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REVIEW ARTICLE

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Molecular mechanisms of embryonic implantation in mammals: Lessons from the gene manipulation of mice

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Abstract

Background: Human infertility has become a serious and social issue all over the world, especially in developed countries. Numerous types of assisted reproductive technology have been developed and are widely used to treat infertility. However, pregnancy outcomes require further improvement. It is essential to understand the cross-talk between the uterus (mother) and the embryo (fetus) in pregnancy, which is a very complicated event.

Methods: The mammalian uterus requires many physiological and morphological changes for pregnancy-associated events, including implantation, decidualization, placentation, and parturition, to occur. Here is discussed recent advances in the knowledge of the molecular mechanisms underlying these reproductive events—in particular, embryonic implantation and decidualization—based on original and review articles.

Main findings (Results): In mice, embryonic implantation and decidualization are regulated by two steroid hormones: estrogen and progesterone. Along with these hormones, cytokines, cell-cycle regulators, growth factors, and transcription factors have essential roles in implantation and decidualization in mice.

Conclusion: Recent studies using the gene manipulation of mice have given considerable insight into the molecular mechanisms underlying embryonic implantation and decidualization. However, as most of the findings are based on mice, comparative research using different mammalian species will be useful for a better understanding of the species-dependent differences that are associated with reproductive events, including embryonic implantation.

KEYWORDS

decidualization, gene manipulation, implantation, pregnancy, uterus

1 | INTRODUCTION

Human infertility has developed into a serious social problem all over the world, especially in developed countries. Numerous types of assisted reproductive technology (ART); for example, artificial insemination,[1] in vitro fertilization,[2] and intracytoplasmic sperm injection,[3] have been developed and are now used widely to treat human infertility. The cryopreservation of germ cells, such as sperm,[4] oocytes,[5] and embryos,[6,7] is an important alternative technology that is used routinely in human infertility clinics. The results from basic research in mice suggest that germ cells that are derived from induced pluripotent stem cells and embryonic stem

cells can be produced and such technologies might be useful for the treatment of human infertility.[8,9] Despite the application of these types of ART and great efforts by physicians, researchers, and embryologists, the infertility of ~50% of couples who desire a baby cannot be improved by the current treatments. Additional research and improved knowledge of embryonic implantation is required to establish new technologies to address these shortcomings.

In most mammalian species, including humans, female germ cells (oocytes) are arrested at metaphase II (MII) in the antral follicles and then ovulated, followed by a luteinizing hormone surge.[7,10] After ovulation, the oocytes reach the oviductal ampulla and then are fertilized with sperm. Sperm penetration triggers the release of the arrest at the MII stage via repetitive rises of intracellular Ca^{2+}, which are called "Ca^{2+} oscillations."[11-13] Thereafter, the oocytes progress to the embryonic stages and then transit to the uterus through the oviduct. When the embryos have moved to the uterus, the embryonic stage is called the "blastocyst."

A hatched blastocyst can implant at the epithelium in a species-dependent manner. The uterus requires considerable physiological and morphological changes during pregnancy. A successful pregnancy is associated with implantation, decidualization, placentation, and parturition.[14,15] The success of these events is indispensable for the birth of offspring. In humans, it is believed that 75% of incomplete pregnancies are associated with implantation failure[16] because implantation is the event of the first contact between the embryo (fetus) and the maternal tissue and a failure at this point never results in subsequent pregnancy-associated events (ie, decidualization, placentation, and parturition).[14,15]

In the uterus, the endometrium is composed of the luminal epithelium (LE), glandular epithelium (GE), and stromal cells (SCs) (Figure 1). The changes in uterine compartments are orchestrated primarily by estrogen and progesterone (P4),[17] which has pivotal roles in the SC proliferation and suppression of epithelial cell proliferation through the expression of Indian hedgehog homolog (IHH) and heart- and neural crest derivatives-expressed protein 2 (Hand2).[18-21] Estrogen is essential for the proliferation of epithelial cells, the suppression of apoptosis, and the regulation of the expression of Muc1 and lactoferrin, which are both critical for normal uterine function.[22-25] Under the functions of estrogen and P4, many molecules, including cytokines, growth factors, homeobox transcription factors, lipid mediators, and ion transporters, function through autocrine, paracrine, and juxtracrine interactions in order to accomplish the complex process of implantation.

Regarding the molecular mechanisms underlying embryonic implantation, a better understanding of estrogen- and P4-dependent pathways will contribute to further improvements of clinical treatments. Recent studies using genetically modified mice have obtained considerable evidence that helps to clarify these molecular mechanisms. This review summarizes the recent advances that are related to implantation, focusing on the roles of estrogen- and P4-dependent signaling.

2 | DEFINITION OF EMBRYONIC IMPLANTATION

Implantation is a complicated process and it is very difficult to define the starting point of embryonic implantation. In a broad sense, it is thought that implantation proceeds through at least five stages: (i) embryo spacing; (ii) apposition; (iii) orientation; (iv) attachment; and (v) invasion. Even among mammalian species, there are large differences at these stages. For example, blastocysts implant with their inner cell mass (ICM) oriented toward the lumen in rodents,[15] whereas in humans the blastocysts are oriented with their ICM toward the LE.[26] In the mouse, the deletion of lysophosphatidic acid receptor (LPA3) resulted in delayed implantation and embryo crowding, suggesting that LPA3 signaling regulates the embryo spacing.[27] As for apposition and orientation, the precise molecular mechanisms are not well understood. The attachment and invasion are collectively called "implantation." The duration that embryos can implant to the uterus is called the "implantation window."

3 | IMPLANTATION WINDOW CONCEPT

In mice, there are three phases of uterine sensitivity for receiving the embryo: (i) the "perceptive" phase (days 1-3, with the day of the vaginal plug observed being defined as day 1); (ii) the "receptive" phase (days 4-5); and (iii) the "refractory" phase[14-17] (beyond the afternoon of day 5) (Figure 1). Only during the receptive phase can embryos implant into the uterine epithelium. This specific period of time during which implantation is possible is called the "implantation window."[28] In humans, a specific morphologic marker was proposed to be associated with the implantation window: the appearance of pinopodes.[29] In both humans and rodents, pinopodes can be observed by scanning electron microscopy around the period in which embryonic implantation would be expected to occur. The pinopodes appear as smooth bulging cells on the apical surface of the endometrium.[30]

However, the presence of well-formed pinopodes in humans from day 20 to day 28 of the menstrual cycle has been reported, with no apparent increase in their appearance during the predicted window of receptivity.[31,32] It also has been demonstrated that pinopodes in both fertile and infertile patients covered between 1% and 50% of the viewed surface area. The entire surface of the endometrium was never covered by pinopodes, with most of the samples showing 5%-20% coverage.[30] The authors of those studies concluded that the presence of pinopodes alone cannot be an indicator of the implantation window.

In contrast to humans, the stricter time period of the implantation window in mice has been well studied with the use of embryo transfer techniques. One study showed that when mouse embryos were transferred at 09:00 hours, 14:00 hours, or 18:00 hours on day 4, successful implantation was confirmed on day 5.[33] A later study showed that a mouse embryo that was transferred at 09:00 hours on day 5 also can be implanted, but not a mouse embryo that was transferred at 21:00 hours on the

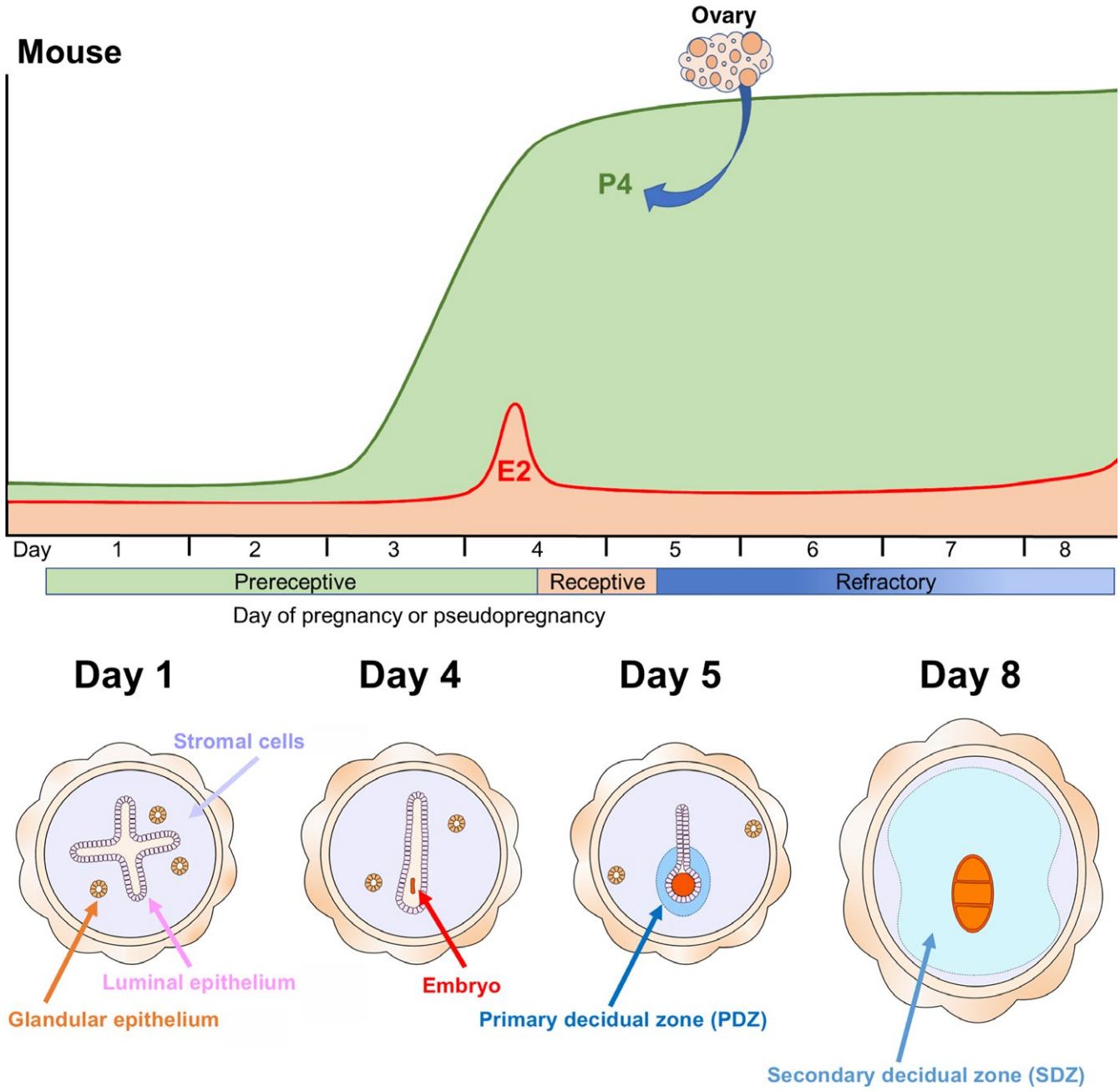

FIGURE 1 Estrogen and progesterone (P4) orchestrate the implantation window in mice, in which uterine sensitivity for accepting the embryo is composed of "perceptive" (days 1-3; with the day of the vaginal plug observed being defined as day 1), "receptive" (day 4), and "refractory" (day 5 afternoon). On day 4, an increase in the estrogen level is observed prior to the receptive stage (top). Morphological changes of the uterus from days 1-8 during pregnancy in mice (bottom). E2, Estradiol

same day.[34] These results suggest that the receptive phase starts around the morning of day 4 and is maintained until the morning of day 5. On the afternoon of day 5, the receptive phase eventually transits to the refractory phase. However, a P4 injection on the morning of day 5 can extend the receptive phase because when the mouse embryos were transferred to P4-primed recipients at 09:00 hours on day 6, implantation was confirmed.[34] Thus, it is thought that the implantation window is primarily orchestrated by estrogen and P4.

Estrogen and P4 bind to their nuclear receptors at different times and different cell types in the uterus can induce on-time functions in the uterine receptivity of mammals.[35,36] In the mouse uterus, an estrogen receptor (*Esr1*: ERα) and two types of P4 receptors (*Pgr*: PR-A and PR-B) are expressed.[37] In mice, the deletion of ERα resulted in defective phenotypes during reproductive events, including implantation.[38] Other studies demonstrated that PR-A and PR-B double knockout mice, but not single PR-B knockout mice, were infertile.[39,40] These results clearly showed

that ERα and PR-A are essential for at least embryonic implantation in mice.

During ovulation in mice, estrogen that is secreted from the ovaries induces a proliferation of uterine epithelial cells in the uterus via ERα.[23] In the epithelial-specific deletion of ERα (*Wnt7*$^{Cre/+}$; *Esr1*$^{flox/flox}$) in the mouse uterus, this proliferation of epithelial cells and the PR distribution were not affected, suggesting that stromal ERα has a major role in these events.[23] At the transition from the prereceptive day 3 to the receptive day 4 stage, P4 is newly secreted from the corpus lutea. Results from epithelial-specific PR (*Wnt7*$^{Cre/+}$; *Pgr*$^{flox/flox}$) knockout mice demonstrated that the role of PR in the epithelial cells is to inhibit epithelial estrogen action.[21] An earlier study showed that a slight increase in the estrogen level occurred prior to the receptive stage before noon of day 4.[41]

In several species other than rodents, ovarian estrogen is important, but dispensable, for embryonic implantation, whereas a high level of P4 is required for embryonic implantation in all species studied to date.[14] Ovariectomized mice on the morning of day 4 (just prior to the increase of the estrogen level) were used as a model of delayed implantation and embryonic dormancy.[33] After an ovariectomy, a continuous P4 injection can maintain the dormancy of the embryos for several days.[42,43] By the priming of estrogen after such a P4 injection, implantation can be induced. These results suggest that a slight increase in the level of estrogen can regulate the induction of embryonic implantation.

Using this model of delayed implantation, the effect of different concentrations of estrogen on embryonic implantation was examined. Priming with estrogen at a high concentration (>10 ng/mouse) rapidly induced the transition to the refractory stage, bypassing the receptive stage.[33] However, an injection of estrogen at a low concentration eventually can induce the transition to the receptive stage. These results strongly suggest that an optimal concentration of estrogen is required for on-time implantation.

4 | MOLECULAR MECHANISMS OF EMBRYONIC IMPLANTATION

4.1 | Estrogen-dependent signaling

Although estrogen and P4 signaling are both essential for embryonic implantation and although their signaling in mammals is complicated, it has been well documented that the major mediators of estrogen and P4 action are leukemia inhibitory factor (LIF) and IHH, respectively.[18,19,44,45] The LIF is a member of the interleukin (IL)-6 family of cytokines[46] and its deletion in mice causes sterility due to complete implantation failure, suggesting that LIF is indispensable for embryonic implantation.[45]

The LIF binds its receptor (LIFR) and IL-6 signal transducer, Gp130.[46] In situ hybridization of sections of mouse uterus from day 4 of pregnancy revealed that the LIFR messenger (m)RNA was highly and mainly expressed in the LE; Gp130 mRNA was highly expressed in the GE and at lower levels in the LE.[47] Although mice with the deletion of the LIFR and Gp130 knockout showed embryonic lethality,[48,49] mice with both the uterine epithelium-specific deletion of the LIFR (*Ltf*$^{Cre/+}$; *Lifr*$^{flox/flox}$) and the uterine-specific deletion of Gp130 (*Wnt7*$^{Cre/+}$; *Gp130*$^{flox/flox}$) showed severe defects in implantation.[50,51] The uterine-specific knockout of a downstream target of Gp130 and the LIFR; that is, a signal transducer and activator of transcription 3 (Stat3) also caused the failure of implantation.[51]

The epithelium-specific deletion of Stat3 (*Wnt7*$^{Cre/+}$; *Stat3*$^{flox/flox}$) also was reported recently to show implantation failure, followed by the downregulation of fibroblast growth factors (FGFs) and a cell-cell adhesion protein, cadherin,[52] whereas a stromal-specific deletion (*Amhr2*$^{Cre/+}$; *Stat3*$^{flox/flox}$) simply showed the phenotype with a decreased number of pups,[53] suggesting that the epithelial LIF signaling pathway is indispensable for implantation via FGF signaling. In humans, it was reported that a slight increase in LIF expression was observed at the endometrium before implantation[54] and some clinical studies demonstrated that the LIF expression around the time point of implantation was higher in fertile women, compared to infertile women.[55,56] However, in mammalian species other than mice, the question of whether LIF is an indispensable and sole factor for implantation remains unanswered.

A comparison of wild-type and LIF knockout mice revealed evidence that a homeobox transcription factor, Msx1, has an essential role during implantation.[57-59] The Msx1 was shown to be expressed transiently in both the LE and GE around the time of receptivity and its expression reached a maximal level on the morning of day 4.[58] The expression of Msx1 was not detected in the uterus of pregnant mice at day 5 (after implantation). The uterine-specific deletion of Msx1 (*Pgr*$^{Cre/+}$; *Msx1*$^{flox/flox}$) showed partial implantation failure, but a double knockout of Msx1 and Msx2, another member in the homeobox transcription factor family in mice (*Pgr*$^{Cre/+}$; *Msx1/Msx2*$^{flox/flox}$), resulted in infertility due to complete implantation failure via a suppression of cyclooxygenase-2 and bone morphologic protein 2 (BMP2).[58] As Msx2 expression was upregulated in the Msx1 null mice but not in the wild-type mice, it has been concluded that Msx2 has a compensatory role for Msx1. The Msx1 and Msx2 were involved in the polarity of the LE at the attachment of embryos.[58] In the uterine-specific Msx1/Msx2 knockout mice (*Pgr*$^{Cre/+}$; *Msx1/Msx2*$^{flox/flox}$), Wnt5a (a traditionally non-canonical Wnt and a mediator of cell polarity) was upregulated in the LE and SCs.[58] In addition, in the uterus of the Msx1/Msx2 knockout mice, E-cadherin, a Ca^{2+}-dependent transmembrane adhesion molecule, was persistently upregulated, even during the implantation period, whereas in the normal mice, E-cadherin was highly expressed in the LE prior to implantation, but transiently downregulated before the blastocyst's invasion into the stroma, suggesting that the remodeling of the adhesion junctions between epithelial cells is a critical event during embryonic implantation.[60-63]

Some studies showed that the loosening of cell-cell junctions in the mouse uterine epithelium through a downregulation of

E-cadherin was a prerequisite for blastocyst attachment.[64,65] Other recent investigations revealed that downstream factors of Wnt5a; that is, receptor tyrosine kinase-like orphan receptor 1/2 (Ror1/2) and Vangl 1/2, were both essential and that the disruption of Wnt5a-Ror-Vangl signaling results in disorderly epithelial projections, crypt formation, and embryo spacing, and impaired implantation.[66,67] Another recent study showed that Rbbj, the nuclear transducer of Notch signaling, conferred an on-time uterine lumen shape transformation by physically interacting with uterine ERα in a Notch pathway-independent manner.[68] It is understood that the estrogen-dependent signaling is required for normal mammalian embryo-uterus interaction via growth factors, cell-cell adhesion, and cell polarity pathways.

4.2 | Progesterone-dependent signaling

In all mammalian species studied to date, the indispensability of P4 for implantation has been confirmed. As a high P4 level also is required for later reproductive events (eg, decidualization[69] and the maintenance of pregnancy),[70] P4 generally is called the "pregnancy hormone." It has been well documented that PR knockout mice show defective phenotypes, such as disrupted ovulation, impaired luteinization, and incomplete decidualization.[39] An epithelial-specific deletion of PR (*Wnt7a*$^{Cre/+}$; *Pgr*$^{flox/flox}$) did not suppress epithelial proliferation.[21] In contrast, a stromal-specific PR deletion (*Amhr2*$^{Cre/+}$; *Pgr*$^{flox/flox}$) was shown to be able to induce the proliferation of the epithelium.[71] These results suggest that stromal PR is essential for the suppression of estrogen action.[21] These knockout female mice also showed infertility, which was attributed to incomplete uterine receptivity with a reduced expression of IHH.

It has been reported that PR can bind directly to the IHH promoter, resulting in the induction of the proliferation of SCs.[21] Another study demonstrated that stromal PR mediated the induction of IHH in the uterine epithelium and its downstream targets in the uterine stroma.[72] Chicken ovalbumin upstream promoter-transcription factor 2 (COUP-TFII), also known as "NR2F2," is a downstream target of IHH signaling. It was expressed in the subepithelial stroma, but not in the epithelial cells at day 5 of pregnancy.[73] The uterine deletion of COUP-TFII (*Pgr*$^{Cre/+}$; *Nr2f2*$^{flox/flox}$) caused implantation failure with excessive estrogenic action in the epithelium.[73] A P4-induced transcription factor, Hand2, was expressed in the stroma and has been reported as a regulatory factor for uterine receptivity and implantation.[20] The uterine deletion of Hand2 (*Pgr*$^{Cre/+}$; *Hand2*$^{flox/flox}$) resulted in excessive estrogenic activity and a proliferation of epithelial cells via a high expression of FGFs.[20] These results suggest that a major role of Hand2 in the SCs is the suppression of epithelial proliferation via a FGF signaling pathway.

It is well known that another P4-inducible factor, FKBP52, is required for modulating PR activity.[74-76] The FKBP52 knockout mice showed unsuccessful implantation due to impaired uterine P4 responsiveness and enhanced estrogen-like signaling. The deletion of FKBP52 increased the sensitivity to oxidative stress, followed by a reduced expression of a unique antioxidant enzyme, peroxiredoxin 6.[77] However, because this infertility was rescued by the injection of antioxidants, it is suggested that FKBP52 is dispensable for implantation under normal conditions.

5 | MOLECULAR MECHANISMS OF DECIDUALIZATION

Following embryonic implantation in mice, the SCs surrounding the implanted embryo progress to proliferation and subsequently differentiate into decidual cells.[78,79] Decidual cells are characterized as polyploidy cells.[15] In contrast to mice, in humans, implantation itself cannot trigger decidualization.[25] With embryonic implantation, the subepithelial SCs initially form an avascular primary decidual zone (PDZ) encasing the fetus around the afternoon of day 5.[80,81] The differentiated SCs other than those in the PDZ continue to proliferate and then further differentiate to form a well-vascularized secondary decidual zone (SDZ). In mice, the process of decidualization is regulated by many factors, such as transcription factors, growth factors, and cell-cycle regulators.

Progesterone signaling via PR-A is essential for the proliferation and differentiation of SCs into decidual cells.[82] It is thought that under progesterone signaling, homeobox genes are important for implantation and decidualization. Homeobox genes are highly conserved in many species.[83-85] Homeobox a (Hoxa) genes, *Hoxa10* and *Hoxa11*, are highly expressed in uterine SCs. The deletion of these genes (*Hoxa10*$^{-/-}$ and *Hoxa11*$^{-/-}$) resulted in severe implantation failure and insufficient decidualization.[84,86,87] The *Hoxa11*$^{-/-}$ mice showed a more severe phenotype than the *Hoxa10*$^{-/-}$ mice.[84] In humans, it also was reported that the expressions of Hoxa10 and Hoxa11 in the endometrium increased significantly in the mid-luteal phase, when the uterus is receptive to embryo attachment,[88,89] and that these expressions were significantly lower in infertile women.[89-92]

The BMPs belong to the transforming growth factor-beta superfamily of growth modulators[93] and transcripts that correspond to several BMP family members are expressed in mouse uteri.[94,95] In all the expressed BMPs in the uteri, only BMP2 was induced in response to P4, with intense expression in the SCs surrounding the implanted embryo.[94] Some studies showed that the in vitro supplementation of BMP2 to the undifferentiated SCs induced the decidualization of the SCs via a Smad signaling pathway.[96,97]

Female mice with a uterine-specific deletion of BMP2 (*Pgr*$^{Cre/+}$; *Bmp2*$^{flox/flox}$) were completely infertile.[96] In these mice, embryonic attachment was normal as in the control mice, but the uterine stroma was incapable of undergoing the decidual reaction to support further embryonic development.[96] Wnt4 has been identified as a downstream target of BMP2-induced decidualization[97] and was expressed primarily in the LE during the prereceptive phase and then it relocalized to the SCs surrounding the implanting embryo and expanded its expression to the deciduas.[57,98] Mice with the uterine-specific deletion of Wnt4 (*Pgr*$^{Cre/+}$; *Wnt4*$^{flox/flox}$) showed the phenotype of subfertility due to defective embryonic implantation and subsequent decidualization.[99] Transcriptome analyses showed that both

BMP2- and Wnt4-induced decidualization were regulated via epidermal growth factor receptor (EGFR), although the mice with a conditional deletion of EGFR ($Pgr^{Cre/+}$; $Egfr^{flox/flox}$) were subfertile.[100] These results indicate that BMP2- and Wnt4-induced decidualization have a complicated mechanism.

As polyploidization is a hallmark of decidualization that occurs via a specialized cell-cycle progression, many molecules that are associated with the cell cycle have been reported as regulators of decidualization.[17,101] The cell-cycle regulator, cyclin D3, is well known to be important for SC proliferation, differentiation, and polyploidization.[101,102] Indeed, a cyclin D3 deficiency in mice (cyclin $D3^{-/-}$) significantly compromised the pregnancy outcomes due to defective decidualization.[101] Hoxa10 was highly expressed at the decidual cells and the mice with its deletion ($Hoxa10^{-/-}$) exhibited impaired decidualization with an aberrant regulation of cyclin D3 and the loss of the region-specific expression of cyclin-dependent kinase (CDK)4 and CDK6 in the decidua bed.[103]

Another study showed that the deletion of IL-11 receptor a resulted in decidual degeneration with derailed endoreplication due to reduced cyclin D3 expression.[104-107] The death of ectodomain-containing protein, which can stabilize cyclin D3, was reported to be indispensable for uterine decidualization, as its deletion leads to impaired decidual development accompanied by attenuated polyploidy.[108,109] In light of these results, it is believed that cyclin D3 has a central role in decidual cells' proliferation and polyploidization.

6 | OUTSTANDING ISSUES

6.1 | Is leukemia inhibitory factor the only factor downstream of the estrogen signal that is necessary for successful implantation in mammals?

In the authors' unpublished study, the results that were obtained by another study were confirmed: in a mouse model of delayed implantation, an injection of estrogen at 3 ng/mouse could induce embryonic implantation.[110] In both studies, Institute of Cancer Research (ICR) cluster of differentiation 1 (CD-1) (outbred) mice were used. Interestingly, the injection of the same concentration of estrogen never resulted in the induction of embryonic implantation in the C57BL/6 mice (which is the most commonly used inbred strain in various research fields) when this strain was used as a model of delayed implantation (M. Kamioka, J. Ito, N. Kashiwazaki, unpublished). High-dose estrogen (10 ng/mouse) enabled the induction of embryonic implantation in the C57BL/6 strain. These results suggest that the estrogen level that is required for embryonic implantation is different between these two mouse strains.

This review's observations might be supported by a study that was performed in 2011.[111] Anti-LIF antibody was injected into C57BL/6 and ICR mice in order to block embryonic implantation.[111] In the C57BL/6 mice, embryonic implantation was inhibited completely, whereas embryonic implantation was inhibited only partially in the ICR mice. Another study used other strains (ddY, BALB/c, DBA/2Cr, and MF1 strains) in addition to the above two strains to test the inhibitory effect of an injection of anti-LIF antibody on embryonic implantation in those strains.[112] Their results demonstrated that the inhibition of LIF during the implantation period caused a severe disruption of embryonic implantation in the C57BL/6 and MF1 mice,[112] whereas implantation was only partly disrupted in the other strains (some embryos could still be implanted).

An injection of cardiotrophin-1 (an IL-6 family member, as is LIF) can induce successful implantation without LIF in mice with delayed implantation (ICR and B6) via the phosphorylation of STAT3 in the LE.[112] In the authors' preliminary study, the uterine-specific LIFR conditional knockout mice that were derived from the C57BL/6 strain ($Pgr^{Cre/+}$; $Lifr^{flox/flox}$) were completely infertile due to implantation failure, suggesting that the LIFR is indispensable for embryonic implantation—at least in C57BL/6 mice (K. Matsuo, J. Ito, N. Kashiwazaki, unpublished). As the LIF-null mice in both the C57BL/6 and ICR (CD-1) strains were infertile, there is no doubt that the LIF-LIFR pathway has an essential role in embryonic implantation in the mouse.[45,113] However, other factor(s) might compensate for the functions that are induced by LIF in some mouse strains.

6.2 | Limitations of knockout mice

In studies of genetically modified mice, estrogen- or P4-dependent factors have been identified as essential factors that are involved in implantation in mammals. However, one must consider that most of the previously reported data are from knockout mice and are not specific to the uterus (Table 1). For example, in most of those studies, Pgr^{Cre} transgenic mice (in which Cre recombinase is expressed under the *PR* promoter) were used to generate mice with uterine-specific gene knockout.[114] The PR is expressed not only in the uterine cells but also the ovarian cells, including the corpus luteum, which is a source of P4 production.[115] It has been shown that the conditional deletion of some genes; for example, *Lgr5*, caused infertility due to the deletion, not in the uterus but in other tissues.[70]

In addition, $Wnt7a^{Cre}$ and $Amhr2^{Cre}$ transgenic mice were used for epithelial-specific and SC-specific deletion, respectively.[23,53] The deletion of Wnt7a or Amhr2 itself caused a failure of the reproductive organs, suggesting that the phenotype of knockout mice with infertility might be a secondary effect. *Lactoferrin*-iCre (Ltf^{Cre}) transgenic mice were developed for the specific deletion of the gene at the epithelium of adult female mice.[116] In these mice, Cre recombinase is first expressed in the uterine epithelium after day 30 postbirth.[116] By using this new transgenic mouse line, it might be possible to more precisely clarify the molecular mechanisms underlying implantation.

Genome editing systems, such as CRISPR/Cas9, recently became available for the production of knockout animals other than mice.[117] It was shown very recently that genome editing systems are also available for generating conditional knockout animals.[118] The previous observations from knockout animals are mainly from mice, but many differences exist, even among mammalian species; for example, the source of estrogen secretion,

TABLE 1 Knockout mice show an impaired reproductive phenotype in the uterus

Gene	Gene product	Knockout	Knockout phenotype in female mice	Reference
Alk3	Activin-like kinase 3	Pgr-Cre	Implantation failure	129
Bmp2	Bone morphogenetic protein 2	Pgr-Cre	Incapable of undergoing the decidual reaction	96
Cdh1	E-cadherin	Pgr-Cre	Implantation failure; failed to artificially induced decidualization	130
Ctnnb1	β-catenin	Pgr-Cre	Implantation failure	131
Dicer	Dicer	Pgr-Cre	Enhanced stromal apoptosis; impaired uterine stromal cell proliferation in response to progesterone	132
Errfi1	ERBB receptor feedback inhibitor 1	Pgr-Cre	Implantation failure due to enhanced ER activity in epithelium	133
Esr1	Estrogen receptor 1	Wnt7a-Cre	Infertile	23
Fkbp52	FK506-binding protein-4	Systemic	Compromised P4 activity; impaired implantation and decidualization	75, 134
Foxa2	Forkhead box A2	Pgr-Cre	Implantation failure, severe impairment to respond to the artificially induced decidualization	135
		Ltf-Cre	Defective implantation and stromal cell decidualization	146
Cja1	Connexin 43	Pgr-Cre	Comprised decidualization; neovascularization defects	136
Ccnd3	Cyclin D3	Systemic	Defective decidualization	101
Dedd	Death effector domain-containing protein	Systemic	Infertile due to defective decidualization	109
Egfr	Epidermal growth factor receptor	Pgr-Cre	Implantation site demise due to a failure in the maintenance and progression of decidualization	100
Gp130	Glycoprotein 130	Pgr-Cre	Implantation failure	51
Hbegf	Hepahn-binding EGF-like growth factor	Pgr-Cre	Subfertile with deferred implantation	137
Hand2	Heart and neural crest derivatives expressed tanscript 2	Pgr-Cre	Impaired PR function	20
Hoxa10	Homeobox gene Hoxa-10	Systemic	Severe implantation failure and defective decidualization	83
Hoxa11	Homeobox gene Hoxa-11	Systemic	Severe implantation failure and defective decidualization	85
IHH	Indian hedgehog homolog	Pgr-Cre	Implantation failure	18
Il11ra	Interleukin-11 receptor-1	Systemic	Defective decidualization	104, 107
LIF	Leukemia inhibitory factor	Systemic	Implantation failure	45
LIFr	Leukemia inhibitory factor receptor	Ltf-Cre	Severe implantation failure	50
Src2	Steroid receptor coactivator 2	Pgr-Cre	Infertile due to impaired PR function mediated by SRC2	138, 139
Klf5	Kruppel-like factor 5	Pgr-Cre	Defective implantation; comprised decidualization	51
Msx1/2	Muscle segment homeobox gene (Msx) family members 1/2	Pgr-Cre	Implantation failure as altered uterine luminal epithelial cell polarity	58, 59
Nodal	NODAL	Pgr-Cre	Abnormal decidua basalis at mid-gestation and aberrant placental development	140
Notch1	Notch 1	Pgr-Cre	Comprised decidualization	141
Nr2f2	Chicken ovalbumin upstream promoter transcription factor II	Pgr-Cre	Implantation failure	73
p53	Transformation-related protein 53	Pgr-Cre	Uterine decidual senescence; preterm birth	142
Pgr	Progesterone receptor	Wnt7a-Cre	Implantation failure	21
		Amhr2-Cre	Reduction of litter size	71

(Continues)

TABLE 1 (Continued)

Gene	Gene product	Knockout	Knockout phenotype in female mice	Reference
Rbpj	Recombining binding protein suppressor of hairless	Pgr-Cre	Subfertile due to abnormal instructing of the initial embryonic-uterine orientation	68
Rea	Repressor of estrogen receptor activity	Pgr-Cre	Implantation and decidualization failure due to uterine development defects	143
Ror1/2	Retinotc acid receptor-related orphan receptor1/2	Pgr-Cre	Implantation failure due to abnormal cell polarity	66
Smo	Smoothened	Pgr-Cre	Uterine hypertrophy; luminal epithelial stratification; impaired decidualization	144
Stat3	Signal transducer and activator of transcription 3	Pgr-Cre	Implantation failure	51
		Wnt7a-Cre	Implantation failure	52
		Amhr2-Cre	Implantation failure	53
Vangl1/2	Vertebrate regulator of planar cell polarity Van Gogh-like 1/2	Pgr-Cre	Implantation failure due to abnormal cell polarity	66, 67
Wnt4	Wingless-related MMTV integration site 4	Pgr-Cre	Implantation defect failed to undergo the artificially induced decidual response	99
Wnt7a	Wingless-related MMTV integration site 7a	Pgr-Cre	Implantation failure	145

EGF, epidermal growth factor; ER, estrogen receptor; MMTV, mouse mammary tumor virus; PR, progesterone receptor.

the orientation of the blastocyst for implantation, and the structure of the placenta. Deletions of a specific gene by genome editing will help to resolve the many pregnancy-associated mysteries with findings that can be expected to differ among mammalian species.

6.3 | Uterine aging

The oocyte quality is known to decrease in an age-dependent manner. For example, the frequency of chromosome segregation errors during meiosis I in mouse oocytes increased with age.[119] Aged oocytes were associated with low fertility,[120] low developmental ability,[121] and aberrant kinetics of the epigenome.[122] In addition, ovarian aging, including the follicles themselves and granulosa cells, affected the reproductive outcomes in many species, including humans.[123-125] A recent study clearly showed that abnormal embryonic development in aged female mice was associated with severe placentation defects, which resulted from major deficits in the decidualization response of the uterine stroma.[126] The same study also revealed that the defect was rooted in a blunted estrogen and P4 responsiveness of the aging uterus. Importantly, that study also demonstrated, using an embryo transfer technique, that a young uterine environment can restore normal placental and embryonic development. The study provided the first evidence at the molecular level of the pivotal, albeit under-appreciated, impact of maternal age on the uterine adaptability to pregnancy as a major contributor to the decline in the reproductive success of older mice.

In humans, the use of a surrogate mother as an option for women who are infertile due to implantation failure and recurrent abortion is very limited from the viewpoint of law and ethics. For these patients, uterine transfer[127] and uterine matrix transplantation[128] can be alternative treatments to regenerate and restore an aged or genetically based impaired uterine environment.

7 | CONCLUSION

Embryonic implantation involves very complicated reproductive events and many molecules are involved with implantation. The results from animal models (in particular, gene-modified mice) have provided clear evidence at the molecular level. Most of these data are from mice and comparative research using other mammalian species will be useful to increase the understanding of the species-dependent differences that are associated with reproductive events, including embryonic implantation.

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