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REVIEW ARTICLE

Uterine receptivity and embryo–uterine interactions in embryo implantation: lessons from mice

Mahiro Egashira · Yasushi Hirota

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Abstract Implantation is a process of the first feto-maternal encounter in the uterus. A competent blastocyst and a receptive uterus are critical for successful implantation. For an acquisition of uterine receptivity, the following conditions need to be satisfied in the uterine environments: the endometrial preparation with stromal proliferation and epithelial differentiation in the pre-receptive phase and proper interactions between the uterus and blastocyst later in the phase. Focusing on these points and primarily referring to the mouse in vivo evidence, this review article has shown detailed molecular mechanisms for successful implantation.

Keywords Cytokines · Embryo implantation · Embryo–uterine interactions · Ovarian steroid hormones · Uterine receptivity

Introduction

Pregnancy is a complicated physiological phenomenon comprising a series of these processes: ovulation, fertilization, implantation, embryonic growth, decidualization, feto-placental growth and parturition. Each process is strictly coordinated and essential for successful pregnancy. Implantation, a process of the first feto-maternal encounter in the uterus, consists of the following three steps: apposition, adhesion, and invasion of the embryo. Successful implantation is the result of appropriate molecular communication between the uterus and the blastocyst during these steps. Animal studies, especially mouse studies, have taken the lead in implantation research [1]. Notably, it is no wonder that recent studies using different kinds of genetically-altered mice have given us valuable information in this research field. Since the current major concepts in embryo implantation have primarily arisen from mouse studies, here we principally refer to the mouse studies.

What are mandatory components for successful implantations? There are two essentials. One is an implantation-competent blastocyst because poor embryo quality is likely to be one of the major causes of implantation failure [2]. On the other hand, "uterine receptivity" is also a significant factor, defined as uterine capacity in order to accommodate the competent blastocyst [1]. This capacity allows an appropriate endometrial preparation with stromal proliferation and epithelial differentiation stimulated by ovarian steroids in advance before the phase of embryo–uterine interactions (Fig. 1). In this process, progesterone-dependent morphological changes in endometrial stroma are observed, which we call "pre-decidualization" (Fig. 1) [3]. The small spike of ovarian estrogen is followed by an acquisition of the endometrial status, and then, the endometrium provides the embryo with adhesion activity. Thus, the uterus enters into the receptive phase. This acquisition of adhesion activity in the dormant blastocyst by endometrium-derived factors is called "blastocyst activation" (Fig. 1) [1]. Further, the blastocyst adhesion onto the uterus induces an endometrial

M. Egashira · Y. Hirota (✉)
Department of Obstetrics and Gynecology, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655, Japan
e-mail: yhirota-tky@umin.ac.jp

M. Egashira
Laboratory of Animal Breeding, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113-8657, Japan

Y. Hirota
PRESTO, Japan Science and Technology Agency, Saitama 332-0012, Japan

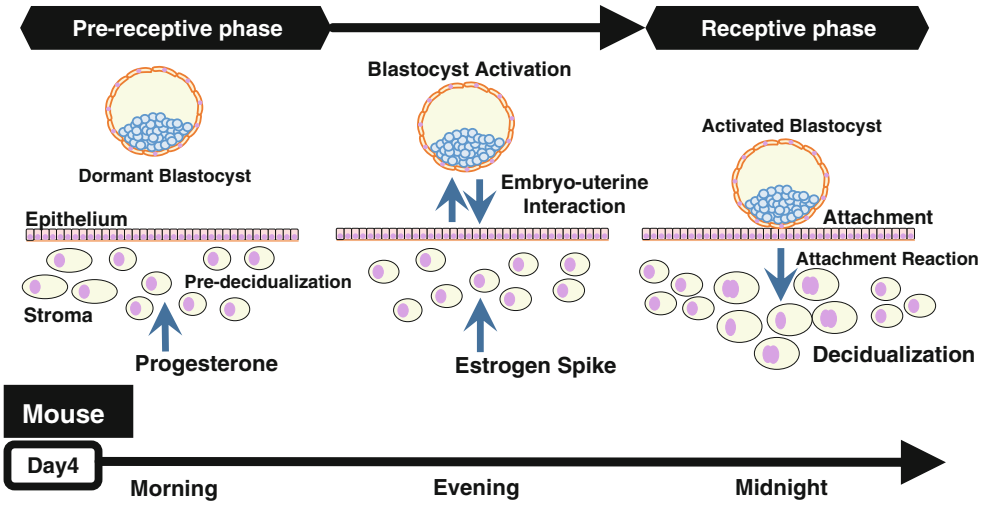

Fig. 1 The process of embryo implantation

attachment reaction, in which stromal cells surrounding the blastocyst start to differentiate concurrently with polyploid formation, which is called "decidualization" (Fig. 1) [3]. The receptive phase of the uterus is transient, and unless the blastocyst adhesion occurs, the endometrium enters into the refractory phase, when any functional blastocysts are incapable of adhesion to the endometrium. Thus, the endometrium can allow blastocyst adhesion in the restricted period, which is usually regarded as the "implantation window" (Fig. 1) [3]. This sequence of events is critical for starting implantation.

Ovarian steroid hormones produce and maintain uterine receptivity throughout implantation processes. Under the influences of progesterone and estrogen, there are important mediators for cell-to-cell communications in the uterine microenvironments during implantation: cytokines and growth factors such as leukemia inhibitory factor (LIF) and heparin-binding epidermal growth factor-like growth factor (HB-EGF). For example, maternal LIF is essential for successful implantation [4], and HB-EGF plays a key role in a two-way communication between the embryo and the uterus [5]. These factors are considered to be crucial for uterine receptivity and blastocyst activation. In this review article, we describe the acquisition process of uterine receptivity and embryo–uterine molecular interactions through the major secreted mediators.

Implantation in mice and humans

Since a time-line and hormonal conditions in the peri-implantation period look comparable between mice and humans (Fig. 2), we have much to learn about embryo implantation from the previous mouse studies [1]. In mice, a vaginal plug is observed in the morning on the day after ovulation and mating, which we define as day 1 of

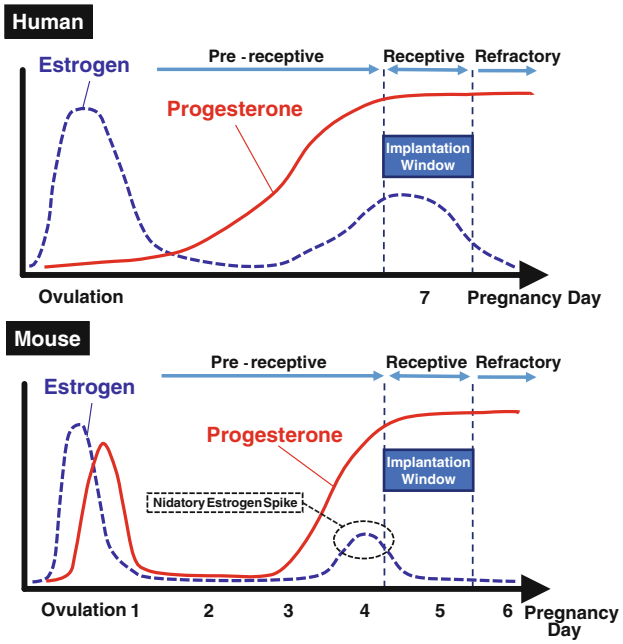

Fig. 2 The hormonal status in early pregnancy

pregnancy. Luminal epithelium strongly proliferates and the uterus looks swollen under the influence of an estrogen surge. On day 3 of pregnancy, corpora lutea are newly formed, and start to produce progesterone. Progesterone becomes completely dominant by the morning of day 4 when heightened progesterone makes endometrial stromal cells proliferate, called pre-decidualization, and this phenomenon is similarly observed in humans. At the same time, the luminal epithelium ceases proliferation and differentiates for the blastocyst attachment reaction. Late on the morning of day 4, a small estrogen surge occurs as a starting signal of implantation. This estrogen spike leads to stromal edema and luminal closure placing the blastocyst in close apposition with the luminal epithelium, and makes

the uterus produce some blastocyst activators like osteopontin (OPN) [6]. It is followed by an intimate adherence of the blastocyst trophectoderm to the luminal epithelium, marking the first discernible sign of implantation on the night of day 4 (2200–2400 h). Immediately after the implantation, stromal cells surrounding the blastocyst start differentiation, change their stromal morphology into epithelioid type with polyploidy, and form a new layer around the embryo. This process is known as decidualization. The attachment reaction coincides with an increased stromal vascular permeability at the site of the blastocyst. Embryo-derived trophoblast cells invade the endometrium, and finally, embryo implantation is completed [1].

As described above, the present general concepts in the embryo implantation are gained from mouse studies. Since both in vivo and in vitro experiments, especially about blastocyst activation and decidualization (not pre-decidualization), are technically and ethically difficult to perform in humans, mouse models are the most powerful approach to understand embryo implantation in vivo, and are globally applied in the current research of reproduction.

Ovarian hormones: estrogen and progesterone

Estrogen and progesterone play crucial roles throughout pregnancy. The following two processes under the control of ovarian steroids are needed for successful implantation: preparation of endometrial proliferation and differentiation, and appropriate embryo–uterine communication. In the pre-receptive phase, the endometrium must keep specific differentiation status in which luminal epithelium ceases proliferation and subluminal stroma start to proliferate in a progesterone-dominant condition. Next, a small spike of estrogen occurs just before the receptive phase. This nidatory estrogen with consistent influences of progesterone gives starting signals for embryo–uterine interactions to the uterus, the dormant blastocyst is activated, and the uterus turns to be receptive. Thus, the implantation-competent blastocyst and the receptive uterus are prepared through the molecular communications between the embryo and uterus under the influence of ovarian hormones [1].

The endometrial proliferation and differentiation in the pre-receptive phase is influenced by ovarian hormones, dominantly by progesterone which is known as a "hormone of pregnancy". Progesterone acts on the uterus via progesterone receptors (PR) throughout the pregnancy [7]. PR deficient mice show impaired reproductive phenotypes in ovulation, implantation and decidualization and a compromised status of endometrial proliferation and differentiation on day 4 of pregnancy [7]. In addition, mice with deletion of PR cochaperone FKBP52 have uterine progesterone resistance and impaired induction of progesterone-responsive genes on the morning of day 4 [8]. The mutant females also have an impaired uterine status of proliferation and differentiation in the pre-receptive phase, and further cause implantation failure [8]. Taken together, progesterone signaling is a major pathway regulating the endometrial preparation for appropriate differentiation and proliferation in the pre-receptive phase.

Constant influences of progesterone are essential in the uterus during and after implantation, while transient estrogen influences are needed for the induction of implantation. Among the estrogen-responsive genes, it is known that LIF and OPN are functionally important secreted proteins during implantation [4, 6]. Estrogen injection up-regulates both genes in the glandular epithelium in the delayed implantation mouse model [6, 9]. These findings suggest that nidatory estrogen induces LIF and OPN in glandular epithelium, and these factors participate in embryo–uterine interactions to continue the subsequent implantation process. In addition, HB-EGF is the most famous secretory factor for embryo–uterine interactions [5]. The detailed functions of these secreted factors are separately described in the following sections.

Leukemia inhibitory factor

The presence of various cytokines and their receptors in the uterus and the embryo during early pregnancy suggests their roles in implantation [1]. It is known that several cytokines such as LIF and M-CSF are critical for normal female fertility by studies using gene-altered mice [4, 10].

LIF, a cytokine in the interleukin-6 family, is expressed in the uterus and plays critical roles in embryo implantation. LIF deficient mice reveal complete implantation failure [4]. In addition, the phenotype of implantation failure is reversed by recombinant LIF injection into the mutant females [4, 11]. LIF null embryos can develop normally and implant in the wild-type uteri after blastocyst transfer to wild-type recipients; however, wild-type embryos do not implant in LIF deficient uteri after blastocyst transfer to the null females [4, 12, 13]. These findings indicate that maternal LIF is critical for successful implantation.

Then how does LIF work in the uterus during implantation? Since LIF expression rapidly increases after estrogen injection in the uteri of ovariectomized mice, LIF is considered to be an estrogen responsive gene in the mouse uterus. Therefore, estrogen might be a major regulator of uterine LIF expression during implantation [9, 14]. In fact, LIF is expressed at the highest level on day 1 of pregnancy when the uterus is under the influence of a preovulatory estrogen surge. Thereafter, it is expressed in uterine glands on the morning of day 4, and then in the stroma

surrounding the blastocyst at the time of the attachment reaction on the night of day 4 and persists through the morning of day 5 [9]. Thus, LIF is expressed in day 4 pregnant uteri at two different times in two different cell types, and its expression is at low basal levels during the post-implantation period [9]. These findings indicate that LIF is not required for pregnancy maintenance but for implantation. Although previous studies show such evidence, the precise effects of maternal LIF on implantation, especially on blastocyst activation, at the molecular level remain unclear.

In our recent study, the mice with uterine specific p53 deletion show normal implantation in spite of the reduction of LIF expression on the morning of day 4 [15]. The stromal LIF expression pattern surrounding the blastocyst at the time of attachment at midnight on day 4 is normal in the mutant females [15], suggesting that the reduced LIF expression levels in p53-deleted uteri on the morning of day 4 is not a limiting factor for implantation. In addition, the CD1 mice with deficiency of PR cochaperone FKBP52 show implantation failure because of progesterone resistance, and have the reduced LIF expression at glandular epithelium on the morning of day 4 and at stroma on the night of day 4 [16]. Progesterone supplementation to the mutant mice can reverse both the phenotype of defective implantation and the stromal LIF expression at midnight on day 4, although the LIF expression at glandular epithelium on the morning of day 4 is still reduced after progesterone treatment [16]. These findings also suggest that the stromal LIF at midnight on day 4 might be more important than the epithelial one on the morning of day 4. Nonetheless, it is controversial where and when uterine LIF is expressed more critically on day 4 of pregnancy, and further investigations are required to clarify this issue (Fig. 3).

Recently, MSX homeobox genes are reported to be essential transcriptional regulators which morphologically modulate luminal epithelium and control normal implantation in mice [17]. Uterine deletion of both Msx1 and Msx2 completely inhibits blastocyst implantation [17]. LIF reduces uterine Msx1 expression and deficiency of Msx1/2 reduces LIF expression [17]. These findings suggest that MSXs are critical modulators in the system of uterine LIF expression in the peri-implantation period.

LIF binds LIF receptor which dimerizes with glycoprotein gp130, the common signaling receptor for IL-6 family cytokines, to activate several signaling pathways including the Jak–Stat pathway, the Ras–Raf–ERK pathway, and the PI3K–AKT pathway. In mouse embryonic stem (ES) cells, LIF up-regulates Klf4 through Jak–Stat3 pathway and Tbx3 through PI3K–AKT pathway, and strongly stimulates the expressions of Sox2 and Nanog to maintain the Oct3/4 expression [18]. In contrast, LIF also activates the Ras–Raf–ERK pathway to inhibit Tbx3 activity, suggesting that these downstream pathways of LIF coordinately regulate the differentiation of ES cells [18]. Compared with this, LIF does not activate ERK but Stat3 in luminal epithelium on day 4 morning [19], suggesting the tissue-selective activation of signaling pathways by LIF.

Osteopontin

Osteopontin, known as secreted phosphoprotein 1, is a glycoprotein and is involved in bone remodeling, leukocyte migration and endothelial cell attachment, and apoptosis [20]. OPN has an Arg-Gly-Asp (RGD) motif recognized by integrins, transmembrane heterodimeric cell-adhesion

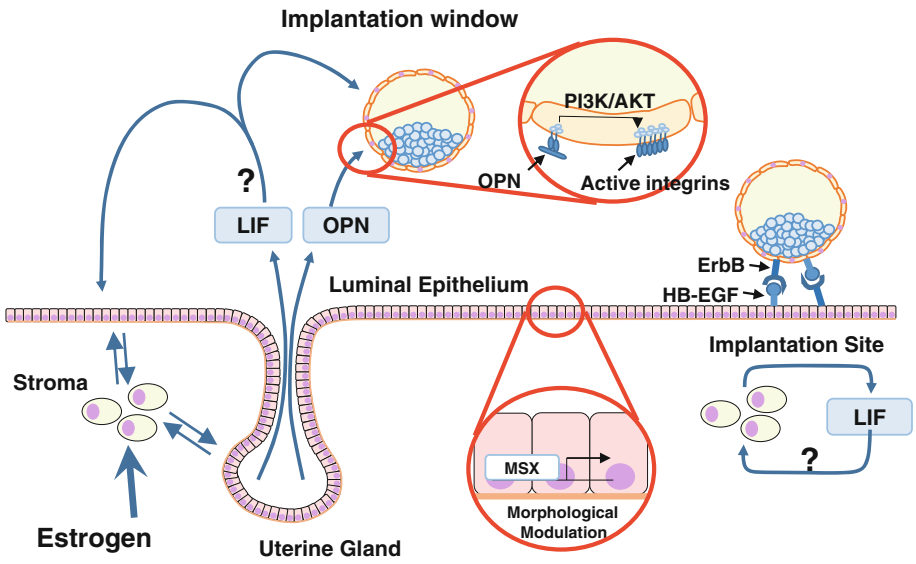

Fig. 3 The secretory factors control embryo implantation

proteins, to bridge cell-to-cell adhesion [20]. In pigs, trophoblast-derived estrogen stimulates OPN secretion from the uterus [21], and OPN bridges trophoblast-endometrial attachment through integrin αvβ6 of trophoblast and αvβ3 of luminal epithelium [22]. OPN is also expressed in rabbits, ewes, and humans in the peri-implantation period [23–25]. In mice, OPN is expressed at uterine glands in an estrogen-dependent manner immediately before implantation, stimulates integrins on the cell surface of trophoblast to activate an intracellular PI3K/AKT signaling pathway (Fig. 3) [6]. Since an activated blastocyst has more active integrins on the surface of trophectoderm than a dormant one [6], it is speculated that a major role of OPN in integrin is to activate a blastocyst. However, OPN null females do not show any reproductive defects and, therefore, other integrin activators must induce blastocyst activation corporately with OPN. In fact, both fibronectin and entactin, RGD motif-containing proteins, promote mouse blastocyst adhesion [26, 27]. Although OPN is recently reported to be one of mediators in embryo–uterine interactions, HB-EGF is the one which was initially identified and is the most well-known [1], and is fully described in the section "Heparin-binding epidermal growth factor-like growth factor".

Heparin-binding epidermal growth factor-like growth factor

Hormonal preparation for the receptive status with appropriate endometrial differentiation enables the uterine environment to proceed to the next phase of bidirectional molecular communications between the embryo and receptive uterus.

HB-EGF, one of the EGF family members, is known as a key player in the embryo–uterine interactions with the subsequent uterine attachment reaction [5]. It is expressed in the luminal epithelium located around active blastocysts some hours before the attachment [28]. HB-EGF is produced in soluble and transmembrane forms, and both forms affect blastocyst functions in an autocrine, paracrine, and/or juxtacrine manner [28, 29] via the EGF family of receptors which is expressed on the cell surface of trophectoderm [30, 31]. The soluble forms help to grow blastocysts [28], and the cell with transmembrane ones can adhere to the activated blastocyst [31] (Fig. 3). In addition, a recent mouse study shows that systemic deletion of HB-EGF leads to perinatal lethality [32], and its uterine deletion defers implantation and reduces litter size [32], emphasizing its importance in implantation.

HB-EGF and other EGF family members such as EGF, TGFα, betacellulin, epiregulin, neuregulin, and amphiregulin interact with the receptor subtypes of the ErbB family, ErbB1, ErbB2, ErbB3, and ErbB4, which have a tyrosine kinase domain for signal transduction. ErbBs form primarily homodimers or heterodimers to be activated by the ligands. Among these ErbB family members, ErbB1 and ErbB4 on the cell surface of trophectoderm can interact with uterine HB-EGF in embryo implantation in mice [30, 31]. The expression of both ErbB1 and ErbB4 is down-regulated in dormant blastocyst but is markedly up-regulated in the activated blastocyst [30, 33]. The activated blastocyst also expresses HB-EGF, which can induce HB-EGF transcripts in the uterus. These findings suggest the presence of a molecular feed-forward loop between the embryo and the uterus for the attachment reaction. Moreover, many studies also show significant roles of HB-EGF in human implantation. For example, the endometrial expression level of HB-EGF is the highest in the receptive epithelium [34, 35]. The cells expressing the transmembrane form of HB-EGF can adhere to human blastocyst displaying cell surface ErbB4 [36]. Taken together, HB-EGF is critical for embryo–uterine interactions during embryo implantation.

Conclusion

This review article described uterine receptivity and embryo–uterine interactions through key players such as ovarian hormones, HB-EGF, LIF and OPN. Since their detailed mechanisms remain vague as described above, many further investigations are required to clarify them. New future findings are expected to be applied in a clinical setting for infertility treatment and contraception.

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Conflict of interest The authors declare that they have no conflict of interest.

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