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Uterine Luminal Epithelium as the Transient Gateway for Embryo Implantation

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Abstract

Uterine luminal epithelium (LE) is the first maternal contact for an implanting embryo. Intrauterine fluid resorption, cessation of LE proliferation and apoptosis, and conducive LE structural changes are prerequisites in establishing transient uterine receptivity for embryo implantation. Vesicle trafficking in LE and receptor-mediated paracrine and autocrine mechanisms are critical for LE preparation as well as LE communications with an embryo and stroma during embryo implantation initiation. This review mainly covers recent *in vivo* studies in LE of mouse models from 0.5 days post-coitus (D0.5) to ~D4 20 h when the trophoblasts pass through the LE layer for embryo implantation. It is organized in three interconnected parts: preimplantation LE preparation for embryo attachment, embryo-LE communications, and LE-stroma communications.

Keywords

Uterine luminal epithelium; vesicle trafficking; intrauterine fluid resorption; LE morphological changes; embryo-LE communications; LE-stroma communications

Overview

Embryo implantation (see Glossary) is a mandatory step in mammalian reproduction. It requires synchronized readiness of a uterus and an embryo as well as their timely dialogues. How the uterus transiently transforms into a receptive state for embryo implantation is far from well-understood. The uterine epithelium includes luminal epithelium (LE) and glandular epithelium (GE), which extends from the LE into the stromal layer. The LE is the first maternal contact for an implanting embryo and serves as the transient gateway for embryo implantation and subsequent embryo development in the uterus. GE secretes molecules critical for embryo implantation. Defective embryo implantation accounts for ~3/4 of pregnancy loss in the general population, in which the pregnancy rate per menstrual cycle is ~30% and ~1/3 of the pregnancies is lost during gestation [1]. Altered **uterine receptivity** (see Glossary), which can be caused by a non-receptive endometrium and

possibly by a shifted receptive window, is likely the main contributing factor for the low implantation rate in *in vitro* fertilization and embryo transfer (IVF-ET) despite a high success rate of IVF for embryo preparation [2]. Understanding mechanisms in establishing uterine receptivity will provide foundations for detecting and treating defective uterine receptivity for embryo implantation.

Dr. Koji Yoshinaga comprehensively reviewed the history of research on embryo implantation that employed various models, including humans, primates, farm animals, rodents, and *in vitro* models [3]. Because of ethical issues and certain conserved mechanisms in embryo implantation expected between humans and mice [4], mice have been widely employed as *in vivo* models for investigating the embryo implantation process. This review mainly focuses on the recent original *in vivo* studies in LE of mouse models. To be consistent in dating early pregnancy, mating night is designated as 0 **d**ay post-coitus (**D0**). Embryo attachment normally occurs ~D4.0 in mice [5] and embryo implantation in the majority of healthy pregnancies occurs ~8-10 days after ovulation in humans [6]. This review mainly covers key events in mouse LE from D0.5 to ~D4 20 h (Fig. 1) and is organized into three interconnected major aspects: 1. Preimplantation LE preparation for embryo attachment; 2. Embryo-LE communications; and 3. LE-stroma communications.

1. Preimplantation LE preparation for embryo attachment

The first maternal-embryo contact occurs between the LE and the trophoblasts, both of which are polarized cells. The apical membranes of polarized cells do not normally interact with each other directly. The LE cells have to go through cellular and molecular changes, e.g., loss of apical microvilli, modifications of apical adhesiveness, alterations in the basolateral membranes, changes in the intracellular vesicle trafficking, and development of immune tolerance, etc., to become conducive for the initial embryo attachment. These LE changes, accompanying with cessation of LE proliferation and apoptosis and resorption of uterine fluid (Fig. 1), are prerequisites for embryo attachment in mice. A brief discussion about the appropriateness of using "differentiation", "transformation", and "loss of apical-basal polarity" in the literature for LE changes is in Box 1.

1.1.   Preimplantation LE proliferation and apoptosis—LE proliferation is induced by pre-ovulatory estrogen and becomes almost undetectable by D3.5 (Fig. 1H). LE apoptosis peaks on D1.5, coincident with the peak endometrial inflammation after mating [7], also becomes almost undetectable by D3.5 (Fig. 1I) [8]. Decreased LE cell proliferation and increased LE apoptosis reduce the uterine lumen lining, concurring with the reduction of uterine fluid (Fig. 1).

Preimplantation LE proliferation and apoptosis are regulated by estrogen receptor alpha (*Esr1*/ERα) and progesterone receptor (*Pgr*/PR) via coordinated autocrine and paracrine mechanisms. Estrogen-induced LE proliferation is mediated by stromal ERα [9], while estrogen-reduced LE apoptosis is mediated by uterine epithelial ERα [10, 11]. Progesterone counteracts estrogen-induced LE proliferation and promotes LE morphological changes. These effects of progesterone are medicated through uterine epithelial PR [12], and most likely stromal PR, in which HAND2 is involved [13]. Studies in genetic mouse models have

implicated multiple factors in the autocrine and paracrine regulation of LE proliferation, such as growth factors (e.g., IGF1, FGFs) [9, 13, 14], cell-cycle related proteins (e.g., MAD2L1, CDKN1A, and CEBPB) [9], and transcription factors (e.g., KLF4 and KLF15) [15–17]. Most likely multiple factors are involved because LE proliferation retains in IGF1-deficient mice [18].

Increased LE proliferation during peri-implantation period is associated with defective embryo implantation, with or without obvious intraluminal fluid retention (Table 1). Peri-implantation intraluminal fluid retention correlates with increased ratio of estrogen signaling over progesterone signaling in several mouse models, which have uterine or uterine epithelial deletion of genes, such as *Alk3* [15], *Arid1a* [17], *Fst* [16], Gα$_{q/11}$ [19], *Gata2* [20], *Hand2* [13] *NCOA6* [21], *Pgr* [12], *SHP2* [22], and *Stat3* [23–25]. However, overexpression of epithelial PRA, which is assumed to increase progesterone signaling, also causes intraluminal fluid retention but no enhanced LE proliferation [26]. Rbpsuh (RBPJ) is mainly detected in the stroma and decidua of the peri-implantation mouse uterus. Deletion of uterine *Rbpj* in mice increases LE proliferation but appears to have no adverse effect on embryo implantation (e.g., implantation timing, embryo spacing, and number of implantation sites) detected by blue dye reaction on D4.5. However, there is increased peri-implantation LE proliferation associated with abnormal LE folds and embryo orientation at the implantation chamber, which leads to increased post-implantation death [27]. A fine balance of uterine local estrogen signaling and progesterone signaling is essential for timely establishment of uterine receptivity. Enhanced LE proliferation and increased uterine estrogen sensitivity can be counteracted by ER antagonist ICI-182780 in *PR$^{Cre/+}$NCOA6$^{flox/flox}$* mice [21], *PR$^{Cre/+}$COUP-TFII$^{flox/flox}$* mice [28], and *PR$^{Cre/+}$Rbpj$^{flox/flox}$* mice [27], while PR antagonist RU486 or exogenous estrogen could counteract enhanced progesterone signaling in *Lpar3*-deficient uterus [29] (Table 1).

It has been observed in several genetic mouse models that when there is enhanced LE proliferation and/or apoptosis around the time of embryo attachment, there is defective LE remodeling and failed embryo attachment (Table 1) [12, 14–16, 19, 20, 22–25, 30–33]. Therefore, the reported abnormal LE morphological changes during implantation window in these animal models is most likely a secondary effect of abnormal preimplantation LE proliferation and/or possibly apoptosis too. One exception on the correlation between enhanced LE proliferation and failed embryo attachment is seen in *PR$^{Cre/+}$Rbpj$^{flox/flox}$* mice [27], in which embryo implantation occurs. Since RBPJ expresses in the stroma and decidua but not LE during peri-implantation, it may indicate that local factors in LE for embryo attachment are functional in the *PR$^{Cre/+}$Rbpj$^{flox/flox}$* mice.

1.2. *LE plasma membrane remodeling* (Fig. 2)—The LE plasma membrane undergoes sequential changes during early pregnancy to prepare for embryo implantation. Studies in ovariectomized, pseudopregnant, delayed implantation, and natural pregnant animal models have demonstrated that the LE plasma membrane changes are under the control of ovarian hormones and appear to be conserved in different species (reviewed in [34]).

1.2.1.   Apical plasma membrane remodeling: The LE microvilli are long and thin under the influence of the pre-ovulatory estrogen on D0.5, they become progressively shorter and irregular with the increasing progesterone from the newly formed corpora lutea during the following days. LE apical protrusions (pinopods/uterodomes) appear prior to embryo attachment. They seem to be the initial contact for trophoblasts and are considered as morphological indicators for the establishment of uterine receptivity [3, 34, 35]. Upon embryo attachment, the LE surface becomes smoother [36].

1.2.2.   LE apical adhesiveness: Main classes of cell adhesion molecules (CAMs) include IgCAMs, cadherins, integrins, selectins, and mucins. Microarray analysis of D3.5 and D4.5 LE shows downregulation of *Jam2* and *Muc1* in D4.5 LE, no differential expression of the most highly expressed cadherins (*Cdh1* and *Cdh11*) and integrins (*Itga6, Itgav, Itgb1, Itgb4, Itgb5*, and *Itgb8*), and low levels of selectins [37]. MUC1, a component of glycocalyx on apical LE, has to be cleared in the entire LE (in mice) or specially the LE at the embryo attachment site (in humans) [4]. MUC1 retention is consistently observed in animal models with enhanced LE proliferation and defective embryo attachment (Table 1). Secreted phosphoprotein 1 (SPP1/osteopontin) interacts with integrins on surfaces of LE and trophoblasts to facilitate embryo-LE attachment [38]. Different species may have different CAMs for embryo-LE attachment [39].

1.2.3.   Lateral plasma membrane remodeling: Tight junctions, adherens junctions, desmosomes, and gap junctions, etc., are present on LE lateral plasma membrane. During early pregnancy in multiple animal models, tight junctions become progressively deeper and more geometrically complex; while adherens junction and its associated terminal web as well as desmosomes are downregulated, accompanying with a more tortuous lateral plasma membrane [34]. The latter changes would weaken the inter-epithelial interactions, which could be broken during specimen preparation for transmission electron microscopy, resulting in the visible inter-epithelial cell spaces despite interdigitated opposing LE layers upon artificial decidualization [40]. Claudins are essential tight junction proteins. *Cldn3, Cldn4*, and *Cldn7* are the most highly expressed Claudins in D3.5 LE, while *Cldn1* and *Cldn10* are the most highly upregulated Claudins in D4.5 LE [37]. Deletion of *Msx1* and *Msx2* leads to upregulation of Claudin-1 in the D3.5 LE [41]. The dominant gap junction protein in the LE, GJB2/*Gjb2* [37], is only upregulated at the implantation chamber upon embryo attachment and the upregulation gradually extends to the nearby LE [40].

1.2.4.   Basal plasma membrane changes: LE basal plasma membrane rests on a basement membrane, which is a specialized extracellular scaffold that is fine-tuned with BM toolkit (collagen IV, laminin, perlecan, nidogen, peroxidasin, and Goodpasture antigen-binding protein (GPBP), etc.) [42]. Focal adhesions anchor LE to the basal lamina. Controlled disassembly of basal focal adhesions [43] is expected to facilitate the detachment of LE basal plasma membrane from basement membrane and LE removal at the implantation site. LE basement membrane is a continuous and winding layer detected on D3.5; it becomes less winding and the lamina densa appears less uniform and morphologically frayed at the embryo attachment site detected on D4.5 [42]. However, after the removal of the LE cells at

the entrance site (~D4 20 h), the basement membrane becomes irregular and tortuous, and the lamina densa frequently appears flocculent and diffuse [44].

1.3.   LE intracellular changes

1.3.1.   Differential gene expression in LE during peri-implantation initiation: ERα and PR are the master transcription factors regulating uterine gene expression during embryo implantation. Although gene expression in D3.5 and D4.5 LE (peri-implantation initiation) of ERα and PR knockout mice has not been systemically profiled, it is expected that ERα and PR in both uterine epithelium and stroma coordinately regulate LE gene expression during embryo implantation initiation [9–12]. Microarray analysis (GSE44451) reveals 382 upregulated and 245 downregulated genes in D4.5 LE compared with D3.5 LE (fold change 2, P< 0.05, difference in intensity values >200) of wild type mice. DNA-dependent transcription, proteolysis, transmembrane transport are among the top categories in Gene Ontology annotation, while biosynthesis and metabolism are among the top signaling pathways of the differentially expressed genes [37]. Available studies have shown three patterns of temporal differential expression in relation to embryo attachment ~D4.0: 1) differential expression starts in the LE layer before embryo attachment, e.g., upregulation of *Atp6v0d2* [45] (Box 2); 2) differential expression initiates only in the LE cells at the implantation chamber upon embryo attachment, e.g., upregulation of GJB2 [40]; and 3) differential expression correlates with decidualization process, which occurs hours after embryo attachment, e.g., downregulation of PR [5].

1.3.2.   LE cytoskeleton remodeling: A cytoskeleton is a complex and dynamic network of interlinking protein filaments extending from nucleus to plasma membrane, including microfilaments, microtubules, and intermediate filaments. They are formed by polymerization of their distinct protein subunits, some of which have dynamic expression in the LE, e.g., α-parvin and β-parvin, which are detected in both cytoplasm and nuclear region on D0.5 but more dominantly in nuclear region in D5.5 rat LE [46]. Cytoskeleton remodeling is correlated with plasma membrane remodeling. For example, the replacement of thick and continuous terminal webs by thinner and irregular actin bands correlates with the progressive loss of LE apical microvilli; the individually distinguishable filaments connecting actin meshworks correspond with the remaining apical plasma membrane protrusions [47]. LE microtubules become fragmented, aligned perpendicular to the apical plasma membrane, and associated with large number of vesicles in LE apical cytoplasm of a receptive rat uterus [48].

1.3.3.   LE intracellular organelles: Although the spatiotemporal changes of different intracellular organelles have not been systemically analyzed in LE during early pregnancy, ultrastructural studies in LE of different rodent models have revealed changes in various intracellular organelles. Vesicle trafficking, which involves endocytic vesicles, multivesicular bodies and dense bodies (lysosomes), but not Golgi apparatus, of intravenously-injected tracer is detected in D0.5 and D4.5 LE, and that of intraluminal administered tracer in D4.5 LE but not D0.5 LE [49]; upon activation of delayed implantation, there appears to have increased discharge of content of LE apical vesicles into uterine lumen [36]; upon estrogen treatment in spayed mice, there are diminished lipid granules, enlarged vacuoles in Golgi

apparatus, and presence of α-cytomembranes [50]. Uterine epithelial acidification upon embryo attachment implies functional changes of acidic organelles (e.g., lysosomes) (Box 2) [45].

1.4.   LE in uterine fluid resorption—Intrauterine fluid secreted from GE and LE could facilitate sperm migration to the oviduct for fertilization, assist intrauterine embryo development and transport, and relay maternal signals to preimplantation embryos, and vice versa [51, 52]. Excessive intrauterine fluid during the implantation window impairs embryo implantation in both mice and humans [52]. The volume of uterine fluid is the net result of secretion and resorption. LE is expected to be the main site for intrauterine fluid resorption in mice because GE-deficient mice show no obvious intrauterine fluid accumulation during peri-implantation [53]. GE contribution to intrauterine fluid resorption could be limited by its minimal contact with intrauterine fluid in mice. Because of limited paracellular flow in the LE layer [49], LE transcelluar flow involving vesicle trafficking and membrane transporters, such as **aquaporins** (see Glossary) and ion channels, are expected to play important roles in intrauterine fluid resorption (Box 3). Vesicle trafficking and channels in regulating intrauterine fluid pH and compositions (e.g., nutrients, exosomes, etc.) [54] and emerging roles of uterine microbiota in regulating uterine receptivity [55] are not covered here.

1.5.   LE immune status—An embryo is semi-allogenic to the biological mother-to-be and allogenic to a surrogate woman. LE has a unique task of accepting an embryo for implantation while handling pathogens, commensal microbiota, and other foreign antigens. The mechanisms of immune tolerance in LE for the very initial maternal-embryo interaction at embryo attachment are largely unknown. Seminal fluid from mating can induce endometrial inflammation [56], in which IFN in seminal plasma plays an important role [7]. On D0.5, lack of LE apical endocytosis may prevent uptake of intraluminal foreign materials (e.g., seminal fluid) to induce local immune attack on sperm [49]. On D1.5, there is peak leukocyte infiltration in the LE, coincident with peak LE apoptosis [8]. By D3.5, the embryos are in the uterus and classic immune cells are absent in the LE layer. Innate immunity is the first line of defense. Several uterine epithelial genes involved in innate immunity and upregulated by estrogen via uterine epithelial ERα (GSE53812) [31], such as *Muc1, Pigr, Sftpd*, and *Spp1*, are downregulated from D1.5 to D3.5. Interestingly, *Sftpd*, and *Spp1* are then upregulated in LE from D3.5 to D4.5 [37]. It has been suggested that upregulation of SPP1/osteopontin may limit maternal complement activation to protect an embryo during the initial embryo-maternal contact [39, 57].

2.   Embryo-LE communications

Upon embryo attachment, there is increased endometrial vascular permeability at the attachment site detected by blue dye reaction ~D4.0, followed by stromal edema and subsequent decidualization by D4 6 h [5]. LE removal and trophoblast invasion occur ~D4 20 h [58] (Fig. 1).

2.1.   Embryo positioning—Uterine blood vessels are branched from the **mesometrium** (see Glossary). In monotocous species such as humans, an embryo tends to implant in the

uterine fundus (see Glossary); in polytocous species such as mice, embryos evenly implant in the anti-mesometrial (AM) side along the uterine horns. This spatial arrangement is most likely determined by signaling gradient, such as Wnt signaling on the anti-mesometrial side and DKK2, an inhibitor of Wnt signaling, on the mesometrial (M) side [59].

When mouse embryos enter a uterine horn ~D3.0, they tend to group together and gradually separate along the longitudinal axis. Genetic and pharmacological approaches have demonstrated the roles of cPLA2α/*Pla2g4*, LPA$_3$/*Lpar3*, β2-adrenoceptor, adrenomedullin, and nicotine in rodent embryo spacing [60]. Although myometrial contraction appears to be the determinant factor for embryo spacing (Box 4) [29, 60–64], unknown embryonic mediators, e.g., extracellular vesicles, likely play roles in inter-embryo communications for even embryo spacing.

2.2. Implantation chamber formation—Embryo spacing and positioning along the LE finalizes a few hours (~D3 19 h) before embryo attachment (~D4.0) [64]. A competent **blastocyst** (see Glossary) initiates LE evagination to form an implantation chamber [65] that involves LE planar cell polarity (PCP) signaling [66]. LE folds in the MAM axis are absent through implantation chamber but retained in inter-implantation region, and shallower along oviductal-cervical axis [67]. LE folds at the implantation chamber could affect embryo orientation in the implantation chamber demonstrated in the $PR^{Cre/+}Rbpf^{flox/flox}$ mice [27]. 3D confocal imaging of D4.5 uterus reveals reorganization of uterine glands upon decidualization, including decreased glandular ductal angle, elongated glandular ducts, and bending of the uterine glands surrounding the implantation site [67]. Deletion or overexpression of uterine *Wnt5a*, deletion of uterine *Ror1* and *Ror2* [68] or PCP gene *Vangl2* in uterine epithelium [65], can lead to disorderly implantation chamber formation, delayed implantation thus delayed decidualization and associated uterine gland reorganization. *Rac1* deficiency also causes defective implantation chamber formation at some implantation sites [33].

2.3. *Embryo-LE mediators* (Fig. 2)—One well established embryo-LE mediator is heparin-binding epidermal growth factorlike growth factor (HB-EGF) [65, 69]. Although other potential mediators have been reported in the literature, none of them has been demonstrated elegantly and conclusively as HB-EGF for mediating the initial embryo and LE communications. HB-EGF from a competent blastocyst can induce HB-EGF expression in the contacted LE, and this effect can be mimicked by HB-EGF-soaked beads. Embryo-derived HB-EGF binds to EGF receptors, such as ErbB4, on LE; and LE-derived HB-EGF reciprocally influences the embryo. In addition to the paracrine effects, HB-EGF signaling also involves autocrine and juxtracrine mechanisms. A prerequisite for HB-EGF function as an embryo-LE mediator is leukemia inhibitory factor (LIF), which is produced in GE and binding to LIFR on LE to exert its essential role in embryo implantation [65, 69, 70]. HB-EGF signaling has cross-talk with lysophosphatidic acid (LPA) signaling [69]. Since LPA is a small lipid signaling molecule abundant in the serum, serum components can be delivered to the uterine lumen via LE basolateral endocytosis and apical exocytosis [49], and LE LPA$_3$-mediated LPA signaling is important for on-time uterine receptivity [62], LPA could

possibly function as an LE-embryo mediator. Multiple mediators are expected to work collaboratively for the initial embryo-LE dialogues.

Extracellular vesicles (see Glossary), including exosomes and microvesicles, with LE or embryo origins, have been identified in intrauterine fluid and LE mucus of different species, and recognized as new players in embryo-LE communications [54] (Fig. 2). Proteins associated with uterine receptivity are detected in exosomes of human endometrium [57]. **MicroRNAs** (see Glossary), either free or in extracellular vesicles, are considered as a new class of embryo-LE mediators [71–73]. Since exosomes are stable and contain signature molecules from trophoblasts and LE, they could be potentially used as biomarkers in assisted reproductive treatments, such as IVF-ET [74]. In addition to the emerging roles as cargo carriers for LE-embryo communications, LE-derived extracellular vesicles most likely also play roles in LE intracellular trafficking (e.g., exosomes) and plasma membrane remodeling (e.g., microvesicles) for establishing uterine receptivity. Emerging roles of epigenetic mechanisms (e.g., microRNAs, DNA methylation, and histone modifications) in regulating uterine functions are not covered here [75–77].

2.4.   Embryo attachment to LE—Embryo attachment involves trophoblasts and LE cells. Morphologically, the polarized LE cells have to go through a series of plasma membrane remodeling (1.2) to become receptive for direct physical interactions with the polarized trophoblasts. Local molecules in cell-cell adhesion (1.2.2) have been suggested to play roles in embryo attachment [4, 38, 39]. Immunologically, the LE cells have to be in a state not rejecting a semi-allogenic or an allogenic embryo (1.5).

Uterine epithelial ERα or PR are essential for embryo-LE attachment because their deficiency causes embryo attachment failure [10, 12]. ERα remains highly expressed in LE during embryo attachment but shows downregulation at the implantation site after attachment. PR expression remains in the LE during embryo attachment and decreases a few hours after embryo attachment in both mice and hamsters [5] (Fig. 1). However, overexpression of uterine epithelial PRA also disrupts embryo-LE attachment [26], indicating the importance of finely balanced spatiotemporal progesterone signaling for embryo attachment. Receptors-mediated estrogen signaling and progesterone signaling regulate downstream molecules, such as transcription factors and membrane receptors (Table 1), to orchestrate embryo attachment to LE [2, 4, 11, 78, 79].

2.5.   Embryo penetration through LE and basement membrane—Embryo penetration through LE at the implantation site is a prerequisite for successful pregnancy in mammals with a hemochorial placenta (humans, mice, etc.) or an endotheliochorial placenta (dog, cat, etc.), but not those with an epitheliochorial placenta (pig, sheep, cow, etc.) (http://www.trophoblast.cam.ac.uk/Resources/enders) [4]. In humans, trophoblasts infiltrate through LE at a very restricted area and expand under the resealed LE. In rodents, LE cells at the entrance site are removed by trophoblasts [58].

Around D4 20 h in mice (~D5 9 h in rats), the LE cells surrounding the blastocyst are phagocytosed by protrusions of invading trophoblasts, with [8, 33, 58], or without [80] evidence of apoptosis. Disassembly of adhesive complexes, which might be an indication of

LE apoptosis, in the LE cells at the trophoblast entry site is required for trophoblast invasion through LE [81]. LE at the implantation chamber fails to go through apoptosis and removal in uterine Rac1 deficient mice, in which LE TNFα-Rac1-P38 signaling cascade is involved [33]; and in uterine FOXO1 deficient mice, in which the LE is able to transmit stimulus for decidualization [32]. These observations suggest that the LE cells at the trophoblast invasion site have to go through certain changes, such as controlled disassembly of adhesive complexes and apoptosis to facilitate LE removal by the invading trophoblasts.

It is interesting to note that LE tight junctions might be correlated with embryo penetration patterns. Limited data from human LE show reduced tight junctions from the beginning of luteal phase to the middle of luteal phase (corresponding to early pregnancy period) [82]. The reduction of tight junctions could potentially make it easier for trophoblasts passing by the LE cells. While in rodents, the LE tight junctions are deepened and more complex approaching embryo implantation [34]. Enhanced tight junctions limit paracellular flow, and consequently, indirectly facilitate transcellular flow in the LE for the initial embryo-maternal communications, which must have included messages for LE modifications that enable LE removal by the invading trophoblasts.

After having phagocytosed the LE cells on the way, the invading trophoblasts have to break through the remaining tortuous basal plasma membrane, which is perforated at multiple discrete loci simultaneously on both sides by the cytoplasmic processes from trophoblasts and decidual cells [44]. With the removal of the basement membrane, the trophoblasts and decidual cells are in close contact to continue the journey together, which will not be covered here.

3. LE-stroma communications

3.1. LE sensing stimuli—A prerequisite for successful embryo implantation in rodents is the differentiation of stromal cells to decidual cells. Decidualization happens in the implantation site hours after embryo attachment during natural pregnancy or upon other stimuli in a receptive uterus in mice (Fig. 1L) [5]. LE is an obligatory transmitter of intraluminal stimuli (e.g., embryo, bead, oil, and scratching) for decidualization because uterine segments with removed/detached LE are incapable of decidualization [83]. Artifical decidualization can also be induced by physical pinch of a receptive uterus [5]. The mechanisms of how the LE cells sense the stimuli are largely unknown. Since all the known stimuli have shapes and/or forces, mechanosensors (e.g., mechanosensitive ion channels) on the LE are expected to play important roles in sensing the stimuli, which will be subsequently transmitted to the subepithelial stromal cells.

3.2. LE to stroma communications—Uterine epithelial ERα and PR are both essential for LE-stroma communications because their deficiencies cause failed embryo attachment and decidualization [10–12]. Uterine epithelial ERα regulates LIF-LIFR, STAT3, and HB-EGF signaling, which coordinately upregulate cyclooxygenase 2 (COX-2) expression in the LE and subepithelial stroma at the implantation site [84]. COX-2 is the rate-limiting enzyme for synthesis of prostaglandins, which promote decidualization. LE HB-EGF can bind to EGFR in stromal cells to promote decidualization. Both uterine

epithelial ERα and PR coordinately regulate expression of epithelial Indian hedgehog (IHH). IHH binds to its receptor PTCH1 in stromal cells and activates signaling cascades involving COUP-TFII, BMP2, and WNT4 to promote decidualization. Therefore, HB-EGF-EGFR, **LIF-LIFR, IHH-PTCH1, COUP-TFII, STAT3** (see Glossary), and prostaglandins are key players for LE to stroma paracrine communications [10–12, 23–25, 31, 70, 78, 79, 85, 86].

3.3.   Stroma to LE communications—On the other hand, the stroma also sends feedback to the LE. Stromal ERα controls estrogen-induced uterine epithelial proliferation, this paracrine effect might involve growth factors (e.g., IGF1) and cell-cycle related proteins (e.g., MAD2L1, CDKN1A, and CEBPB) [9]. PR-regulated stromal HAND2 controls uterine epithelial cell proliferation via inhibition of stromal expression of FGFs, therefore suppressing the paracrine activation of FGFR-ERK1/2 pathway that promotes uterine epithelial proliferation [13, 78]. Genetic mouse models have demonstrated or implied other stromal factors on sending stromal feedback to LE, such as COUP-TFII [87], SHP2 [22], RBPJ [27], and molecules in TGFβ signaling [88].

3.4.   Vesicle trafficking in LE-stroma communications—During embryo implantation process, LE vesicle trafficking is active on both directions [49] (Fig. 2). Ultrastructural studies in rat uteri during early pregnancy revealed peaking basal pinocytotic invagination in D4.5 LE (~time of embryo attachment in rats) and LE apical vesicles, likely for exocytosis to uterine lumen [89]. Although direct *in vivo* evidence is still lacking, vesicle trafficking, including that involves extracellular vesicles, likely plays important roles in the transport of mediators for the LE-stroma paracrine communications.

Concluding Remarks

LE is the transient gateway for embryo implantation. Preimplantation preparations of LE for this essential role involve endocrine, paracrine, and autocrine mechanisms. Defects in LE at implantation window are most likely derived from impaired preimplantation preparations, in which many questions remain to be answered (see Outstanding Questions).

Acknowledgements

Glossary

Aquaporins

They are a family of transmembrane water channels that allow transmembrane water movement along an osmotic gradient.

Blastocyst

It is an embryo at the last preimplantation stage, with a transparent spherical shell called zona pellucida, an outer layer of trophoblasts, an inner cell mass, and a fluid-filled cavity called blastocoel. It hatches from zona pellucida before embryo implantation.

Colchicine

It is a drug that can bind to tubulin to inhibit microtubule polymerization. Because tubulin is essential to mitosis, colchicine can inhibit mitosis to arrest cell division for karyotyping.

Embryo implantation

It is a process in which a blastocyst makes direct contact with the transiently receptive LE to establish the very first maternal-embryo interface, and subsequently either penetrates through the LE (e.g., human, mice, etc.) or remains direct contact with the LE (e.g., pig, sheep, etc.) for post-implantation pregnancy events.

Extracellular vesicles

They are lipid bilayer-enclosed particles, including exosomes (30-150 nm) and microvesicles (100-1500 nm), loaded with proteins, lipids, and nucleic acids (e.g., miRNAs), etc., and released from cells into extracellular spaces. They can be transported to different tissues and internalized by target cells via fusion or endocytosis to modulate diverse biological processes. Exosomes are released by fusion of the multivesicular body, which is an organelle of the endocytic pathway and contains intraluminal vesicles, with the plasma membrane; while microvesicles are shed by outward blebbing of the plasma membrane. Therefore, they can play roles not only in cell-to-cell communications as cargo carriers, but also potentially in intracellular trafficking and plasma membrane remodeling.

LIF-LIFR, IHH-PTCH1, COUP-TFII, STAT3

Leukemia inhibitory factor (LIF) is a cytokine produced in GE and a specific ligand for LIF receptor (LIFR) in LE. LIF-LIFR acts synergistically with progesterone-PR to upregulate Indian hedgehog (IHH) in LE. IHH is a secreted ligand binding the transmembrane receptor Patched1 (PTCH1) in stroma to exert LE-stroma crosstalks. Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII/NR2F2) in stromal cells promotes stromal cell proliferation and differentiation for embryo implantation. Signal transducer and activator of transcription 3 (STAT3) can be activated by LIF-LIFR in LE and induce downstream signaling cascades for LE-stroma crosstalks.

Mesometrium

It is a contiguous set of tissues attaching and supply blood vessels to the uterus. In a mouse uterine horn, the uterine side connected with mesometrium is called mesometrial side and the opposite side is called antimesometrial side where embryo implantation occurs.

MicroRNAs

They are a class of small (~22 nucleotides) noncoding RNAs. Their biogenesis requires multiple key proteins: Drosha/DGCR8 complex, Exportin-5, Dicer, and Argonaute (AGO). They bind to the 3'-untranslated region of target mRNAs to silence target gene expression via mRNA degradation and/or translational repression.

Uterine fundus

It is the top dome-like area of the human uterus connected by two fallopian tubes. It is equivalent to the antimesometrial side in mice.

Uterine receptivity

It is an estrogen and progesterone-controlled transient uterine state when the uterus is capable of accepting a competent embryo for implantation. It normally occurs ~4 days in mice and 8-10 days in women after ovulation. It can be disrupted by inappropriate estrogen and/or progesterone signaling.

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Box 1.

Discussion on the appropriateness of using the words "differentiation", "transformation", and "loss of apical-basal polarity" for the alterations in the uterine luminal epithelial (LE) cells during early pregnancy

Based on the definitions, "differentiation" of a cell involves the change of a cell type, such as from a stem cell to an epithelial cell; while "transformation" of a mammalian cell involves immortality, and most likely changes in the DNA also, such as mutations related to oncogenic transformation. It is known that the uterine stromal cells go through differentiation to become decidual cells during menstrual cycle in women and during embryo implantation in mice (Fig. 1L). Both "differentiation" and "transformation" have been used in the literature for the structural changes in the LE during early pregnancy to prepare for embryo implantation. Since the LE cells remain to be E-cadherin positive epithelial cells (Fig. 1N), which are not expected to become immortal during the normal implantation process, and the changes in LE cells are reversible [34], it is inaccurate to use "differentiation" and "transformation" for the changes in the LE cells during early pregnancy. However, in the term "plasma membrane transformation" [34], which was coined by Dr. Christopher Murphy, the "transformation" means changes in the entire LE plasma membrane and it does not necessarily imply immortality of the LE cells. Therefore, it might be still suitable to use "transformation" in "plasma membrane transformation". To reduce confusion, it might be more appropriate to use "plasma membrane remodeling" for describing the changes in the LE plasma membrane. However, Dr. Christopher Murphy thinks that "'changes' or 'remodeling' just don't have the same ring as 'transformation' when combined with plasma membrane" (personal communication).

The use of "loss of apical-basal polarity" for the changes in the LE during early pregnancy is also questionable. Loss of apical-basal polarity in epithelial cells is usually associated with epithelial-mesenchymal transition (EMT) to gain migratory capacity, such as during physiological development and pathological cancer metastasis. Although the LE cells at the embryo entrance site in rodents are eventually phagocytosed by trophoblasts [58], the LE cells remain as epithelial cells prior to embryo penetration through the LE layer, indicated by the strong E-cadherin staining throughout early pregnancy (Fig. 1N). The LE cells also retain their structural polarity, evidenced by the deeper and more geometrically complexed tight junctions on the apical side but weakened inter-epithelial physical interactions on the rest of the lateral plasma membrane [34]; as well as functional polarity, evidenced by the differential apical to basolateral and basolateral to apical intracellular vesicle trafficking in the LE during embryo implantation in rodents (Fig. 2) [49, 89]. Therefore, "differential polarization" or "modified polarity" could be more suitable to describe the LE remodeling leading to embryo implantation (personal communications with Dr. Christopher Murphy and Dr. John Aplin).

Box 2.

Acidification of uterine epithelium upon embryo attachment

Microarray analysis of D3.5 and D4.5 LE reveals dramatic upregulation of Atpase, H^+ transporting, lysosomal VO subunit D2 (*Atp6v0d2*) [37], a gene encoding a tissue-specific d subunit of vacuolar-type H^+-ATPase (V-ATPase), in D4.5 LE upon embryo implantation initiation. V-ATPase is a highly conserved evolutionarily ancient enzyme. It has V0 and V1 domains. The transmembrane integral V0 domain is composed of 6 subunits (a, c, c', c", d and e) and involved in proton translocation, while the cytoplasmic peripheral V1 domain contains 8 subunits (A-H) and is responsible for ATP hydrolysis. The ATP dependent proton transport by V-ATPase is from the cytoplasmic compartment to the opposite side of the membrane, which can be either the lumen of intracellular organelles (e.g., lysosomes, endosomes, and secretory vesicles) or the lumen of extracellular environment (e.g., epithelial cells), to acidify the intracellular organelles or the extracellular environment, respectively. Different tissue distributions, cellular localizations, and utilizations of specific subunit(s) may contribute to the diverse functions of V-ATPase.

The upregulation of *Atp6v0d2* led to a novel finding of uterine epithelial acidification during implantation initiation detected by LysoSensor Green DND-189 (pKa ~5.2). LysoSensor Green DND-189 is an acidotropic fluorescence probe. It will become fluorescent only when it is inside acidic compartments and its fluorescence intensity correlates with the intracellular acidity thus serves as an intracellular pH indicator. Uterine epithelial acidification is also seen in the uterine epithelium upon artificial decidualization but not in the uterus during estrous cycle [45]. Since the lysosomes are the most acidic intracellular organelles within the pH range (pH<5) detectable by LysoSensor Green DND-189, the uterine epithelial acidification detected by LysoSensor Green DND-189 indicates lysosomal acidification, although acidification of other intracellular organelles (e.g., endosomes) couldn't be ruled out.

Since lysosomes are critical for vesicle trafficking and digesting recycled materials, and lysosomal acidity is essential for lysosomal activity, lysosomal acidification in the uterine epithelium upon embryo attachment supports enhanced vesicle trafficking and material recycling to accommodate uterine epithelial functions. LE apical vesicle trafficking may also facilitate LE apical plasma membrane remodeling for embryo attachment, and potentially relay embryonic messages to LE and stroma, etc. The underlying mechanisms for uterine epithelial lysosomal acidification upon embryo implantation awaits to be investigated.

Box 3.

LE in regulating uterine fluid volume

LE apical endocytosis but not basolateral endocytosis is under the control of progesterone. Uptake of intraluminal horseradish peroxidase (HRP) tracer is lacking on D0.5 but extensive on D4.5 20 min after injection; no presence of tracer in the lateral intercellular spaces confirms limited paracellular flow [49]. Pinopods appear before embryo attachment and have pinocytosis activity in mice [34]; they are promoted by adrenomedullin [93]. Intravenously injected HRP reaches LE 2 hours after injection via basolateral to apical vesicle trafficking on both D0.5 and D4.5. LE vesicular trafficking is dependent on microtubules, evidenced by **colchicine** (see Glossary) treatment [94] and transmission electron microscopy [48].

Aquaporins in LE and GE, such as aquaporins 5 and 8, play important roles in estrogen (mice deficient of *Aqp5* and/or *Aqp8* have reduced intrauterine fluid accumulation upon estrogen treatment) or ovarian hyperstimulation-induced intraluminal fluid accumulation [52, 95], and may contribute to intrauterine fluid retention in uterine epithelium PRA overexpressed mice [26]. Under the influence of pre-ovulatory estrogen, aquaporins facilitate water transport to uterine lumen; during embryo implantation, water transport across LE presumably occurs bidirectionally [93].

Although ion channels are indispensable for intrauterine fluid homeostasis, *in vivo* pharmacological or genetic evidence is still lacking to validate *in vivo* roles of a specific ion channel in regulating intrauterine fluid volume. Inhibition of uterine epithelial sodium channel (ENaC) by amiloride (IC50: 0.1 μM) or siRNA does not appear to cause intrauterine fluid accumulation [85]. Amiloride at 100 μM in uterine lumen could reduce >50% of intrauterine fluid resorption rate in progesterone-primed uterus but not estrogen-primed uterus [96]. Since amiloride has ENaC-independent effects [97] and can target other channels and inhibit endocytosis (e.g., macropinocytosis) [98], and LE apical endocytosis is upregulated by progesterone but not estrogen [49], the inhibitory effect of amiloride on intrauterine fluid resorption rate at this high concentration could not be solely contributed to ENaC and most likely involves LE apical endocytosis. Although many CFTR (cystic fibrosis transmembrane conductance regulator, a cAMP-activated C1 channel) mutation mouse models are available, none could conclusively demonstrate functions of CFTR in regulating intrauterine fluid volume. Transient receptor potential cation channel subfamily v member 6 (TRPV6), a Ca^{2+} channel, is the most upregulated and highly expressed transmembrane transport channel in D4.5 LE [37]. TRPV6 deficiency does not appear to affect female fertility [99]. It is most likely that multiple membrane transporters (e.g., ion channels, anion exchangers, and cotransporters [100], etc.) coordinately regulate not only the intraluminal fluid volume, but also its pH and contents.

Box 4.

Embryo spacing in mice

Studies in $Lpar3^{-/-}$ mice clearly demonstrate maternal factors in embryo spacing. $Lpar3$ expression peaks in D3.5 LE and is upregulated by progesterone [61, 62]. $Lpar3^{-/-}$ females have delayed embryo implantation, embryo crowding, reduced uterine levels of $Ptgs2$ (prostaglandin-endoperoxide synthase 2, encoding cyclooxygenase-2, or COX2) and COX-derived prostaglandins [62], and a decreased ratio of estrogen signaling / progesterone signaling in the uterus [29]. Although PGE_2+cPGI, RU486 (PR antagonist), and estrogen treatments can all restore implantation timing, these treatments have no effect on embryo crowding in the $Lpar3^{-/-}$ uterus [29, 62, 63]. However, 11-deoxy PGF2α, a thromboxane A2 receptor agonist that induces myometrial contraction, can partially alleviate embryo crowding in the $Lpar3^{-/-}$ uterus [63], indicating the role of uterine contraction on embryo spacing [63]. This observation is consistent with abolished LPA$_3$ agonist-induced uterine contraction in the D3.5 $Lpar3^{-/-}$ uterus [64]. $Lpar3$ is almost exclusively expressed in the LE with peak expression on D3.5 when embryo spacing occurs. How LPA$_3$-mediated signaling in LE is relayed through the stromal layer to the myometrium for contraction during D3.5 remains elusive. Since $Lpar3$ deficiency reduces uterine COX-derived prostaglandins, COX-derived prostaglandins could override the effect of $Lpar3$ deficiency on embryo implantation, and prostaglandins are important for stromal and myometrial functions, prostaglandins could be candidate mediators to relay LPA$_3$ signaling in LE to stroma and myometrium. Other known factors in embryo spacing, cPLA2α, β2-adrenoceptor, adrenomedullin, and nicotine, all have effects on muscle contraction [60]. How would the uterus know the number of embryos there and make them evenly spaced? Would the final embryo attachment sites be determined by communications among the embryos also? Could extracellular vesicles from D3.5 blastocysts communicate signals among the blastocysts themselves and with the LE to decide on the attachment sites? If embryonic factors are indeed involved in the intrauterine embryo distribution, how the embryonic signals are sensed by the LE and transmitted to the myometrium to guide muscular movement in real-time remains to be explored.

Highlights

- Intrauterine fluid resorption, cessation of LE proliferation and apoptosis, and conducive LE structural changes are prerequisites for establishment of uterine receptivity.

- Receptive LE can sense intraluminal stimuli and most likely involves mechanosensors.

- Vesicle trafficking in LE, and ERα and PR receptor-mediated paracrine and autocrine mechanisms are critical for LE remodeling and LE communications with an embryo and stroma during embryo implantation initiation.

- Extracellular vesicles, HB-EGF, and other potential mediators coordinately relay the paracrine signals between embryo and LE, and between LE and stroma.

- LE immune tolerance is essential for embryo attachment but the mechanisms involved are largely unknown.

- LE planar cell polarity signaling contributes to implantation chamber formation and subsequent implantation events.

- LE remodeling is necessary for LE removal at implantation site by invading trophoblasts.

Outstanding Questions Box

- How to regulate LE functions to prevent intraluminal fluid retention at implantation window?

- What roles does LE vesicle trafficking play during different stages of early pregnancy?

- What are the molecular mechanisms involved in LE vesicle trafficking?

- How to suppress LE proliferation to allow LE remodeling for embryo implantation?

- Stromal growth factors are potential candidates for mediating the paracrine effect of estrogen-stromal ERα signaling on LE proliferation. What are the key paracrine mediators? How to target the paracrine mediators for treating related infertility and for contraception?

- How does an embryo choose an implantation site? How to control the site of embryo implantation to prevent related clinical issues, such as placenta previa?

- Is delayed implantation always associated with disorganized implantation chamber formation?

- What molecules on the LE apical membrane sense the presence of an implanting embryo? Could mechanical sensors be involved?

- What molecules in LE relay embryo attachment signal to the subepithelial stromal cells?

- How do mediators, such as extracellular vesicles and microRNAs, travel between cells to exert paracrine effects?

- How to determine uterine local fine balance of estrogen signaling and progesterone signaling?

- How to detect the readiness of LE for embryo attachment? What biomarkers in uterine secretion, blood, and urine can be monitored in real-time to pinpoint the uterine receptive window?

- How to synchronize a competent embryo with a receptive uterus?

- How is immune tolerance established in the LE for embryo attachment, the very first maternal-embryo interface?

- How does the immune status in the LE differ from that in the stroma during early pregnancy? How do they coordinately regulate embryo attachment to LE and embryo invasion into stroma?

- What roles do microbiota in uterine lumen and apical LE play in LE remodeling and in embryo-LE communications for embryo attachment to LE?

- How do epigenetic mechanisms regulate LE remodeling and LE communications with an implanting embryo and with the subepithelial stromal cells?
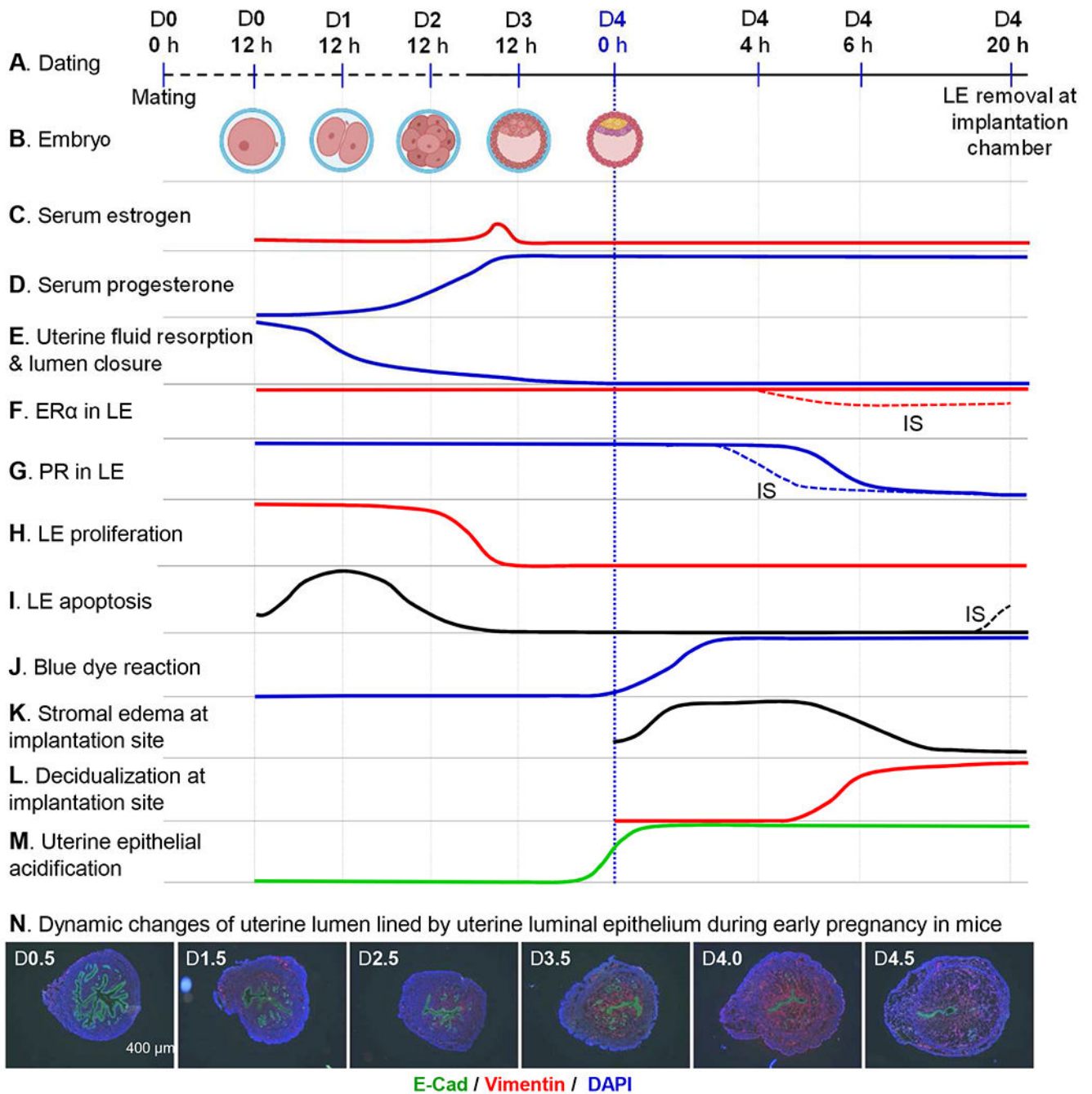
Figure 1. Key events during early pregnancy in mice.

A. Dating of early pregnancy [5]. Mating night is defined as 0 day post-coitus (D0). Fertilization and early embryo development to morula stage occur in the oviduct (dotted line). Embryos reach the uterus (solid line) ~D3.0 and continue developing to blastocysts. Embryos hatch from the zona pellucida and attach to the uterine luminal epithelium (LE) ~D4.0. LE cells at the implantation chamber are removed by trophoblasts ~D4 20 h [58]. B. Preimplantation embryo development. D0 12 h (D0.5), fertilized oocyte; D1 12 h (D1.5), two-cell embryo; D2 12 h (D2.5), 8-cell embryo; D3 12 h (D3.5), blastocyst; D4 0 h (D4.0),

hatched blastocyst; blue ring, zona pellucida. Created using BioRender. C. Serum estrogen level. A small surge of estrogen occurs before D3 12 h that is often difficult to be measured but verified in experimental settings [3]. D. Serum progesterone level. Progesterone is mainly produced in the newly formed corpora lutea that are normally developed in the ovulated follicles [3]. E. Uterine fluid resorption & lumen closure (also see Figs. 1N, 2A, 2B). F. ERα expression in LE. There appeared to have reduced expression in the LE at the implantation site (IS, dotted curve) [29]. G. PR expression in LE. It remains expressed in LE when embryo attachment occurs ~D4.0, and decreases in the LE at the implantation site (IS, dotted curve) then the rest LE when decidualization becomes apparent [5]. H. LE proliferation [8, 29]. I. LE apoptosis. Dotted curve, LE apoptosis at implantation site (IS) [8]. J. Blue dye reaction upon embryo attachment [5]. K. Stromal edema at implantation site [5]. L. Decidualization at implantation site [5]. M. Uterine epithelial acidification detected by LysoSensor Green DND-189 (pKa ~5.2) [45]. N. Dynamic changes of uterine lumen during early pregnancy in mice. Frozen cross sections; uterine lumen lined by luminal epithelium (LE, white arrow) that is stained with epithelial cadherin (E-Cad), green staining; red staining, vimentin; blue staining, DAPI; D0.5-D4.5, 0.5-4.5 days post-coitum; scale bar, 400 μm. This set of original data was generated by Dr. Rong Li.
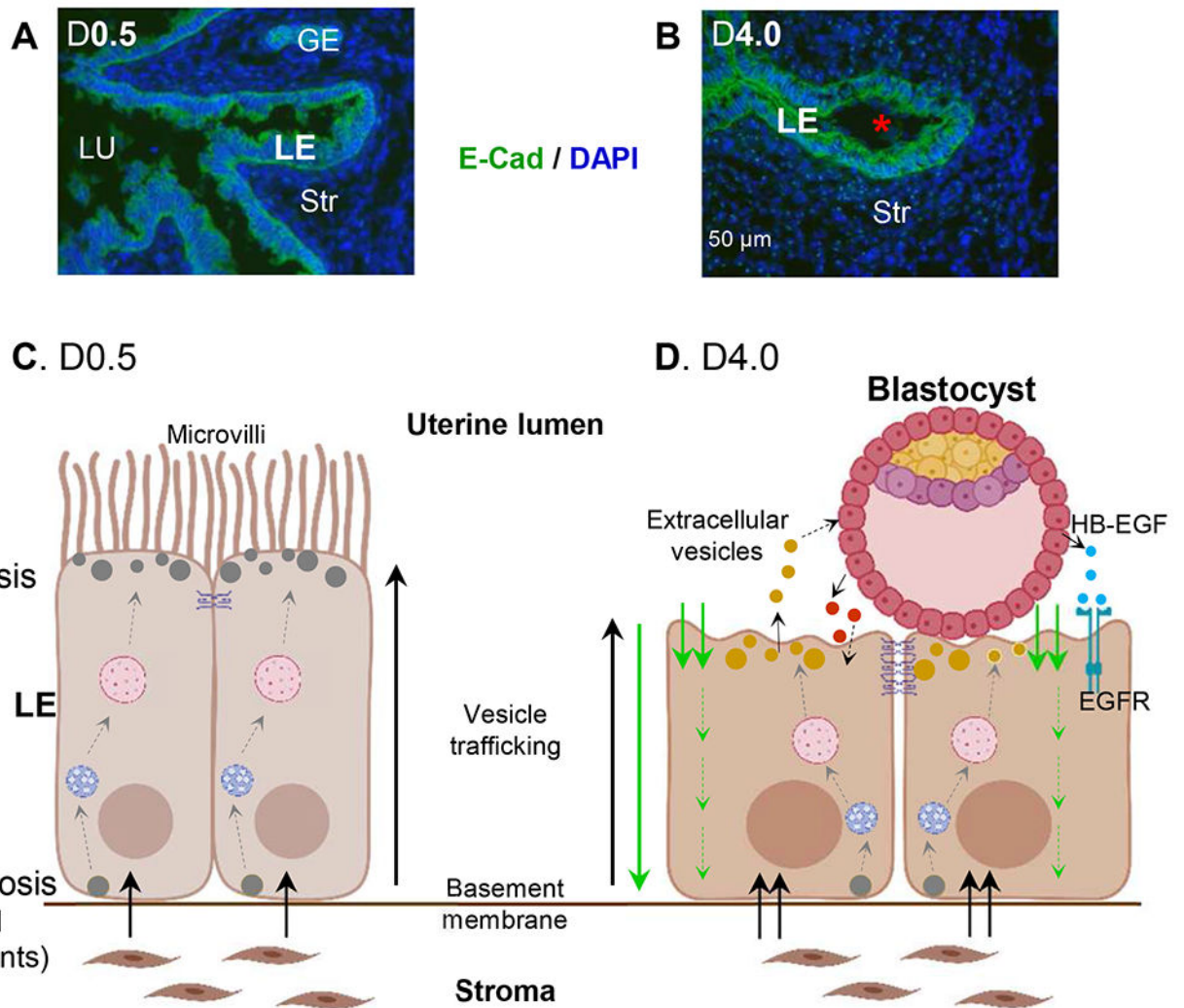
Figure 2. Key cellular and molecular changes in uterine luminal epithelium (LE) upon embryo attachment in mice.

A. An image enlarged from D0.5 in Fig. 1N, showing enlarged uterine lumen (LU) and folding LE layer. B. An image enlarged from D4.0 in Fig. 1N, showing sealed uterine lumen and an embryo (*) wrapped by LE. A & B: green E-Cad staining, LE and GE; Str, stroma; GE, glandular epithelium; scale bar, 50 μm. C. A carton of D0.5 LE. There is a lack of LE apical endocytosis but basolateral endocytosis and apical exocytosis; microvilli are long and thin under the influence of pre-ovulatory estrogen. D. A carton of D4.0 LE, about the time of embryo attachment. LE has gone through plasma membrane remodeling, such as loss of microvilli, deeper tight junctions but reduced other lateral junctions, thus increased paracellular space. LE intracellular vesicle trafficking enhances and may peak on both endocytosis and exocytosis on both apical and basolateral sides. It is expected to facilitate LE-blastocyst and LE-stroma communications, as well as relay signals from the implanting blastocyst to the stromal layer and beyond. Extracellular vesicles with LE-origin and blastocyst-origin are released into the uterine lumen to mediate LE-blastocyst communications. Ligand-receptor mediated mechanisms (HB-EGF-EGFR as an example)

are critical players in the LE communications with the surrounding cells [69]. Stromal cells are yet to be differentiated into decidual cells on D4.0 in mice [5]. Solid long arrows outside of LE cells: directions of intracellular trafficking; solid arrows on plasma membrane: endocytosis; vesicles on apical cytoplasm: exocytosis; dashed arrows in cytoplasm: intracellular vesicle trafficking; blue intracellular vesicles: endosomes; pink intracellular vesicles: lysosomes; orange extracellular vesicles: LE origin; red extracellular vesicles: blastocyst origin; HB-EGF (blue dots): heparin-binding epidermal growth factor; EGFR: epidermal growth factor receptor; symbols connecting two LE cells: tight junctions. For more comprehensive illustrations of embryo-LE interactions, please refer to the cited review papers [2, 4, 11, 69, 78, 79]. Fig. 2C and 2D were created using BioRender.

Table 1.

Uterine local factors balancing uterine estrogen signaling and progesterone signaling during peri-implantation in mice (D3.5-D4.5)

Gene deletion or overexpression (a)	Uterine expression	Fluid retention (D3.5)	Lumen closure (D4.5)	Embryo attachment (D4.5 / D5.5)	Decidualization	LE penetration / removal	LE proliferation (D3.5 / D4.5)	Sustained PR expression in LE(D4.5)	Ratio of uterine E2/P4 signaling	Reference
Alk3 (*Pgr-cre*)	D3.5 LE GE > Stroma	+	+/− (b)	−	−	−	+	+/− −↑	↑	[15]
Arid1a (*Pgr-cre*)	LE, GE, stroma	+	−	−	−	−	+	ND ↓ in D3.5 LE	↑	[17]
COUP-TFII (*Pgr-Cre*)	Stroma	?	+/−	−	−	−	+	ND	↑	[28]
Egr1 (Global)	Dynamic: LE GE stroma decidua	− (c)	−	−	30% down	ND	+	No expression on D3.5 No change	↑	[30]
Esr1/ERα (*Wnt7a-Cre*)	LE, GE, stroma	− (c)	ND	− (c)	− (c)	− (c)	+ (c)	− (c)	?	[10, 31]
Esr1/ERα (*Amhr2-Cre*)	LE, GE, stroma	−	+	+	+/−	+/−	−	ND	?	[9]
Foxo1 (nuclear) (*Pgr-Cre*)	D4.5 LE GE Blood vessel	+/−	+/− (b)	−	+	−	+	+	?	[32]
Fst (*Pgr-Cre*)	D3.5 LE, GE, stroma	+	−	−	50% down	ND	+	ND	↑	[16]
Gata2 (*Pgr-Cre*)	LE, GE, stroma	+	−	ND	−	ND	+ stratified squamous epithelium	ND Down in D3.5 LE	↑	[20]
Gnaq (*Pgr-Cre*) *Gna11*−/− (Global)	Uterus	+	−	−	−	−	+	ND	↑	[19]
Hand2 (*Pgr-Cre*)	Stroma	+	−	−	−	−	+	ND	↑	[13]
Hdac3 (*Pgr-Cre*)	LE, GE, stroma	+	−	−	−	−	+	ND	?	[90]
Ihh (*Pgr-Cre*)	LE, GE	?	?	−	−	−	− (c)	ND	?	[91]
Lpar3 (Global)	D3.5 LE	−	+	+ Delayed	+	+	−	+	↓	[29]
Mig-6/ERRFI1	Ubiquitous	+/−	+/−	ND	−	−	ND	ND	↑	[92]
NCOA6 (*Pgr-Cre*)	LE, GE > stroma, myometrium	+ (d)	− (d)	−	−	−	+	ND	↑	[21]
Ndst1 (*Pgr-Cre*) *Ndst2*−/− *Ndst3*−/− (Global)	*Ndst1* & *Ndst2*: LE GE > stroma *Ndst3*: low	?	−	+	−	−	+	ND	?	[14]
Pgr (*Wnt7a-Cre*)	LE, GE, stroma/ decidua	+	−	−	−	−	+	N/A	↑	[12]
PR-A (a) (*Wnt7a-Cre*)	LE, GE, stroma/ decidua	+	−	−	−	−	−	+ PRA	↓	[26]

Gene deletion or overexpression (a)	Uterine expression	Fluid retention (D3.5)	Lumen closure (D4.5)	Embryo attachment (D4.5 / D5.5)	Decidualization	LE penetration / removal	LE proliferation (D3.5 / D4.5)	Sustained PR expression in LE(D4.5)	Ratio of uterine E2/P4 signaling	Reference
Rac1 (*Pgr-Cre*)	LE, GE, stroma, decidua	−	+	+ Delayed	ND	−	+	ND	?	[33]
Rbpj (*Pgr-Cre*)	LE, GE (D0.5); Stroma/ decidua (D3.5-D7.5)	−	+/−	+	+	+	+	ND (No difference on D3.5)	↑	[27]
SHP2 (*Pgr-Cre*)	D4.5 LE & stroma / decidua	+	−	−	−	−	+	Up in D3.5 GE?	↑	[22]
Stat3 (*Pgr-Cre*)	LE, GE & stroma	+	−	−	−	−	+	No change?	↑	[23–25]

• All the effects are not associated with ovarian functions, either normal estrogen and progesterone levels or corrected estrogen and progesterone levels.

• Conditional gene deletion/overexpression: *Pgr-Cre* (progesterone receptor) for uterine cells, *Wnt7a-Cre* (Wnt Family Member 7A) for uterine epithelial cells, and *Amhr2* (anti-Mullerian hormone receptor type 2) for stromal cells (mainly in the anti-mesometrial pole [9]). In *Pgr-Cre* mice, Cre recombinase was inserted into exon 1 of the *Pgr* gene (https://doi.org/10.1002/gene.20098), therefore, the conditional knockout mice generated by using *Pgr-Cre* were also heterozygotes for the progesterone receptor. Because of the essential role of the progesterone receptor in embryo implantation, it is unclear how much of the phenotypes in these conditional knockout mice was contributed by the loss of function of one *Pgr* allele.

• Gene abbreviations: *Alk3/BMPR1A*, bone morphogenetic protein receptor, type IA, a receptor for BMPs; *Arid1a*, AT-rich interactive domain 1A gene; *COUP-TFII*, COUP transcription factor 2), also known as NR2F2; *EGR1*, early growth response 1; *Esr1/NR3A1/ERα*, estrogen receptor α; *Foxo1*, forkhead box O1; *Fst*, follistatin; *Gnaq* & *Gna11*, G-protein $G\alpha_{q/11}$; *Hand2*, heart and neural crest derivatives expressed 2; *Hdac3*, histone deacetylase 3; *IHH*, Indian hedgehog; *Lpar3*, the third receptor for lysophosphatidic acid; *Mig-6/ERRFI1*, mitogen-inducible gene 6 / ERBB receptor feedback inhibitor 1; *NCOA6*, nuclear receptor coactivator-6; *Ndst1-3*, N-deacetylase and N-sulfotransferase 1-3; *PGR/NR3C3*, progesterone receptor; *PR-A*, progesterone receptor A; *Rac1*, Ras-related C3 botulinum toxin substrate 1; *Rbpj* (Rbpsuh), recombination signal binding protein for immunoglobulin kappa j region, a transcriptional regulator important in the Notch signaling pathway; *SHP2/Ptpn11*, Src homology 2 domain containing protein tyrosine phosphatase.

• Annotations: a. gene overexpression; b. unsealed implantation chamber and no obvious fluid retention on D4.5; c. information deducted from E2 and E2+P4-treated ovariectomized mice; d, information deducted from consistent and extensive folding of LE on D3.5 uterine cross sections; ND, no data; N/A, not applicable; ? lack of conclusive data despite supportive data for potential roles in regulating uterine local estrogen signaling and/or progesterone signaling; +, yes; −, no; +/−, mixed results; ↓, decreased; ↑, increased.

• This is not a complete list of known uterine genes in regulating uterine estrogen signaling and progesterone signaling during peri-implantation in mice ([2, 4, 11, 78, 79]) .