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Deciphering the Molecular Basis of Uterine Receptivity

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SUMMARY

Uterine receptivity is defined as a limited time period during which the uterus enters into an appropriately differentiated state that is ready for the initiation of implantation by competent blastocysts. Although various cellular aspects and molecular pathways involved in uterine receptivity have been identified by gene expression studies and genetically engineered mouse models, a comprehensive understanding of the window of uterine receptivity is still missing. This review focuses on the recent progress in this area, with particular focus on the molecular basis of stromal-epithelial dialogue and crosstalk between the blastocyst and the uterus during implantation. A better understanding of the underlying mechanisms governing the window of uterine receptivity is hoped to generate new strategies to correct implantation failure and to improve pregnancy rates in women.

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"Successful implantation is the result of reciprocal interactions between the implantation-competent blastocyst and the receptive uterus."

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INTRODUCTION

A new life begins upon the union of an egg with a sperm, a process known as fertilization. Following fertilization, pre-implantation development of early embryos in eutherian mammals occurs within the female reproductive tract, first in the oviduct and then in the uterus at later developmental stage. Embryos at the blastocyst stage initiate the first physical and physiological interaction with the endometrium, eventually implanting into the endometrial bed (Wang and Dey, 2006). Successful implantation requires a competent blastocyst and a uterus that accepts and accommodates the implanting conceptus. Communication of competency by both parties must occur in a short, self-limited period, namely the window of implantation during

Abbreviations: COX, cyclooxygenase; ER [KO], estrogen receptor [knockout]; gp130, glycoprotein 130; HB-EGF, heparin-binding EGF-like growth factor; IVF, in vitro fertilization; *Klf5*, Krüpple-like factors 5; LIF, leukemia inhibitory factor; MUC1, mucin-1; PR, progesterone receptor.

which the uterus is able to receive the blastocyst, also called the period of ''uterine receptivity'' (Yoshinaga, 1988).

The concept of receptivity was first established in rats by using asynchronous transfer of embryos into the uteri of pseudopregnant females, and was later reported in other species including mouse, hamster, guinea-pig, rabbit, and farm animals (Yoshinaga, 1988). Immediately after the receptive state is terminated, the uterus automatically enters into the refractory phase, independent of whether or not implantation occurs (Dey et al., 2004). The uterus in this refractory phase is indifferent, even toxic, for embryos (Yoshinaga, 1988). In mice, for example, the uterus is receptive on Day 4 of pregnancy (Day 1 = day of the vaginal plug) or pseudopregnancy, a period when embryo transfer can induce a normal embryo–uterine attachment reaction. But on the afternoon of Day 5, when the uterus enters the refractory phase, the transferred blastocysts fail to attach to the uterus (Song et al., 2002) and the blastocysts retrieved 24 hr after transfer degenerate when cultured in vitro (Yoshinaga, 1988). In humans, the receptivity period spans between days 20 and 24 of a regular menstrual cycle (7-11 days after the luteinizing hormone (LH) surge that triggers ovulation) prior to this period, the uterus is considered ''pre-receptive'' and becomes refractory thereafter (Psychoyos, 1973; Rashid et al., 2011).

In recent years, gene expression studies and genetically engineered mouse models have provided valuable clues to the implantation process with respect to specific growth factors, cytokines, lipid mediators, adhesion molecules, and transcription factors (Dey et al., 2004). Although the cellular events that confer uterine receptivity have been described, the molecular pathways that are crucial to this process, and how they interact, are not clearly understood. In this regard, we present the current understanding of implantation events in various model systems and in humans, primarily focusing on the molecular and morphological markers, and the embryo-uterus dialogues, and the stromal-epithelial interactions during endometrial receptivity. The knowledge might enable investigators to improve this critical step in modern reproductive therapies.

HORMONAL REQUIREMENTS FOR ESTABLISHMENT OF UTERINE RECEPTIVITY

Ovarian progesterone and estrogen are principal hormones that direct uterine receptivity. Although hormonal requirements for receptivity are species-dependent, progesterone is essential in nearly all mammals studied. Progesterone alone is adequate for inducing implantation in species such as guinea pig, rhesus monkey, and golden hamster (Heap and Deanesly, 1967; Harper et al., 1969; Kwun and Emmens, 1974), whereas ovarian estrogen is required to establish the uterine receptivity for implantation in other species such as rat and mouse (McCormack and Greenwald, 1974; Heap et al., 1981). Whether or not blastocyst-uterus attachment during implantation requires ovarian estrogen in humans is still uncertain (Wang and Dey, 2006; Su et al., 2012).

In mice, estrogen is essential for uterine receptivity in the progesterone-primed uterus. On Day 1 of pregnancy, uterine epithelial cells undergo extensive proliferation under the influence of pre-ovulatory ovarian estrogen and this epithelial proliferation, to some extent, continues through Day 2. Rising progesterone levels secreted from the newly formed corpus luteum initiate stromal cell proliferation from Day 3 onward (Huet et al., 1989; Huet-Hudson et al., 1989). In the morning of Day 4, when the uterus enters the pre-receptive stage, a small amount of estrogen is crucial for the uterus to attain receptivity (Tranguch et al., 2005b). Ovariectomy immediately before this pre-implantation estrogen secretion plus daily progesterone supplementation beginning on Day 5 results in blastocyst dormancy and inhibition of implantation, whereas a single injection of physiological levels of 17β-estradiol can induce the appropriate uterine differentiation from the neutral phase into the receptive state, and renders the reactivation of blastocyst implantation (Whitten, 1955; Yoshinaga and Adams, 1966; McLaren, 1968). Based on these hormone profiles during the pre-implantation period, exogenous estrogen and progesterone can also confer a receptive-stage uterus in ovariectomized mice (Paria et al., 1999b).

Estrogen and progesterone function in uteri primarily through nuclear estrogen receptors (ER) and progesterone receptors (PR), respectively. Both the receptor types have two isoforms, respectively known as ERα and ERβ and PRA and PRB (Edwards, 2005; Hewitt et al., 2005). Pharmacological and genetic evidence has revealed the necessity of the ER and PR for the preparation of uterine receptivity. Both ER and PR antagonist administered before implantation efficiently abolish uterine receptivity (Harper and Walpole, 1967; Major and Heald, 1974; Roblero et al., 1987; Vinijsanun and Martin, 1990). Previous studies using knockout mice for ER and PR have demonstrated their differential functions in uterine biology. The αERKO uterus is hypoplastic and unable to support implantation (Lubahn et al., 1993; Curtis Hewitt et al., 2002), whereas the βERKO uterus retains biological functions that allow for normal implantation (Krege et al., 1998; Wada-Hiraike et al., 2006; Lee et al., 2012). The uteri also express PRA and PRB (Mote et al., 2006), and mice lacking both PRA and PRB are infertile with many defects in ovarian and uterine functions (Lydon et al., 1995). PRB-deficient females are fertile, however, with normal ovarian and uterine responses (Mulac-Jericevic et al., 2000), indicating that essential progesterone-regulated functions in uteri are primarily mediated by PRA.

MOLECULAR CHANGES IN THE EPITHELIUM DURING UTERINE RECEPTIVITY

Uterine tissue consists of three major layers: an outer muscle layer, the inner luminal layer, and a stromal bed in between. Uterine epithelium is the first cell-layer to have physical and physiological contact with the blastocyst trophectoderm (Murphy, 2004). Under the coordination of estrogen and progesterone, endometrial epithelial cells

undergo structural and functional changes that establish uterine receptivity. Morphological changes of the luminal epithelium include apical microvilli retraction and the emergence of large apical protrusions (pinopodes) (Paria et al., 2002); functional changes are mediated by several factors such as adhesion molecules, cytokines, and homeotic proteins. Many of these signaling molecules have been identified as potential markers of uterine receptivity.

The glycoproteins expressed in the luminal epithelium are thought to act as a uterine barrier that inhibits the interaction between the trophoblasts and luminal epithelium at the time of attachment (Dey et al., 2004). Unmasking of these glycoproteins at the implantation site correlates with increased blastocyst adhesiveness to the uterus (Paria et al., 2002). For example, MUC1, a mucin-type glycoprotein, is integrally located in the apical plasma membrane of the luminal epithelium before implantation, whereas its expression is timely down-regulated during the receptive period (Meseguer et al., 1998). In humans, on the other hand, expression of MUC1 remains at high levels during the implantation window, which seems to contradict the anti-adhesion function of MUC1. One explanation is that the embryo utilizes MUC1-associated glycans, which has been demonstrated in rabbit implantation (Horne et al., 2005). Yet, in vitro experiment using human blastocyst and endometrial epithelial cells indicates that the embryo induces paracrine degeneration of epithelial-expressed MUC1 at the implantation site (Meseguer et al., 2001). Thus, it appears that MUC1 must be locally removed at the implantation site prior to successful blastocyst attachment.

Cytokines produced by trophoblast cells and the uterine epitheliums are important for transforming the uterus into a receptive state as they regulate the expression of various adhesion molecules. Leukemia inhibitory factor (LIF), which binds to the LIF receptor and shares gp130 as a common signal-transduction partner with other cytokines (Wang and Dey, 2006), is critical for implantation (Stewart et al., 1992). The expression of LIF is biphasic on Day 4, found in uterine glands in the morning and transitioning to stromal cells surrounding the blastocyst during attachment in the afternoon (Song et al., 2000). This specific expression pattern indicates that LIF has dual roles: initially in uterine preparation and later in the attachment reaction (Stewart et al., 1992; Song et al., 2000). *Lif*-deficient female mice showed implantation failure that can be rescued by supplementing with exogenous LIF. The role of LIF signaling in implantation is further reinforced by the phenotype of implantation failure upon inactivation of gp130 through deleting its STAT (signal transducer and activators of transcription) binding sites (Ernst et al., 2001). The potential mechanism underlying how LIF executes its effects on implantation is not clear, however. In humans, LIF is expressed at a high level in the glandular epithelium of the secretory endometrium (Rashid et al., 2011). It has also been reported that an optimum level of LIF is required for blastocyst implantation (Menkhorst et al., 2011; Terakawa et al., 2011), a finding that complements clinical evidence showing that insufficient levels or a deficiency in LIF is associated with unexplained recurrent abortions and infertility in women (Hambartsoumian, 1998; Ernst et al., 2001; Dey et al., 2004). These findings suggest that LIF is crucial for successful implantation in women.

Msx1, a homeobox gene, is transiently expressed in the mouse luminal epithelium and glandular epithelium on the morning of pregnancy Day 4, but its expression is dramatically down-regulated to undetectable levels upon the termination of uterine receptivity as well as the initiation of blastocyst implantation (Pavlova et al., 1994; Daikoku et al., 2004). In $Lif^{-/-}$ mice, however, *Msx1* is consistently expressed in the uterine epithelium even on pregnancy Day 6, suggesting that LIF signaling is essential for the down-regulation of *Msx1* that precedes uterine receptivity (Daikoku et al., 2004). This is further confirmed by observations of a sustained *Msx1* expression in uteri with conditional depletion of gp130, a LIF receptor partner (Daikoku et al., 2011). Recent studies further demonstrated that conditional deletion of *Msx1* in uteri leads to reduced fertility due to impaired implantation. Histological analysis of $Msx1^{-/-}$ implantation sites reveals that the luminal epithelium lacks well-defined crypts for blastocyst homing and attachment (Daikoku et al., 2011). Moreover, double deletion of uterine *Msx1* and *Msx2* results in complete implantation failure with altered uterine luminal epithelium cell polarity and impaired stromal-epithelial dialogue (Daikoku et al., 2011; Nallasamy et al., 2012), pointing toward a compensatory role for Msx2 in establishment of uterine receptivity in the absence of Msx1. Nonetheless, these results suggest that *Msx1*/*Msx2* genes are critical for conferring uterine epithelial integrity, and thus uterine receptivity, in mice. Dynamic expression of Msx1 in the human endometrium around the time of implantation indicates that Msx1 may play potential roles in determining uterine receptivity in women as well (Mirkin et al., 2005).

Following blastocyst attachment, the luminal epithelial cells surrounding the invading blastocyst undergo apoptosis whereas those distal from implantation site remain intact (Parr et al., 1987). Apoptosis of luminal epithelium plays a critical role in transmitting embryonic signals to underlying stromal cells, and the failure of these cells to undergo apoptosis affects normal implantation. For example, Krüppel-like factor 5 (*Klf5*), a zinc finger-containing transcription factor, is persistently expressed in the luminal epithelium throughout the pre-implantation stage; genetic loss of KLF5 in uterine epithelial leads to female infertility because the epithelium at the site of blastocyst apposition fails to degenerate (Sun et al., 2012).

EPITHELIAL-MESENCHYMAL INTERACTIONS CONFER UTERINE RECEPTIVITY

Synchronization of estrogen and progesterone directs the uterus into a receptive state that is accompanied by obvious morphological and functional changes in the epithelium. Increasing attention has been paid to address the issues regarding how these two hormones execute their differential function on two major uterine cell-types, and what the underlying molecular basis of stromal-epithelial interactions essential for uterine

receptivity is. The orchestrations describing synergic or antagonistic interactions of ovarian progesterone and estrogen during uterine cell proliferation versus differentiation are summarized in Figure 1, and are discussed below.

Estrogen Acts on Stromal ERα Stimulating the Proliferation of Uterine Epithelium via Paracrine Factors

ER is expressed in both epithelial and stromal cells of adult uteri, and it was initially assumed that estrogen acts directly through the ER in the corresponding compartments (Cooke et al., 1998). The crucial finding that estrogen stimulated epithelial proliferation in neonatal mouse uterus, which does not express ER, indicated that estrogen might affect mitogenesis indirectly (Cooke et al., 1998). Employing ER-negative αERKO mouse models and stromal-epithelial separation/recombination systems (Cunha, 2008), an early study demonstrated that estrogen could not stimulate epithelial proliferation in genetically recombined tissue that lacks stromal ERα, even in the presence of epithelial ERα (Cooke et al., 1997). Newly developed tissue-specific knockout techniques provide an excellent model for further studying the effect of estrogen on uterine responsiveness. Selective deletion of ERα in the uterine epithelium (UtEpiaERKO) using Wnt7a-Cre and Esr1-loxp mouse models proved that stromal ERα is responsible for estrogen-induced epithelial proliferation (Winuthayanon et al., 2010).

Yet, how does the estrogen-ERα activity in the stroma induce the epithelial proliferation? Paracrine actions of polypeptide growth factors, such as Insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), or transforming growth factor α (TGFα), are believed to be an integral component of the uterine response to estrogens. IGF-1, a key growth factor induced and activated in the uterine stroma upon treatment with estrogen, is necessary for estrogen-induced uterine epithelial DNA synthesis through IGF-1 receptor signaling in the luminal epithelium (Chen et al., 2005; Kurita et al., 2005; Zhu and Pollard, 2007). The Igf1 knockout mice fail to respond to the estrogen-stimulated proliferation of uterine epithelial cells, suggesting the role of IGF1 in mediating estrogen action in the endometrium (Adesanya et al., 1999; Sato et al., 2002). These studies collectively support a paracrine mechanism of estrogen-mediated epithelial proliferation that solely requires functional ERα in the underlying stroma. Moreover, following estrogen treatment, PR is dramatically down-regulated in the epithelium and increased in the stroma in wild-type and UtEpiaERKO mice, whereas ICI (an ER antagonist) could inhibit the effect in both genotypes (Winuthayanon et al., 2010), suggesting that stromal ERα is also required for estrogen-induced down-regulation of uterine epithelial PR (Kurita et al., 2001).

Differentiation of the Uterine Epithelium Requires Functional ERα in Both the Epithelium and Stroma

Although uterine epithelial ERα is dispensable for estrogen-induced epithelial proliferation, it is essential for complete biological and biochemical responses. Selective deletion of uterine epithelial ERα resulted in compromised

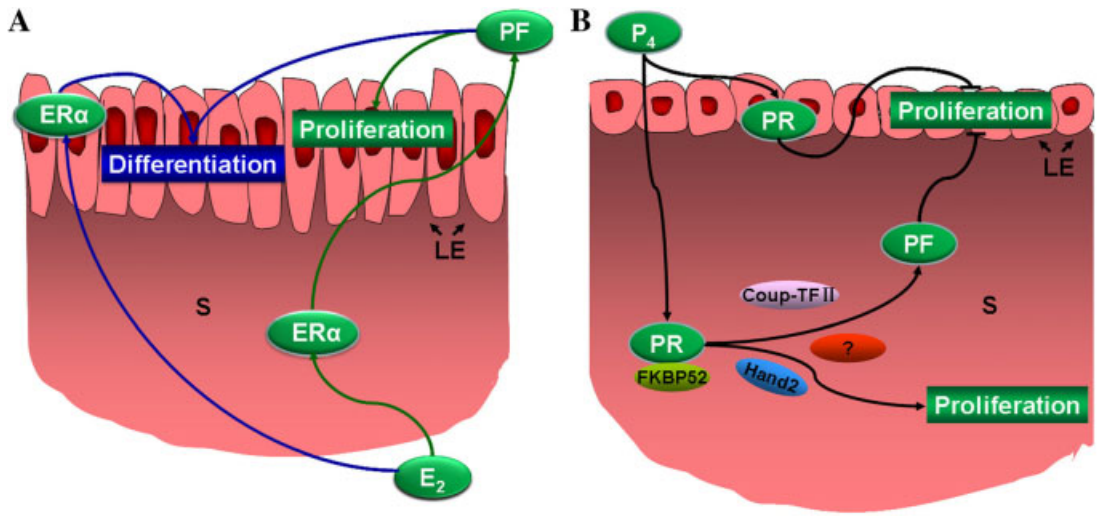

Figure 1. Putative mechanisms of uterine proliferation and differentiation in response to ovarian steroid hormones. **A**: The proliferation of uterine epithelium in response to estrogen requires stromal estrogen receptor alpha (ERα) and occurs via paracrine factors whereas the differentiation of uterine epithelium requires both epithelial and stromal ERα and occurs in a paracrine/autocrine manner. **B**: Progesterone acts through stromal and epithelial PRs to inhibit estrogen-induced epithelial proliferation while inducing proliferation of the underlying stroma. This effect is mediated by numerous progesterone receptor (PR) target genes. COUP-TF II, chicken ovalbumin upstream promoter transcription factor II; Hand2, Heart and neural crest derivatives-expressed protein 2; E_2, 17β-estradiol; ERα, nuclear estrogen receptor-α; FKBP52, FK506 binding protein-4; LE, luminal epithelium; P_4, progesterone; PF, paracrine factor; PR, progesterone receptor; S, stroma.

uterine weight induced by estrogen and in epithelial apoptosis after initial proliferation (Winuthayanon et al., 2010). Differentiation of the uterine epithelium, as indicated by secretory products such as lactoferrin (LF), complement component C3, and MUC-1, requires functional ERα in both the stroma and epithelium, and may be a direct effect of ERα signaling or a paracrine/autocrine effect guided by the synthesis of secreted factors (Buchanan et al., 1999; Kurita et al., 2000).

Progesterone Acts Through Stromal PR to Antagonize the Proliferative Response of the Epithelium to Estrogen, While Inducing Proliferation of the Stroma

PR-null uteri revealed a phenotype similar to ovariectomized mice exposed to prolonged estrogen treatment, which ascribed an essential mode of PR activity in the uterus (Lydon et al., 1995). Recombination experiments using uterine tissue from PR-null mice demonstrated that stromal PR is required to decrease estrogen's proliferative effect on the endometrial epithelium (Kurita et al., 1998). In recent years, numerous genes have been identified that mediate progesterone-PR signaling.

Immunophilin FK506 binding protein-4 (FKbp52), a co-chaperone required for appropriate uterine PR function (Daikoku et al., 2005), shows overlapping expression with PR in uterine stroma. Fkbp52$^{-/-}$ mice exhibit implantation failure and reduced progesterone function with exaggerated estrogenic influence in the epithelium (Tranguch et al., 2005a; Yang et al., 2006). At the histological and cellular level, Fkbp52$^{-/-}$ mice displayed aberrant epithelial proliferation and lower stromal proliferation than controls in Day 4 uteri, consistent with defects in progesterone-governed events. Yet, ER activity was unaffected, and the implantation defect could be rescued by the treatment of high dose of progesterone alone in the transgenic mice with CD1 background (Tranguch et al., 2007).

Chicken ovalbumin upstream promoter transcription factor II (Coup-TF II, also known as NR2F2), a member of the nuclear receptor super family, is highly expressed in the uterine stroma (Takamoto et al., 2005) and its expression is controlled by progesterone-Indian hedgehog (IHH)-Patched signaling from the epithelium to the stroma (Kurihara et al., 2007). Uterine conditional knockout of the Coup-TF II gene results in implantation failure and enhanced epithelial ER activity. This finding reveals that stromal Coup-TF II is an essential PR mediator that inhibits epithelial function (Kurihara et al., 2007; Simon et al., 2009; Lee et al., 2010).

The basic helix-loop-helix transcription factor, heart and neural crest derivatives expressed transcript 2 (Hand2) was identified by microarray gene profiling analysis of progesterone-responsive transcription at the implantation window in the mouse (Li et al., 2011). Progesterone induces the expression of Hand2 in the uterine stroma. Selective ablation of the *Hand2* gene in uterine cells showed implantation failure and continued induction of fibroblast growth factors (FGFs), which act as paracrine mediators to stimulate estrogen-induced epithelial proliferation. This indicates that Hand2 is a critical regulator of the uterine stromal-epithelial communication that directs proper steroid regulation conducive for the establishment of pregnancy.

Epithelial PR Mediates Progesterone Action by Inhibiting Estrogen-Induced Epithelial Proliferation

Despite the well-established concept that stromal PR mediates the antagonistic activity of progesterone on the proliferative response of the epithelium to estrogen, specific roles of epithelial PR in uterine biology were largely ignored. A recent study using Wnt7a-Cre/PR^{loxp} mouse models to ablate uterine epithelium PR has demonstrated that epithelial PR is essential for uterine stromal-epithelial crosstalk. Loss of epithelial PR results in complete pregnancy failure due to impaired uterine receptivity. Epithelial PR inhibited estrogen-dependent epithelial proliferation by directly targeting IHH signaling (Franco et al., 2011). This finding clearly demonstrated that epithelium PR is an essential player regulating the stromal-epithelial interaction for normal uterine physiology (Fig. 1).

CROSSTALK BETWEEN THE RECEPTIVE UTERUS AND THE BLASTOCYST

Successful implantation is the result of reciprocal interactions between the implantation-competent blastocyst and the receptive uterus (Dey et al., 2004). In addition to the physical interaction of the embryonic trophoblast cells with the uterine luminal epithelial cells prior to the attachment reaction, this embryo-uterine communication is undoubtedly influenced by multiple genes and factors (Fig. 2).

Heparin-binding EGF-like growth factor (HB-EGF) has been highlighted as an early molecular marker of embryo–uterine crosstalk during implantation (Das et al., 1994; Wang et al., 1994; Lim and Dey, 2009). It is produced as soluble and transmembrane forms and is expressed in the uterine luminal epithelium at the site of blastocyst apposition several hours before the attachment reaction in mice (Das et al., 1994). Molecular and genetic evidence show that HB-EGF functions via an auto-induction loop to mediate the crosstalk between the blastocyst and uterus. For example, the implantation-competent blastocysts express an increased amount of HB-EGF, which in turn induces its own gene expression in the uterine epithelium surrounding the blastocyst in a paracrine manner (Hamatani et al., 2004). Moreover, uterine-produced HB-EGF facilitates blastocyst trophectoderm differentiation in a paracrine and/or juxtacrine manner by interacting with epidermal growth factor receptors ErbB1 and ErbB4 on the blastocyst cell surface (Paria et al., 1999a). This auto-induction loop is recapitulated by Affi-gel bead transfer experiments that demonstrate implantation-like reactions of beads preabsorbed with purified HB-EGF and transferred into receptive uteri on Day 4 of pseudopregnancy (Paria et al., 2001a). Maternal deficiency of HB-EGF in the uterus

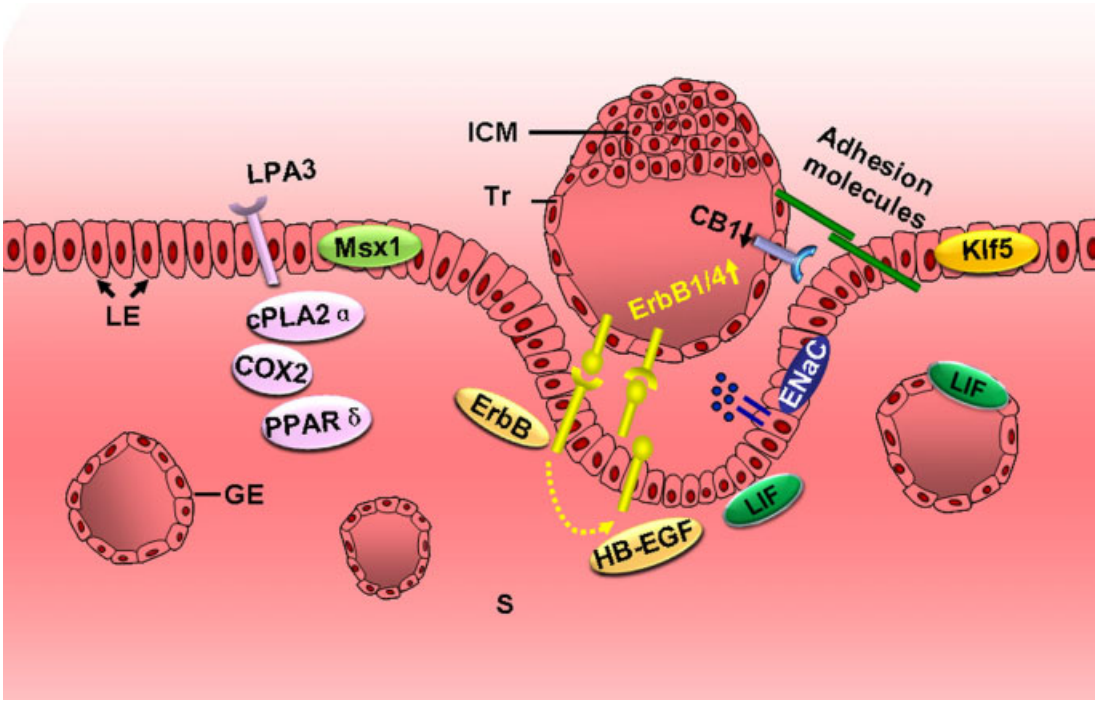

Figure 2. Signaling pathways participating in embryo–uterus crosstalk. During implantation, the synchronization of ovarian estrogen and progesterone induce an intricate cascade of molecular interactions involving growth factors, cytokines, transcription factors, and vasoactive mediators and their receptors. Timely regulation of the expression of these molecules is necessary for transforming the uterus into receptive state. CB1, brain-type cannabinoid receptor-1; COX-2, cyclooxygenase-2; cPLA2α, cytosolic phospholipase A2α; ENaC, epithelial Na^+ channel; ErbB, EGF-receptor family; FGF, fibroblast growth factor; GE, glandular epithelium; HB-EGF, heparin-binding EGF-like growth factor; ICM, inner cell mass; LE, luminal epithelium; LIF, leukemia inhibitory factor; LPA3, lysophosphatidic-acid receptor-3; PPARδ, peroxisome-proliferator-activated receptor-δ; S, stroma; Tr, trophectoderm.

delays the window of implantation, leading to a compromised pregnancy outcome, while amphiregulin, another heparin-binding growth factor of the EGF family member, can partially compensate for the loss of HB-EGF during implantation (Xie et al., 2007). In humans, expression of HB-EGF is high in the receptive endometrium, indicating that HB-EGF may also play an important role in mediating human implantation (Leach et al., 1999; Wang and Dey, 2006). A similar adhesion ligand-receptor signaling between the embryo and uterus is the selectin-based system utilized during human implantation (Genbacev et al., 2003). Selectin oligosaccharide ligands expressed in the receptive uterine epithelium are significantly elevated during the receptive phase, while complementary L-selectin receptors are expressed in the trophoblast cells (Wang et al., 2008). This unique expression pattern is critical to facilitate firm adhesion of the trophectoderm to the endometrium, and to therefore initiate attachment in the implantation process.

Lipids biosynthesized from a precursor released from the plasma membrane are also known to contribute to signaling processes of implantation (Wang and Dey, 2005). Cytoplasmic phospholipase A 2α (cPLA2α), for example, can selectively release arachidonic acid for prostaglandin (PG) biosynthesis, and were thus speculated to play a role in regulating embryo implantation. In fact, mice null for cPLA2-α consistently exhibited on-time implantation failure, highlighting the physiological significance of the PG signaling axis in implantation (Song et al., 2002). The rate-limiting enzyme for converting arachidonic acid to PGH2 is cyclooxygenase (COX), which exists in two isoforms, COX-1 and COX-2. In mice, COX-1 is expressed in uterine luminal and glandular epithelial cells on the morning of pregnancy Day 4, but becomes undetectable in the luminal epithelial cells after attachment occurs. In contrast, with the onset of attachment reaction on midnight of Day 4, COX-2 is initially expressed in the luminal epithelium and later restricted in the subepithelial stromal cells at the anti-mesometrial pole exclusively surrounding the blastocyst (Chakraborty et al., 1996), indicating an essential role of COX-2 during implantation. This is further supported by observations of implantation failure in COX-2 null mutant mice (Lim et al., 1997). The defects in COX-2 deficient females are genetic background dependent, however, and COX-1 can compensate for COX-2 to improve infertility in the CD1 background (Wang et al., 2004). Moreover, in the absence of Klf5, luminal epithelial COX-2 expression is absent, the

epithelium around the implantation chamber is retained, and embryonic growth is arrested, together suggesting that epithelium-expressed COX-2 plays a role in the degeneration of the luminal epithelium for blastocyst invasion (Sun et al., 2012).

Among various PGs, prostacyclin I2 (PGI2) is major PG produced at the implantation site in mice. The necessity of cPLA2α-COX-2-PGI2 in embryo implantation is further supported by observations that PGI2 supplementation can restore normal embryo implantation in COX-2 knockout mice, and that the null mutant of the PGI2 nuclear receptor, peroxisome proliferator activated receptor δ (PPARδ), delays the implantation window (Lim et al., 1999; Wang et al., 2007). In addition, it is interesting to note that the lysophosphatidic acid 3 (LPA3) signaling pathway may interact with the cPLA2α-COX2-PG signaling axis. *Lpa3*$^{-/-}$ females showed defects similar to those exhibited by *cPla2*$\alpha^{-/-}$ mice (Ye et al., 2005). For example, aberrant expression of COX-2, although treatment with PGs can restore the on-time implantation in LPA3-null females (Song et al., 2002). The physiological significance of PG signaling during human embryo implantation is evidenced from previous studies showing that both COX-1 and COX-2 are expressed in the endometrium during the implantation period, and reduced PG synthesis in the human endometrium leads to poor endometrial receptivity (Marions and Danielsson, 1999; Achache et al., 2010).

Another lipid signaling molecule that mediates an embryo–uterine dialogue during implantation is anandamide, a major endogenous cannabinoid, that can function through G-protein-coupled cannabinoid receptors CB1 and CB2 (Wang et al., 2006). Previous mouse studies provided evidence that low levels of anandamide are crucial to implantation since the levels of uterine anandamide and blastocyst CB1 are coordinately down-regulated in the receptive uterus and the activated blastocysts, respectively (Paria et al., 2001b; Guo et al., 2005). In fact, anandamide within a very narrow range regulates blastocyst function and implantation by differentially modulating mitogen-activated protein kinase (MAPK) signaling and Ca^{2+} channel activity via CB1 receptors. For example, anandamide at a low concentration induces the activation of MAPK signaling (Wang et al., 2003), while anandamide at a higher concentration inhibits Ca^{2+} channel activity and blastocyst competency for implantation without influencing MAPK signaling. Thus, it is conceivable that critical levels of uterine-derived endocannabinoids interact with appropriately expressed blastocyst CB1 in synchronizing blastocyst activation with uterine receptivity for implantation, whereas aberrant levels of uterine endocannabinoids and/or blastocyst CB1 interfere with these processes, resulting in pregnancy termination. It is worth noting that spontaneous pregnancy losses are associated with elevated anandamide levels in women (Maccarrone et al., 2000; Habayeb et al., 2008), reinforcing that endocannabinoid signaling is an important player determinant of embryo implantation.

Apart from physical signals, many different molecules have also been implicated as chemical signals for embryo implantation. Amiloride-sensitive epithelial Na^{+} channel (ENaC), encoded by SCNN1 genes within the degernerin/ENaC superfamily, is critical in electrolyte and water reabsorption (Ruan et al., 2012). In mice, ENaC is localized in the apical membrane of uterine endometrial epithelium and is up-regulated during the pre-implantation period (Ruan et al., 2012). Therefore, it was hypothesized that the up-regulation of ENaC may be responsible for the disappearance of uterine fluid or uterine luminal closure. The invading embryos can release trypsin, a serine protease known to activate ENaC (Vallet et al., 1997; Kleyman et al., 2009). Recently, Ruan et al. (2012) demonstrated that activation of ENaC in the murine uterus regulates prostaglandin production and release, thereby affecting implantation. Blocking or knockdown of uterine ENaC in mice resulted in implantation failure. Notably, it is useful to cross-reference a previous study on SGK1 (serum- and glucocorticoid-inducible kinase), a key regulator of sodium transport in mammalian epithelia (Fejes-Toth et al., 2008). SGK1 functions by directly activating and stabilizing pools of ENaC by inhibiting the ubiquitin ligase NEDD4-2 (Lang et al., 2006). In mice, *sgk1* mRNA levels transiently decline in the luminal epithelium during the window of endometrial receptivity (Fisher and Giudice, 2011; Salker et al., 2011). Intraluminal delivery of an overexpressing *sgk1* vector abolishes normal implantation with markedly up-regulated levels of the ENaC α-subunit. This result indicates that overexpression of uterine ENaC may also be detrimental to implantation.

FLEXIBILITY OF UTERINE RECEPTIVITY

Although uterine receptivity only occurs during a short, limited period, it can be modified under different hormonal environments. Revealing the underlying mechanism may help to develop new strategies to extend the window of receptivity in clinical practice.

Estrogen Is a Critical Determinant Specifying the Duration of Uterine Receptivity for Implantation

In rodents, estrogen is essential for the preparation of a progesterone-primed uterus to the receptive state. Ovariectomy conducted before pre-implantation estrogen secretion on the morning of Day 4 results in blastocyst dormancy and inhibition of implantation, also known as delayed implantation. This neutral uterine phase can be maintained by continued progesterone treatment, but is terminated by estrogen injection (Paria et al., 1992; Song et al., 2002).

The impacts of different doses of estrogens on the length of implantation window have been explored using a delayed implantation model (Ma et al., 2003). For example, estrogen at a low threshold level extends the window of uterine receptivity, whereas estrogen at physiological higher levels rapidly shuts off the implantation window, transforming the uterus into a refractory state that is accompanied by aberrant uterine expression of implantation-related genes, such as LIF (Ma et al., 2003). The model that high levels of estrogen are detrimental to pre-implantation events is

further supported by findings that ovarian hyper-stimulation leads to implantation failure and embryo resorption (Ertzeid and Storeng, 2001; Shapiro et al., 2011). In humans, the lifespan of fully developed pinopodes last maximally 48 hr, suggesting a transient cell state in the receptive endometrium (Nikas et al., 1999). Following ovarian stimulation with clomiphene citrate and human chorionic gonadotropin (hCG), pinopodes formed 1–2 days earlier than in the natural cycles (Cavagna and Mantese, 2003). Early pinopode formation caused by ovarian stimulation may have a role in shifting the window of receptivity, and it is thus reasonable to postulate that reduced implantation at in vitro fertilization (IVF) cycles could be due to asynchrony between the endometrium and blastocyst that result from exposure to high-levels of estrogen (Devroey et al., 2004).

Progesterone Supplementation Extends the Window of Uterine Receptivity

In mice, blastocysts can initiate implantation out of the normal ''window'' of uterine receptivity (Song et al., 2007). For example, blastocysts can still initiate attachment in a non-receptive uterus when transferred on Day 5 of pseudopregnancy, but implantation will not occur when normal blastocysts are transferred into Day 6 pseudopregnant uteri. Exogenous progesterone supplementation can prolong the implantation window to Day 6, which might be due to sustained LIF expression (Song et al., 2007). Deferred embryo implantation beyond the normal ''window'' of uterine receptivity leads to embryonic demise before birth in mice, however (Song et al., 2002; Wang and Dey, 2006), and is often associated with higher risk of early pregnancy losses in humans (Wilcox et al., 1999).

IMPLICATIONS FOR HUMAN INFERTILITY

Despite significant developments in IVF and embryo transfer technology in humans, pregnancy success rates remain disappointingly low; implantation failure due to inappropriate uterine receptivity is one of the major causes (Miller et al., 2012). Since the study of human uterine endometrium has many restrictions, including ethics and lack of an ideal cell culture system for studying intricate cell–cell interactions, current progress in fertility treatment relies predominantly on animal models, in particular, mouse models (Lim and Wang, 2010). Indeed, studies in mouse models have provided important insights into the molecular basis of human implantation. Some critical molecules for mouse implantation have been regarded as prospective markers for assessing human uteri quality and stage (Table 1) (Giudice, 1999; Cavagna and Mantese, 2003; Achache and Revel, 2006). For example, LIF, interleukin-11 (IL-11), HB-EGF, COX2, and homeobox (HOX) family members, which are important at different stages of implantation in mice, are also thought to be involved in human implantation (Salamonsen et al., 2009; Menkhorst et al., 2011). The expression of these genes is disturbed in the endometrium of infertile women (Laird et al., 2006; Lim and Wang, 2010). Further insights into these essential regulatory molecules might help to improve pregnancy success as well as aid the design of new contraceptives (Salamonsen et al., 2009). In fact, specific inhibitors of LIF and IL-11 have been developed to block implantation: a complete block of implantation was obtained with a LIF inhibitor (White et al., 2007) while a complete block of pregnancy due to decidual deficiency was achieved by treating with an IL-11 inhibitor when tested in mice (Menkhorst et al., 2009). The advent of

TABLE 1. Molecules Associated With Endometrial Receptivity

Molecules		Potential role	References
Hormones	Estrogen Progesterone	Coordinate proliferation and differentiation of endometrial, stromal, and epithelial cells	Huet-Hudson et al. (1989), Lydon et al. (1995)
Adhesion molecules	MUC1 L-selectin cadherins integrins	Facilitate blastocyst capture and attachment; promote interaction between the epithelium and trophectoderm	Stewart et al. (1992), Meseguer et al. (2001), Horne et al. (2005)
Cytokines	LIF IL6 IL11	Regulate functions of endometrial cells and embryo–maternal interactions during attachment and decidualization	Stewart et al., (1992), Salamonsen et al., (2009), Menkhorst et al. (2011)
Growth factors	HB-EGF IGF TGFβ	Locally mediate the hormone's effects on uterine cell proliferation and differentiation	Paria et al., (2001a), Chen et al. (2005), Kurita et al. (2005), Zhu and Pollard (2007)
Homeobox gene	HOXA10 HOXA11 MSX1/2	Determine the early reproductive tract development and regulate post-implantation uterine development Maintain uterine readiness to implantation; Regulate uterine luminal epithelial cell polarity	Wang and Dey (2006), Lim and Wang (2010), Daikoku et al. (2011), Nallasamy et al. (2012)
Lipids	cPLA2 COX2 PPAR LPA3	Regulate prostaglandin production and mediate prostaglandin intracellular function, increase vascular permeability, promote implantation, promote adhesiveness of uterus	Lim et al. (1997, 1999), Song et al. (2002), Wang et al. (2004, 2007), Ye et al. (2005)
Other factors	MMPs	Degenerate the components of extracellular matrix for uterine remolding	Kao et al., (2002), Skrzypczak et al. (2007), Rashid et al. (2011), Pabona et al. (2012)
	DKK1	Mediate epithelial-embryo and/or epithelial-stromal interactions for preparation of uterine receptivity	

newly developed -omics approaches, such as proteomics and secretomics, have been used to screen for novel biomarkers to date the endometrium during the estrous cycle (Haouzi et al., 2009; Diaz-Gimeno et al., 2011), resulting in a broad dissection of differentially expressed genes and proteins in the receptive and non-receptive phase of the endometrium (Carson et al., 2002; Kao et al., 2002; Borthwick et al., 2003; Riesewijk et al., 2003; Pabona et al., 2012). Whether or not such a differential profile can be used to inform clinical treatment and optimize IVF protocols needs to be further determined in women.

CONCLUSIONS AND PROSPECTS

The uterus is one of the most fascinating tissues in mammals, whose major purpose to accept implantation-competent blastocysts during a relatively short period of uterine receptivity. It has been generally accepted that uterine receptivity is one of the key events determining the success of pregnancy. Moreover, derailed endometrial receptivity also largely accounts for low pregnancy success rates in assistant reproductive technique programs (Wilcox et al., 1999; Diedrich et al., 2007; Miller et al., 2012).

Despite all recent advances in understanding the nature of uterine receptivity, the molecular basis of uterine receptivity and crosstalk between the blastocyst and the uterus during implantation remains largely unknown. On the one hand, the list on implantation-associated molecules is still expanding, so the signaling pathways and mechanism of these newly identified regulators need to be further deciphered. On the other hand, many defined genes that are expressed in an implantation-specific manner and appear to be important for implantation cannot be studied in depth because deletion of these genes often results in embryonic lethality or developmental defects. Thus, it is of paramount importance to define the precise hierarchical arrangements of the genes involved in implantation through inducible cell- and stage-specific silencing or activation of candidates. Since the duration of the implantation window depends on timely regulated expression of a wide range of genes, the integration of proteomics, genomics, and metabolomics with system biology approaches should be adopted for a better, holistic understanding the molecular signature of uterine receptivity and embryo-uterine dialog. Only when endometrial receptivity is better understood at the molecular and physiological level will it be possible to manipulate the uterine environment to improve fertility and to develop new non-hormonal contraceptives for humans.

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