Progesterone governs endometrial proliferation-differentiation switching and blastocyst implantation

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Abstract. Blastocyst implantation contains the following three processes: apposition, attachment, and invasion of the blastocyst. Ovarian hormone progesterone (P₄) regulates these processes exquisitely. P₄-induced molecular communications between the endometrial epithelium and stroma as well as endometrial proliferation-differentiation switching (PDS) until blastocyst attachment are fundamental steps in blastocyst implantation. Based on the knowledge obtained from the previous studies of mouse models by my group and others, this article outlines how P₄ directs the uterus to complete blastocyst implantation.

Key words: Blastocyst implantation, Uterine receptivity, Progesterone, Endometrial proliferation-differentiation switching

Introduction

Infertility and recurrent miscarriage are one of global health problems. Despite enormous efforts by medical experts, their underlying mechanisms remain unclear. Blastocyst Implantation is a process of the first embryomaternatal encounter in pregnancy. It contains the following three processes: apposition, attachment, and invasion of the blastocyst. A blastocyst attaches to the receptive uterine luminal epithelium to start pregnancy. Then, the blastocyst invades into the endometrial stroma under the epithelium, and the stroma becomes decidua, which supports growth and survival of blastocyst [1-7]. Because it is estimated that implantation failure causes 75% of unsuccessful conceptions [8], it should be an obligatory mission to elucidate the mechanism and solutions of implantation failure.

Successful blastocyst implantation is the result of suitable molecular crosstalk between the uterus and the blastocyst during these processes. During these processes, the ovarian steroid hormone progesterone (P₄), that is called a “hormone of pregnancy”, plays an essential role by thoroughly managing uterine functions [1, 9-12]. P₄ is produced by ovarian corpus luteum after ovulation, and later, the main source of P₄ secretion changes from ovary to trophoblast during first trimester of pregnancy. In the clinical situation, luteal support by P₄ improves the implantation rate [13-15], indicating significant role of P₄ in human embryo implantation.

Studies using mouse models are frequently conducted in the research field of embryo implantation [3, 6]. Particularly, some genetically-engineered mice provide us valuable piece of information to elucidate molecular functions of P₄ in blastocyst implantation [9-12]. This article presents the literature about the role of P₄ in mouse blastocyst implantation by my group and others in order to support better comprehension of molecular and cellular basis for human blastocyst implantation. The current article is separated into three parts; (1) biological function of P₄ during mouse blastocyst implantation, (2) molecular mechanisms of P₄ action in mouse blastocyst implantation, (3) key molecular mechanisms in mouse blastocyst implantation beyond P₄ action.

Biological Function of P₄ during Mouse Blastocyst Implantation

Uterine receptivity to the blastocyst in mice

An implantation-competent blastocyst is one of two indispensable components for embryo implantation, because a major reason of implantation failure is a poor-quality embryo [16]. The other essential element is uterine receptivity defined as a capacity to accommodate the competent blastocyst in the uterus [3, 6]. The receptive mouse uterus displays stromal proliferation and epithelial
differentiation as an indicator of proper arrangement for blastocyst implantation [17], which is governed by ovarian hormones. P4-dependent morphological alteration termed “pre-decidualization” occurs during this step [5, 6]. Increased production of ovarian steroid hormones estradiol-17β (E2) and P4 provides the uterus with a capacity of blastocyst attachment after pre-decidualization. Consequently, blastocyst attachment takes place in the uterus. Then, stromal cells surrounding the embryo begin to differentiate concomitantly with polyloid formation. This stromal differentiation is termed “decidualization” [5, 6]. If the blastocyst attachment fails, any competent blastocysts are never able to stick to the uterus (Fig. 1). The uterus permits blastocysts to attach to itself within the limited period. This limited period is termed “implantation window” [5, 6]. Luminal epithelium around the blastocyst exfoliates, and trophoblast begins to invade into the stroma, which is termed “blastocyst invasion” (Fig. 1) [5, 18]. In this way, uterine receptivity is involved in embryo implantation.

**P4 directs mouse blastocyst implantation**

Mouse models are employed most commonly among the animal experimental models [3, 6]. Vaginal plug is noticed in the morning on the next day of ovulation and mating, and this day is defined as day 1 of pregnancy. The uterus appears swollen under the influence of E2 surge on day 1, and luminal epithelial cells proliferate outstandingly. Circulating P4 levels rise on day 3 due to its production from newly-formed corpus luteum. Elevated P4 takes over E2 and becomes a dominant ovarian hormone by day 4 morning. Notably, P4 provides uterine receptivity, and changes uterine cell proliferation status dynamically. The stromal cells begins to proliferate, and in contrast, the luminal epithelial cells declines to proliferate and begins to differentiate [17], which I here name “endometrial proliferation-differentiation switching (PDS)”. These changes in time- and cell-specific proliferation and differentiation is accompanied by the expression of several genes critical for uterine receptivity. Concomitant ovarian secretion of E2 under continuous effects of P4 on day 4 morning provides initiating signals to the uterus for the communications between the blastocyst and the uterus on day 4 evening. Dormant blastocyst is activated by E2-derived uterine factors, and the uterus turns to be receptive. Under the influence of ovarian hormones, the implantation-competent blastocyst as well as the receptive uterus is prepared through the molecular communications between the blastocyst and uterus [3, 6]. The blastocyst trophectoderm adheres to the luminal epithelium on day 4 midnight, and stromal cells surrounding the blastocyst initiate differentiation, change their stromal morphology into epithelioid type containing polyloid, and form a new endometrial layer around the blastocyst as a process of decidualization. Stromal vascular permeability is markedly increased at the attachment sites surrounding the blastocyst. An intravenous injection of Chicago Blue Dye solution can visualize the attachment site with increased vascular permeability. Trophoblast cells invade into the stroma on day 5 evening, and blastocyst implantation is completed [3, 6, 18].

![Fig. 1 Blastocyst attachment and invasion as important steps of mouse blastocyst implantation](image)
Molecular Mechanisms of P₄ Action in Mouse Blastocyst Implantation

**P₄-PR signaling in mouse blastocyst implantation**

P₄ acts through progesterone receptor (PR), which regulates the expression of P₄ responsive genes transcriptionally and the critical pathways for pregnancy event including ovulation and implantation. Genetic alteration of PR and its related molecules in female mice has exposed the roles of P₄ in pregnancy. PR knockout female mice showed infertility due to ovulation failure [9], suggesting that P₄-PR signaling is essential for ovulation. Although PR knockout mice are very powerful and useful to analyze the molecular pathway in ovulation, but not to dissect the role of P₄ on blastocyst implantation.

Stability of PR complex impacts on PR function. Functionally-mature PR complex contains a receptor monomer, a 90-kDa heat shock protein (Hsp90) dimer, the cochaperone p23, and one of four cochaperones which include tetratricopeptide repeat (TPR) repeat that binds to Hsp90 [19]. The immunophilin cochaperone 52-kDa FK506 binding protein (FKBP52) is one of TPR-containing chaperones, binding both Hsp90 and PR, stabilizing the structure of PR complex, thus reinforcing P₄-PR signaling [11, 19]. Deficiency of FKBP52 deteriorates uterine P₄-PR signaling, but does not pose complete demise, because minimal binding of P₄ to PR is retained [11, 19]. P₄ supplementation rescues uterine PR signaling on the CD1 background, which is a noteworthy aspect of FKBP52 knockout mice unlike PR knockout mice [12]. Furthermore, FKBP52 knockout mice show normal ovulation with normal P₄ secretion [12]. Based on the findings of FKBP52 knockout mice, P₄-PR signaling is essential in the process of blastocyst implantation.

**Endometrial PDS is governed by P₄-PR signaling**

As mentioned above, endometrial stroma begins to proliferate, and luminal epithelium attenuates proliferation and begins to differentiate [17], which is named “endometrial PDS”. In general, cell differentiation is compatible with poor cell proliferation, and distinct switching between proliferation and differentiation occurs in many cell types [20-23]. Our study clearly demonstrated that an injection of PR antagonist RU486 hampers endometrial PDS and blastocyst implantation in wild-type (WT) mice [17]. FKBP52 knockout uterus has uterine P₄ resistance with persistent epithelial cell proliferation without much stromal cell proliferation on day 4, which is recovered by P₄ supplementation [17]. These findings indicate that P₄-PR signaling directs the uterus and encourages endometrial PDS in the peri-implantation period (Fig. 2). In addition, PDS in the receptive uterus occurs not only in mice but in humans [17]. As far as I know from the literature, all types of genetically-modified mice lacking endometrial PDS show implantation failure [12, 17-19, 24-28].

PR has two isoforms, PR-A and PR-B, and PR-A is primarily responsible for uterine function during pregnancy, contributing to endometrial PDS [10, 29]. However, it is expected that PR-B does not have critical function in pregnancy, because systemic ablation of PR-B does not lead to any problems in pregnancy outcome [10, 29]. These findings suggest that P₄-PR-A signaling serves endometrial PDS and uterine receptivity.

**Balance between E₂ and P₄ signaling fine-tunes endometrial PDS and uterine receptivity**

Both lack and excess of E₂ levels can prevent blastocyst implantation [30]. Implantation failure due to the superioriority in E₂ effects is also observed in knockout mouse models other than FKBP52 knockout mice. Uterine specific deletion of nuclear receptor co-activator 2 (NCOA2) encoding steroid receptor co-activator 2 (SRC2) causes implantation failure through the disorder of the optimization of PR function by NCOA2 in mice [31]. Another mouse study demonstrated that nuclear receptor co-activator 6 (NCOA6) does not act as a coactivator but promotes the ubiquitination and degradation of ERα, attenuating E₂-ERα signaling in the peri-implantation period [26]. Uterine NCOA6 knockout mice causes the accumulation of ERα and enhances E₂ sensitivity, leading to implantation failure [26]. Imbalanced hormonal signaling as well as implantation failure are rescued by the injection of estrogen receptor antagonist ICI-182780 [26]. Protein tyrosine phosphatase Src homology 2 domain containing protein tyrosine phosphatase-2 (SHP2), a classic cytoplasmic protein, is primarily located in the nuclei of uterine cells during implantation, and nuclear SHP2 enhances SRC kinase-mediated ERα tyrosine phosphorylation, facilitates ERα binding to PR promoter, and promotes the ERα transcription activity in the peri-implantation mouse uterus [27]. A recent mouse study revealed that B lymphoma Moloney insertion region 1 homolog (BMI1), a component of the polycomb repressive complex-1 (PRC1), regulates PR ubiquitination in a polycomb complex-independent manner, and uterine deletion of BMI1 causes implantation failure due to the disruption of uterine P₄ responsiveness [28]. In women with history of miscarriage, low expression levels of endometrial BMI1 is related to poor PR responsiveness [28]. These findings suggest that BMI1 controls endometrial PR function by post-transcriptional regulation of PR, leading to successful implantation in both mice and humans.

Mice with uterine deficiency of signal transducer and
activator of transcription 3 (STAT3), known as a down¬
stream molecule of leukemia inhibitory factor (LIF)
during blastocyst implantation [32], demonstrated im¬
plantation failure with relatively higher uterine influence
of E2-ERα than P4-PR signaling [33], but it is not fully
elucidated how STAT3 influences E2/P4 signaling in the
peri-implantation uterus.

P4-induced endometrial PDS is modified by HAND2
and IHH in mice
A basic helix-loop-helix transcription factor, heart and
neural crest derivatives-expressed protein 2 (HAND2), is
expressed in the endometrial stroma under the influence
of P4-PR signaling and hinders endometrial PDS through
suppression of fibroblast growth factor, despite no con-
tribution to stromal proliferation [25]. HAND2 acts as
a blocker of epithelial E2 signaling during blastocyst
implantation. Uterine HAND2 knockout mice shows
defective blastocyst attachment [25], suggesting that
stromal HAND2 causes blastocyst attachment by way of
P4-induced differentiation of luminal epithelium.

Indian hedgehog (IHH), a downstream factor of PR, is
strongly expressed in the luminal epithelium of mouse
uterus immediately before blastocyst attachment [34,
35]. IHH acts through its receptor Patched-1 (PTCH1).
PTCH1 is localized in the stroma, and induces stromal
proliferation [34-36]. One of two major downstream tar-
gets in IHH signaling is a transcriptional factor GLI [34],
and the other is a nuclear receptor chicken ovalbumin
upstream promoter-transcriptional factor (COUP-TFII)
GLI contributes to stromal proliferation [34] and COUP-TFII keeps the balance between E₂/P₄ signaling [37]. Taken together, the interactions between the endometrial epithelium and stroma under the hormonal control is involved in endometrial PDS (Fig. 2).

**Epigenetic regulation of P₄-PR signaling by microRNA is involved in endometrial PDS**

Our previous study showed that endometrial PDS occurs in a spatial manner, between the uterus and cervix [17]. Blastocysts do not implant in the cervix but in the uterus in normal pregnancy. The mouse uterus displays endometrial PDS, and in contrast, the cervix does not exhibit any changes of proliferation and differentiation both in the epithelium and stroma. The human uterus also presents dynamic endometrial PDS between the proliferative phase and the secretory phase, unlike the human cervix with no significant changes in the proliferation status [17]. Based on these findings, we hypothesized that there is the different machinery for the reduced P₄-PR signaling in the cervix unlike the uterus. Indeed, P₄-PR signaling weakens in the cervix due to microRNA (miR)-200a in two different pathways. One is the reduction of PR levels in the cervix by posttranscriptional regulation by miR-200a [17]. The other is local metabolism of P₄ in the cervix, in which miR-200a upregulates 20α-hydroxysteroid dehydrogenase, a P₄-metabolizing enzyme, through down-regulation of STAT5 [17, 38]. Moreover, the expression levels of miR-200a are low in the receptive uterus compared to the pre-receptive one (unpublished observation), indicating that miR-200a makes uterine P₄-PR signaling appropriate for successful implantation.

**Molecular Mechanisms of Blastocyst Implantation beyond P₄ Action**

**Involvement of LIF and FOXA2 in mouse blastocyst attachment**

Uterine P₄-PR signaling is critical not only in the process of uterine receptivity acquisition but even after blastocyst attachment throughout pregnancy (Fig. 3). Recent studies performed by us and other groups clarified key regulators of blastocyst attachment and invasion other than P₄-PR signaling. Forkhead box protein A2 (FOXA2) regulates blastocyst attachment [39], and hypoxia inducible factor 2α (HIF2α) controls blastocyst invasion [18]. As mentioned above, E₂ is an initiator for blastocyst attachment. Leukemia inhibitory factor (LIF), an IL-6 family cytokine, is secreted from the endometrium in response to E₂ secreted by ovaries and plays a crucial role in blastocyst attachment. In a delayed implantation mouse model with ovariectomy on day 4 of pregnancy and later hormone supplementation, LIF can induce blastocyst attachment on behalf of E₂ [40]. However, it remains unclear how LIF allow blastocyst attachment. FOXA2 is necessary for development of endometrial glands in mouse newborn pups [39]. FOX transcription factors are involved in cell proliferation, differentiation and longevity of many tissues and organs. FOXA2 is expressed in the glandular epithelium of mouse [39]. In mice, deletion of FOXA2 in the entire uterus shows complete loss of endometrial gland [39]. In addition, deletion of FOXA2 in uterine epithelium shows reduction of LIF expression and failure of blastocyst attachment which LIF supplementation can recover [39], suggesting critical roles of E₂-FOXA2-LIF pathway in blastocyst attachment.
Involvement of HIF2α in mouse blastocyst invasion

Uterine molecules associated with blastocyst invasion have rarely been investigated. Oxygen concentration of endometrial surface of the early human pregnancy is known to be lower than that of inner endometrium [41], suggesting the uterine cavity and endometrial surface are hypoxic state during blastocyst implantation. Hypoxia inducible factor (HIF) is a major transcriptional factor inducible by low oxygen tension [42] and HIF2α is expressed in the mouse uterus during blastocyst implantation [43]. Our recent study revealed that mice with deletion of HIF2α in the entire uterus are infertile due to implantation failure [18]. Treatment with P₄ and LIF rescues decidual growth arrest and aberrant position of implantation sites in uterine HIF2α knockout mice, respectively, but does not restore pregnancy failure. Uterine HIF2α knockout mice show persistence of the intact luminal epithelium [18], which blocks direct contact between stroma and blastocyst, inactivation of PI3K-AKT pathway (embryonic survival signal), and failed blastocyst invasion [18]. Mice with stromal deletion of HIF2α are infertile due to impaired blastocyst invasion, and those with epithelial deletion of HIF2α show normal fertility, suggesting the critical role of stromal HIF2α in blastocyst invasion. Taken together, our study revealed that stromal HIF2α allows trophoblast invasion through detachment of the luminal epithelium and activation of an embryonic survival signal. Thus, HIF2α is one of few uterine molecules regulating blastocyst invasion (Fig. 3).

Summary

To improve fertility rate, there remain many problems to solve. One of these issues is recurrent implantation failure regardless of good-quality embryo transfer [4, 44]. Several molecules which are employed within very limited time are involved in the opening of implantation window. Basic research is required to dissect the mechanism of implantation failure and to establish its effective treatment. One possible mechanism of implantation failure is “P₄ resistance” [12, 19]. P₄ supplementation for fertility treatment is very common in humans, and its effectiveness on patients with luteal insufficiency is established [13]. However, P₄ treatment does not always help infertile patients suffering from implantation failure. Thus, the current fertility therapy can not cure patients with severe P₄ resistance. FKBP52 knockout mouse is a well-designed experimental model which reflects the influence of P₄ resistance on implantation failure, and therefore, this mouse model may assist our comprehension of human infertility. In addition, it is notable that our group discovered uterine HIF2α knockout mice as a new mouse model for the investigation of blastocyst invasion. I believe that better understanding of P₄ functions must be of great help in developing novel approaches of infertility and contraception.

Acknowledgements

This work was supported by JSPS KAKENHI (grant numbers 16H04679, 16H05469, 16K15700, 16K15701, 15H04979, 15K10660, 17K16833, 16K10668, 17H06640, 15K15597, 18K19601, 18K19600, 18H02943, 18K09284), AMED-PRIME (JP18gm5910010), Takeda Science Foundation, and Bayer Grant.

Disclosure

The authors declare that they have no conflict of interest.

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