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Review

Molecular complexity in establishing uterine receptivity and implantation

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Abstract. Implantation is the process by which the blastocyst comes into intimate physical and physiological contact with the uterine endometrium. This process is governed by an intimate cross-talk between the activated blastocyst and the receptive uterus. An increased understanding of mammalian implantation has been gained through the use of the mouse model. This review highlights the more recently defined signaling cascades involved in this dialogue, focusing specifically on cyclooxygenase-2-derived prostaglandins, endocannabinoids, Wnt proteins, homeotic transcription factors, and immunophilins. Unraveling the nature of these signals and discovering additional molecular cascades may lead to strategies to correct implantation failure and improve pregnancy rates in women.

Key words. Implantation; uterus; blastocyst; cannabinoids; immunophilins; growth factor.

Introduction

One of the prerequisites for mammalian reproduction is an effective reciprocal interaction between the implantation-competent blastocyst and the receptive uterus. Unless this dialogue at the molecular and cellular level is established, the embryo will not implant. Although numerous signaling pathways crucial to implantation have been described, comparatively little is known about the hierarchy of events that trigger the attachment of a blastocyst to a receptive uterine luminal epithelium. This review will discuss recent developments in the field, primarily using mice as an animal model.

The processes essential to implantation include synchronized preimplantation embryo development to the blastocyst stage, escape of the blastocyst from the zona pellucida, and differentiation of the uterus to the receptive state [1, 2]. The establishment of a differentiated uterus to support blastocyst implantation primarily depends on the coordinated effects of estrogen and progesterone (P_4) [2]. In rodents, the first conspicuous sign for the initiation of implantation is an increased endometrial vascular permeability at the site of blastocyst attachment. This process can be visualized in the uterus as discrete blue bands after an intravenous injection of a blue dye solution [1, 2]. In mice, the attachment reaction occurs in the evening (2000–2400 h) on day 4 of pregnancy (day 1 = vaginal plug) [3]. This is preceded by uterine luminal closure, which results in an intimate apposition of the blastocyst trophectoderm with the luminal epithelium [1, 3–5]. Blastocyst attachment is followed by luminal epithelial apoptosis and localized stromal decidualization at the site of implantation [6]. This results in subsequent adherence and penetration by trophoblast cells through the stroma in a regulated manner [7–9].

Steroid hormonal regulation of early pregnancy

The uterus is comprised of heterogeneous cell types that respond differentially to estrogen and P_4. For a successful

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pregnancy to occur, fertilized eggs must develop to blastocysts, transit to the uterus, establish an intimate contact with uterine tissues, be nurtured through embryogenesis, and delivered at birth. On the maternal side, the uterus must be transiently receptive for implantation and undergo extensive remodeling to decidualization.

In mice and rats, estrogen stimulates proliferation and differentiation of luminal and glandular epithelia, whereas in the stroma, these processes require both estrogen and P_4 [1, 10]. On day 1 of pregnancy in mice, uterine epithelial cells undergo proliferation under the influence of preovulatory ovarian estrogen secretion and this proliferation, to some extent, continues through day 2. Rising P_4 levels secreted from the newly formed corpora lutea initiate stromal cell proliferation from day 3 onward. The stromal cell proliferation is further stimulated by a small amount of ovarian estrogen secreted on the morning of day 4. These coordinated effects of estrogen and P_4 result in the cessation of uterine epithelial cell proliferation, initiating differentiation [10]. After implantation has been initiated on the night of day 4, stromal cells surrounding the implanting blastocyst begin to proliferate extensively and differentiate into decidual cells [10].

Window of uterine receptivity

In mice, uterine sensitivity with respect to steroid hormonal requirements and implantation is divided into prereceptive, receptive, and nonreceptive (refractory) phases as defined by embryo transfer experiments in pseudopregnant mice [1, 2]. During normal pregnancy, the presence of an active blastocyst in the uterus is the stimulus for implantation. However, various nonspecific stimuli, such as intraluminal infusion of oil, air, or mechanical stimuli can also initiate certain aspects of the decidual cell reaction in pseudopregnant or steroid hormonally prepared uteri [11].

Uterine receptivity only occurs for a limited period during pregnancy or pseudopregnancy. In normal pregnant or pseudopregnant mice, the prereceptive uterus on days 1–3 becomes fully receptive on day 4 (the day of implantation), while by late day 5, the uterus becomes refractory and fails to initiate implantation [2]. It is well accepted that the concentration of estrogen within a very narrow range determines the duration of the window of receptivity. The uterus can only be rendered receptive to blastocyst implantation if exposed to a small amount of estrogen after 24–48 h of P_4 priming [12].

Delayed implantation is a process by which implantation is postponed for a certain period of time, and it occurs naturally in a variety of species. The uterus remains in a quiescent state, and embryos at the blastocyst stage become dormant [13]. In mice and rats, ovariectomy before the presumed estrogen secretion in the morning of day 4 of pregnancy results in the failure of implantation and initiates a state of dormancy of the blastocyst within the uterine lumen [14, 15]. This condition is referred to as delayed implantation and can be maintained for many days by continued treatment with progesterone. The process of implantation with blastocyst activation can be rapidly initiated by a single injection of estrogen in the P_4-primed uterus [14, 15]. The mechanisms by which estrogen mediates the processes of blastocyst activation and implantation are poorly understood. Delayed implantation also occurs naturally (facultative) during lactation after postpartum ovulation and fertilization of eggs in mice and rats [16, 17]. Whether this phenomenon occurs in humans is not yet known. The delayed-implantation models in mice and other species are often exploited to better understand the molecular signaling that occurs between an implantation-competent blastocyst and the receptive uterus.

Ovarian P_4 and estrogen tightly condition the uterus for implantation and P_4 is specifically required for pregnancy maintenance. The actions of P_4 include holding the proliferative effects of estrogen in check and inhibiting uterine smooth-muscle contraction until birth. Progesterone and estrogen act primarily through the nuclear progesterone receptor (PR) and estrogen receptor-α (ERα), respectively, to activate transcription of genes involved in uterine receptivity and decidualization. Genes activated through PR and/or ERα include homeobox transcription factors, growth factors, cytokines, and a variety of signaling molecules [18]. Definitive evidence for the roles of these genes in implantation and/or decidualization has been forthcoming from an array of mouse gene knockout models [19].

Recent evidence suggests that a short delay in the attachment reaction produces an adverse ripple effect throughout pregnancy, with aberrant spacing of embryos, defective placentation, resorption, and retarded development of fetuses, ultimately giving rise to a poor pregnancy outcome [20–22]. This concept of timing as a crucial component of normal feto-placental development and determinant of pregnancy outcome comprises a novel theme whereby early embryo-uterine interaction directs the developmental programming for the remainder of gestation.

Implantation is a complex process involving spatiotemporally regulated endocrine, paracrine, autocrine, and juxtacrine modulators that span cell-cell and cell-matrix interactions. The embryo also functions as an active unit with its own molecular program of cell growth and differentiation. Thus, deficiencies in uterine receptivity, embryo development, or the embryo-uterine dialogue will compromise fertility. However, the precise sequence and details of the molecular interactions involved have not yet been defined. This review focuses primarily on the recently identified signaling network involving lipid signaling, growth factors, Wnt proteins, homeotic transcription factors, and immunophilins (fig. 1). Because each of

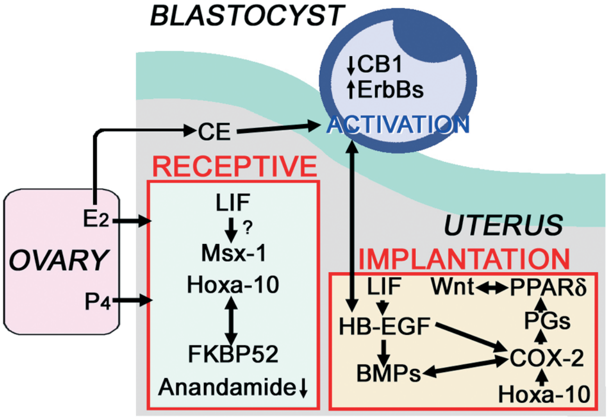

Figure 1. Aspects of the molecular signaling cascade in uterine receptivity and implantation in the mouse. CE, catecholestrogen; PGs, prostaglandins; HB-EGF, heparin-binding epidermal growth factor; BMPs, bone morphogenetic proteins; LIF, leukemia inhibitory factor.

these signaling pathways plays an important role in the embryo-uterine dialogue during implantation, any alteration in them is likely to alter dramatically the implantation process and, therefore, decrease the probability of a successful pregnancy outcome.

Prostaglandin signaling

Cylooxygenase-1 (Cox-1) and Cox-2 mediate the conversion of arachidonic acid into prostaglandin H_2 (PGH_2) which is then converted to various PGs by specific synthases [23]. Although Cox-1 and Cox-2 isoforms are encoded by separate genes, *Ptgs1* and *Ptgs2*, respectively, they share similar structural and kinetic properties and show distinct cell-specific expression and regulation [23]. Cox-1 is normally thought to serve ‘housekeeping’ functions as a constitutive enzyme, whereas Cox-2 is highly inducible by diverse stimuli including cytokines, growth factors, mitogens, and tumor promoters, and regulates inflammation, differentiation, mitogenesis, and angiogenesis [23].

The processes of ovulation and implantation are considered analogous to proinflammatory responses, hence the speculation that PGs play a role in these events. Specifically, PGs play a role in follicular rupture during ovulation, endometrial vascular permeability during implantation, and decidualization [24, 25]. The unique expression pattern of Cox-1 and Cox-2 genes in the periimplantation mouse uterus further suggests an important role for PGs in these processes [24]. Cox-1 is expressed in uterine luminal and glandular epithelial cells on the morning of day 4 of pregnancy, but its expression becomes very low to undetectable in epithelial cells by the time of the attachment reaction. In contrast, Cox-2 is expressed in the luminal epithelium and underlying stromal cells solely at the site of blastocyst attachment. This expression pattern suggests that Cox-2 expression during the attachment reaction is critical to implantation [24]. Indeed, gene-targeting experiments have demonstrated that Cox-2-derived PGs are essential for implantation and decidualization [26–28]. In contrast, Cox-1$^{-/-}$ mice have normal fertility, albeit showing defects in parturition [26, 29]. Interestingly, experiments with Cox-1$^{-/-}$ mice suggest that the loss of Cox-1 is compensated by the expression of Cox-2 for implantation [29]. Conversely, depending on the genetic background, Cox-1 can rescue female infertility in Cox-2-deficient mice [30]. The increasing evidence of altered phenotypes depending on the genetic background of mice has significant implications, since nonsteroidal antiinflammatory drugs (NSAIDs) and Cox-2-selective inhibitors are used without regard for genetic diversities among human populations, while if used more carefully to target specific populations could perhaps exert their effects without the serious side effects.

The roles of PGs in reproduction are further illustrated by the reduced fertility of female mice lacking cytoplasmic phospholipase $A_{2\alpha}$ ($cPLA_{2\alpha}$), which is involved in the liberation of arachidonic acid from membrane phospholipids for PG synthesis by the Cox system [31–33]. The reduced fertility in these females is due to deferral of on-time implantation, leading to subsequent retarded feto-placental development and reduced litter size [20]. This phenotype indicates that the $cPLA_{2\alpha}$-Cox-2 axis is crucial to implantation. More important, studies with $cPLA_{2\alpha}^{-/-}$ mice instigated the novel ripple effect hypothesis during the course of pregnancy, i.e., blastocysts implanting beyond the normal window of receptivity create an adverse ripple effect throughout the course of pregnancy. This ripple effect leads to aberrant uterine spacing of embryos, defective placentation, resorption, and retarded development of fetuses ultimately giving rise to poor pregnancy outcome [20, 21]. Recently, another knockout mouse model exhibiting a similar phenotype has been reported. Lysophosphatidic acid (LPA) is a small bioactive lipid, and mice deficient for LPA_3, one of the G-protein-coupled receptors for LPA, demonstrate a novel and critical role for this lipid in implantation and pregnancy maintenance [22]. While PGs belong to the eicosanoid class, LPA belongs to the lysophospholipid (LP) group. LPs influence a range of processes including angiogenesis, vascular maturation, and neural development by activating cell surface G-protein-coupled receptors [34]. LPA specifically executes its effects by activating at least four G-protein-coupled receptors, LPAs 1–4 [35]. While mice with deletion of LPA_1 or LPA_2 have normal reproduction, Chun and colleagues showed that LPA_3-deficient mice have a strikingly similar phenotype to the $cPLA_2$ knockout mice, exhibiting poor fertility with deferred implantation, retarded fetal development, embryo crowding, sharing of placentas by multiple embryos and reduced litter size. These mice, when treated with PGs, resume on-time implantation, but embryo crowding remains [22]. Similar results have been observed with cPLA$_{2\alpha}$- and Cox-2-deficient mice [20, 28, 30], suggesting that LPA$_3$ converges onto the LPA$_3$/Cox-2 signaling axis. One unresolved issue concerns the aberrant spacing of embryos. While this phenotypic evidence implies that an LPA$_3$-cPLA$_{2\alpha}$-Cox-2 signaling axis is important for embryo spacing, no evidence has yet been found to rescue the spacing defect. The molecular mechanism that initiates the ripple effect also remains unknown.

Cannabinoid/endocannabinoid signaling

Exposure to marijuana and its cannabinoid derivatives results in many adverse effects on reproductive functions including reduced fertilizing capacity of sperm, retarded development of embryos, fetal loss, and pregnancy failure [36–39]. The effects are primarily mediated by two G-protein-coupled cannabinoid receptors CB1 and CB2 [40, 41]. The discovery of CBs was followed by the identification of two major endogenous cannabinoid ligands, arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG) [42, 43]. Both anandamide and 2-AG bind and activate either CB1 or CB2, and mimic most of the effects of natural and synthetic cannabinoids.

The mouse uterus synthesizes anandamide, and its levels fluctuate in the uterus during early pregnancy coincident with the window of uterine receptivity for implantation [44]. Thus, anandamide levels are lower in the receptive uterus and at implantation sites, but higher in the nonreceptive uterus and at interimplantation sites [44, 45]. The progesterone-treated delayed implanting uterus also shows elevated levels of anandamide, but these levels are down-regulated with the termination of delayed implantation by estrogen [9]. Higher anandamide levels, therefore, are associated with uterine nonreceptivity.

Functional CB1 is expressed in preimplantation embryos [7, 46]. This suggests that embryonic CB1 is a potential target for both natural cannabinoids and endocannabinoids. Indeed, exposure of early embryos to cannabinoids is detrimental to blastocyst formation, zona hatching, and trophoblast outgrowth, and these effects are mediated via CB1 [7, 8, 44, 47]. However, some of these effects are dependent on ligand concentrations and developmental stages of the embryo. While a lower dose of anandamide accelerates trophoblast outgrowth in vitro, this growth is attenuated at a higher dose [47, 48]. These results show that a narrow range of cannabinoid/endocannabinoid concentrations regulates embryonic development. Since uterine anandamide levels vary depending on the stages of uterine receptivity and nonreceptivity, these observations provide evidence that endocannabinoid effects are biphasic and differentially executed depending on the embryonic stage and endocannabinoid levels in the uterine microenvironment [45, 47]. Thus, a regulated level of uterine anandamide and embryonic CB1 during early pregnancy is important for preimplantation embryonic development and implantation. This speculation is consistent with the recent observations of asynchronous preimplantation embryo development in CB1 mutant mice, and inhibition of implantation in wild-type mice, but not in CB1$^{-/-}$ mutant mice, with experimentally induced sustained levels of cannabinoids [9].

Collectively, the expression of cannabinoid receptors in the preimplantation mouse embryo, synthesis of anandamide in the uterus, and the dose- and stage-specific effects of anandamide on embryo development and implantation suggest that ligand-receptor signaling with endocannabinoids/cannabinoids is important for these events. However, the mechanism by which biphasic effects of endocannabinoid ligand-receptor signaling are mediated has yet to be defined. One possibility is that lower, but not higher, levels of uterine endocannabinoids render blastocysts implantation competent by stimulating MAP kinase signaling, which is known to stimulate cell proliferation and differentiation [49].

Preimplantation embryo development and uterine preparation for implantation are important processes that impact women's health issues, including infertility and marijuana use. Basic research to better understand these events will help alleviate problems of infertility. Recent investigations indicating the embryo and uterus as targets for cannabinoid signaling in mice is now also thought to be important for pregnancy outcome in humans. For example, the observation that heightened levels of cannabinoids inhibit implantation in mice subsequently led to the discovery that elevated peripheral levels of anandamide induce spontaneous pregnancy losses in women [50, 51].

Wnt signaling corroborates with homeobox signaling during implantation

The proteins of the Wnt family are highly conserved secreted signaling molecules involved in cell-cell interactions during embryogenesis and tumorigenesis [52, 53]. Wnt proteins bind to the Frizzled (Fzd) family of cell surface receptors [53]. The classical canonical pathway involves several cytoplasmic components with the end result consisting of the liberation of β-catenin, which enters the nucleus to form a complex with T cell factor to activate transcription of Wnt target genes [54]. However, Wnt proteins also signal through noncanonical pathways including Wnt/Ca^{2+} and Wnt/Jun N-terminal kinase pathways. Until now, 19 Wnt and 8 Fzd proteins have been identified in the mouse, suggesting distinct and overlapping functions of Wnt signaling. Targeted deletion of several Wnt genes leads to specific developmental defects. For example, deletion of Wnt 2 results in placental defects [55], while deletion of Wnt 7a results in defects in limb polarity, female infertility, and abnormal uterine patterning [56–58]. Interestingly, Wnt 7a is expressed in the uterine glandular and luminal epithelium in newborn mice, and in the luminal epithelium in adult females [57].

Fzd proteins resemble G-protein-coupled receptors with a serpentine structure containing seven transmembrane helical domains and a cytoplasmic carboxy terminal. They are endowed with cysteine-rich domains, known as Fzd domains [59]. Several proteins have strong homology with the cysteine-rich domain of Fzd proteins, but lack the transmembrane domain. These molecules are termed secreted Fzd-related proteins (sFRPs). Five sFRPs (sFRPs 1–5) have been identified in the mouse. sFRPs inhibit Wnt signaling by competing with Fzd for Wnt ligands or in a dominant-negative fashion by forming a nonsignaling complex with Fzds [53]. We have recently observed aberrant uterine expression of sFRP4 and Wnt 4 in Hoxa-10$^{-/-}$ mice, suggesting an important interaction between Wnt and homeobox signaling during implantation [60]. One emerging theme is that sFRPs, acting as anti-Wnts, participate in the regulation of morphogenetic gradients or zones of Wnt signaling. There is also evidence that similar gradient zones created for bone morphogenetic protein (BMP) signaling by its antagonists Noggin and Chordin collaborate with Wnt gradients in specifying cell fates and demarcating boundaries [53, 61].

In both mice and humans, stromal cells surrounding the implanting blastocyst undergo decidualization, eventually embedding the embryo into the stromal bed. However, the mechanism by which the blastocyst is directed to the antimesometrial luminal epithelium or by which the orientation of the blastocyst is achieved at the time of implantation remains elusive [62, 63]. Wnt 4 signaling in collaboration with those of BMP and fibroblast growth factor (FGF) is speculated to help in orienting the implantation chamber in the antimesometrial-mesometrial direction and specifies these boundaries during implantation and decidualization. The unique expression patterns of BMPs and FGFs in the uterus during implantation lend support to this theory [64]. For example, Wnt 4 is undetectable in the uterus on the morning of day 4 of pregnancy, but appears in the decidualizing stromal cells surrounding the embryo at the onset of attachment at midnight on day 4, with a further increase on day 5. This expression then spreads into the cells forming the secondary decidual zone (SDZ) on day 8 in parallel with the expression of sFRP4 in a thin layer of undifferentiated stromal cells separating the myometrium from the SDZ. Together, these localization patterns implicate the importance of Wnt 4 signaling in establishing this boundary [60, 64]. An inverse relationship has been shown with respect to the expression of BMP2 and its antagonist Noggin during implantation and decidualization. Furthermore, antimesometrial expression of FGF2 in contrast to that of FGF10 at the mesometrial pole contributes to the tenet that antimesometrial-mesometrial orientation of the uterus during early pregnancy is associated with differential gene expression [60, 64]. The expression pattern of FGF2 eventually overlaps with those of BMP2 and Wnt4 [64]. While these developmental genes are well known to play crucial roles in establishing boundaries and polarities during embryogenesis, the aforementioned recent studies suggest that they are also important for establishing the orientation of the growing implantation chamber and creating boundaries to prevent undifferentiated stromal cells from decidualization and restoring these undifferentiated precursor stromal cells to provide normal stromal tissue after delivery [60]. More insight into the molecular mechanisms involved in delineating these boundaries awaits further studies.

Msx-1 (formerly named Hox-7.1) is an important homeobox gene [65]. Mouse Msx-1, an ortholog of the *Drosophila msh*, is involved in several developmental processes, including craniofacial and tooth development [66–68]. Regulation of Msx-1 is thought to depend on coordinated interactions between the epithelial and mesenchymal compartments involving BMP and Wnt signaling [68]. Although Hox genes have critical roles during embryogenesis, their functions during adult life are limited due to the reduced or absent developmental plasticity of most tissues. One of the exceptions is the female reproductive tract, initially rudimentary at birth but then undergoing extensive morphological and functional changes during the reproductive cycles and pregnancy in adult life. Because of this developmental plasticity, it is expected that developmental genes encoding the members of the homeobox, Wnt, and BMP families have temporal and cell-specific functions in the uterus during pregnancy when it undergoes extensive morphological and functional changes. Msx-1 was shown to be expressed in uteri of nonpregnant mice but drastically down-regulated during pregnancy [67]. However, our recent study shows that Msx-1 is also expressed during pregnancy but in a temporal and cell-specific fashion with respect to implantation [60]. Specifically, although the expression of Msx-1 in the uterine epithelium on the morning of day 4 is coincident with epithelial differentiation for the preparation of implantation, its down-regulation in the evening of day 4 with approaching implantation, and further decreases with the initiation and progression of implantation suggest that a tightly regulated transient expression of Msx-1 is critical to both uterine receptivity and implantation. This is consistent with the recent finding that mice lacking leukemia inhibitory factor (LIF) with implantation failure have elevated expression of Msx-1 in the evening of day 4 and past the anticipated time of implantation [60].

In contrast to Msx-1 expression in the epithelium, Hoxa-10 and Hoxa-11 are expressed in uterine stromal cells before implantation and then in decidual cells after implantation. Because Hoxa-10 is correctly expressed in the LIF$^{-/-}$ uterus, and Msx-1 is correctly expressed in the Hoxa-10$^{-/-}$ uterus, Msx-1 expression has been suggested to be independent of Hoxa-10 but responsive to LIF in the pregnant mouse uterus [60, 69]. Collectively, these recent studies suggest that whereas a tightly regulated transient expression of Msx-1 in the uterus is important for uterine receptivity and implantation, coordinated Wnt 4 signaling in collaboration with Hoxa-10 signaling is also important for maintaining boundaries of the uterine tissue compartments that undergo extensive remodeling during implantation and for establishing the antimesometrial-mesometrial orientation of the implantation chamber. The definitive role of Msx-1 in uterine biology and implantation will require cell-specific conditional knockouts in the uterus because genome-wide mutation of this gene produces homozygous null pups that die soon after birth due to craniofacial defects [66].

The immunophilin-steroid receptor signaling axis cooperates with homeotic factors

The pathway for assembly of steroid receptor/chaperone complexes is complicated, involving heat shock proteins (Hsps), specifically Hsp70 and Hsp90, and a variety of cochaperones. Nuclear steroid receptor/chaperone complexes are differentiated functionally and structurally based on their association with immunophilins, such as FKBP51, FKBP52, cyclophilin 40 (Cyp40) or protein phosphatase 5 (PP5). Recent reports show that the glucocorticoid receptor (GR), ER, and PR, when complexed with Hsp90, display preferential binding with specific immunophilins [70]. For the PR, the mature complex containing Hsp90 should be maintained for the receptor to bind its ligand with high affinity. At steady-state, the PR exists in the mature complex with an Hsp90 dimer, the Hsp-binding protein p23, and either FKBP51 or FKBP52. There is a constant assembly and disassembly of these components, so that different stages of the complex, termed early and intermediate, are present within a cell at any given time [70]. However, only the mature receptor complex is capable of binding hormone with both high efficiency and affinity. The mature receptor complex is repressed until hormone binding promotes the dissociation of chaperones, thereby derepressing the receptor.

FKBP52 belongs to the subclass of immunophilin FK506-binding proteins based on its ability to bind an immunosuppressive drug, FK506. FKBP52, FKBP51, and Cyp40 were initially characterized as Hsp90-binding proteins because of their conserved tetratricopeptide repeat (TPR) domains [71]. These domains characterize the Hsp90-binding immunophilins and target the immunophilin to Hsp90 receptor complexes. While the FKBPs and Cyp40 lack sequence homology, they share immunosuppressant-sensitive peptidyl-prolyl cis-trans isomerase (PPIase) activity, thought to be a rate-limiting step in protein folding [72]. While roles for Hsp90 and p23 are relatively well-defined, functions of immunophilins in the receptor complexes are not yet well-characterized.

FKBP52 not only influences PR complex formation, but FKBP52 is also suggested to facilitate rapid translocation of the PR from the cytoplasm to the nucleus following hormone binding [73]. This is evident from binding of FKBP52 to a nuclear localization signal (NLS) and to dynein [73–75]. These data collectively suggest that FKBP52 plays a role in shuttling the PR complex along microtubules within the cytoplasm or in nucleocytoplasmic transport. Interestingly, FKBP52 and FKBP51 compete for a common binding site on Hsp90 and thus compete for assembly with receptor complexes [76]. The existence of functional differences between these two immunophilins is surprising given the 70% homology in their amino acid sequences [77]. Specifically, FKBP52 elevates the hormone-binding affinity of the PR in an Hsp90- and PPIase-dependent manner, while FKBP51 antagonizes the actions of FKBP52 and reduces PR-binding affinity, thereby reducing secondary responses to hormones [76]. The infertile phenotype of FKBP52 mutant mice implies an important role for FKBP52 in reproductive functions, while FKBP51 mutant mice display no reproductive defects [31].

Hoxa-10 is an abdominalB-like homeobox gene whose role in development has been defined by gene-targeting experiments [78, 79]. Hoxa-10-deficient mice exhibit female infertility, with the proximal region of the uterus showing partial homeosis into an oviduct-like structure. However, the cause of infertility in these females is rather due to impaired stromal cell proliferation and decidualization [80]. Furthermore, embryo transfer experiments have conclusively shown the maternal requirement for Hoxa-10 in both implantation and decidualization [79, 81].

Stromal cell proliferation in Hoxa-10$^{-/-}$ mice in response to ovarian P_4 and estrogen is severely compromised, while epithelial cell proliferation remains normal in response to estrogen [81]. That stromal cell responsiveness to P_4 with respect to cell proliferation is impaired in this mouse model suggests that Hoxa-10 induces genes that are vital for stromal cell proliferation and differentiation and that Hoxa-10 functions as a mediator of P_4 effects in implantation. We have recently shown that FKBP52 is a downstream target of Hoxa-10, contributing to the regulation of stromal cell proliferation and differentiation during uterine receptivity, implantation, and decidualization [82]. The mouse uterus shows a unique temporal and cell-specific expression of FKBP52 around the time of implantation, suggesting that this immunophilin is intimately involved with the implantation process. Since P_4 effects are correlated with epithelial cell differentiation and stromal cell proliferation on day 4, perhaps FKBP52 expression in the epithelium and stroma directs these events in the context of cell types. Indeed, FKBP52 expression patterns correlate well with nuclear PR expression patterns [18, 82]. Preliminary evidence showing infertility in FKBP52 null mice further suggests that a Hoxa-10-FKBP52 signaling axis is critical to uterine receptivity and implantation. Collectively, these studies suggest that FKBP52 is important for the attainment of uterine receptivity and implantation.

Concluding remarks

Although a wealth of knowledge on the roles of growth factors, cytokines, homeotic genes, transcription factors, and lipid mediators in embryo-uterine interactions during implantation has been generated, their hierarchical blueprint in directing uterine and embryonic function during implantation remains to be deciphered. The ripple effect concept asserts that the quality of implantation determines the quality of pregnancy and fetal well-being. Therefore, failure to achieve on-time implantation is a risk factor for normal pregnancy outcome. This is clinically relevant as well, since implantation beyond the normal window of implantation in humans leads to spontaneous pregnancy losses [21]. An arduous task before us is to unravel the intricate nature of the signaling pathways in implantation. We need to understand whether these pathways function independently, in parallel, or converge to a common signaling pathway to establish the network of cross-talk between the embryo and uterus that is necessary for implantation. Gene-targeting experiments in mice have identified a large number of genes that are important for female fertility. However, this list is not exhaustive and is likely to expand enormously with the emergence of rapidly evolving new technologies.

Although numerous molecules involved in the implantation process have been identified, our understanding of this process is still far from complete. For example, many of the 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 results in embryonic lethality. Uterine- or embryo-specific conditional knockout of genes of interest is urgently needed to better understand the definitive roles played by these genes in uterine biology and implantation. Our failure to identify suitable uterine-cell-specific promoters has hindered the achievement of this objective. One additional difficulty in identifying the critical roles of signaling molecules within a gene family is the redundant or compensatory functions of the gene products within the family.

Strategies comparing global gene expression profiles between the implantation and interimplantation sites have identified novel genes in the implantation process. Thus, a genome-wide screening approach coupled with functional assays will help elucidate these complex signaling pathways. Recently, microarray analysis was conducted to investigate differences between active and dormant blastocysts under defined experimental conditions [82]. This analysis compared the gene expression profiles between activated and dormant blastocysts, indicating that distinctions exist at the gene expression level, suggesting that specific molecular mechanisms are involved in maintaining blastocyst dormancy and in activating blastocysts for implantation. These insights into the molecular basis of blastocyst dormancy provided information that pathways involving the cell cycle, energy metabolism, and Ca^{2+} signaling all participate in the resumption of the blastocyst dormant state, whereas chromatin remodeling, adhesion signaling, and heparin binding-epidermal growth factor-like growth factor participate in embryo-uterine interactions required for activation of the blastocyst and subsequent implantation [82]. The results obtained from these experiments may help uncover new signaling molecules and pathways not previously identified, providing a landscape of gene interactions during early pregnancy. The application of proteomics is also likely to provide information on the interactions among various molecular pathways in specifying the molecular road map to implantation [83].

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