts. Domest Anim Endocrinol 3:185–198

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

Hettinger AM, Allen MR, Zhang BR, Goad DW, Malayer JR, Geisert RD (2001) Presence of the acute phase protein, bikunin, in the endometrium of gilts during estrous cycle and early pregnancy. Biol Reprod 65:507–513

Hilton WA (1902) The morphology and development of intestinal folds and villi in vertebrates. Am J Anat 1:459–504

Humphries JD, Byron A, Humphries MJ (2006) Integrin ligands at a glance. J Cell Sci 119:3901–3903

Hynes RO (1987) Integrins: a family of cell surface receptors. Cell 48:549–554

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

Jenkins RG, Su X, Su G, Scotton CJ, Camerer E, Laurent GJ, Davis GE, Chambers RC, Matthay MA, Sheppard D (2006) Ligation of protease-activated receptor 1 enhances alph(v)beta6 integrin-dependent TGF-beta activation and promotes acute lung injury. J Clin Invest 116:1601–1614

Johansson S, Svineng G, Wennerberg K, Armulik A, Lohikangas L (1997) Fibronectin-integrin interactions. Front Biosci 2:d126–d146

Johnson GA, Spencer TE, Burghardt RC, Bazer FW (1999a) Ovine osteopontin. I. Cloning and expression of mRNA in the uterus during the peri-implantation period. Biol Reprod 61:884–891

Johnson GA, Burghardt RC, Spencer TE, Newton GR, Ott TL, Bazer FW (1999b) Ovine osteopontin II. Osteopontin and alpha(v)beta(3) integrin expression in the uterus and conceptus during the peri-implantation period. Biol Reprod 61:892–899

Johnson GA, Burghardt RC, Bazer FW, Spencer TE (2003) Minireview: osteopontin: roles in implantation and placentation. Biol Reprod 69:1458–1471

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

Johnson GA, Burghardt RC, Bazer FW (2014) Osteopontin: a leading candidate adhesion molecule for implantation in pigs and sheep. J Anim Sci Biotechnol 5:56–70

Joyce MM, White FJ, Burghardt RC, Muñiz JJ, Spencer TE, Bazer FW, Johnson GA (2005a) Interferon stimulated gene 15 conjugates to endometrial cytosolic proteins and is expressed at the uterine-placental interface throughout pregnancy in sheep. Endocrinology 146:675–684

Joyce MM, Gonzalez JF, Lewis S, Woldesenbet S, Burghardt RC, Newton GR, Johnson GA (2005b) Caprine uterine and placental osteopontin expression is distinct among epitheliochorial implanting species. Placenta 26:160–170

Joyce MM, Burghardt RC, Geisert RD, Burghardt JR, Hooper RN, Ross JW, Ashworth MD, Johnson GA (2007a) 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, Jaeger LA, Spencer TE, Bazer FW, Johnson GA (2007b) Pig conceptuses increase uterine interferon regulatory factor-1 (IRF-1), but restrict expression to stroma through estrogen-induced IRF-2 in luminal epithelium. Biol Reprod 77:292–302

Joyce MM, Burghardt JR, Burghardt RC, Hooper RN, Bazer FW, Johnson GA (2008) Uterine major histocompatibility class I molecules and beta 2 microglobulin are regulated by progesterone and conceptus interferons during pig pregnancy. J Immunol 181:2494–2505

Kaczynski P, Kowalewski MP, Waclawik A (2016) Prostaglandin F2alpha promotes angiogenesis and embryo-maternal interactions during implantation. Reproduction 151:539–552

Keys JL, King GL (1980) Microscopic examination of porcine conceptus-maternal interface between days 10 and 19 of pregnancy. Am J Anat 188:221–238

Kimber SJ, Spanswick C (2000) Blastocyst implantation: the adhesion cascade. Semin Cell Dev Biol 11:77–92

Kimber SJ, Illingworth IM, Glasser SR (1995) Expression of carbohydrate antigens in the rat uterus during early pregnancy and after ovariectomy and steroid replacement. J Reprod Fertil 103:75–87

Kimmins S, Lim HC, MacLaren LA (2004) Immuohistochemical localization of integrin alpha v and beta 3 and osteopontin suggests that they do not interact during embryo implantation in ruminants. Reprod Biol Endocrinol 2:19–32

Kling D, Fingerle J, Harlan JM (1992) Inhibition of leukocyte extravasation with a monoclonal antibody to CD18 during formation of experimental intimal thickening in rabbit carotid arteries. Arterioscler Thromb 12:997–1007

Knight JW, Bazer FW, Wallace HD (1974) Effect of progesterone induced increase in uterine secretory activity on development of the porcine conceptus. J Anim Sci 39:743–746

Knight JW, Bazer FW, Thatcher WW, Franke DE, Wallace HD (1977) Conceptus development in intact and unilaterally hysterectomized ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci 44:620–637

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

Larsen M, Artym VV, Green JA, Yamada KM (2006) The matrix reorganized: extracellular matrix remodeling and integrin signaling. Curr Opin Cell Biol 18:463–471

Lawrence DA (1996) Transforming growth factor-β: a general review. Eur Cytokine Netw 7:363–374

Lefèvre F, Martinat-Botté F, Locatelli A, De Niu P, Terqui M, La Bonnardière C (1998a) Intrauterine infusion of high doses of pig trophoblast interferons has no antiluteolytic effect in cyclic gilts. Biol Reprod 58:1026–1031

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

Leiser R, Dantzer V (1994) Initial vascularization in the pig placenta: II. Demonstration of gland and areola-gland subunits by histology and corrosion casts. Anat Rec 238:326–334

Lessey BA (2002) Adhesion molecules and implantation. J Reprod Immunol 55:101–112

Massuto DA, Kneese EC, Johnson GA, Hooper NH, Burghardt RC, Ing NH, Jaeger LA (2009a) Transforming growth factor beta (TGFB) signaling is activated during porcine implantation: proposed role for latency associated peptide-integrins at the conceptus-maternal interface. Reproduction 139:465–478

Massuto DA, Hooper RN, Kneese EC, Johnson GA, Ing NH, Weeks BR, Jaeger LA (2009b) Intrauterine infusion of latency associated peptide (LAP) during early porcine pregnancy affects conceptus elongation and placental size. Biol Reprod 82:534–542

Mathew DJ, Newsom EM, Guyton JM, Tuggle CK, Geisert RD, Lucy MC (2015) Activation of the transcription factor nuclear factor-kappa B in uterine luminal epithelial cells by interleukin 1 beat 2L a novel interleukin 1 expressed by the elongating pig conceptus. Biol Reprod 107:1–13

Meyer AE, Pfeiffer CA, Brooks KE, Spate LD, Benne JA, Cecil R, Samuel MS, Murphy CN, Behura S, McLean MK, Ciernia LA, Smith MF, Whitworth KM, Wells KD, Spencer TE, Prather RS, Geisert RD (2019) New perspective on conceptus estrogens in maternal recognition and pregnancy establishment in the pig. Biol Reprod 101:148–161

Mirando MA, Harney JP, Beers S, Pontzer CH, Torres BA, Johnson HM, Bazer FW (1990) Onset of secretion of proteins with antiviral activity by pig conceptuses. J Repord Fertil 88:197–203

Mirkin S, Arslan M, Churikov D, Corica A, Diaz JI, Williams S, Bocca S, Oehninger S (2005) In search of candidate genes critically expressed in the human endometrium during the window of implantation. Hum Reprod 20:2104–2117

Mossman HW (1937) Comparative morphogenesis of the foetal membranes and accessory uterine structures. Contrib Embryol 26:129–246

Mulac-Jericevic B, Conneely OM (2004) Reproductive tissue selective actions of progesterone receptors. Reproduction 128:139–146

Pfeiffer CA, Meyer AE, Brooks KE, Chen PR, Milano-Foster J, Spate LD, Benne JA, Cecil RF, Samuel MS, Ciernia LA, Spinka CM, Smith MF, Wells KD, Spencer TE, Prather RS, Geisert RD (2020) Ablation of conceptus PTGS2 expression does not alter early conceptus development and establishment of pregnancy in the pig. Biol Reprod 102(2):475–488

Pontzer CH, Torres BA, Vallet JL, Bazer FW, Johnson HM (1988) Antiviral activity of the pregnancy recognition hormone ovine trophoblast protein-1. Biochem Biophys Res Commun 152:801–807

Powell JK, Glasser SR, Woldesenbet S, Burghardt RE, Newton GR (2000) Expression of carbohydrate antigens in the goat uterus during early pregnancy and on steroid-treated polarized uterine epithelial cells *in vitro*. Biol Reprod 62:277–284

Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M, Fisher SJ (2004) Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. J Clin Invest 114:744–754

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

Roberts RM, Bazer FW (1988) The functions of uterine secretions. J Reprod Fertil 82:875–892

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

Ross JW, Ashworth MD, Hurst AG, Malayer JR, Geisert RD (2003) Analysis and characterization of differential gene expression during rapid trophoblastic elongation in the pig using suppression subtractive hybridization. Reprod Biol Endocrinol 1:23

Ross JW, Ashworth MD, White FJ, Johnson GA, Aoubi PJ, DeSilva UE, Whitworth KM, Green JA, Prather RS, Geisert RD (2007) Premature estrogen exposure alters endometrial gene expression to disrupt pregnancy in the pig. Endocrinology 148:4761–4773

Ruoslahti E, Pierschbacher MD (1987) New perspectives in cell adhesion: RGD and integrins. Science 238:491–497

Sastry SK, Burridge K (2000) Focal adhesions: a nexus for intracellular signaling and cytoskeletal dynamics. Exp Cell Res 261:25–36

Senger DR, Perruzzi CA, Papadopoulos-Sergiou A, Van de Water L (1994) Adhesive properties of osteopontin: regulation by a naturally occurring thrombin-cleavage in close proximity to the GRGDS cell-binding domain. Mol Biol Cell 5:565–574

Seo H, Li X, Wu G, Bazer FW, Burghardt RC, Bayless KJ, Johnson GA (2020) Mechanotransduction drives morphogenesis to develop folding at the uterine-placental interface of pigs. Placenta 90:62–70

Simons BD (2013) Development. Getting your gut into shape. Science 342:203–204

Sodek J, Ganss B, McKee MD (2000) Osteopontin. Crit Rev Oral Biol Med 11:279–303

Song G, Bailey DW, Dunlap KA, Burghardt RC, Spencer TE, Bazer FW, Johnson GA (2010) Cathepsin B, cathepsin L and cystatin C in the porcine uterus and placenta: potential roles in endometrial/placental remodeling and in fluid-phase transport of proteins secreted by uterine epithelia across placental areolae and neonatal gut. Biol Reprod 82:854–864

Spencer TE, Bartol FF, Bazer FW, Johnson GA, Joyce MM (1999) Identification and characterization of glycosylation dependent cell adhesion molecule 1 (GlyCAM-1) expression in the ovine uterus. Biol Reprod 60:241–250

Spencer TE, Johnson GA, Bazer FW, Burghardt RC (2004) Implantation mechanisms: insights from the sheep. Reproduction 128:656–668

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

Spencer TE, Johnson GA, Bazer FW, Burghardt RC (2007b) Fetal-maternal interactions during the establishment of pregnancy in ruminants. Reproduction 64:379–396

Steinhauser CB, Bazer FB, Burghardt RC, Johnson GA (2017) Expression of progesterone receptor in the porcine uterus and placenta throughout gestation: correlation with expression of uteroferrin and osteopontin. Domestic Anim Endocrinol 58:1–11

Sukjumlong S, Dalin A-M, Sahlin L, Persson E (2005) Immunohistochemical studies on the progesterone receptor (PR) in the sow uterus during the oestrous cycle and in inseminated sows at oestrus and early pregnancy. Reproduction 129:349–359

Tuo WB, Bazer FW (1996) Expression of oncofetal fibronectin in porcine conceptuses and uterus throughout gestation. Reprod Fertil Dev 8:1207–1213

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

Vallet JL, Freking BA (2007) Differences in placental structure during gestation associated with large and small pig fetuses. J Anim Sci 85:3267–3275

Vallet JL, Miles JR, Freking BA (2009) Development of the pig placenta. Soc Reprod Fertil Suppl 66:265–279

Veldius JP, Klase P, Hammond JM (1980) Characteristic of changes in volume, osmolarity and electrolyte composition of porcine fetal fluids during gestation. Biol Reprod 22:1168–1180

Vonnahme KA, Malayer JR, Spivey HO, Ford SP, Clutter A, Geisert RD (1999) Detection of kallikrein gene expression and enzymatic activity in porcine endometrium during the estrous cycle and early pregnancy. Biol Reprod 61:1235–1241

Waclawik A, Jabbour HN, Blitek A, Ziecik AJ (2009a) Estradiol-17beta, prostaglandin E2 (PGE2), and the PGE2 receptor are involved in PGE2 positive feedback loop in the porcine endometrium. Endocrinology 150:3823–3832

Waclawik A, Blitek A, Kaczmarek MM, Kiewisz J, Ziecik AJ (2009b) Antiluteolytic mechanisms and the establishment of pregnancy in the pig. Soc Reprod Fertil Suppl 66:307–320

Waclawik A, Kaczemarek MM, Blitek A, Kaczynski P, Ziecik AJ (2017) Embryo-maternal dialogue during pregnancy establishment and implantation in the pig. Mol Reprod Dev 84:842–855

Wetendorf M, DeMayo FJ (2014) Progesterone receptor signaling in the initiation of pregnancy and preservation of a healthy uterus. Int J Dev Biol 58:95–106

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

White FJ, Burghardt RC, Hu J, Joyce MM, Spencer TE, Johnson GA (2006) Secreted phosphoprotein 1 (osteopontin) is expressed by stromal macrophages in cyclic and pregnant endometrium of mice, but is induced by estrogen in luminal epithelium during conceptus attachment for implantation. Reproduction 132:919–929

Whyte JJ, Meyer AE, Spate LCD, Benne JA, Cecil R, Samuel MS, Murphy CN, Prather RS, Geisert RD (2018) Inactivation of porcine interleukin-1β results in failure of rapid conceptus elongation. Proc Natl Acad Sci U S A 115:307–312

Wildeman DE, Chen C, Erez O, Grossman LI, Goodman M, Romero R (2006) Evolution of the mammalian placenta revealed by phylogenetic analysis. PNAS 103:3203–3208

Wozniak MA, Modzelewska K, Kwong L, Keely PJ (2004) Focal adhesion regulation of cell behavior. Biochim Biophys Acta 1692:103–119

Ziecik AJ, Waclawik BM (2008) Conceptus signals for establishment and maintenance of pregnancy in pigs – lipid singnaling system. Exper Clin Endocrinol Diabetes 116:443–449