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Cell signaling in trophoblast-uterine communication

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ABSTRACT Intricate and precise communication between the blastocyst and the uterus orchestrates embryo implantation. However, many questions remain unanswered regarding the molecular complexities of implantation. On-time implantation requires a receptive uterus and a mature blastocyst with trophoblast cells capable of adhering to and invading the endometrium. Defects in uterine receptivity or embryo/uterine signaling can cause implantation failure or early pregnancy loss, whereas deficient trophoblast differentiation can generate placental abnormalities that produce adverse pregnancy outcomes. This review will discuss several examples of signaling pathways that regulate trophoblast and uterine development during this period. Leukemia inhibitory factor is involved in uterine priming for implantation. The epidermal growth factor signaling system contributes to trophoblast-uterine communication, as well as trophoblast adhesion and invasion. Indian hedgehog signaling synchronizes tissue compartments within the uterus, and WNT signaling mediates numerous interactions within the implantation site and developing placenta. The autocrine, paracrine and juxtacrine interactions mediated by these signaling pathways contribute significantly to the establishment of pregnancy, although there are many other known and yet to be discovered factors that synchronize the maternal and embryonic developmental programs.

KEY WORDS: *growth factor, trophoblast, endometrium, blastocyst, signaling*

Introduction

Successful implantation and placentation in humans and other eutherian mammals depend upon complex interactions between the embryo and a receptive uterus. Steroid hormones, particularly estrogen and progesterone, are well established as having a critical role. However, an abundance of evidence shows that various cytokines and growth factors are crucial for implantation to succeed; in part, because they mediate the actions of the steroid hormones (Zhang *et al*., 2013a).

In this brief review, several key signaling systems will be discussed that coordinate the distinct developmental programs of the endometrium and embryonic trophoblast during the constitution of a viable conceptus. The mouse, which is amenable to genetic and experimental approaches, has been a useful model for understanding the molecular dialogue between embryo and maternal tissue. However, there are important physiological differences between murine and human reproductive biology that are beginning to surface through the increasing impact of translational studies with human tissues and cell lines. Where possible, signaling pathways critical to human reproduction will be highlighted.

Leukemia inhibitory factor

Leukemia inhibitory factor (LIF), a pleiotropic cytokine, is essential in the early stages of pregnancy. LIF is a member of the interleukin-6 family that is highly glycosylated and exerts its physiological effects by interacting with the LIF receptor (LIFR) and gp130 to activate the Jak/STAT downstream pathway (Heinrich *et al*., 1998). The first evidence linking LIF to implantation was obtained in experiments with transgenic mice. Blastocyst implantation does not occur in LIF gene deficient mice; however, their embryos are able to implant in non-LIF deficient mice (Stewart *et al*., 1992). Furthermore, delivering exogenous LIF to LIF deficient dams enables implantation (Stewart *et al*., 1992). It has also been shown that inhibition of the LIF-dependent STAT 3 pathway in mice significantly reduces embryo implantation (Catalano *et al*., 2005).

In humans, evidence suggests that LIF plays a crucial role in

Abbreviations used in this paper: HBEGF, heparin binding EGF-like growth factor; LIF, leukemia inhibitory factor.

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implantation and is secreted maximally during the luteal phase. LIF mRNA is detected in the human endometrium, increasing during the secretory phase (Arici *et al.*, 1995). More specifically, LIF and LIFR are maximally expressed during the "window of implantation" and correspond with the presence of pinopodes in endometrial biopsies (Aghajanova, 2004). Immunohistochemical analysis of LIF in 89 biopsies of fertile women reveals its highest expression during the mid-luteal phase in the luminal epithelium, significantly greater than in glands or stroma (Leach *et al.*, 2012). Thus, LIF is positioned for signaling directly to the peri-implantation blastocyst. LIF secretion by the glandular endometrium depends on progesterone, as treatment with mifepristone, a progesterone receptor antagonist, reduces LIF secretion from glandular endometrium (Danielsson *et al.*, 1997). Endometrial LIF transcription and biosynthesis is complex and is regulated by various growth factors and cytokines, including interleukin-1, tumor necrosis factor (TNF), platelet-derived growth factor and epidermal growth factor (EGF), while it is inhibited by interferon gamma (Lass *et al.*, 2001).

LIF plays a critical role in uterine communication to the embryo, as it is secreted from the endometrium and human embryos contain LIFR (Charnock-Jones *et al.*, 1994). Earlier in embryonic development, LIF is secreted from the ampullary portion of the fallopian tube (Keltz *et al.*, 1996). Furthermore, LIF concentration in follicular fluid positively correlates with embryo quality (Arici *et al.*, 1997). Interestingly, supplementation of serum-free culture medium with LIF increases human blastocyst formation, as compared to non-supplemented controls (Sargent *et al.*, 1998). These findings suggest that LIF could regulate embryonic growth and development in preparation for implantation.

Any aberration in LIF secretion is likely to have an adverse effect on fertility. In fertile patients, LIF increases two-fold between proliferative and secretory phases, whereas it fails to increase in infertile women (Hambartsoumian, 1998). Even during the proliferative phase, overall endometrial LIF levels decrease in infertile women as compared to fertile women (Wu *et al.*, 2013). It has also been suggested that heterozygous mutations of the LIF gene could lead to decreased availability or decreased biological activity of LIF in the uterus and contribute to infertility in women (Giess *et al.*, 1999). Significantly better *in vitro* fertilization (IVF) outcomes are associated with elevated LIF expression in the endometrium of infertility patients (Serafini *et al.*, 2009).

The presence of LIF and LIFR in decidua and placenta implicates a role not only in implantation, but also in placentation (Kojima *et al.*, 1994, Sharkey *et al.*, 1999). Moreover, LIFR deficient mouse embryos display significant placental defects and growth abnormalities in other organs (Ware *et al.*, 1995).

LIF is required for expression of the EGF family of growth factors during implantation, illustrated by uteri of LIF knockout mice that are deficient in the subepithelial expression of EGF-like growth factors at implantation sites (Song *et al.*, 2000). Conversely, the upregulation of LIF expression by human uterine epithelial cells cultured with heparin binding EGF-like growth factor (HBEGF) suggests that LIF expression is dependent upon the EGF signaling system (Lessey *et al.*, 2002). Studies of human preimplantation embryos cultured in growth factors indicate that LIFR expression is dependent on HBEGF signaling, and that the HBEGF receptor ERBB4 is upregulated by LIF (Kimber *et al.*, 2008). These data highlight the complexity of cross talk amongst growth factors during implantation and placentation.

Epidermal growth factor (EGF) signaling system

The EGF family of growth factors and their receptors regulate a variety of biological processes that include proliferation, differentiation and survival. Growth factors in the EGF superfamily include EGF, HBEGF, transforming growth factor-alpha (TGFA), amphiregulin (AREG), betacellulin (BTC), epiregulin (EREG), epigen and the neuregulins (NRG) (Holbro and Hynes, 2004, Riese and Stern, 1998). Recent studies have examined the roles of each EGF family member in implantation and placentation.

The EGF-like growth factors activate receptor tyrosine kinases in the plasma membrane that include epidermal growth factor receptor (EGFR/ERBB1) and related ERBB family proteins (ERBB2, ERBB3, ERBB4). Growth factor binding initiates an intrinsic protein-tyrosine kinase activity, autophosphorylation of the cytoplasmic domain and receptor dimerization to generate a downstream signaling cascade (Holbro and Hynes, 2004). The EGF-like growth factors are synthesized as membrane bound proteins that signal to their receptors on adjacent cells, also called juxtacrine signaling, or they are secreted through proteolytic cleavage of their extracellular domain, carried out by metalloproteinases, for autocrine or paracrine signaling (Riese and Stern, 1998).

Mouse implantation

The importance of the EGF signaling system for blastocyst implantation is suggested by the EGFR knockout (Miettinen *et al.*, 1995, Sibilia and Wagner, 1995, Threadgill *et al.*, 1995), which, depending on the genetic background of host mice, has a perinatal-lethal phenotype. However, the picture is less clear from studies of embryos with deletion of EGF family growth factors where several knockouts produce mice that are viable and fertile, while others have roles in early post-implantation embryonic development that make interpretation difficult. It remains to be determined whether the EGF signaling system has a specific role in the process of implantation or if it is more involved in embryonic survival and differentiation. Alternative approaches provide evidence to support the hypothesis that this complex signaling system directly influences the success of blastocyst implantation.

Experiments in mice establish that HBEGF is present in the uterus shortly before embryo implantation, providing the earliest known signaling between the blastocyst and maternal cells (Lim and Dey, 2009). *In situ* hybridization demonstrates that HBEGF is expressed in the luminal epithelium specifically at sites surrounding blastocysts 6-7 hours prior to the attachment reaction (Das *et al.*, 1994). HBEGF is also expressed by blastocysts prior to implantation (Liu and Armant, 2004), suggesting its role in a two-way dialogue. Experimentally induced delayed implantation in ovariectomized mice reveals no HBEGF expression at implantation sites; however, HBEGF becomes upregulated in both the uterus (Das *et al.*, 1994) and blastocyst (Hamatani *et al.*, 2004) with administration of estrogen to activate the blastocyst to implant. Its receptors ERBB1 and ERBB4 are concomitantly regulated by estrogen along with HBEGF in the blastocyst (Paria *et al.*, 1993, Paria *et al.*, 1999). HBEGF signaling between the trophoblast and uterine epithelial cells is most likely initiated by embryonic HBEGF shedding, as depicted in Fig. 1A.

Crosstalk with other signaling pathways mobilizes HBEGF from intracellular stores in the trophectoderm and demonstrates the complexity of the maternal-embryo dialogue. Lysophosphatidic

acid (LPA) produced in the maternal reproductive tract accelerates blastocoel formation at the morula stage and trophoblast differentiation at the early blastocyst stage through downstream intracellular Ca^{2+} signaling (Liu and Armant, 2004, Stachecki and Armant, 1996). LPA activates cognate G protein-coupled receptors that signal through phospholipase C to generate inositol-1,4,5-trisphosphate and diacylglycerol that, in turn, mobilize cytoplasmic free Ca^{2+} and activate protein kinase C (PKC), respectively (Contos *et al.*, 2000). Signaling by both cytoplasmic Ca^{2+} and PKC is known to stimulate HBEGF shedding (Dethlefsen *et al.*, 1998, Dong and Wiley, 2000) and the subsequent transactivation of ERBB signaling (Umata *et al.*, 2001). Immunofluorescence microscopy demonstrates that HBEGF is sequestered from the plasma membrane during blastocyst development, but accumulates transiently at the embryo surface in response to LPA-induced intracellular Ca^{2+} release (Liu and Armant, 2004). Therefore, uterine LPA secretion could activate HBEGF shedding from blastocysts in apposition to the uterine wall, signaling its presence to the adjacent uterine epithelium (Fig. 1A).

Several studies support the hypothesis that expression of HBEGF in the peri-implantation endometrial epithelium is induced by embryo derived HBEGF signaling. HBEGF is expressed in both mouse and hamster preimplantation blastocysts (Hamatani *et al.*, 2004, Liu and Armant, 2004, Wang *et al.*, 2002). Interestingly, when growth factor-soaked beads the approximate size of blastocysts are transferred into the uteri of pseudopregnant mice only HBEGF and insulin-like growth factor-1 (IGF1) provoke discrete implantation-like responses (Paria *et al.*, 2001). Furthermore, highly localized expression of HBEGF occurs in the uterine epithelium adjacent to apposition stage blastocysts (Das *et al.*, 1994). These studies suggest a positive feed-forward regulation of uterine HBEGF expression initiated by the blastocyst during the apposition stage of implantation. While HBEGF secreted by the embryo is sufficient to induce HBEGF expression in the luminal epithelium, other factors secreted by embryos (e.g., IGF1) could have equivalent activity and perhaps compensate in HBEGF deficient embryos, which are able to implant normally when transferred to wild type pseudopregnant dams (Xie *et al.*, 2007).

HBEGF protein appears on the surface of the uterine epithelium early on gestation day 5 in mice as the attachment reaction commences (Das *et al.*, 1994). The primary target of HBEGF in the luminal epithelium is the trophoblast (Fig. 1B). Late on day 4, HBEGF signaling in the blastocyst becomes robust as a result of ERBB4 trafficking to the trophoblast surface (Wang *et al.*, 2000). ERBBs 1-3 are already localized at the apical surface as the blastocyst forms. However, ERBB4 is the preferred receptor for HBEGF in blastocysts (Paria *et al.*, 1999). Thus, ERBB1 and ERBB4 both become available on the trophoblast at approximately the time when uterine HBEGF first appears. These events position HBEGF and its receptors to mediate blastocyst adhesion and trophoblast differentiation.

HBEGF-induced ERBB autophosphorylation advances trophoblast adhesive differentiation (Das *et al.*, 1994, Paria *et al.*, 1999, Wang *et al.*, 2000). In blastocysts cultured serum-free, HBEGF induces trafficking of integrin subunits, including ITGA5 (Fig. 1B), into the plasma membrane of trophoblast cells approximately 24 hours earlier than in control embryos, which promotes their adhesion to fibronectin in the maternal extracellular matrix (Wang *et al.*, 2000). *In vitro* experiments suggest that after apoptosis of the apposing luminal epithelial cells, ligation of the integrin ITGA5/ITGB1 by the exposed basement membrane activates phospholipase Cγ (Wang *et al.*, 2007), leading to trafficking of ITGA2B into the plasma membrane (Rout *et al.*, 2004), which strengthens cell adhesion as trophoblast invasion commences (Fig. 1C).

Experiments have been reported suggesting that HBEGF supports the attachment reaction during implantation in addition to its ability to advance hatching and trophoblast differentiation. Using engineered cells that express membrane bound HBEGF to simulate receptive uterine epithelial cells, it has been demonstrated that cell surface HBEGF binds to day 4 mouse blastocysts, but not to delayed blastocysts (Raab *et al.*, 1996). HBEGF signaling activates Ca^{2+} influx across the plasma membrane through N-type voltage-gated Ca^{2+} channels (Fig. 1B) that stimulates PKC

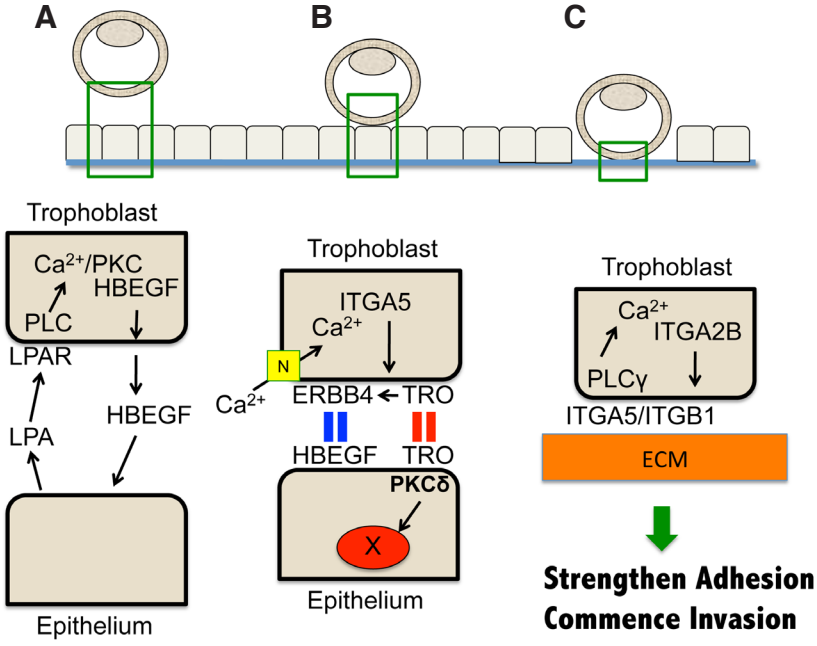

Fig. 1. Heparin binding EGF-like growth factor (HBEGF)-signaling and cell differentiation during implantation in mice. *Blastocyst-uterine interactions shown at the top of the diagram illustrate the* **(A)** *apposition,* **(B)** *attachment and* **(C)** *invasion stages of implantation. The regions within the green boxes are expanded below to summarize signaling pathways that orchestrate trophoblast and uterine epithelial cell differentiation at each stage, as discussed in the text. Arrows depict growth factor secretion or shedding, binding to receptors, intracellular trafficking, ion influx, and de-repression in the case of TRO and ERBB4. Double lines indicate juxtacrine signaling and/or cell-cell adhesion. ECM, extracellular matrix; HBEGF, heparin-binding EGF-like growth factor; ITGA, integrin alpha subunit; ITGB, integrin beta subunit; LPA, lysophosphatidic acid; LPAR, LPA receptor; N, N-type voltage-gated Ca^{2+} channel; PKC, protein kinase C; PLC, phospholipase C; TRO, trophinin; X, indicates apoptosis induced by nuclear localization of PKCδ.*

and calmodulin, consequently accelerating trophoblast invasion (Wang *et al.*, 2000). It appears that there are complex autocrine, paracrine and juxtacrine pathways that integrate HBEGF signaling with input from other growth factors, as well as through cell-cell interactions between the trophectoderm and uterine epithelium during implantation (Fig. 1).

Maternal HBEGF deficiency targeted to the uterus defers the window of implantation and compromises pregnancy outcome, thus, implicating the importance of two-way communication between the embryo and the uterus (Xie *et al.*, 2007). However, another heparin-binding member of the EGF family, AREG, partially compensates for HBEGF loss. Progesterone upregulates AREG throughout the uterine luminal epithelium of the mouse early on gestation day 4 independently of the blastocyst (Das *et al.*, 1995), but it becomes restricted to the implantation site by day 5. Studies conducted to clarify the mechanism of AREG signaling at the embryonic-maternal interface suggest that the presence of fertilized embryos in the reproductive tract elevates expression of AREG on day 4 (Lee *et al.*, 2006a). Therefore, the presence of embryos in an HBEGF-deficient uterus could induce partially-compensating production of AREG, which can activate only ERBB1 (Riese and Stern, 1998).

Another EGF family member, TGFA, has extensive sequence homology to EGF and comparable affinity for ERBB1, its only receptor. Immunohistochemical, *in situ* hybridization, northern blot and RT-PCR analyses clearly demonstrate TGFA expression in the uterus and embryo (Rappolee *et al.*, 1988, Tamada *et al.*, 1991). Additionally, a reduction in the number of implantation sites in rats after administration of intraluminal anti-TGFA injections supports the involvement of TGFA in implantation (Tamada *et al.*, 1997). Addition of TGFA to *in vitro* culture medium is favorable for preimplantation embryo development and trophoblast differentiation (Haimovici and Anderson, 1993, Machida *et al.*, 1995). However, this involvement is controversial, as TGFA knock out mice have normal implantation (Luetteke *et al.*, 1993, Mann *et al.*, 1993). Similarly, AREG and EGF are not essential for implantation, indicated by mice deficient in AREG and triple knockout mice lacking TGFA, AREG and EGF, which are all fertile (Luetteke *et al.*, 1999). These studies support the concept of a compensatory response by other EGF family members, perhaps HBEGF, during implantation.

Another group of EGF-like growth factors that mediate cell-cell interactions, the NRGs, are encoded by four distinct genes, NRG-1, NRG-2, NRG-3 and NRG-4 (Burden and Yarden, 1997, Falls, 2003). There is evidence for 15 splice variants of NRG-1 that are grouped into three subtypes (Ben-Baruch and Yarden, 1994, Meyer *et al.*, 1997). Of the three subtypes, Type 1 is comprised of *neu* differentiation factor (NDF), heregulin and acetylcholine receptor-inducing activity (Ben-Baruch and Yarden, 1994). Type 2 consists of the glial growth factor (GGF) and type 3 comprises the sensory and motor neuron derived factor (SMDF). (Brown *et al.*, 2004). NDF and its isoforms are expressed in the preimplantation mouse uterus (Reese *et al.*, 1998). Mice with delayed implantation do not express NDF; however, upon administration of estrogen, the expression of NDF returns. Alternately there is evidence that SMDF has a similar cellular distribution to NDF and its expression becomes prominent at the time of implantation (Brown *et al.*, 2004). However, no significant expression of GGF is observed in the peri-implantation uterus. In the blastocyst, both ERBB3 and ERBB4 are present on trophoblast cells, suggesting the capacity for signaling with NRG family members (Wang *et al.*, 2000). A parallel study observed a similar expression pattern of the EGF family members BTC and EREG (Das *et al.*, 1997), suggesting that optimal expression of NDF, BTC and EREG requires an activated blastocyst. Therefore, it remains possible that these EGF family members contribute to mouse implantation.

Human implantation

In contrast to its regulation in mice that have a short estrus cycle, HBEGF is regulated independently of the presence of an embryo in the stromal and epithelial compartments during the menstrual cycle of the human endometrium (Chobotova *et al.*, 2002a, Leach *et al.*, 1999, Yoo *et al.*, 1997). There is evidence that HBEGF mediates maternal-blastocyst signaling and attachment in humans as its does in rodents owing to the switch in expression of HBEGF mRNA and protein from the subepithelial stroma during early secretory phase to glandular and luminal epithelium in the mid-secretory phase (Leach *et al.*, 1999). ERBB4 is expressed by the trophoblast cells of human peri-implantation blastocysts, suggesting that HBEGF present in the luminal epithelium could stimulate the blastocyst through paracrine or juxtacrine signaling (Chobotova *et al.*, 2002b). HBEGF not only accelerates the development of human embryos to the blastocyst stage, but also aids in hatching from the zona pellucida (Martin *et al.*, 1998).

Evidence indicates that proliferation in human endometrial stromal cells is regulated by both the soluble and transmembrane forms of HBEGF, along with other EGF family members, EGF and BTC, suggesting key functions for the EGF family of growth factors in regeneration and maturation of the human endometrium and to prepare the embryo for implantation (Chobotova *et al.*, 2002a). However, this induction is under the cooperative effect of TNF. Another study shows that the endometrial stromal cells produce both the soluble and transmembrane forms of HBEGF to regulate TNF and TGFB (Chobotova *et al.*, 2005). This provides a survival function that prevents apoptosis of endometrial cells exposed to apoptotic factors, which highlights the importance of HBEGF and its receptors in the human endometrium.

HBEGF appears to participate in a broader dialogue that includes other molecules, exemplified by trophinin-bystin signaling. Both human trophoblast cells and the uterine epithelium express the transmembrane protein trophinin that mediates cell adhesion through homophilic binding (Suzuki *et al.*, 1999). Trophinin forms a complex with its cytoplasmic partner bystin and ERBB4, which inhibits ERBB tyrosine phosphorylation required for invasive trophoblast differentiation (Fig. 1B). However, during the attachment reaction between the blastocyst and luminal epithelium, homophilic trophinin binding releases bystin, thus derepressing ERBB4, which can then become activated by HBEGF to promote trophoblast differentiation (Sugihara *et al.*, 2007, Tamura *et al.*, 2011). Meanwhile, in the luminal epithelium, homophilic trophinin binding disrupts its tethering of protein kinase Cδ to the plasma membrane, freeing the latter to enter the nucleus where it induces apoptosis (Tamura *et al.*, 2011). Thus, trophinin signaling removes the cellular barrier to blastocyst implantation as it licenses activation of trophoblast adhesion competence.

Human placentation

Most components of the EGF signaling system are present throughout pregnancy. Transcripts from placental tissues obtained in all three trimesters demonstrate expression of HBEGF, with the

highest abundance in first trimester decidua (Yoo *et al.*, 1997). Strong staining for HBEGF is observed in villous and extravillous trophoblast cells in both normal placentas and those delivered preterm (Leach *et al.*, 1999, Leach *et al.*, 2002). Other EGF family members and ERBB receptor tyrosine kinases are expressed in placental trophoblast populations (Hofmann *et al.*, 1992, Tanimura *et al.*, 2004), indicating that the EGF signaling system is highly active during placental development.

There is evidence to support the hypothesis that HBEGF, and possibly other EGF family members, promote trophoblast invasion (Jessmon *et al.*, 2009). The capacity for invasion weakens as gestation progresses (Damsky *et al.*, 1994, Librach *et al.*, 1991), indicating the importance of the EGF signaling system in first trimester trophoblast cells (Bass *et al.*, 1994). EGF, TGFA and HBEGF can stimulate first trimester trophoblast cells to become invasive and differentiate to the extravillous phenotype (Bass *et al.*, 1994, Leach *et al.*, 2004). However, the effectiveness of EGF diminishes with trophoblast cells obtained from the second or third trimester (Bass *et al.*, 1994), suggesting that their loss of invasion competence is intrinsic rather than a product of their environment. Invading extravillous trophoblast cells are necessary for adequate remodeling of the uterine spiral arteries that perfuse the developing placenta (Norwitz *et al.*, 2001).

The first 10 weeks of pregnancy are a period of low O_2 within the conceptus (Burton and Jauniaux, 2004, Burton *et al.*, 1999). Intrauterine measurements report low (~2%) O_2 concentrations in placental tissues compared to the surrounding decidua (Jauniaux *et al.*, 2001, Rodesch *et al.*, 1992). This environment favors the proliferation of the trophoblast cells, while repressing invasion (Genbacev *et al.*, 1996, Genbacev *et al.*, 1997). As a result, trophoblast cells accumulate and occlude the maternal blood vessels, which maintains the relatively hypoxic state (Burton and Jauniaux, 2004). The most distal trophoblast cells contact maternal blood, exposing them to higher O_2 concentrations, which promotes the invasive phenotype. As gestation progresses beyond 10 weeks, the spiral arteries are remodeled to an extent that reperfuses and oxygenates the placenta by dislodging the occluding trophoblast cells. The widespread expression of HBEGF in placental trophoblast cells and surrounding decidua (Leach *et al.*, 1999) could facilitate invasion and contribute to remodeling of the spiral arteries. It has been demonstrated that when the HTR-8/SVneo human first trimester cytotrophoblast cell line is cultured at 2% O_2, HBEGF, but not other EGF family members, is upregulated and secreted (Armant *et al.*, 2006). However, trophoblast cells in villous explants from term placentas do not elevate HBEGF in response to low oxygen and their survival is compromised by hypoxia (Imudia *et al.*, 2008). Addition of HBEGF to term villous explants cultured at 2% O_2 inhibits apoptosis, demonstrating its capacity as a survival factor.

The placenta is programmed to survive at a low O_2 tension during the first trimester, while trophoblast invasion progresses slowly at the vascular interface in preparation for the full onset of placental perfusion (Norwitz *et al.*, 2001). However, if reoxygenation does not occur on time, placental insufficiency ensues, which can increase risk for obstetric disorders that include pregnancy loss, intrauterine growth restriction or preeclampsia (Burton and Jauniaux, 2004). These disorders are associated with poor trophoblast invasion and increased apoptosis (Brosens *et al.*, 1972, DiFederico *et al.*, 1999), two functions that are strongly regulated by HBEGF signaling (Jessmon *et al.*, 2009). Placental tissues from preeclamptic pregnancies have reduced expression of HBEGF compared to gestational age-matched normotensive placentas, as well as reduced HBEGF mRNA expression (Leach *et al.*, 2002). These findings suggest the hypothesis that the reduction in expression of HBEGF could contribute to the shallow trophoblast invasion and poor survival observed in preeclampsia. It remains to be established whether the reduced expression of HBEGF precedes the onset of preeclampsia or if it is merely associated with late placental demise. There is also clinical evidence of HBEGF disruption associated with infertility in women (Aghajanova *et al.*, 2008). The decreased HBEGF expression in infertile couples was not confirmed in a larger clinical study, but it was noted that a significant increase between the early and mid secretory phases was absent in the infertile patients (Leach *et al.*, 2012). The clinical significance of HBEGF signaling during human pregnancy will not be fully appreciated without more sophisticated experimental approaches that take into account its interactions with other signaling pathways.

Indian hedgehog

Another secreted factor shown to be necessary for implantation and placentation is Indian Hedgehog (IHH). In humans, blastocyst implantation is progesterone dependent (Lee *et al.*, 2006b). IHH is a key element in the progesterone priming of the uterus for implantation (Zhang *et al.*, 2013a). The highly conserved hedgehog family of genes was discovered in 1980 in a study of abnormalities in the *Drosophila* body plan (Nusslein-Volhard and Wieschaus, 1980). The requirement of IHH for mouse development was first described in 1993 (Echelard *et al.*, 1993). Since then IHH has been linked to the formation of many organs, including bone, intestines, and heart (Ingham and McMahon, 2001). In mice, IHH increases in response to progesterone in the uterine glandular epithelium during the implantation window (Takamoto *et al.*, 2002). The hedgehog receptor, PATCHED (PTC), and GLI-Kruppel family transcription factors, GLI1, 2 and 3, are simultaneously upregulated in the underlying stroma (Matsumoto *et al.*, 2002). The epithelial-stromal interaction mediated by progesterone and IHH is regulated by chicken ovalbumin upstream promoter transcription factor II (Coup TF II), a transcription factor found in the uterine stroma of mice (Kurihara *et al.*, 2007). Deletion of COUP TFII results in decidualization and implantation failure, similar to that of IHH deletion, indicating that IHH may work in a paracrine fashion within the uterus to orchestrate progesterone-induced inhibition of epithelial proliferation necessary for implantation (Matsumoto *et al.*, 2002). The uterine effects of IHH knockout mice cannot be studied due to its lethality; however, targeted ablation of IHH in progesterone positive uterine cells shows that these mice are infertile due to impaired implantation, resembling mice with progesterone receptor knockout uteri (Lee *et al.*, 2006b).

In the human endometrium, IHH is upregulated in conjunction with progesterone receptor during the secretory phase (Talbi *et al.*, 2006). IHH, PTC, GLI1 and GLI2 all increase during the secretory phase (Wei *et al.*, 2010). In contrast to the mouse, IHH mRNA has been found in both the stroma and epithelium in human endometrium. This provides evidence that in humans, similar to mice, IHH is likely upregulated by progesterone and plays an essential role in uterine decidualization and implantation. Interestingly, it has been shown that IHH is abnormally expressed in the endometrium of endometriosis patients as compared to healthy controls, implicating

a role in the decline of fertility experienced by endometriosis patients and possibly others, as well (Smith *et al.*, 2011). Pathology occurs throughout the reproductive tract, causing decreased fertility rates in these patients. Recently, impaired endometrial receptivity has been postulated (Wei *et al.*, 2009). It would be instructive to know whether IHH signaling is disrupted in patients with progesterone resistance (Al-Sabbagh *et al.*, 2012).

Wnt signaling

A critical pathway that regulates embryo-uterine interactions and placentation is Wnt signaling. The term "Wnt" combines the Wg (Wingless) and Int1 genes, which were found to be homologous (Nusse *et al.*, 1991). The Wnt pathway includes a large number of ligands and receptors (19 and 10 respectively), resulting in 190 different potential interactions, which can be enhanced or attenuated by additional co-receptors and co-activators with multiple signaling activities (van Amerongen *et al.*, 2012). The Wnt signaling pathway is pleiotropic, as abnormalities in Wnt signaling are associated with cancers, osteoperosis, degenerative diseases and developmental disorders (Sonderegger *et al.*, 2010). Wnt signaling operates through both canonical and non-canonical pathways.

The canonical pathway functions through β-catenin, as shown in Fig. 2. In its unstimulated state, β-catenin is complexed with adenomatous polyposis coli (APC), Axin, GSK3β and CK1α, which mediates constitutive degradation of β-catenin (Liu *et al.*, 2002). Upon binding of Wnt ligands to transmembrane frizzled receptors (FZD) and low density lipoprotein receptor-related protein co-receptors-5/6 (LRP5/6), β-catenin is liberated from its complex (Metcalfe and Bienz, 2011), accumulates intracytoplasmically and translocates into the nucleus where it directs transcription of target genes (Behrens *et al.*, 1996). Several diverse non-canonical pathways operate independently of β-catenin (Semenov *et al.*, 2007).

Wnt pathways are crucial for blastocyst-uterine communication and subsequent implantation. Mouse morula and blastocyst stage embryos express Wnt genes in response to uterine factors induced by estrogen prior to implantation (Mohamed *et al.*, 2004). In mice, the canonical β-catenin pathway is dynamically activated in the uterus, initially in the circular smooth muscle of the myometrium, followed by activation in the luminal epithelium directly opposed to the blastocyst at implantation sites, both of which require the presence of a blastocyst (Mohamed *et al.*, 2005). Inhibition of the Wnt/β-catenin pathway prevents implantation (Mohamed *et al.*, 2005). Interestingly, it has been shown that interruption of nuclear β-catenin signaling in the developing embryo does not adversely affect the development of the embryo to the blastocyst stage; however it does interfere with implantation (Xie *et al.*, 2008). Through embryo transfer experiments, these investigators found that implantation failure is due to silencing of the canonical pathway in the blastocyst rather than its deficiency in the acquisition of uterine receptivity.

Wnt signaling is also necessary in uterine decidualization. Uterine Wnt4 expression is absent prior to implantation, but increases during the period of receptivity for implantation and continues to be expressed in the decidua (Paria *et al.*, 2001). Additionally, Wnt 5a, Wnt 7a, Wnt 11, and Wnt 16 are expressed in the mouse uterus during the window of implantation (Hayashi *et al.*, 2009).

Wnt signaling systems are critical for proper placentation and trophoblast function in mice. Wnt 2 deficient mice have a 50% prenatal mortality due to placental defects with histological findings of increased fibrinoid and decreased capillary formation within the placentas (Monkley *et al.*, 1996). Furthermore, mice lacking R-spondin3, an activator of the WNT/β-catenin pathway, die prenatally due to improper placental development (Aoki *et al.*,

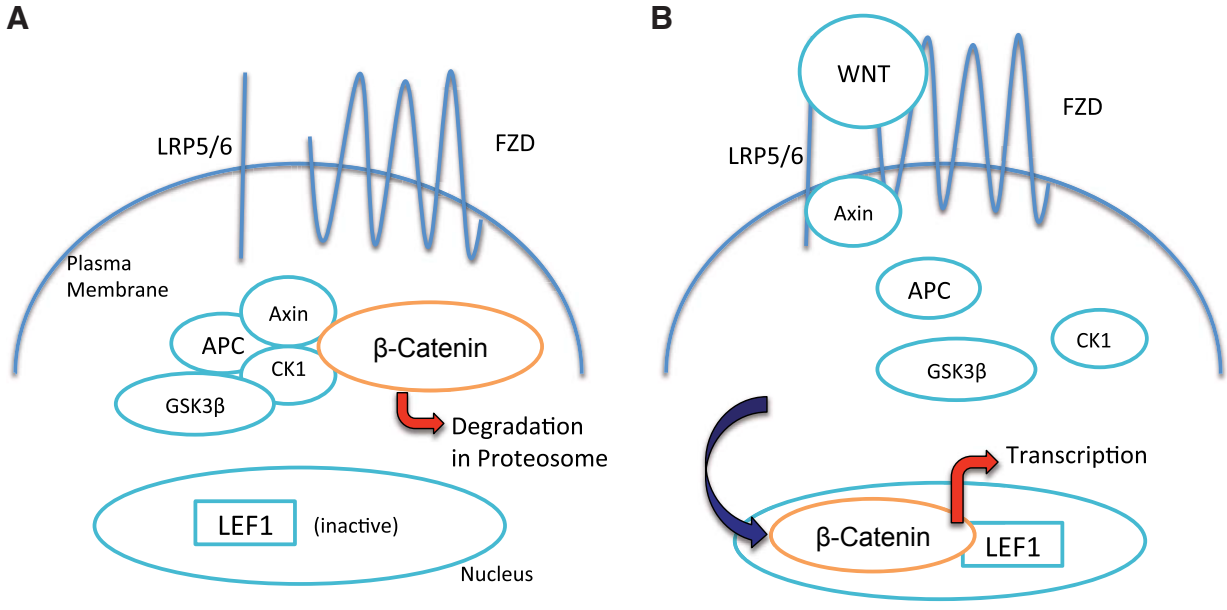

Fig. 2. Canonical Wnt signaling. *General schematic of a cell in the unstimulated state* **(A)** *and activation of the canonical pathway by WNT binding* **(B)***, as discussed in the text. In the absence of WNT, β-catenin in the cytoplasm is complexed with adenomatous polyposis coli (APC), Axin, glycogen synthase kinase-3β (GSK3β) and casein kinase 1 (CK1), leading to its phosphorylation, ubiquitinization and degradation in the proteosome. In the absence of β-catenin, lymphoid enhancer-binding factor-1 (LEF1) inhibits transcription of WNT target genes. WNT binding to a frizzled receptor (FZD) and a low density lipoprotein receptor-related protein co-receptor (LRP) frees β-catenin from the complex and prevents its degradation. β-catenin then translocates to the nucleus and when paired with LEF1 activates transcription of WNT target genes.*

2007), and FZD5 knockout mice die prenatally with placental abnormalities (Ishikawa *et al.*, 2001). Wnt7b is required for fusion of the chorion and allantois and Wnt7b deficient mice die prenatally due to placental defects (Parr *et al.*, 2001).

In humans, numerous Wnt ligands and receptors have been identified in the endometrium, indicating their importance for endometrial function (Tulac *et al.*, 2003). Wnt signaling in human endometrium during implantation differs from mice. In mice, proper uterine decidualization and implantation is estrogen dependent, and estrogen dependent Wnt signaling and β-catenin expression is crucial. In humans, however, decidualization and implantation are progesterone driven. Studies have shown that during the early luteal to mid luteal phase of human endometrium, Dickkopk1 (DKK1), an inhibitor of the canonical Wnt pathway, is significantly upregulated (Carson *et al.*, 2002). This is likely progesterone driven, as progesterone can increase DKK1 expression in human endometrial stromal cells. Furthermore, after treatment with mifepristone, a progesterone antagonist, an increase in β-catenin, Wnt ligands and Wnt receptors have been observed in human endometrium (Catalano *et al.*, 2007), suggesting that progesterone driven human decidualization down regulates the canonical Wnt pathway.

Abnormalities in Wnt signaling could contribute to infertility. Significantly higher levels of β-catenin expression have been observed in the endometrium of the mid-secretory phase in infertile patients with endometriosis, compared to fertile counterparts (Matsuzaki *et al.*, 2010). Impaired Wnt signaling could also contribute to the etiology of endometriosis, as DKK1 mRNA expression was significantly decreased in endometriotic fibroblast of endometriosis patients compared to women without disease (Aghajanova *et al.*, 2010). This deficiency could play a role in the development of endometriosis and in maintaining a proliferative phase of the lesions.

As found in mice, Wnt signaling is essential for human placentation. Expression of 14 out of 19 Wnt ligands and 8 out of 10 FZD receptors has been documented in human placentas (Sonderegger *et al.*, 2007). Interestingly, certain Wnts, including Wnt1, Wnt7b, Wnt10a, and Wnt10b, were absent in term placentas, although they are expressed in first trimester placentas, suggesting a role in the critical early period of placental development and trophoblast differentiation (Sonderegger *et al.*, 2007). Wnt3a can stimulate trophoblast invasion, which could be blocked by DKK1 (Pollheimer *et al.*, 2006). Methylation of certain genes encoding inhibitors of Wnt signaling has been found in human placentas and trophoblast cells, further supporting a role of Wnt signaling in trophoblast invasion (Novakovic *et al.*, 2008). Interestingly, hypermethylation of the APC gene promoter, an inhibitor of β-catenin, has been reported in choriocarcinoma trophoblast cell lines (Wong *et al.*, 2008). Similarly, DKK1 is decreased in choriocarcinoma cell lines, and its over-expression induces mitotic arrest (Peng *et al.*, 2006). Nuclear localization of β-catenin is significantly elevated in invasive trophoblasts of complete hydatidiform mole, compared to normal placentas (Pollheimer *et al.*, 2006), suggesting that although activation of the Wnt system is critical for placentation, over activation could cause excessive invasiveness that may be associated with carcinomas. Conversely, repression of the canonical Wnt pathway could lead to pregnancy related complications associated with abnormal placentation. Wnt2 and β-catenin are both decreased in placentas of women with preeclampsia, whereas DKK1 is increased (Zhang *et al.*, 2013b). High levels of DKK1 were also found in patients with recurrent miscarriages (Bao *et al.*, 2013).

Wnt signaling plays crucial roles in many aspects of reproduction and in humans its inhibition and activation must be properly balanced for normal reproductive function. Inhibition of the Wnt canonical pathway is critical for proper endometrial decidualization and implantation, whereas excessive activation is linked with infertility. In placentation, activation of the canonical pathway is necessary for normal placental development and differentiation; however, overexpression may lead to rare placental carcinomas.

Conclusions

Significant advancements have been made in unraveling the molecular mechanisms governing growth factors involved in implantation and trophoblast invasion. The mouse has provided a powerful experimental model; however, its correlation with human implantation and trophoblast development is not entirely consistent. Infertility affects approximately 15% of reproductive age women and in the past four decades, IVF has become a viable and widely used treatment option for infertile couples, with over 1.5% of babies in the U.S currently conceived through IVF (Sunderam *et al.*, 2013). Although relatively successful compared to natural fecundity, one of the major barriers to higher IVF implantation rates is a non-receptive endometrium (Li and Jin, 2013). A better understanding of the molecular mechanisms governing the expression of growth factors in implantation and placentation could better define the window of implantation and contribute towards higher IVF pregnancy rates. Furthermore, this additional information could provide a foundation for development of new treatments for clinical pathologies such as recurrent pregnancy loss and placental insufficiencies that reduce fetal growth rates or cause preeclampsia. Therefore, continued investigation of growth factors and the molecular mechanisms that ensure successful implantation and placentation has significant clinical relevance.

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References

AGHAJANOVA L. (2004). Leukemia inhibitory factor and human embryo implantation. *Ann N Y Acad Sci* 1034: 176-183.

AGHAJANOVA L, BJURESTEN K, ALTMAE S, LANDGREN B M and STAVREUS-EVERS A. (2008). HB-EGF but not amphiregulin or their receptors HER1 and HER4 is altered in endometrium of women with unexplained infertility. *Reprod Sci* 15: 484-492.

AGHAJANOVA L, HORCAJADAS J A, WEEKS J L, ESTEBAN F J, NEZHAT C N, CONTI M and GIUDICE L C. (2010). The protein kinase A pathway-regulated transcriptome of endometrial stromal fibroblasts reveals compromised differentiation and persistent proliferative potential in endometriosis. *Endocrinology* 151: 1341-1355.

AL-SABBAGH M, LAM E W and BROSENS J J. (2012). Mechanisms of endometrial progesterone resistance. *Mol Cell Endocrinol* 358: 208-215.

AOKI M, MIEDA M, IKEDA T, HAMADA Y, NAKAMURA H and OKAMOTO H. (2007). R-spondin3 is required for mouse placental development. *Dev Biol* 301: 218-226.

ARICI A, ENGIN O, ATTAR E and OLIVE D L. (1995). Modulation of leukemia inhibitory factor gene expression and protein biosynthesis in human endometrium. *J Clin Endocrinol Metab* 80: 1908-1915.

ARICI A, ORAL E, BAHTIYAR O, ENGIN O, SELI E and JONES E E. (1997). Leukaemia inhibitory factor expression in human follicular fluid and ovarian cells.

Hum Reprod 12: 1233-1239.

ARMANT D R, KILBURN B A, PETKOVA A, EDWIN S S, DUNIEC-DMUCHOWSKI Z M, EDWARDS H J, ROMERO R and LEACH R E. (2006). Human trophoblast survival at low oxygen concentrations requires metalloproteinase-mediated shedding of heparin-binding EGF-like growth factor. *Development* 133: 751-759.

BAO S H, SHUAI W, TONG J, WANG L, CHEN P and DUAN T. (2013). Increased Dickkopf-1 expression in patients with unexplained recurrent spontaneous miscarriage. *Clin Exp Immunol* 172: 437-443.

BASS K E, MORRISH D, ROTH I, BHARDWAJ D, TAYLOR R, ZHOU Y and FISHER S J. (1994). Human cytotrophoblast invasion is up-regulated by epidermal growth factor: evidence that paracrine factors modify this process. *Dev Biol* 164: 550-561.

BEHRENS J, VON KRIES J P, KUHL M, BRUHN L, WEDLICH D, GROSSCHEDL R and BIRCHMEIER W. (1996). Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* 382: 638-642.

BEN-BARUCH N and YARDEN Y. (1994). Neu differentiation factors: a family of alternatively spliced neuronal and mesenchymal factors. *Proc Soc Exp Biol Med* 206: 221-227.

BROSENS I A, ROBERTSON W B and DIXON H G. (1972). The role of the spiral arteries in the pathogenesis of preeclampsia. *Obstet Gynecol Annu* 1: 177-191.

BROWN N, DEB K, PARIA B C, DAS S K and REESE J. (2004). Embryo-uterine interactions via the neuregulin family of growth factors during implantation in the mouse. *Biol Reprod* 71: 2003-2011.

BURDEN S and YARDEN Y. (1997). Neuregulins and their receptors: a versatile signaling module in organogenesis and oncogenesis. *Neuron* 18: 847-855.

BURTON G J and JAUNIAUX E. (2004). Placental oxidative stress: from miscarriage to preeclampsia. *J Soc Gynecol Investig* 11: 342-352.

BURTON G J, JAUNIAUX E and WATSON A L. (1999). Maternal arterial connections to the placental intervillous space during the first trimester of human pregnancy: the Boyd collection revisited. *Am J Obstet Gynecol* 181: 718-724.

CARSON D D, LAGOW E, THATHIAH A, AL-SHAMI R, FARACH-CARSON M C, VERNON M, YUAN L, FRITZ M A and LESSEY B. (2002). Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. *Mol Hum Reprod* 8: 871-879.

CATALANO R D, CRITCHLEY H O, HEIKINHEIMO O, BAIRD D T, HAPANGAMA D, SHERWIN J R, CHARNOCK-JONES D S, SMITH S K and SHARKEY A M. (2007). Mifepristone induced progesterone withdrawal reveals novel regulatory pathways in human endometrium. *Mol Hum Reprod* 13: 641-654.

CATALANO R D, JOHNSON M H, CAMPBELL E A, CHARNOCK-JONES D S, SMITH S K and SHARKEY A M. (2005). Inhibition of Stat3 activation in the endometrium prevents implantation: a nonsteroidal approach to contraception. *Proc Natl Acad Sci USA* 102: 8585-8590.

CHARNOCK-JONES D S, SHARKEY A M, FENWICK P and SMITH S K. (1994). Leukaemia inhibitory factor mRNA concentration peaks in human endometrium at the time of implantation and the blastocyst contains mRNA for the receptor at this time. *J Reprod Fertil* 101: 421-426.

CHOBOTOVA K, KARPOVICH N, CARVER J, MANEK S, GULLICK W J, BARLOW D H and MARDON H J. (2005). Heparin-binding epidermal growth factor and its receptors mediate decidualization and potentiate survival of human endometrial stromal cells. *J Clin Endocrinol Metab* 90: 913-919.

CHOBOTOVA K, MUCHMORE M E, CARVER J, YOO H J, MANEK S, GULLICK W J, BARLOW D H and MARDON H J. (2002a). The Mitogenic Potential of Heparin-Binding Epidermal Growth Factor in the Human Endometrium Is Mediated by the Epidermal Growth Factor Receptor and Is Modulated by Tumor Necrosis Factor-alpha. *J Clin Endocrinol Metab* 87: 5769-5777.

CHOBOTOVA K, SPYROPOULOU I, CARVER J, MANEK S, HEATH J K, GULLICK W J, BARLOW D H, SARGENT I L and MARDON H J. (2002b). Heparin-binding epidermal growth factor and its receptor ErbB4 mediate implantation of the human blastocyst. *Mech Dev* 119: 137-144.

CONTOS J J, ISHII I and CHUN J. (2000). Lysophosphatidic acid receptors. *Mol Pharmacol* 58: 1188-1196.

DAMSKY C H, LIBRACH C, LIM K H, FITZGERALD M L, MCMASTER M T, JANATPOUR M, ZHOU Y, LOGAN S K and FISHER S J. (1994). Integrin switching regulates normal trophoblast invasion. *Development* 120: 3657-3666.

DANIELSSON K G, SWAHN M L and BYGDEMAN M. (1997). The effect of various doses of mifepristone on endometrial leukaemia inhibitory factor expression in the midluteal phase--an immunohistochemical study. *Hum Reprod* 12: 1293-1297.

DAS S K, CHAKRABORTY I, PARIA B C, WANG X N, PLOWMAN G and DEY S K. (1995). Amphiregulin is an implantation-specific and progesterone- regulated gene in the mouse uterus. *Mol Endocrinol* 9: 691-705.

DAS S K, DAS N, WANG J, LIM H, SCHRYVER B, PLOWMAN G D and DEY S K. (1997). Expression of betacellulin and epiregulin genes in the mouse uterus temporally by the blastocyst solely at the site of its apposition is coincident with the "window" of implantation. *Dev Biol* 190: 178-190.

DAS S K, WANG X N, PARIA B C, DAMM D, ABRAHAM J A, KLAGSBRUN M, ANDREWS G K and DEY S K. (1994). Heparin-binding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: a possible ligand for interaction with blastocyst EGF-receptor in implantation. *Development* 120: 1071-1083.

DETHLEFSEN S M, RAAB G, MOSES M A, ADAM R M, KLAGSBRUN M and FREEMAN M R. (1998). Extracellular calcium influx stimulates metalloproteinase cleavage and secretion of heparin-binding EGF-like growth factor independently of protein kinase C. *J Cell Biochem* 69: 143-153.

DIFEDERICO E, GENBACEV O and FISHER S J. (1999). Preeclampsia is associated with widespread apoptosis of placental cytotrophoblasts within the uterine wall. *Am J Pathol* 155: 293-301.

DONG J and WILEY H S. (2000). Trafficking and proteolytic release of epidermal growth factor receptor ligands are modulated by their membrane-anchoring domains. *J Biol Chem* 275: 557-564.

ECHELARD Y, EPSTEIN D J, ST-JACQUES B, SHEN L, MOHLER J, MCMAHON J A and MCMAHON A P. (1993). Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* 75: 1417-1430.

FALLS D L. (2003). Neuregulins: functions, forms, and signaling strategies. *Exp Cell Res* 284: 14-30.

GENBACEV O, JOSLIN R, DAMSKY C H, POLLIOTTI B M and FISHER S J. (1996). Hypoxia alters early gestation human cytotrophoblast differentiation/invasion *in vitro* and models the placental defects that occur in preeclampsia. *J Clin Invest* 97: 540-550.

GENBACEV O, ZHOU Y, LUDLOW J W and FISHER S J. (1997). Regulation of human placental development by oxygen tension. *Science (WashDC)* 277: 1669-1672.

GIESS R, TANASESCU I, STECK T and SENDTNER M. (1999). Leukaemia inhibitory factor gene mutations in infertile women. *Mol Hum Reprod* 5: 581-586.

HAIMOVICI F and ANDERSON D J. (1993). Effects of growth factors and growth factor-extracellular matrix interactions on mouse trophoblast outgrowth in vitro. *Biol Reprod* 49: 124-130.

HAMATANI T, DAIKOKU T, WANG H, MATSUMOTO H, CARTER M G, KO M S and DEY S K. (2004). Global gene expression analysis identifies molecular pathways distinguishing blastocyst dormancy and activation. *Proc Natl Acad Sci USA* 101: 10326-10331.

HAMBARTSOUMIAN E. (1998). Endometrial leukemia inhibitory factor (LIF) as a possible cause of unexplained infertility and multiple failures of implantation. *Am J Reprod Immunol* 39: 137-143.

HAYASHI K, ERIKSON D W, TILFORD S A, BANY B M, MACLEAN J A, 2ND, RUCKER E B, 3RD, JOHNSON G A and SPENCER T E. (2009). Wnt genes in the mouse uterus: potential regulation of implantation. *Biol Reprod* 80: 989-1000.

HEINRICH P C, BEHRMANN I, MULLER-NEWEN G, SCHAPER F and GRAEVE L. (1998). Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem J* 334 (Pt 2): 297-314.

HOFMANN G E, DREWS M R, SCOTT R T, JR., NAVOT D, HELLER D and DELIGDISCH L. (1992). Epidermal growth factor and its receptor in human implantation trophoblast: immunohistochemical evidence for autocrine/paracrine function. *J Clin Endocrinol Metab* 74: 981-988.

HOLBRO T and HYNES N E. (2004). ErbB receptors: directing key signaling networks throughout life. *Annu Rev Pharmacol Toxicol* 44: 195-217.

IMUDIA A N, KILBURN B A, PETKOVA A, EDWIN S S, ROMERO R and ARMANT D R. (2008). Expression of heparin-binding EGF-like growth factor in term chorionic villous explants and its role in trophoblast survival. *Placenta* 29: 784-789.

INGHAM P W and MCMAHON A P. (2001). Hedgehog signaling in animal development: paradigms and principles. *Genes Dev* 15: 3059-3087.

ISHIKAWA T, TAMAI Y, ZORN A M, YOSHIDA H, SELDIN M F, NISHIKAWA S and TAKETO M M. (2001). Mouse Wnt receptor gene Fzd5 is essential for yolk sac and placental angiogenesis. *Development* 128: 25-33.

JAUNIAUX E, WATSON A and BURTON G. (2001). Evaluation of respiratory gases and acid-base gradients in human fetal fluids and uteroplacental tissue between 7 and 16 weeks' gestation. *Am J Obstet Gynecol* 184: 998-1003.

JESSMON P, LEACH R E and ARMANT D R. (2009). Diverse functions of HBEGF during pregnancy. *Mol Reprod Dev* 76: 1116-1127.

KELTZ M D, ATTAR E, BURADAGUNTA S, OLIVE D L, KLIMAN H J and ARICI A. (1996). Modulation of leukemia inhibitory factor gene expression and protein biosynthesis in the human fallopian tube. *Am J Obstet Gynecol* 175: 1611-1619.

KIMBER S J, SNEDDON S F, BLOOR D J, EL-BAREG A M, HAWKHEAD J A, METCALFE A D, HOUGHTON F D, LEESE H J, RUTHERFORD A, LIEBERMAN B A and BRISON D R. (2008). Expression of genes involved in early cell fate decisions in human embryos and their regulation by growth factors. *Reproduction* 135: 635-647.

KOJIMA K, KANZAKI H, IWAI M, HATAYAMA H, FUJIMOTO M, INOUE T, HORIE K, NAKAYAMA H, FUJITA J and MORI T. (1994). Expression of leukemia inhibitory factor in human endometrium and placenta. *Biol Reprod* 50: 882-887.

KURIHARA I, LEE D K, PETIT F G, JEONG J, LEE K, LYDON J P, DEMAYO F J, TSAI M J and TSAI S Y. (2007). COUP-TFII mediates progesterone regulation of uterine implantation by controlling ER activity. *PLoS genetics* 3: e102.

LASS A, WEISER W, MUNAFO A and LOUMAYE E. (2001). Leukemia inhibitory factor in human reproduction. *Fertil Steril* 76: 1091-1096.

LEACH R E, JESSMON P, COUTIFARIS C, KRUGER M, MYERS E R, ALI-FEHMI R, CARSON S A, LEGRO R S, SCHLAFF W D, CARR B R, STEINKAMPF M P, SILVA S, LEPPERT P C, GIUDICE L, DIAMOND M P and ARMANT D R. (2012). High throughput, cell type-specific analysis of key proteins in human endometrial biopsies of women from fertile and infertile couples. *Hum Reprod* 27: 814-828.

LEACH R E, KHALIFA R, RAMIREZ N D, DAS S K, WANG J, DEY S K, ROMERO R and ARMANT D R. (1999). Multiple roles for heparin-binding epidermal growth factor-like growth factor are suggested by its cell specific expression during the human endometrial cycle and early placentation. *J Clin Endocrinol Metab* 84: 3355-3363.

LEACH R E, KILBURN B A, WANG J, LIU Z, ROMERO R and ARMANT D R. (2004). Heparin-binding EGF-like growth factor regulates human extravillous cytotrophoblast development during conversion to the invasive phenotype. *Dev Biol* 266: 223-237.

LEACH R E, ROMERO R, KIM Y M, CHAIWORAPONGSA T, KILBURN B A, DAS S K, DEY S K, JOHNSON A, QURESHI F, JACQUES S and ARMANT D R. (2002). Pre-eclampsia and expression of heparin-binding EGF-like growth factor. *Lancet* 360: 1215-1219.

LEE D S, YANAGIMOTO UETA Y and SUZUKI H. (2006a). Expression of amphiregulin during the pre- and post-implantation period in the mouse reproductive tract. *J Reprod Dev* 52: 781-787.

LEE K, JEONG J, KWAK I, YU C T, LANSKE B, SOEGIARTO D W, TOFTGARD R, TSAI M J, TSAI S, LYDON J P and DEMAYO F J. (2006b). Indian hedgehog is a major mediator of progesterone signaling in the mouse uterus. *Nat Genet* 38: 1204-1209.

LESSEY B A, GUI Y, APPARAO K B, YOUNG S L and MULHOLLAND J. (2002). Regulated expression of heparin-binding EGF-like growth factor (HB-EGF) in the human endometrium: a potential paracrine role during implantation. *Mol Reprod Dev* 62: 446-455.

LI M Q and JIN L P. (2013). Ovarian stimulation for *in vitro* fertilization alters the protein profile expression in endometrial secretion. *Int J Clin Exp Pathol* 6: 1964-1971.

LIBRACH C L, WERB Z, FITZGERALD M L, CHIU K, CORWIN N M, ESTEVES R A, GROBELNY D, GALARDY R, DAMSKY C H and FISHER S J. (1991). 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. *J Cell Biol* 113: 437-449.

LIM H J and DEY S K. (2009). HB-EGF: a unique mediator of embryo-uterine interactions during implantation. *Exp Cell Res* 315: 619-626.

LIU C, LI Y, SEMENOV M, HAN C, BAEG G H, TAN Y, ZHANG Z, LIN X and HE X. (2002). Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell* 108: 837-847.

LIU Z and ARMANT D R. (2004). Lysophosphatidic acid regulates murine blastocyst development by transactivation of receptors for heparin-binding EGF-like growth factor. *Exp Cell Res* 296: 317-326.

LUETTEKE N C, QIU T H, FENTON S E, TROYER K L, RIEDEL R F, CHANG A and LEE D C. (1999). Targeted inactivation of the EGF and amphiregulin genes reveals distinct roles for EGF receptor ligands in mouse mammary gland development. *Development* 126: 2739-2750.

LUETTEKE N C, QIU T H, PEIFFER R L, OLIVER P, SMITHIES O and LEE D C. (1993). TGF alpha deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. *Cell* 73: 263-278.

MACHIDA T, TAGA M and MINAGUCHI H. (1995). Effects of epidermal growth factor and transforming growth factor alpha on the mouse trophoblast outgrowth in vitro. *Eur J Endocrinol* 133: 741-746.

MANN G B, FOWLER K J, GABRIEL A, NICE E C, WILLIAMS R L and DUNN A R. (1993). Mice with a null mutation of the TGF alpha gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. *Cell* 73: 249-261.

MARTIN K L, BARLOW D H and SARGENT I L. (1998). Heparin-binding epidermal growth factor significantly improves human blastocyst development and hatching in serum-free medium. *Hum Reprod* 13: 1645-1652.

MATSUMOTO H, ZHAO X, DAS S K, HOGAN B L and DEY S K. (2002). Indian hedgehog as a progesterone-responsive factor mediating epithelial-mesenchymal interactions in the mouse uterus. *Dev Biol* 245: 280-290.

MATSUZAKI S, DARCHA C, MALEYSSON E, CANIS M and MAGE G. (2010). Impaired down-regulation of E-cadherin and beta-catenin protein expression in endometrial epithelial cells in the mid-secretory endometrium of infertile patients with endometriosis. *J Clin Endocrinol Metab* 95: 3437-3445.

METCALFE C and BIENZ M. (2011). Inhibition of GSK3 by Wnt signalling--two contrasting models. *J Cell Sci* 124: 3537-3544.

MEYER D, YAMAAI T, GARRATT A, RIETHMACHER-SONNENBERG E, KANE D, THEILL L E and BIRCHMEIER C. (1997). Isoform-specific expression and function of neuregulin. *Development* 124: 3575-3586.

MIETTINEN P J, BERGER J E, MENESES J, PHUNG Y, PEDERSEN R A, WERB Z and DERYNCK R. (1995). Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* 376: 337-341.

MOHAMED O A, DUFORT D and CLARKE H J. (2004). Expression and estradiol regulation of Wnt genes in the mouse blastocyst identify a candidate pathway for embryo-maternal signaling at implantation. *Biol Reprod* 71: 417-424.

MOHAMED O A, JONNAERT M, LABELLE-DUMAIS C, KURODA K, CLARKE H J and DUFORT D. (2005). Uterine Wnt/beta-catenin signaling is required for implantation. *Proc Natl Acad Sci USA* 102: 8579-8584.

MONKLEY S J, DELANEY S J, PENNISI D J, CHRISTIANSEN J H and WAINWRIGHT B J. (1996). Targeted disruption of the Wnt2 gene results in placentation defects. *Development* 122: 3343-3353.

NORWITZ E R, SCHUST D J and FISHER S J. (2001). Implantation and the survival of early pregnancy. *N Engl J Med* 345: 1400-1408.

NOVAKOVIC B, RAKYAN V, NG H K, MANUELPILLAI U, DEWI C, WONG N C, MORLEY R, DOWN T, BECK S, CRAIG J M and SAFFERY R. (2008). Specific tumour-associated methylation in normal human term placenta and first-trimester cytotrophoblasts. *Mol Hum Reprod* 14: 547-554.

NUSSE R, BROWN A, PAPKOFF J, SCAMBLER P, SHACKLEFORD G, MCMAHON A, MOON R and VARMUS H. (1991). A new nomenclature for int-1 and related genes: the Wnt gene family. *Cell* 64: 231.

NUSSLEIN-VOLHARD C and WIESCHAUS E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287: 795-801.

PARIA B C, DAS S K, ANDREWS G K and DEY S K. (1993). Expression of the epidermal growth factor receptor gene is regulated in mouse blastocysts during delayed implantation. *Proc Natl Acad Sci USA* 90: 55-59.

PARIA B C, ELENIUS K, KLAGSBRUN M and DEY S K. (1999). Heparin-binding EGF-like growth factor interacts with mouse blastocysts independently of ErbB1: a possible role for heparan sulfate proteoglycans and ErbB4 in blastocyst implantation. *Development* 126: 1997-2005.

PARIA B C, MA W, TAN J, RAJA S, DAS S K, DEY S K and HOGAN B L. (2001). Cellular and molecular responses of the uterus to embryo implantation can be elicited by locally applied growth factors. *Proc Natl Acad Sci USA* 98: 1047-1052.

PARR B A, CORNISH V A, CYBULSKY M I and MCMAHON A P. (2001). Wnt7b regulates placental development in mice. *Dev Biol* 237: 324-332.

PENG S, MIAO C, LI J, FAN X, CAO Y and DUAN E. (2006). Dickkopf-1 induced apoptosis in human placental choriocarcinoma is independent of canonical Wnt signaling. *Biochem Biophys Res Commun* 350: 641-647.

POLLHEIMER J, LOREGGER T, SONDEREGGER S, SALEH L, BAUER S, BILBAN M, CZERWENKA K, HUSSLEIN P and KNOFLER M. (2006). Activation of the canonical wingless/T-cell factor signaling pathway promotes invasive differentiation of human trophoblast. *Am J Pathol* 168: 1134-1147.

RAAB G, KOVER K, PARIA B C, DEY S K, EZZELL R M and KLAGSBRUN M. (1996). Mouse preimplantation blastocysts adhere to cells expressing the transmembrane form of heparin-binding EGF-like growth factor. *Development* 122: 637-645.

RAPPOLEE D A, BRENNER C A, SCHULTZ R, MARK D and WERB Z. (1988). Developmental expression of PDGF, TGF-alpha, and TGF-beta genes in preimplantation mouse embryos. *Science (WashDC)* 241: 1823-1825.

REESE J, BROWN N, DAS S K and DEY S K. (1998). Expression of neu differentiation factor during the periimplantation period in the mouse uterus. *Biol Reprod* 58: 719-727.

RIESE D J and STERN D F. (1998). Specificity within the EGF family/ErbB receptor family signaling network. *Bioessays* 20: 41-48.

RODESCH F, SIMON P, DONNER C and JAUNIAUX E. (1992). Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. *Obstet Gynecol* 80: 283-285.

ROUT U K, WANG J, PARIA B C and ARMANT D R. (2004). alpha5beta1, alphaVbeta3 and the platelet-associated integrin alphaIIbbeta3 coordinately regulate adhesion and migration of differentiating mouse trophoblast cells. *Dev Biol* 268: 135-151.

SARGENT I L, MARTIN K L and BARLOW D H. (1998). The use of recombinant growth factors to promote human embryo development in serum-free medium. *Hum Reprod* 13 Suppl 4: 239-248.

SEMENOV M V, HABAS R, MACDONALD B T and HE X. (2007). SnapShot: Noncanonical Wnt Signaling Pathways. *Cell* 131: 1378.

SERAFINI P C, SILVA I D, SMITH G D, MOTTA E L, ROCHA A M and BARACAT E C. (2009). Endometrial claudin-4 and leukemia inhibitory factor are associated with assisted reproduction outcome. *Reprod Biol Endocrinol* 7: 30.

SHARKEY A M, KING A, CLARK D E, BURROWS T D, JOKHI P P, CHARNOCK-JONES D S, LOKE Y W and SMITH S K. (1999). Localization of leukemia inhibitory factor and its receptor in human placenta throughout pregnancy. *Biol Reprod* 60: 355-364.

SIBILIA M and WAGNER E F. (1995). Strain-dependent epithelial defects in mice lacking the EGF receptor [published erratum appears in Science 1995 Aug 18; 269(5226):909]. *Science (WashDC)* 269: 234-238.

SMITH K, ALNIFAIDY R, WEI Q and NIEMAN L K. (2011). Endometrial Indian hedgehog expression is decreased in women with endometriosis. *Fertil Steril* 95: 2738-2741 e2731-2733.

SONDEREGGER S, HUSSLEIN H, LEISSER C and KNOFLER M. (2007). Complex expression pattern of Wnt ligands and frizzled receptors in human placenta and its trophoblast subtypes. *Placenta* 28 Suppl A: S97-102.

SONDEREGGER S, POLLHEIMER J and KNOFLER M. (2010). Wnt signalling in implantation, decidualisation and placental differentiation--review. *Placenta* 31: 839-847.

SONG H, LIM H, DAS S K, PARIA B C and DEY S K. (2000). Dysregulation of EGF family of growth factors and COX-2 in the uterus during the preattachment and attachment reactions of the blastocyst with the luminal epithelium correlates with implantation failure in LIF-deficient mice. *Mol Endocrinol* 14: 1147-1161.

STACHECKI J J and ARMANT D R. (1996). Regulation of blastocoele formation by intracellular calcium release is mediated through a phospholipase C-dependent pathway. *Biol Reprod* 55: 1292-1298.

STEWART C L, KASPAR P, BRUNET L J, BHATT H, GADI I, KONTGEN F and ABBONDANZO S J. (1992). Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature (London)* 359: 76-79.

SUGIHARA K, SUGIYAMA D, BYRNE J, WOLF D P, LOWITZ K P, KOBAYASHI Y, KABIR-SALMANI M, NADANO D, AOKI D, NOZAWA S, NAKAYAMA J, MUSTELIN T, RUOSLAHTI E, YAMAGUCHI N and FUKUDA M N. (2007). Trophoblast cell activation by trophinin ligation is implicated in human embryo implantation. *Proc Natl Acad Sci USA* 104: 3799-3804.

SUNDERAM S, KISSIN D M, CRAWFORD S, ANDERSON J E, FOLGER S G, JAMIESON D J, BARFIELD W D, DIVISION OF REPRODUCTIVE HEALTH N C F C D P and HEALTH PROMOTION C D C. (2013). Assisted reproductive technology surveillance - United States, 2010. *MMWR Surveill Summ* 62: 1-24.

SUZUKI N, NAKAYAMA J, SHIH I M, AOKI D, NOZAWA S and FUKUDA M N. (1999). Expression of trophinin, tastin, and bystin by trophoblast and endometrial cells in human placenta. *Biol Reprod* 60: 621-627.

TAKAMOTO N, ZHAO B, TSAI S Y and DEMAYO F J. (2002). Identification of Indian hedgehog as a progesterone-responsive gene in the murine uterus. *Mol Endocrinol* 16: 2338-2348.

TALBI S, HAMILTON A E, VO K C, TULAC S, OVERGAARD M T, DOSIOU C, LE SHAY N, NEZHAT C N, KEMPSON R, LESSEY B A, NAYAK N R and GIUDICE L C. (2006). Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology* 147: 1097-1121.

TAMADA H, DAS S K, ANDREWS G K and DEY S K. (1991). Cell-type-specific expression of transforming growth factor- alpha in the mouse uterus during the peri-implantation period. *Biol Reprod* 45: 365-372.

TAMADA H, SAKAMOTO M, SAKAGUCHI H, INABA T and SAWADA T. (1997). Evidence for the involvement of transforming growth factor-alpha in implantation in the rat. *Life Sci* 60: 1515-1522.

TAMURA N, SUGIHARA K, AKAMATO O and FUKUDA M N. (2011). Trophinin-mediated cell adhesion induces apoptosis of human endometrial epithelial cells through PKC-delta. *Cell Cycle* 10: 135-143.

TANIMURA K, NAKAGO S, MURAKOSHI H, TAKEKIDA S, MORIYAMA T, MATSUO H, HASHIMOTO K and MARUO T. (2004). Changes in the expression and cytological localization of betacellulin and its receptors (ErbB-1 and ErbB-4) in the trophoblasts in human placenta over the course of pregnancy. *Eur J Endocrinol* 151: 93-101.

THREADGILL D W, DLUGOSZ A A, HANSEN L A, TENNENBAUM T, LICHTI U, YEE D, LAMANTIA C, MOURTON T, HERRUP K, HARRIS R C and *et al.,* (1995). Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science (WashDC)* 269: 230-234.

TULAC S, NAYAK N R, KAO L C, VAN WAES M, HUANG J, LOBO S, GERMEYER A, LESSEY B A, TAYLOR R N, SUCHANEK E and GIUDICE L C. (2003). Identification, characterization, and regulation of the canonical Wnt signaling pathway in human endometrium. *J Clin Endocrinol Metab* 88: 3860-3866.

UMATA T, HIRATA M, TAKAHASHI T, RYU F, SHIDA S, TAKAHASHI Y, TSUNEOKA M, MIURA Y, MASUDA M, HORIGUCHI Y and MEKADA E. (2001). A dual signaling cascade that regulates the ectodomain shedding of heparin-binding epidermal growth factor-like growth factor. *J Biol Chem* 276: 30475-30482.

VAN AMERONGEN R, FUERER C, MIZUTANI M and NUSSE R. (2012). Wnt5a can both activate and repress Wnt/beta-catenin signaling during mouse embryonic development. *Dev Biol* 369: 101-114.

WANG J, MAYERNIK L and ARMANT D R. (2007). Trophoblast adhesion of the peri-implantation mouse blastocyst is regulated by integrin signaling that targets phospholipase C. *Dev Biol* 302: 143-153.

WANG J, MAYERNIK L, SCHULTZ J F and ARMANT D R. (2000). Acceleration of trophoblast differentiation by heparin-binding EGF-like growth factor is dependent on the stage-specific activation of calcium influx by ErbB receptors in developing mouse blastocysts. *Development* 127: 33-44.

WANG X, WANG H, MATSUMOTO H, ROY S K, DAS S K and PARIA B C. (2002). Dual source and target of heparin-binding EGF-like growth factor during the onset of implantation in the hamster. *Development* 129: 4125-4134.

WARE C B, HOROWITZ M C, RENSHAW B R, HUNT J S, LIGGITT D, KOBLAR S A, GLINIAK B C, MCKENNA H J, PAPAYANNOPOULOU T, THOMA B and ET A. (1995). Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. *Development* 121: 1283-1299.

WEI Q, LEVENS E D, STEFANSSON L and NIEMAN L K. (2010). Indian Hedgehog and its targets in human endometrium: menstrual cycle expression and response to CDB-2914. *J Clin Endocrinol Metab* 95: 5330-5337.

WEI Q, ST CLAIR J B, FU T, STRATTON P and NIEMAN L K. (2009). Reduced expression of biomarkers associated with the implantation window in women with endometriosis. *Fertil Steril* 91: 1686-1691.

WONG N C, NOVAKOVIC B, WEINRICH B, DEWI C, ANDRONIKOS R, SIBSON M, MACRAE F, MORLEY R, PERTILE M D, CRAIG J M and SAFFERY R. (2008). Methylation of the adenomatous polyposis coli (APC) gene in human placenta and hypermethylation in choriocarcinoma cells. *Cancer Lett* 268: 56-62.

WU M, YIN Y, ZHAO M, HU L and CHEN Q. (2013). The low expression of leukemia inhibitory factor in endometrium: possible relevant to unexplained infertility with multiple implantation failures. *Cytokine* 62: 334-339.

XIE H, TRANGUCH S, JIA X, ZHANG H, DAS S K, DEY S K, KUO C J and WANG H. (2008). Inactivation of nuclear Wnt-beta-catenin signaling limits blastocyst competency for implantation. *Development* 135: 717-727.

XIE H, WANG H, TRANGUCH S, IWAMOTO R, MEKADA E, DEMAYO F J, LYDON J P, DAS S K and DEY S K. (2007). Maternal heparin-binding-EGF deficiency limits pregnancy success in mice. *Proc Natl Acad Sci USA* 104: 18315-18320.

YOO H J, BARLOW D H and MARDON H J. (1997). Temporal and spatial regulation of expression of heparin-binding epidermal growth factor-like growth factor in the human endometrium: a possible role in blastocyst implantation. *Dev Genet* 21: 102-108.

ZHANG S, LIN H, KONG S, WANG S, WANG H, WANG H and ARMANT D R. (2013a). Physiological and molecular determinants of embryo implantation. *Mol Aspects Med* 34: 939-980.

ZHANG Z, LI H, ZHANG L, JIA L and WANG P. (2013b). Differential expression of beta-catenin and Dickkopf-1 in the third trimester placentas from normal and preeclamptic pregnancies: a comparative study. *Reprod Biol Endocrinol* 11: 17.