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Molecular and cellular events during blastocyst implantation in the receptive uterus: clues from mouse models

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Abstract. The success of implantation is an interactive process between the blastocyst and the uterus. Synchronized development of embryos with uterine differentiation to a receptive state is necessary to complete pregnancy. The period of uterine receptivity for implantation is limited and referred to as the "implantation window", which is regulated by ovarian steroid hormones. Implantation process is complicated due to the many signaling molecules in the hierarchical mechanisms with the embryo-uterine dialogue. The mouse is widely used in animal research, and is uniquely suited for reproductive studies, i.e., having a large litter size and brief estrous cycles. This review first describes why the mouse is the preferred model for implantation studies, focusing on uterine morphology and physiological traits, and then highlights the knowledge on uterine receptivity and the hormonal regulation of blastocyst implantation in mice. Our recent study revealed that selective proteolysis in the activated blastocyst is associated with the completion of blastocyst implantation after embryo transfer. Furthermore, in the context of blastocyst implantation in the mouse, this review discusses the window of uterine receptivity, hormonal regulation, uterine vascular permeability and angiogenesis, the delayed-implantation mouse model, morphogens, adhesion molecules, crosslinker proteins, extracellular matrix, and matricellular proteins. A better understanding of uterine and blastocyst biology during the peri-implantation period should facilitate further development of reproductive technology.
Key words: Blastocyst, Decidualization, Mouse, Steroid hormones, Uterine receptivity

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In mammalian reproduction, crosstalk between the blastocyst and the uterine luminal epithelium is essential for the implantation process [1, 2]. The synchronization of embryonic development with uterine differentiation into a receptive state is essential for a successful pregnancy [1–13]. Uterine receptivity for implantation that supports blastocyst growth, attachment, and the subsequent events of implantation is time-limited. Implantation is a complex process involving spatiotemporally regulated endocrine, paracrine, autocrine, and juxtacrine modulators that mediate cell-cell and cell-matrix interactions [1–13]. The embryo is also an active unit with its own molecular program of cell growth and differentiation. The trophectoderm (TE) of implantation-competent blastocysts alters the embryo's functional programming *via* changes in cell surface molecules. The invasive trophoblasts of mouse blastocysts adhere, spread, and migrate on extracellular matrix (ECM) substrates [14–17] and penetrate three-dimensional ECM structures [18]. The proliferation and differentiation of uterine endometrial cells are also crucial steps during peri-implantation. Many molecules are involved in this process, including ECM, adhesion molecules, lipid mediators, and transcription factors. Successful embryo implantation is dependent on the cellular and molecular crosstalk between the uterus and the embryo; however, elucidation of the underlying molecular pathways has been hindered by their intricacy. The present review focuses on the molecular and cellular events during blastocyst implantation in the receptive uterus in a mouse model. This review first describes why the mouse is the preferred model organism for implantation studies, focusing on uterine morphology and physiological traits (Fig. 1), and then highlights knowledge regarding steroid hormonal regulation for blastocyst implantation and uterine receptivity (Fig. 2A). This review also describes the determinants of blastocyst competency and postimplantation development, i.e., selective proteolysis, adhesion molecules, crosslinker proteins, ECM, and matricellular proteins (Fig. 2B). Furthermore, this review describes the determinants of uterine receptivity and postimplantation uterine function, including morphogens, prostaglandins, and angiogenic factors (Fig. 2C).

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Mouse as the Preferred Model for Implantation Studies

The mouse, *Mus musculus*, is widely used in animal research because of its small size, resistance to infection, relatively rapid generation time, and large litter size [19]. Furthermore, it is uniquely suited for reproductive studies, i.e., the sexual maturity of female mice begins around 6 weeks of age and vaginal cytology shows estrous cycles of 4–5 days [19].

The morphology of the female reproductive tract organs is markedly different among mammalian species, i.e., the mouse has a long duplex uterus with a dual cervix [20–23]. The duplex uterus in the

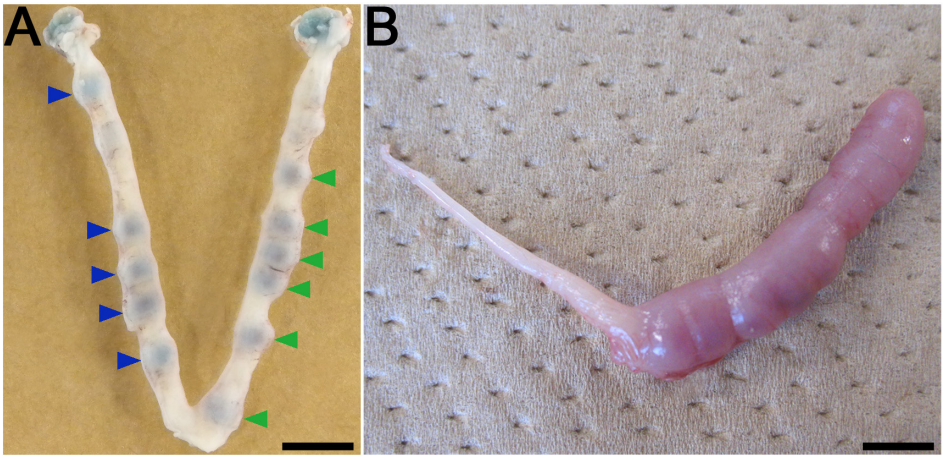

Fig. 1. The mouse uterus traits and the research paradigm for embryonic development and decidualization of uterine cells. (A) Implantation sites of transferred embryos and the uterine blue reaction 2 days after transference of blastocysts on day 6 of pregnancy (vaginal plug = day 1). Untreated control or treated blastocysts were transferred into one (left) or the other uterine horn (right) respectively. Mice have a long duplex uterus and it does not permit transuterine migration of embryos from one horn to the other. This trait allows the study of embryonic potential during the peri-implantation period for embryos transferred to the same recipient. Blue arrowheads and green arrowheads indicate the implantation sites of untreated control and treated embryos respectively. (B) Induced decidualization by artificial stimulus. Infusion of oil resulted in the decidual cell reaction (deciduoma). Pseudopregnant mice were given an intraluminal oil infusion (25 µl) into one uterine horn (right) on day 4 to induce decidualization, followed by estimation on day 8. The induction of uterine decidualization by artificial stimulus is useful to examine the effect of gene deficiency on uterine function and the steroidogenesis potential of transplanted ovarian tissues in ovariectomized mice. Scale bars represent 5 mm.

mouse does not permit transuterine migration of embryos from one horn to the other, and this difference allows for maintenance of pregnancy in one uterine horn while the other horn does not contain embryos [20–22]. This trait is favorable for the study of the embryonic potential of transferred embryos during the peri-implantation period [24, 25], e.g., in the same recipient mouse, the untreated control or treated blastocysts are transferred into one or the other uterine horn, respectively, followed by comparison of the implantation rates (Fig. 1A)[24]. These physiological traits and experiment methodology allow the concurrent obtainment of both the implantation rate of the embryos and the pregnancy rate of the recipient mice.

In mice, blastocyst attachment stimulates the uterine stroma to form a spongy mass of cells known as decidual tissue. The process is known as the decidual reaction, and the mass of decidual cells around a single embryo can be referred as the deciduum or decidua [19]. The decidual reaction involves a rapid increase in the permeability of local capillaries, causing the uterine stroma to become swollen and edematous. The attachment reaction coincides with a localized increase in stromal vascular permeability at the site of the blastocyst, as can be demonstrated by intravenous injection of a macromolecular blue dye (the uterine blue reaction) (Fig. 1A) [26]. The first sign of the attachment reaction in the process of implantation (i.e., the apposition stage) occurs in the mouse towards the end of day 4 of the pregnancy (vaginal plug = day 1) [26, 27].

The decidual reaction only occurs in a uterus appropriately primed with progesterone and estrogen. Blastocysts are the normal inducers of these events, while various nonspecific stimuli, such as the intraluminal infusion of oil, air, and mechanical stimuli, can also initiate certain aspects of the decidual cell reaction (deciduoma) in pseudopregnant or steroid hormonally prepared uteri (Fig. 1B) [28]. Therefore, the induction of uterine decidualization by stimulus is used experimentally as a tool to examine the effect of gene deficiencies on uterine function [1, 2, 29, 30] and the potential for steroidogenesis in ovarian tissues transplanted into ovariectomized mice [31, 32].

Ovarian Steroid Hormones Regulate Blastocyst Implantation and the "Window" of Uterine Receptivity

For successful pregnancy in mice, uterine receptivity for implantation lasts for a limited time (Fig. 2A) [1, 4, 33, 34]. The duration of the receptive stage is also called the "implantation window". At this stage, the uterine environment is able to support blastocyst growth, attachment, and the subsequent implantation events. The ovarian steroids, progesterone (P_4) and 17β-estradiol (E_2), are crucial for implantation in mice (Fig. 2A) [35, 36]. The coordinated actions of P_4 and E_2, which regulate proliferation and/or differentiation of uterine cells in a spatiotemporal manner, establish the implantation window, i.e., on the first day of pregnancy in mice (as indicated by a vaginal plug); preovulatory E_2 secretion induces the proliferation of uterine epithelial cells, and the increase in P_4 levels secreted from the freshly formed corpora lutea initiates stromal cell proliferation from day 3 onward. [37]. The pre-receptive uterus on day 3 of pregnancy becomes receptive on day 4 due to rising P_4 levels and a small elevation in ovarian E_2 secretion (Fig. 2A) [36]. While E_2 stimulates stromal cell proliferation, the coordinated effects of P_4 and E_2 halt uterine epithelial cell proliferation and initiate differentiation [37]. An active blastocyst in the uterus stimulates implantation during a

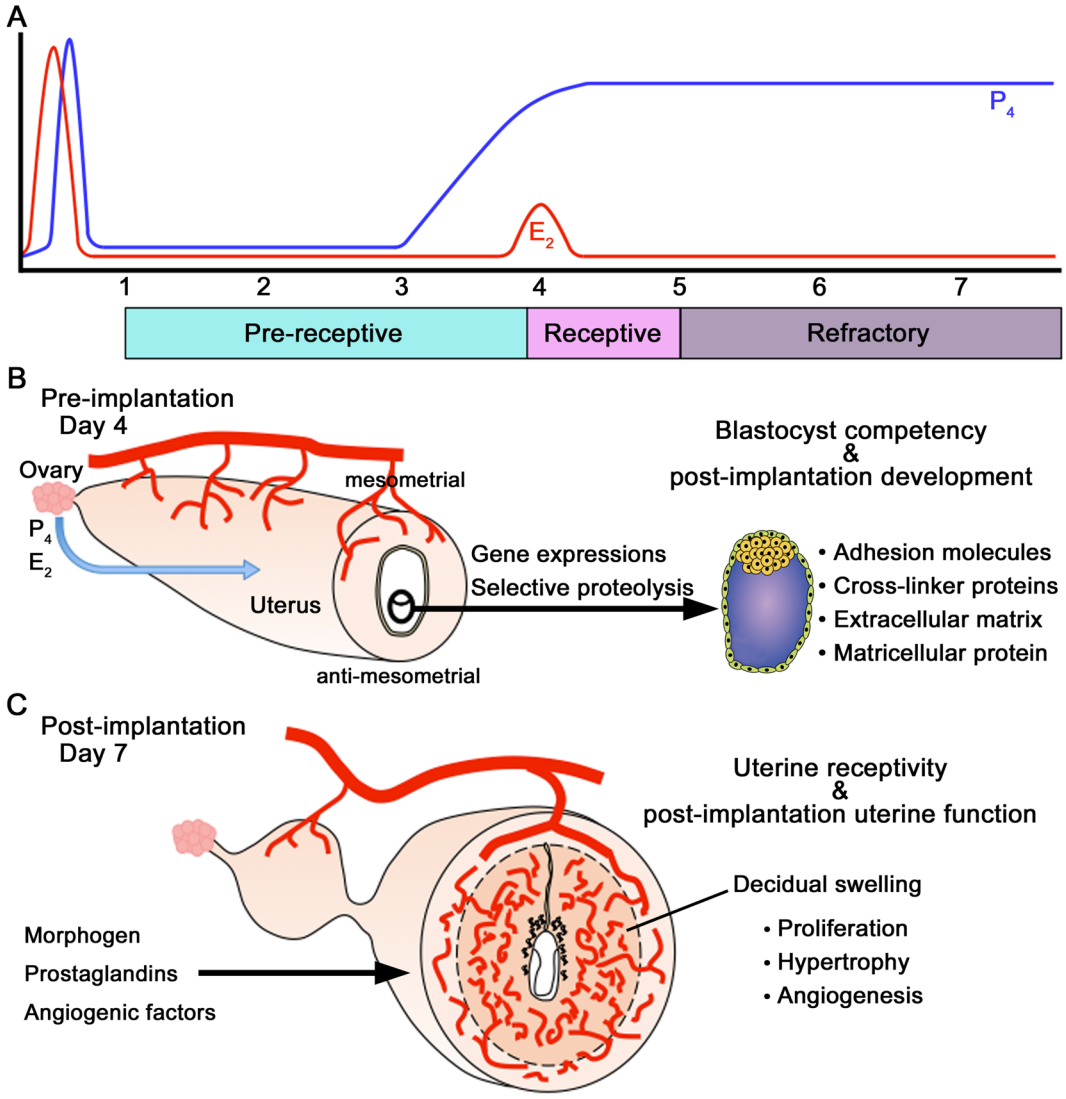

Fig. 2. Regulation of blastocyst implantation, uterine receptivity, and post-implantation development. (A) Regulation of the window for uterine receptivity is achieved by the actions of P_4 and E_2 in the mouse. Uterine sensitivity to implantation is categorized as pre-receptive, receptive, or refractory (nonreceptive) phases. The uterus is pre-receptive on days 1–3 of pregnancy or pseudopregnancy, it is receptive on day 4, and by the afternoon of day 5, it becomes refractory to implantation. The pre-receptive uterus on day 3 of pregnancy becomes receptive on day 4 due to rising P_4 levels and a small elevation in ovarian E_2 secretion. (B) Determinants of blastocyst competency and post-implantation development. This review also describes selective proteolysis, adhesion molecules, crosslinker proteins, extracellular matrix, and matricellular protein. (C) Determinants of uterine receptivity and post-implantation uterine function. This review also describes morphogens, prostaglandins, and angiogenic factors.

normal pregnancy. The first attachment reaction between the blastocyst TE and the uterine luminal epithelium occurs at the end of day 4 of pregnancy in the mouse. After attachment is initiated on day 4 at 2400 h, the stromal cells surrounding the implanting blastocyst begin to proliferate extensively and differentiate into decidual cells (decidualization) [1, 4, 9].

E_2 is a Critical Determinant of the Duration of Uterine Receptivity for Implantation

It has been demonstrated that within a very narrow range, the levels of E_2 determine the duration of the uterine receptivity window for embryo transfer [38]. Although E_2 at different physiological concentrations can initiate implantation, the window of uterine receptivity remains open for an extended period at lower E_2 levels, but rapidly closes at higher levels [38]. The uterine refractoriness that follows the receptive state at high E_2 levels is accompanied by the aberrant expression of implantation-related genes. Therefore, the careful regulation of E_2 levels is an important factor for the improvement of female fertility in *in vitro* fertilization (IVF) and embryo transfer programs.

In contrast, these results suggest that the poor potential of ovar-

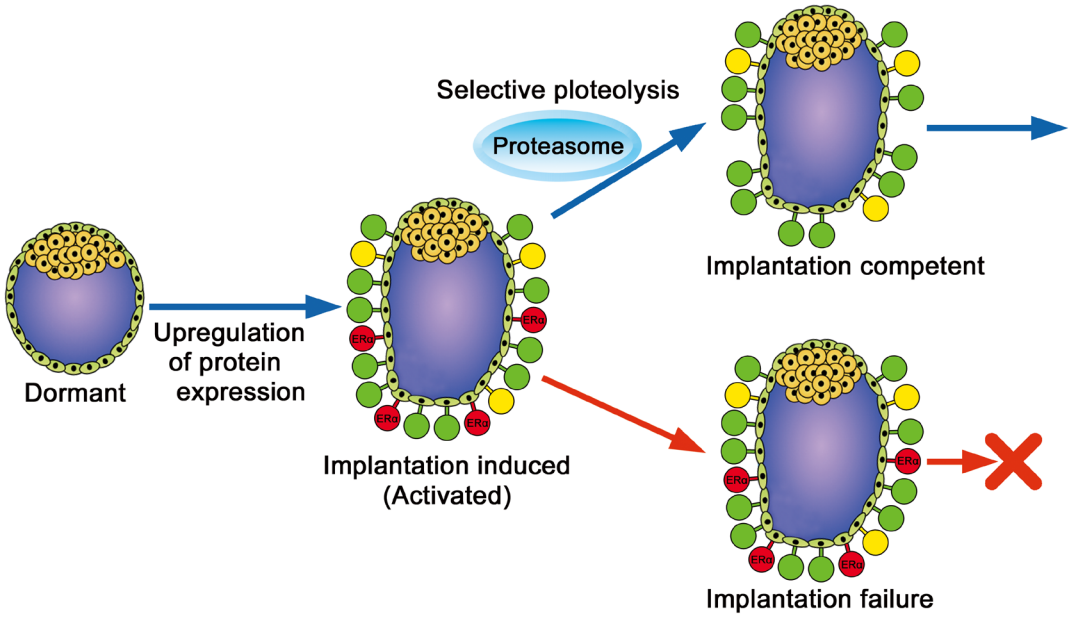

Fig. 3. A schematic diagram of blastocyst implantation competency. Protein expression (green, yellow, and red) is upregulated in implantation-induced (activated) blastocysts. The downregulation of specific proteins (red) in the activated blastocyst is critical for successful implantation. For example, the degradation of ERα is required for blastocyst implantation. Expressed proteins in activated blastocysts are categorized into three groups, i.e., essential (green), expressed but unnecessary (yellow), and obligatorily downregulated (red) to complete blastocyst implantation.

ian steroidogenesis extends the "implantation window". As such, vitrified-warmed ovarian tissue autotransplantation (VOAT) into estrus cycle-ceased ovariectomized mice restored fertility and led to full-term fetal development for the transferred embryos, although steroidogenesis and blood vessel formation in the corpus luteum were less than those in intact mice [31]. Indeed, the rate of live births was similar between VOAT mice that received an embryo transfer on pseudopregnancy day 4 and those that received the transfer on day 5, whereas intact mice that received an embryo transfer on day 5 failed to support pregnancy [32]. For embryo transfer on pseudopregnancy day 5, oocyte warming and IVF can be performed on the day after mating a female with a vasectomized male. If a plug positive female is not obtained, oocyte warming and IVF can be postponed. Therefore, embryo transfer on day 5 could be a useful method for mice with poor ovarian potential for the improvement of female fertility within IVF and embryo transfer programs [32].

Determinants of Blastocyst Competency Using the Delayed-implantation Mouse Model

Delayed implantation is a process in which implantation is postponed for a period of time. This causes the uterus to remain quiescent and an embryo in the blastocyst stage to become dormant. In mice, an ovariectomy early on day 4 (vaginal plug = day 1) prior to pre-implantation E_2 secretion prevents implantation and initiates blastocyst dormancy within the uterine lumen [39]. The delayed implantation can be maintained by continuous P_4 treatment, but can be terminated upon E_2 injection leading to blastocyst activation and subsequent implantation in the uterus approximately 24 h later. For successful implantation in the receptive uterus, the blastocyst must also attain implantation competency, where the activity of the blastocyst determines the window of implantation in the receptive uterus [36, 38]. The delayed implantation model is a powerful tool for defining the molecular signaling components that direct blastocyst activation or dormancy.

An analysis of global gene expression in the delayed implantation model demonstrated that these two different blastocyst physiological states can be distinguished at the molecular level, and that the genes involved control the cell cycle, cell signaling, and energy metabolism (Fig. 2B) [40]. The study also revealed an upregulation of *Hbegf* expression, which encodes heparin-binding EGF-like growth factor (HBEGF), and the HBEGF receptors ERBB1 and ERBB4 in blastocysts [40–42]. Catecholoestrogens produced from primary estrogens in the uterus activate blastocysts [43]. Another lipid-signaling molecule that targets blastocysts is the endocannabinoid anandamide, where endocannabinoid signaling is crucial for implantation in mice [44–46]. Levels of uterine anandamide and blastocyst CB1 are coordinately downregulated with the attainment of uterine receptivity and blastocyst activation respectively, but are elevated in the nonreceptive uterus and dormant blastocyst [46–48]. Anandamide regulates blastocyst functions by differentially modulating mitogen-activated protein kinase (MAPK) signaling and Ca^{2+} channel activity *via* CB1 [48]. This is consistent with findings that the MAPK and phosphatidylinositol 3-kinase/Ca^{2+} signaling cascades are crucial to blastocyst development and activation [49–52].

Degradation of Estrogen Receptor α in Activated Blastocysts is Associated with Implantation

Although estrogen receptor α (ERα, *Esr1*) protein is expressed in blastocysts, its targeted disruption does not affect embryonic development or implantation [53, 54]. Therefore, the expression of ERα in blastocysts is considered unnecessary for the peri-implantation period. In contrast, ERα overexpression results in a decreased number of implantation sites and litter size [55]. These results suggest the possibility of an optimum level of ERα downregulation in blastocyst implantation during the peri-implantation period. Indeed, increased expression of ERα protein in implantation-induced (activated) blastocysts was decreased within 6 h in culture, whereas the expression of other proteins such as breast cancer 1 (BRCA1) was maintained in the blastocysts during culture (Fig. 3) [24]. The selective degradation of ERα expression in activated blastocysts is regulated by the ubiquitin-proteasome pathway (Fig. 2B, 3). Furthermore, downregulation of ERα in the activated blastocyst is associated with the completion of blastocyst implantation [24]. Although the reason for selective proteolysis immediately after expression in activated blastocysts is unclear, elimination of transcription factors to avoid inadequate protein expression could be associated with implantation competent blastocysts.

Adhesion Molecules and Crosslinker Proteins

For the attachment phase of implantation, adhesive signaling systems are required, e.g., numerous glycoproteins and carbohydrate ligands and their receptors are expressed in the TE and luminal epithelium around the time of implantation (Fig. 2B) [56, 57]. CD44 is likely involved in peri-implantation interactions. It recognizes polyanionic glycans including hyaluronan and chondroitin sulfate [58]. Furthermore, CD44 integral membrane proteins crosslink with actin filaments *via* ezrin/radixin/moesin (ERM) proteins in the organization of cortical actin-based cytoskeletons, including microvilli formation [59]. Radixin (also known as RDX) and ezrin (also known as EZR) are involved in the cellular organization of the TE during blastocyst activation prior to implantation in the delayed implantation mouse model, and radixin is particularly involved in preparing the mural TE for implantation, the presumptive site of attachment with the luminal epithelium (Fig. 2B) [60]. In contrast, the ERM-associated adhesive molecules, CD44, CD43 (also known as SPN), ICAM1 and ICAM2, are present in the TE of dormant blastocysts. These findings suggest that in dormant blastocysts prior to activation, adhesive molecules associated with ERM proteins are already positioned in a cell-specific manner to interact with radixin and ezrin in activated blastocysts [60]. Thus, ERM proteins expressed on TE cell surfaces of implantation-induced blastocysts may act as crosslinkers between actin and adhesive molecules and change the cell polarization and/or differentiation for adhesion and attachment with the luminal epithelium.

ECM and Matricellular Protein TINAGL1

The blastocyst is composed of distinct cell types, i.e., the pluripotent inner cell mass (ICM) generates future cell lineages of the embryo proper, while the outer epithelial TE makes the first physical and physiological connection with the maternal uterus for implantation. The invasive trophoblasts of mouse blastocysts adhere, spread, and migrate on ECM substrates [14–17] and penetrate the three-dimensional ECM structures [18]. The TE of the implantation-induced blastocyst alters its functional programming *via* changes in cell surface molecules. The basement membrane consists predominantly of laminins and collagens secreted by the TE and the parietal endoderm of the pre-implantation blastocysts (Fig. 2B) [61] that then enter the implantation stage [62]. The parietal endoderm arises from the ICM in the blastocyst as a result of differentiation events and produces large quantities of ECM proteins to form the Reichert's membrane, which separates the yolk cavity from the maternal tissue [63, 64]. The Reichert's membrane contains laminin and collagen IV [65, 66].

In contrast to the ECM, extracellular matrix proteins that do not contribute directly to the formation of structural elements in vertebrates but serve to modulate cell-matrix interactions and cell function are categorized as matricellular proteins [67]. The mouse ortholog of the gene encoding tubulointerstitial nephritis antigen-like 1 (TINAGL1, also known as adrenocortical zonation factor 1 [AZ-1] or lipocalin 7) has been cloned from mouse adrenocortical cells and is tightly linked with the zonal differentiation of this cell type [68]. TINAGL1 is a matricellular protein that interacts with both structural matrix proteins and cell surface receptors [69]. On the basis of its colocalization and binding ability with laminin 1 and collagens, TINAGL1 was found to be a component of the basal lamina [69]. During the pre-implantation phase of mouse embryonic development, the expression of both *Tinagl1* mRNA and TINAGL1 protein is increased just prior to implantation (Fig. 2B) [66, 70]. In blastocysts, TINAGL1 expression is localized to the TE after hatching from the zona pellucida and is restricted to the basement membrane at the surface of the blastocoele site of the TE just prior to luminal epithelium attachment [66, 70]. In post-implantation embryos, TINAGL1 is an extraembryonic tissue-specific protein and interacts with laminin 1 in the Reichert's membrane. In the uterus, TINAGL1 is expressed in the basement membrane of luminal epithelial cells during the pre-implantation period. During post-implantation, TINAGL1 is markedly expressed in the decidual endometrium, including the uterine capillaries, and it associates with integrins α5 and β1 in the decidualized uterine endometrium [71]. These findings suggest that it plays a physical and physiological role in embryo development and/or decidualization of the uterine endometrium during pregnancy. Indeed, TINAGL1 deficiency affects female mice and results in subfertility phenotypes [72]. In humans, TINAGL1 protein is downregulated in preeclamptic women [73]. Furthermore, the behavior of trophoblasts invading the uterus resembles that of metastatic tumor cells, and recent studies have revealed a novel role for TINAGL1 that is associated with metastasis in cancer cells [74, 75]. Therefore, *Tinagl1* knockout mice would likely substantially contribute to revealing the role of TINAGL1 in reproductive functions and metastasis.

IHH as a Progesterone-responsive Factor Mediating Epithelial–mesenchymal Interactions in the Uterus

The importance of morphogens for uterine receptivity has been reported, including research on hedgehog (HH), WNT, and bone-

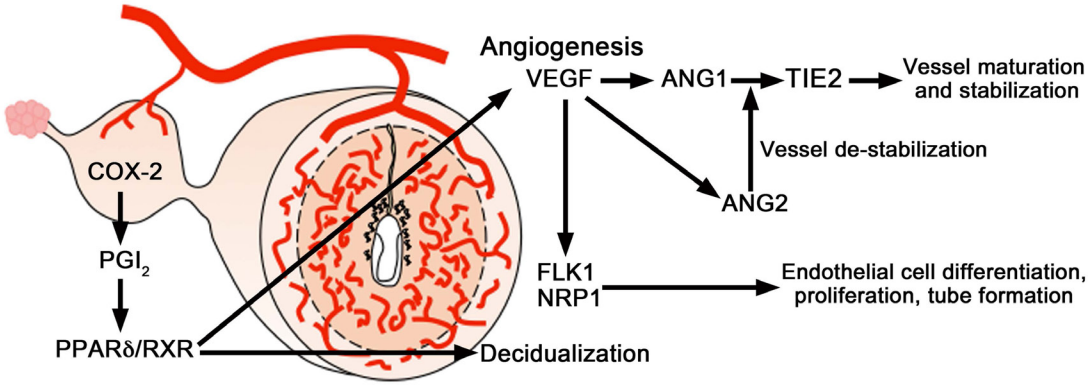

Fig. 4. A schematic diagram of angiogenic signaling in the uterus during implantation. COX2-derived prostaglandins are important for uterine angiogenesis during implantation and decidualization and primarily target the VEGF system, but not the angiopoietin system. The proangiogenic factor VEGF and its receptor FLK1 are important for uterine angiogenesis during the post-implantation period. VEGF in complementation with the angiopoietins (ANG1 and ANG2) and their receptor TIE2 directs angiogenesis during decidualization. ANG1 in collaboration with VEGF induces vessel maturation and maintains vessel leakiness, whereas ANG2 induces vessel destabilization required for further sprouting in the presence of VEGF.

morphogenetic-protein (BMP) signaling (Fig. 2C) [1, 2, 76]. Indian hedgehog (*Ihh*) expression is induced by P_4 in the uterus [77–80]. The genes encoding the components of the HH signalling pathway, *Ihh*, HH-binding protein/receptor Patched (*Ptc*) and the transcription factors *Gli1–3* are expressed in the mouse uterus [77, 80]. *Ihh* expression is P_4-dependent and reaches high levels in epithelial cells on day 4, while that of *Ptc*, *Gli1* and *Gli2* is upregulated in the underlying stroma [77]. In day 4 uterine-explant cultures, recombinant N-sonic hedgehog (N-SHH) stimulates mesenchymal-cell proliferation, a characteristic of the receptive phase [77]. Furthermore, uterine deletion of *Ihh* leads to implantation failure due to poor uterine receptivity [78]. These results suggest that epithelial IHH functions as a paracrine growth factor for stromal cells and that this epithelial–mesenchymal signalling is important for uterine receptivity. The chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII, *Nr2f2*) has been proposed as a downstream target of IHH signaling, and is expressed in the subepithelial stroma [81]. Uterine-specific *COUP-TFII* knockout mice are infertile due to failure of implantation, i.e., epithelial IHH regulates stromal COUP-TFII to control BMP2 and regulate decidualization [81]. In addition, enhanced epithelial estrogen activity impedes the maturation of the receptive uterus in the absence of COUP-TFII [81]. These finding reveal that COUP-TFII plays a critical role in maintaining the balance between E_2 and P_4 activities to establish proper implantation.

Uterine Angiogenesis *via* VEGF and Its Receptors

The control of uterine angiogenesis by angiogenic factors, including vascular endothelial growth factor (VEGF) and its receptors, has been studied to assess its role in uterine angiogenesis during implantation and decidualization [5, 8, 10]. Differential splicing of the *Vegf* gene transcript generates several VEGF isoforms in both humans and mice. $VEGF_{121}$ and $VEGF_{165}$ are the predominant isoforms in humans, whereas $VEGF_{120}$ and $VEGF_{164}$ are the most predominant isoforms in mice [82, 83]. In the mouse uterus, $VEGF_{164}$ mediates vascular changes and angiogenesis in the uterus during implantation and decidualization (Fig. 2C, 4)[83].

The effects of VEGF are primarily mediated by two tyrosine kinase receptors: VEGFR1 [fms-like tyrosine kinase 1(FLT1)] and VEGFR2 [fetal liver kinase 1 (FLK1)/kinase insert domain-containing receptor (KDR)] [84−87]. During the post-implantation period, the expression of *Flk1* was evident in stromal cells close to, but not immediately surrounding, blastocysts on day 5 (Fig. 2, 4). On days 6–8 (Fig. 2, 4), *Flk1* mRNA accumulation occurred in cells in both the mesometrial and anti-mesometrial decidual beds. However, *Flk1* expression was more intense at the mesometrial pole, the presumptive site of placentation and heightened angiogenesis. On day 8, some embryonic cells exhibited a marked accumulation of *Flk1* mRNA. *Flk1* mRNA was absent from the avascular primary decidual zone (PDZ) [83, 88]. During peri-implantation in the mouse uterus, expression levels of *Flt1*, as detected by northern blot hybridization and *in situ* hybridization, were lower than those of *Flk1* [88]. Another multifunctional VEGF receptor is neuropilin-1 (NRP1). The *Nrp1* mRNA expression pattern is similar to that of *Flk1* in the mouse uterus (Fig. 4) [83, 88]. However, it is interesting to note that *Nrp1* mRNA was observed to be more widely distributed than *Flk1*, suggesting that NRP1 is present in stromal cells other than endothelial cells [83]. Collectively, genes encoding murine VEGF isoforms and their receptors, *Flk1*, *Flt1*, and *Nrp1*, are differentially expressed in the mouse uterus in a spatiotemporal manner during implantation, and the predominant $VEGF_{164}$ isoform interacts with FLK1 and NRP1 [83, 88]. These results suggest that the VEGF system is involved in uterine vascular permeability and angiogenesis during implantation (Fig. 4).

Receptor TIE2 and Angiopoietins Substrates

The effects of VEGF are complemented and coordinated by another class of angiogenic factors—the angiopoietins [89]. VEGF acts during the early stages of vessel development [90–92], while

angiopoietin 1 (Ang1, also known as Angpt1) acts later to promote angiogenic remodeling, including vessel maturation, stabilization, and leakiness [93–95]. In contrast to the agonistic functions of ANG1, ANG2 behaves as an antagonist, i.e., ANG1 and ANG2 are naturally occurring positive and negative regulators of angiogenesis, respectively. They interact with an endothelial cell-specific tyrosine kinase receptor called TIE2 [96]. Collectively, VEGF and its receptor FLK1 are primarily important for uterine vascular permeability and angiogenesis before and during the attachment phase of implantation, whereas VEGF, together with the angiopoietins and their receptor TIE2, direct angiogenesis during decidualization after implantation (Fig. 4) [97].

Prostaglandins Derived from COX2 Participate in Uterine Angiogenesis during Implantation and Decidualization

Prostaglandins are also likely to participate in uterine vascular permeability and angiogenesis during implantation and decidualization, i.e., COX2 (also known as PTGS2)-derived prostaglandins participate in uterine angiogenesis during implantation and decidualization (Fig. 2C, 4) [97]. *Cox2*(–/–) mice show implantation and decidualization failure. The attenuation of uterine angiogenesis in these mice is primarily due to defects in VEGF signaling, rather than the angiopoietin system. $Vegf_{164}$ expression is remarkably downregulated in stromal cells at the blastocyst site in *Cox2*(–/–) mice. A prostacyclin (PGI_2) agonist, carbarprostacyclin (cPGI; a more stable analog of PGI_2), functions as a ligand for peroxisome proliferator-activated receptor δ (PPARδ) and facilitates its heterodimerization with the retinoid X receptor (RXR). cPGI together with the RXR agonist, 9-*cis*-retinoic acid (9-*cis*-RA), improves the poor implantation in *Cox2*(–/–) mice [97, 98]. Administration of cPGI and 9-*cis*-RA also restored the expression of *Vegf*, as well as the number of blood vessels, leading to improved implantation. These results suggest COX2-derived prostaglandins influence uterine angiogenesis primarily by affecting the VEGF system during implantation (Fig. 4). In contrast, no significant difference was noted in the expression patterns of angiopoietins and *Tie2* between the *Cox2*(–/–) and wild type mice, although the decidual response was depressed in *Cox2*(–/–) mice. Therefore, the angiopoietin signaling involved in uterine angiogenesis is distinct from that of the COX2-derived prostaglandins. Collectively, COX2-derived prostaglandins direct angiogenesis during implantation and decidualization by differentially regulating VEGF and angiopoietin signaling (Fig. 2C, 4).

Differential Regulation of E_2 and P_4 for Uterine Vascular Permeability and Angiogenesis

The expression of VEGF and its receptors in the uterus is affected by steroid hormones [88]. E_2 rapidly induces uterine vascular permeability and *Vegf* transcription through the nuclear estrogen receptor [88], and the *Vegf* gene contains estrogen response elements [99, 100]. P_4 also upregulates uterine *Vegf* expression through activation of the nuclear progesterone receptor, but at a slower rate [99, 100]. E_2 was widely believed to be a potent stimulator of uterine angiogenesis during normal reproductive processes *in vivo* because vascular permeability is considered a prerequisite for angiogenesis and E_2 rapidly stimulates uterine vascular permeability and *Vegf* expression. However, the evidence from molecular, genetic, physiological, and pharmacological studies has revealed that E_2 and P_4 have different effects *in vivo*. E_2 promotes uterine vascular permeability but profoundly inhibits angiogenesis, whereas P_4 stimulates angiogenesis with little effect on vascular permeability [101]. These effects of E_2 and P_4 are mediated by the differential spatiotemporal expression of proangiogenic factors in the uterus [101].

Differential Expression and Hormonal Regulation of Motin Family Members in the Uterus

Angiomotin (AMOT) is a vascular angiogenesis-related protein that was initially identified as an angiogenesis inhibitor angiostatin-binding protein that can induce endothelial cell migration and tubule formation, and therefore, promote angiogenesis [102–104]. There are also two angiomotin-like proteins, AMOTL1 and AMOTL2. These three proteins belong to the motin family characterized by a highly conserved coil-coil domain, PDZ binding domain, and glutamine-rich domain [103]. AMOTL1 and AMOTL2 also play important roles in cell migration and angiogenesis [105–108]. The expression patterns of motin family members vary during development, i.e., there is a spatiotemporal-dependent expression of *Amot*, *Amotl1*, and *Amotl2* in the mouse uterus during pre-implantation and post-implantation periods [109]. Specifically, ovarian steroid hormones regulate the differential expression of motins. The expression of *Amot* is induced by P_4 in stromal cells. *Amotl1* expression is upregulated by both P_4 and E_2 in stromal cells; however, E_2 increases *Amotl1* expression for only a limited time—12 h after its expression diminishes. In contrast, P_4 regulates the expression of *Amotl2* in stromal cells while E_2 regulates its expression in luminal epithelial cells. Collectively, *Amot*, *Amotl1*, and *Amotl2* are differentially expressed in uterine cells during peri-implantation, and their expression is differentially regulated by P_4 and E_2.

Conclusions

Although many important discoveries have been made in this field, the knowledge of the complex events that occur during implantation is insufficient to prevent infertility caused by implantation failure. This review article describes the molecular and cellular events during blastocyst implantation in the receptive uterus in mouse models. These observations may help to elucidate the mechanisms underlying the completion of blastocyst implantation that allow for the establishment of pregnancy. The implantation rate of IVF-derived blastocysts after embryo transfer remains low, with poor embryo quality among the limiting factors for low pregnancy success in IVF. Therefore, it is possible that the inadequate expression of specific proteins in IVF-derived blastocysts induced by culture contributes to low implantation rates. In this instance, appropriate treatments to induce up- and/or down-regulation *in vitro* culture before embryo transfer may improve the implantation rate and embryonic development during the post-implantation period. However, further investigation is still required to develop strategies to further improve the success of implantation and pregnancy.

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